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Physiology, Phylogeny, and Functions of the TRP Superfamily of Cation Channels

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The transient receptor potential (TRP) protein superfamily consists of a diverse group of Ca^{2+} permeable nonselective cation channels that bear structural similarities to *Drosophila* TRP. TRP-related proteins play important roles in nonexcitable cells, as demonstrated by the recent finding that a mammalian TRPC protein is expressed in endothelial cells and functions in vasorelaxation. However, an emerging theme is that many TRP-related proteins are expressed predominantly in the nervous system and function in sensory physiology. The TRP superfamily can be divided into six subfamilies, the first of which is composed of the "classical TRPs" (TRPC subfamily). These proteins all share the common features of three to four ankryin repeats, ≥30% amino acid homology over ≥750 amino acids, and a gating mechanism that operates through phospholipase C. Some classical TRPs may be store-operated channels (SOCs), which are activated by release of Ca^{2+} from internal stores. The mammalian TRPC proteins are also expressed in the central nervous system, and several are highly enriched in the brain. One TRPC protein has been implicated in the pheromone response. The archetypal TRP, *Drosophila* TRP, is predominantly expressed in the visual system and is required for phototransduction. Many members of a second subfamily (TRPV) function in sensory physiology. These include VR1 and OSM-9, which respond to heat, osmolarity, odorants, and mechanical stimuli. A third subfamily, TRPN, includes proteins with many ankyrin repeats, one of which, NOMPC, participates in mechanotransduction. Among the members of a fourth subfamily, TRPM, is a putative tumor suppressor termed melastatin, and a bifunctional protein, TRP-PLIK, consisting of a TRPM channel fused to a protein kinase. PKD2 and mucolipidin are the founding members of the TRPP and TRPML subfamilies, respectively. Mutations in PKD2 are responsible for polycystic kidney disease, and mutations in mucolipidin result in a severe neurodegenerative disorder. Recent studies suggest that alterations in the activities of SOC and TRP channels may be at the heart of several additional neurodegenerative diseases. Thus, TRP channels may prove to be important new targets for drug discovery.

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Introduction

Stimulation of many nonexcitable cells with growth factors or hormones leads to activation of phospholipase C (PLC), production of inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG), and Ca^{2+} influx across the plasma membrane (*1*, *2*). Such Ca^{2+} influx may play an important role in processes ranging from T cell activation to apoptosis, cell proliferation, fluid secretion, and cell migration (*1*). Because of the prevalence and potential importance of PLC-dependent Ca^{2+} influx pathways, there has been considerable interest in identifying the relevant influx channels. Of particular interest to many investigators are the molecular identities of PLC-dependent channels that are activated by release of Ca^{2+} from internal stores. These so-called store-operated channels (SOCs) display a diversity of properties. Some, such as the CRAC (calcium-release-activated channel), are low-conductance, highly Ca^{2+}-selective channels (*3*, *4*), whereas others display much higher conductances and are nonselective cation channels (*5-7*). Nevertheless, the molecular identities of the relevant channels have been elusive.

Members of the transient receptor potential (TRP) superfamily of proteins have emerged as candidate channel subunits responsible for PLC-dependent Ca^{2+} influx. Mammalian TRPC proteins (*8*, *9*) would be expected to be expressed principally in nonexcitable cells because these are the cell types in which PLC-dependent Ca^{2+} influx has been primarily characterized (*1*, *2*). In fact, one mammalian TRPC is ubiquitously expressed and several are detected in multiple nonexcitable tissues. Nevertheless, all seven mammalian TRPC proteins are expressed in neurons, and several are highly enriched in the brain (*10*). Thus, the question arises as to the function of TRPC proteins in the mammalian nervous system. An equally intriguing question concerns the functions of a growing panoply of distantly related TRP proteins. Many of these proteins are also expressed in the nervous system, and there is evidence that a large proportion of the classical and TRP-related proteins participate in sensory physiology.

The identification of the superfamily of TRP channels has provided new insights into the structure and modes of activation of PLC-dependent channels. Direct interactions have been reported to occur in vitro between at least two TRPC proteins and the IP_3-receptor (IP_3R) (*11-14*) or the ryanodine receptor (*15*). If similar interactions occur in vivo, such phenomena would have important implications concerning the mechanisms through which TRPC channels are activated. Other studies indicate that the physical associations between TRP and other proteins are far more complex than previously imagined. The classical TRP proteins appear to be organized into macromolecular assemblies, the functions of which are just beginning to be understood.

The TRP Superfamily

The TRP superfamily is composed of six subfamilies, all of which include six putative transmembrane domains (Fig. 1A). The fourth transmembrane segment lacks the complete set of positively charged residues necessary for the voltage sensor in many voltage-gated ion channels (*16*). Within each TRP subfamily, there is a high level of primary amino acid sequence similarity; however, the relatedness between subfamilies is limited primarily to the transmembrane segments and a small region COOH-terminal to the sixth transmembrane domain. The number of subfamilies defined here is greater than the three suggested in a recent review (short-TRPs, long-TRPs, and osm-TRPs) (*17*) because there are now additional TRP-related proteins that form distinct groups. Furthermore, two of the previously named subfamilies, short- and long-TRPs (STRP and LTRP), are referred to here as TRPC and TRPM, respectively, because the former nomenclature may create confusion because of overlap in the lengths of the member proteins.

At least five of the six subfamilies include members that are conserved in animals as divergent as *Caenorhabditis elegans*, *Drosophila*, and humans. Representative members of most of the TRP subfamilies have been expressed in vitro, and each appears to be a nonselective cation channel. Nevertheless, the modes by which the various TRPs are activated appear to be quite diverse.

Classical TRPs (TRPC)

Drosophila *TRPs.* TRPC proteins contain three to four ankryin repeats and extensive amino acid homology to *Drosophila* TRP, and are PLC-operated nonselective cation channels. The founding member of the TRP family was discovered as a key component required for the light response in *Drosophila* photoreceptor cells. Mutations in *trp* cause the response to light to be transient (*18*) and result in a ~10-fold decrease in the level of light-induced Ca^{2+} influx (*19*). This phenotype, combined with the observation that fly vision requires PLC (*20*), raised the possibility that *trp* might encode the archetypal PLC-operated Ca^{2+} channel. This hypothesis was confirmed with the cloning and functional characterization of TRP. The gene *trp* encodes an eye-specific protein with four NH_2-terminal ankyrin repeats and an overall predicted topology similar to many members of the superfamily of voltage-gated and second-messenger-gated ion channels (*21*). Consistent with the structural similarities between TRP and known ion channels, in vitro studies have demonstrated that TRP is a cation channel with modest selectivity for Ca^{2+} relative to Na^+ (P_{Ca}:P_{Na} ~10:1) (see below) (*22-24*).

In addition to TRP, there exist two other TRP-related proteins in *Drosophila*, TRPL (*25*) and TRPγ (*26*), each of which shares ~45 to 50% amino acid identity with TRP over the NH_2-terminal 800 to 900 amino acids. The sequence similarity encompasses all six transmembrane segments and decreases after a highly conserved 25-amino acid segment of unknown function, termed the TRP domain (Fig. 1, A and B). The TRP domain includes an invariant sequence referred to as the TRP box (Glu-Trp-Lys-Phe-Ala-Arg) and a proline-rich motif that resembles the binding site for the scaffold protein Homer. The possibility that TRP may contain a Homer binding site is intriguing because Homer associates with the IP_3R (*27*). As is the case with TRP (*21*), both TRPL (*28*) and TRPγ (*26*) are highly enriched in photoreceptor cells. Thus, all the classical TRP family members in *Drosophila* are expressed predominantly in the visual system.

Analyses of loss-of-function and dominant negative forms of the *Drosophila* TRPs indicate that all three contribute to the light-dependent cation influx. Flies devoid of TRPL were originally reported to be indistinguishable from wild type (*28*). However, the light response in *trpl* mutant flies displays several differences from wild type, including changes in the permeability ratios for several cations (*29*) and a reduced response to a light stimulus of long duration (*30*). Double mutants lacking both TRP and TRPL are completely unresponsive to light (*28*), indicating that TRPγ cannot function independently of TRP and TRPL. TRPγ may function in combination with TRPL because the light response is nearly eliminated in *trp* mutant flies expressing a dominant negative form of TRPγ (*26*).

Mammalian TRPC proteins. The identification of TRP as the archetypal PLC-dependent Ca^{2+} channel raised the possibility that mammalian homologs of TRP, if they exist, might account for Ca^{2+} influx coupled to the stimulation of PLC. Of particular interest was whether mammalian TRP related proteins are SOCs and whether any encode the highly Ca^{2+} selective, low-conductance channels (CRAC channels) first characterized in mast cells and T cells.

A total of seven TRP-related isoforms have been described in mammals, referred to as TRPC(1-7) or TRP(1-7) proteins (*8*, *9*, *31-39*). Although both nomenclatures appear in the literature, TRPC is the designation assigned by the HUGO (Human Genome Organization) nomenclature committee and adopted by the Online Mendelian Inheritance in Man (http://www3.ncbi.nlm.nih.gov/Omim/) in order to distinguish them from TRP1, TRP2, and TRP3, the gene names previously established for human transfer RNA proline 1, 2, and 3, respectively. The seven TRPC proteins can be subdivided into four groups on the basis of their primary amino acid sequences (Table 1). As is the case for the three *Drosophila* TRPs, all of the TRPC proteins include three to four ankyrin repeats, six putative transmembrane domains, and amino acid sequence identity (≥30%) over the NH_2-terminal ~750 to 900 amino acids. As is the case with the *Drosophila* TRP proteins, the homology typically ends after the TRP domain, and the sequences of the mammalian TRPC proteins are quite variable in the region COOH-terminal to the TRP domain. However, the lengths of the COOH-terminal tails and the overall size of the mammalian TRPC proteins (Table 1) are typically smaller than the *Drosophila* TRPs (1124 to 1275 residues).

Five Subfamilies of Nonclassical TRP-Related Proteins

A diverse group of distantly TRP-related proteins has been described that can be subdivided into five classes (Table 2). All TRP-related proteins share significant homology to TRP in the transmembrane segments. The modes of activation and characteristics of the currents mediated by many of these channels have been described.

TRPV subfamily. The first class of TRP-related proteins is referred to as TRPV on the basis of the first identified member, *C. elegans* OSM-9 (*40*). This subfamily is the same as the one referrred to recently as OTRP (*17*); however, the nomenclature is changed slightly here because the prefix before "TRP" typically refers to the species designation. The proteins that comprise the TRPV subfamily typically contain three ankyrin repeats and share ~25% amino acid identity to TRPC proteins over a span that includes transmembrane segments V and VI and the TRP box (Fig. 1A). OSM-9 has not been functionally

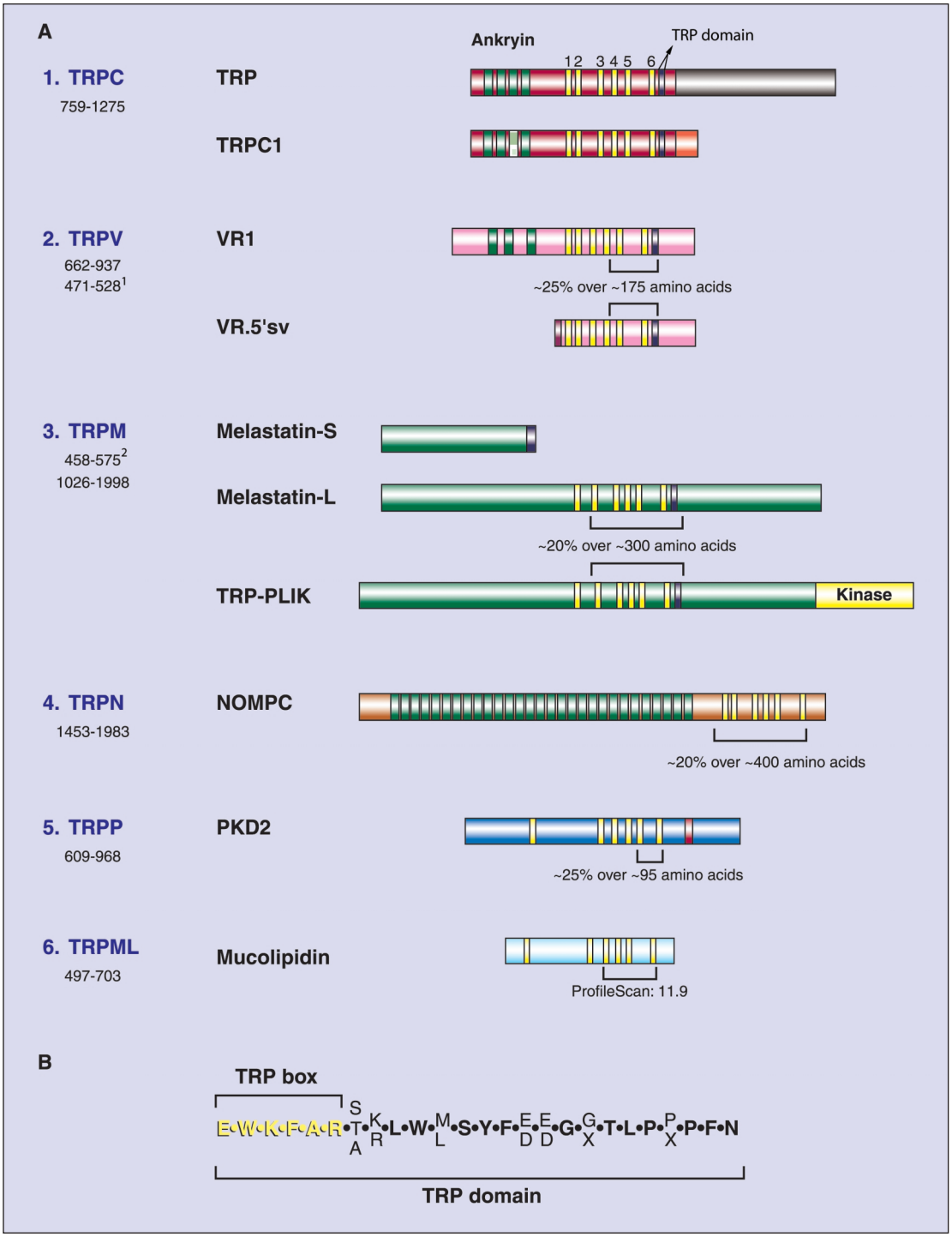

Fig. 1. The TRP superfamily. (**A**) Domain organization of the five TRP subfamilies. Several domains are indicated by small vertical colored rectangles as follows: ankyrin repeats, green; EF-hand, red; transmembrane segments, yellow; TRP box, blue. The third ankyrin repeat in the representation of the TRPC1 protein is highlighted differently to indicate that some TRPC1 isoforms contain three (*8, 9*), rather than four, repeats (*154*). The regions in the TRP-related subfamilies (TRPV, TRPM, TRPN, and TRPP) that share sequence identity to TRPC proteins or similarity to TRPC proteins using the algorithm ProfileScan (TRPML) (*86*) are indicated. The founding members of each subfamily and the lengths of proteins (in amino acids) are listed. [1]VR.5'sv is a truncated form of VR1. [2]Short Melastatin RNAs, which are generated by alternative RNA splicing, encode just a portion of the NH_2-terminal domain and none of the transmembrane segments (Melastatin-S). (**B**) The TRP domain. A highly conserved 25-amino-acid region in TRPC proteins that is COOH-terminal to the transmembrane segments [see (A)]. The nearly invariant TRP box is indicated in blue. X denotes any amino acid.

Group	Members	Length (amino acids)	% ID within same group	% ID to mTRPCs outside group	% ID to fly TRPs	Tissues with highest expression
1	TRPC1	759-810	N/A	31-44	33-36[4]	Heart, brain, testis, ovaries
2	TRPC2	Pseudo[1] 432[2] 876-1172	N/A	33-38	33-34	VNO, testis
3	TRPC3 TRPC6 TRPC7	828-848 815-931 862	71-79[3] 70-72 69-79	37-38 35-37 35-37	33-38 32-37 30-36	Brain Lung, brain Eye, heart, lung
4	TRPC4 TRPC5	836-1077 966-975	65 65	35-45 34-43	42-47 40-43	Brain Brain

Table 1. Four groups of mammalian TRPC proteins. In many cases, the lengths of the TRPC proteins differ due to alternative RNA splicing. With the exception of certain forms of TRPC2, all of the TRPC proteins are predicted to contain six transmembrane domains. The percent identities apply to the NH_2-terminal ~750 to 900 amino acids. This portion of the proteins includes all six transmembrane segments and the TRP domain. [1]Human TRPC2 is a pseudogene (*8*). [2]A bovine form of TRPC2 is predicted to encode only four transmembrane segments (*32*). [3]TRPC3 and TRPC7 share slightly greater amino acid identities to each other than to TRPC6. [4]TRPγ is the most related to each mammalian TRPC protein and then to a lesser degree to TRPL and TRP. TRPγ shares 50% amino acid identity to either TRP or TRPL over the NH_2-terminal ~800 residues. TRP and TRPL are 45% identical over the NH_2-terminal ~900 residues. ID, identity; mTRPC, mouse TRPC.

expressed in vitro; however, the second TRPV protein to be described, the human vanilloid receptor 1 (VR1), is a cation channel with significant preference for divalent cations such as Ca^{2+} and Mg^{2+} (*41*). A fascinating feature of VR1, and the characteristic used to identify the protein through an expression cloning strategy, is that it is activated by vanilloid compounds such as capsaicin that are present in spicy foods (i.e., hot chili peppers) (*41*). In addition, moderate heat (≥43°C) or protons (pH ≤5.9) can activate VR1. Protons decrease the heat threshold for activation of the cation conductance, suggesting that VR1 is a molecular integrator for multiple types of sensory input (*42*).

Recently, there has been a flurry of reports describing new mammalian members within the TRPV subfamily. These proteins are highly related but display distinct modes of activation (*43*). The heat-activated cation channel, VRL-1 (vanilloid receptor-like 1) requires a high heat threshold (≥52°C); however, in contrast to VR1, neither capsaicin nor acid activates it (*44*). A mouse protein that is ~80% identical to the rat VRL-1 growth-factor-regulated channel (GRC), participates in cation influx only after translocating from intracellular pools to the plasma membrane in response to insulin growth factor I (*45*). However, these studies were performed using an in vitro expression system, and it remains to be determined whether GRC displays a similar growth-factor-induced translocation in vivo. OTRPC4 (also referred to as VR-OAC and TRP12) is a human cation channel that is activated by decreases in osmolarity but not by heat or vanilloid compounds compounds (*46-48*). Other TRPV proteins include CaT1 (calcium transport protein 1) (*49*), and the highly related protein EcaC (epithelial Ca^{2+} channel) (*50*). Variations of VR1 have been reported that differ due to alternative mRNA splicing. A truncated isoform of VR1, VR.5′sv, contains all six transmembrane domains but is devoid of nearly the entire NH_2 terminus of VR1 (Fig. 1A) (*51*). VR.5′sv does not appear to function independently as a cation channel; thus, the question arises as to whether it serves as a regulatory subunit. A second truncated TRPV variant contains one rather than three ankyrin repeats. This isoform is a stretch-inactivated channel (SIC) and thus appears to be activated by cell shrinkage in response to hypertonic conditions (*43*). However, there is some question as to the mechanism by which the SIC messenger RNA (mRNA) is generated. The sequences of the NH_2- and COOH-terminal portions of SIC are the same as VR1 and VRL-2, respectively. Because VR1 and VRL-2 are encoded on different chromosomes, SIC may arise through an unconventional mode of trans-RNA splicing between two RNA precursors. Alternatively, the SIC cDNA may be an artifact resulting from recombination between the VR1 and VRL-2 cDNAs.

TRPM subfamily. A second subgroup of TRP-related proteins (TRPM) includes a putative tumor suppressor protein, melastatin (MLSN). *MLSN* was isolated in a screen for genes whose level of expression correlated with the severity of metastatic potential of variants of a mouse melanoma cell line (*52*, *53*). *MLSN* expression in the cell lines and in melanocytic neoplasms is inversely correlated with melanoma aggressiveness (*52*, *54*). Furthermore, down-regulation of MLSN RNA appears to be a prognostic marker for metastasis in patients with localized malignant melanoma (*55*). Another TRPM protein, TRP-p8, is expressed primarily in the prostate and, in contrast to MLSN, expression of TRP-p8 is elevated in tumors (*56*). TRP-p8 is most related to a TRPM protein that was unfortunately named TRPC7 (*57*) and should not be confused with the classical TRP, TRPC7, mentioned above (*39*). To minimize confusion, this TRPM protein will be referred to here as TRPM2. MTR1, which also belongs to this class, appears to be an imprinted gene and maps to a chromosomal region implicated in Beckwith-Wiederman syndrome, a complex disorder that is associated with an increased risk of developing neoplasms (*58*, *59*). Members of the TRPM subfamily also exist in *Drosophila* and *C. elegans*, and one such protein, CED-11, functions in programmed cell death in worms. Another *C. elegans* protein, GON-2, is required for mitotic cell

Subfamily	Protein	Accession numbers
TRPC	TRP (Dm)	AAA28976
	TRPL (Dm)	AAA28979
	TRPY (Dm)	CAB96204, CAB96205
	TRPC1	CAA61447, AAA93252
	TRPC2	X89067[1], AAD17195, AAD17196, AAG29950, AAG29951, AAD31453, CAA06964
	TRPC3	AAC51653
	TRPC4	CAA68125, BAA23599
	TRPC5	AAC13550, CAA06911, CAA06912
	TRPC6	NP_038866
	TRPC7	AAD42069
TRPV	OSM-9 (Ce)	T37241
	VR1, VR.5'sv	AAC53398, AAF28389
	SIC	BAA34942
	VRL-1, GRC	AAD26363, AAD26364, BAA78478
	OTRPC4, VR-OAC, TRP12, VRL-2	AAG17543, AAG16127, AAG28027, AAG28028, AAG28029, CAC20703
	CaT1	AAD47636
	ECaC	CAB40138
TRPM	GON-2 (Ce)	T23707
	CED-11 (Ce)	P34641
	MLSN	AAC13683, AAC80000
	TRPM2	BAA34700
	MTR1	AAF26288
	TRP-PLIK/ChaK	AAF73131
	TRP-p8	AC005538[2]
TRPN	NOMPC (Dm)	AAF59842
	NOMPC (Ce)	CAB61116.1
TRPP	PKD2	AAC50520
	PKD2L, PKDL, PCL	AAD08695, NP_057196
TRPML	ML4, MCOLN1	AAG00797, CAC07813, AAG10422

Table 2. Accession numbers of TRP-related proteins. The table includes only those members of the TRP superfamily that have been characterized and reported in research publications. Many other members of the TRP superfamily, which are predicted to exist on the basis of examination of the sequence databases using the BLAST algorithm, are not included in the compilation. Most of the TRP-related proteins listed are mammalian proteins. Those members of the TRP superfamily that were isolated from *D. melanogaster* (Dm) or *C. elegans* (Ce) are indicated. The accession numbers listed correspond only to the first vertebrate or invertebrate family members reported. Multiple accession numbers are due either to contemporanous publications or to proteins derived from alternatively spliced isoforms. Due to space limitations, accession numbers are not included for homologs in some organisms or for certain isoforms generated by alternative mRNA splicing. [1]Human TRPC2 is a pseudogene; therefore, the accession number corresponds to a nucleotide sequence rather than a protein sequence. [2]No protein or gene accession number is currently available for TRP-p8. The accession number listed for TRP-p8 corresponds to the nucleotide sequence of a cosmid that includes the TRP-p8 gene.

divisions of the gonadal precursor cells (*60*).

TRPM proteins share ~20% amino acid identity to TRP over a ~325 residue region that includes the COOH-terminal five transmembrane segments and the TRP domain (Fig. 1A). The NH_2-terminal domain of TRPM proteins, however, is devoid of ankyrin repeats and is considerably longer (~750 residues) than the corresponding regions in TRPC and TRPV proteins (~325 to 450 residues). The total length of TRPM proteins (~1000 to 2000 amino acids) varies primarily because of considerable diversity in the regions COOH-terminal to the transmembrane segments. However, an exception is MLSN-S, a short protein (~500 residues) encoded by one of the major MLSN mRNAs (Fig. 1A), which consists exclusively of the NH_2-terminal region of MLSN and is devoid of any predicted transmembrane segments (*52*, *61*). Given that NH_2-terminal fragments of the *Drosophila* and mammalian TRPCs can bind to and suppress the activities of full-length TRPC proteins (*24*, *26*, *62*), it is possible that MLSN-S may function to decrease the activity of full-length *Mlsn*.

The most notable variation in the COOH-terminal regions of TRPM proteins occurs in TRP-PLIK, a protein consisting of an NH_2-terminal region highly related to MLSN (>50% identical over 1250 residues) fused to a COOH-terminal protein kinase domain (*63*). The protein kinase domain, which was identified as a PLC-interacting kinase (PLIK), is also expressed as a separate 347-amino-acid protein independent of the MLSN domain. PLIK contains a FYVE (Fab1, YOTB, Vac1, and EEA1) domain zinc finger motif (*64*) and is most related to the atypical α-kinase family (*65*), which includes myosin heavy chain kinase A (*66*) and elongation factor-2 kinase (*67*). The protein kinase in

TRP-PLIK is critical for function because the nonselective cation channel activity displayed by the wild-type protein is obliterated upon mutation of either the ATP binding or the Zn^{2+}-finger motif in the PLIK domain (*62*). Considering that the protein kinase domain can bind to PLC, it is possible, although not proven, that activation of TRP-PLIK is a PLC-dependent phenomenon.

TRPN subfamily. The TRPN subfamily includes putative channels in *Drosophila* (referred to as NOMPC) and *C. elegans* with 29 ankyrin repeats NH_2-terminal to the six transmembrane segments (*68*, *69*). Because of these multiple repeats, NOMPC contains an extended NH_2-terminal domain of ~1150 amino acids and an overall length of ~1600 residues (Fig. 1A). TRPN proteins share ~20% amino acid identity to TRPC proteins over a ~400-amino-acid segment that spans the six transmembrane domains. However, TRPN proteins differ from TRPC, TRPV, and TRPM proteins in that they do not include a TRP domain. Furthermore, in contrast to the other five TRP subfamilies, TRPN proteins may be restricted to invertebrates, because vertebrate members of the TRPN group currently do not appear in the databases.

Drosophila NOMPC is most likely a subunit for a mechanically gated channel because it is expressed in mechanosensory organs and the mechanosensory response is greatly reduced in loss-of-function mutants (*69*). In addition, there exists a *C. elegans* TRPN protein that appears to be expressed in mechanosensory neurons (*69*). However, neither TRPN protein has yet been characterized in vitro, and it is not yet clear whether any of these proteins is capable of functioning independently as a channel.

TRPP subfamily. A TRP subfamily distantly related to the classical TRPs is TRPP, so named because of the founding member, PKD2. PKD2 was discovered as one of the gene products mutated in many cases of polycystic kidney disease (PKD) (*70*). PKD is an autosomal dominant disease that results in kidney failure in ~1 in 1000 individuals (*71*-*73*). TRPP proteins appear to be expressed throughout the animal kingdom and include human PKD2 (*70*), PKD2L (also referred to as PKDL) (*74*, *75*) and a related protein in *C. elegans*, LOV-2 (*76*). TRPP proteins share ~25% amino acid identity to the most closely related TRPC proteins, TRPC3 and TRPC6, over a region spanning transmembrane segments IV, V, and the pore-loop (H5 segment), which is a hydrophobic domain between segments V and VI that contributes to ion selectivity (*77*) (Fig. 1A). Mammalian PKD2 contains a Ca^{2+} binding motif (EF hand) and a coiled-coil domain near the COOH terminus, but does not include any ankyrin repeats or a TRP domain. In addition, TRPP proteins include a large extracellular loop between the first and second presumed transmembrane segments.

Human PKD2 interacts with PKD1 (*78*, *79*), and mutations in one or the other of these two proteins account for ~95% of autosomal dominant PKD (*71*). Moreover, the interaction of PKD2 with PKD1 appears to be critical for function. Introduction of PKD2 into Chinese hamster ovary (CHO) cells does not result in any discernible channel activity. However, co-expression of PKD1 along with PKD2 induces translocation of PKD2 to the plasma membrane and production of a Ca^{2+}-permeable nonselective cation conductance (*80*). PKD2L has also been functionally expressed and shown to be a nonselective cation channel that is positively regulated by intracellular Ca^{2+} (*81*). However, in contrast to PKD2, PKD2L displays Ca^{2+} influx activity in the absence of PKD1. Interestingly, human PKD2 is capable of heteromultimerizing with TRPC1 in vitro (*82*), although the features of a PKD2/TRPC1 heteromultimeric channel have not been described.

TRPML subfamily. The most recently identified subfamily of TRP-related proteins, TRPML, is defined by a human protein, mucolipidin1, encoded by the ML4 gene (*83*-*85*). Mutations in ML4 are responsible for a lysosomal storage disorder, mucolipidosis type IV, which leads to severe neurodegenerative defects. Although the disease primarily affects the nervous system, ML4 RNA is expressed in most tissues. Mucolipidin1 is small (580 residues), relative to other TRP-related proteins, and the level of primary amino acid sequence identity to TRPC proteins is quite limited. However, analysis of mucolipidin1 using ProfileScan (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html), an algorithm that compares proteins to known motifs and patterns (*86*), reveals a stronger relation to TRPCs than to other proteins in the databases. The similarity of mucolipidin1 (amino acids 331 to 521) to TRP spans the region that includes transmembrane segments 3 to 6 and the putative pore-loop region. Mucolipidin1 also has similarities to members of the TRPP subfamily such as a large extracellular loop between transmembrane domains 1 and 2 and ≥20% amino acid identity over a region that includes transmembrane segments 4 to 6. Other notable sequence motifs in mucolipidin1 include a lipase serine active site domain, a bipartite nuclear localization signal, and a putative late endosomal-lysosomal targeting signal.

Members of the TRPML subfamily are conserved in worms and flies (*83*-*85*). *Drosophila* and *C. elegans* each encode a single TRPML family member that shares 44% and 40% amino acid identity, respectively, over most of mucolipidin1. Within a domain encompassing transmembrane segments 3 to 6, the percent identity to these invertebrate homologs rises to nearly 60%. In addition to ML4, there is a second gene encoding a human TRPML protein, mucolipidin2, and this protein is only 497 amino acids in length (*83*-*85*). Nevertheless, as is the case with the other TRPML proteins, this latter protein is predicted to contain six transmembrane domains. Currently, none of the TRPML proteins has been functionally expressed. Given the short cytoplasmic domains NH_2- and COOH-terminal to the transmembrane segments, TRPML may depend on additional subunits for regulated activity.

Function of TRP-Related Proteins in Nonexcitable Cells

Store-operated Ca^{2+} selective and nonselective cation entry channels have been characterized in a wide variety of nonexcitable cells, including mast cells, T lymphocytes, platelets, pancreatic acinar cells, salivary gland cells, and vascular endothelial cells (*1*). As such, there has been considerable interest in determining whether store-operated channels play roles in the physiology of these cells. Mammalian TRP channels are candidates for mediating essential influx pathways because members of this superfamily are cation influx channels, many of which are expressed in nonexcitable cells. Nevertheless, until recently, direct evidence demonstrating functions for TRP channels has been lacking. However, recent studies provide the first indications that TRP channels indeed play critical roles in nonexcitable cells.

TRPC proteins function in vascular endothelial cells. Sustained Ca^{2+} entry in vascular endothelial cells leads to changes in cell shape (*87*) and affects vessel tone and permeability (*88*,

89), angiogenesis (*90*), and leucocyte trafficking (*91*). TRPC channels may mediate these Ca^{2+} entry pathways as different TRPCs are expressed in a variety of endothelial cells (*67*, *87*, *92-94*). Furthermore, a dominant negative form of TRPC3 inhibits store-operated Ca^{2+} entry (SOCE) in umbilical vein endothelial cells (*92*) and oxidant-induced cation influx in aortic endothelial cells (*67*). Most recently, the first mouse knockout of a TRPC protein, TRPC4, provided evidence for a TRPC protein in endothelial cell function (*94*). *TRPC4*$^{-/-}$ mice are viable and reach maturation, but they display impaired vasorelaxation of the aortic rings. This defect may be a consequence of a perturbation in SOCE, because agonist-induced Ca^{2+} influx is virtually eliminated in aorta endothelial cells isolated from the *TRPC4*$^{-/-}$ mice.

TRPC proteins are also expressed in a variety of other nonexcitable cells proposed to be regulated by SOCE. These include pancreatic beta cells (*95*), human platelets (*14*), rabbit portal vein smooth muscle (*96*), and salivary gland cells (97). TRPC6 is expressed in rabbit portal vein myocytes and introduction of TRPC6 antisense oligonucleotides to such primary cells inhibits the nonselective cation channel activated by α1-adrenoreceptor agonists (*96*). Because the α1-adrenoreceptor functions in the control of systemic blood pressure (*98*), it is possible that it may do so through activation of TRPC6. Furthermore, TRPC1 is a candidate for modulating the secretion of fluids and electrolytes in salivary glands as SOCE is reduced in salivary gland cells transfected with antisense TRPC1 RNA (*97*).

Requirement for mouse TRPC2 for the sperm acrosomal reaction. Fertilization of a mammalian egg is a multistep process that begins with association of the sperm with a glycoprotein, ZP3, in the egg's extracellular matrix (*99*). The sperm/ZP3 interaction triggers the release of hydrolytic enzymes from the sperm acrosome and remodeling of the sperm surface. These events, referred to as the acrosomal reaction, are critical for penetration of the egg by the sperm, ultimately leading to zygotic development. Association of the sperm with ZP3 initiates the acrosomal reaction through a signaling cascade that involves trimeric G proteins (heterotrimeric GTP binding proteins) (*100*), PLCδ4 (*101*), and activation of a store-operated, Ca^{2+}-permeable channel (*102*). The identity of the Ca^{2+} influx channel has been elusive; however, it now appears that mouse TRPC2 is an essential subunit of the ZP3 triggered channel. A TRPC2 isoform is highly enriched in the sperm, and antibodies to an extracellular domain of TRPC2 significantly inhibit the ZP3-induced Ca^{2+} influx and acrosomal reaction (*103*). Although TRPC2 appears to participate in the acrosomal reaction in the mouse, a different protein must usurp this function in humans because human TRPC2 is a pseudogene (*8*).

Potential roles of TRPV proteins in the kidneys and small intestines. Several members of the TRPV subfamily are expressed in the kidneys, one of which, OTRPC4 [also VRL-2, VR-OAC (VR-osmotically activated channel), and TRP12], is expressed predominantly in the distal nephron of the kidneys (*46-48*, *104*). OTRPC4 is activated by decreases in osmolarity; thus, it is intriguing that it is expressed in a region of the kidneys that may be exposed to hypotonic fluid (*46-48*). Based on these findings, OTRPC4 may participate in the regulation of electrolyte or fluid transport in distal nephron. The TRPV proteins ECaC and CaT1, which are expressed primarily in the kidneys or small intestines, may play important roles in Ca^{2+} absorption (*49*, *50*). Another TRPV protein, SIC, is also expressed in kidneys and is activated by cell shrinkage (*43*, *105*). This latter protein has been proposed to function in response to the mechanical stress induced by glomerular blood flow or intratubular urinary flow (*43*).

Renal disease due to defects in a TRP family member. Kidney failure in individuals with autosomal dominant PKD (ADPKD) results from the formation of renal cysts as a consequence of mutations in either PKD1 or PKD2 (*71*, *72*). These proteins are widely expressed (*70*, *106-108*), and cyst formation may arise in other tissues as well. Mice with targeted mutations in PKD2 die in utero and display cyst formation in the maturing nephrons and pancreatic ducts (*109*, *110*). In addition, there are defects in the cardiac septum. Thus, the mouse model recapitulates many of the features of human ADPKD. However, the molecular basis for cyst production and the normal functions of PKD2 remain obscure. Nevertheless, it is notable that mutations in either PKD1 or PKD2 result in similar clinical manifestations, and both proteins interact (*78*, *79*) and are required for cation influx (*80*).

Functions of the TRP Superfamily in the Nervous System

Potential functions of mammalian TRPC proteins in neurons. Because PLC- and store-operated Ca^{2+} entry pathways have been characterized mainly in nonexcitable cells (*1*), it was anticipated that TRPC proteins would function primarily in nonexcitable cells. Consequently, the expression patterns of the mammalian TRPC RNAs and proteins are surprising because each is expressed in the brain (*111*) and several, such as TRPC3 (*31*, *112*, *113*), TRPC4 (*113*), and TRPC5 (*36*, *37*), are highly enriched in the brain. Others, such as TRPC1, are expressed in a variety of tissues in addition to the central nervous system (*8*, *9*).

The neuronal expression of all the mammalian TRPC gene products suggests that PLC-dependent Ca^{2+} entry may function widely in the nervous system. One TRPC protein, TRPC2, may play a role in the pheromone response because in rodents it is expressed in the vomeronasal organ (VNO), which functions in the detection of pheromones (*34*, *114*, *115*). It is noteworthy that the VNO may not be functional in humans (*116*), and that human TRPC2 is a pseudogene (*8*).

TRPC3 may participate in activity-dependent changes that occur in the mammalian brain around the time of birth. In support of this proposal, TRPC3 is expressed primarily in the brain immediately before and after birth (*112*). Furthermore, TRPC3 is activated in vivo through a pathway that is initiated with the activation of the transmembrane receptor protein tyrosine kinase TrkB by brain-derived nerve growth factor (BDNF) (*112*). Neurotrophins such as BDNF are well known to initiate signaling pathways that function in neuronal differentiation and survival (*117*, *118*). These long-term effects typically function through changes in transcription and are observed many hours after exposure to the neurotrophins. However, there is now evidence that BDNF is involved in synaptic plasticity and can cause very rapid effects, such as morphological changes at the growth cone and modulation of neurotransmitter release (*119-121*). Because these effects are too rapid to occur through transcriptional induction, they may be mediated by BDNF-stimulated Ca^{2+} influx through TRPC3. Thus, one function of TRPC3 may be to facilitate activity-dependent synaptic plasticity that occurs in the mammalian brain around the time of birth.

Several nonclassical TRP proteins function in sensory physiology. The physiological functions of several nonclassical TRP

proteins have been identified, and an emerging theme is that many members of the TRP superfamily function in sensory perception. In addition to the well-characterized roles of the *Drosophila* TRP proteins in visual transduction, genetic analyses in model organisms demonstrate that many TRPV proteins also function in sensory responses. One such TRPV protein, *C. elegans* OSM-9, appears to expressed in a subset of chemo-, mechano-, and osmosensory neurons, and loss-of-function mutations in *osm-9* result in defects in olfaction, mechanosensation, and osmosensation (*40*). Another TRPV protein, human VR1, is expressed primarily in trigeminal and dorsal root sensory ganglia, both of which contain primary sensory neurons that respond to vanilloid compounds (*42*). Furthermore, mice lacking VR1 display defects in the response to capsaicin, acid, heat (>43°C), and thermal hyperalgesia (*122*, *123*). VRL1, another mammalian protein highly related to VR1, is also expressed in sensory ganglia and is activated by high (≥52°C) temperatures (*44*).

The mammalian osmosensor, OTRPC4 (VR-OAC or TRP12) is most highly expressed in the kidneys, although it may also function in the mammalian nervous system because it is expressed in a variety of neurosensory cells, including those of the central nervous system that respond to osmotic pressure, somatosensory cells, and mechanosensory cells of the inner ear (*46-48*). However, OTRPC4 is probably not the mechanotransduction channel of the inner ear because the properties of the OTRPC4-dependent conductance are inconsistent with those of the transduction channel.

Members of at least two of the remaining three TRP subfamilies may also function in sensory perception. As mentioned above, the TRPN proteins are expressed in mechanosensory organs, and disruption of the *Drosophila nompC* locus severely impairs mechanosensation (*69*). The normal functions of mammalian members of the TRPP subfamily are not known. However, the *C. elegans* homolog of PKD2 is localized to sensory neurons that function in male mating behavior. Currently, there are no mutations in this TRPP protein; although there are loss-of-function mutations in a locus that encodes a *C. elegans* relative of PKD1, *lov-1* (*76*). Mammalian PKD1 and PKD2 interact; thus, it is notable that LOV-1 is expressed in the same neurons as the TRPP family member and mutations in *lov-1* disrupts vulva location. Both LOV-1 and the PKD2-related protein may function in sensory perception; thus, chemo- and mechanosensation may be involved in vulva location.

TRP and neurodegeneration in the mammalian brain. Mutations in the TRPML protein, mucolipidin1 (*83-85*), cause a lysosomal storage disorder, mucolipidosis type IV, which leads to a variety of neurodegenerative defects (*124*, *125*). These include several ophthamolmogic abnormalities, such as retinal degeneration, strabismus, corneal opacity, and severe psychomotor retardation. In contrast to other lysosomal storage disorders, the disease does not appear to result from a disruption in catabolic enzymes (*126*). Instead, there appears to be a defect in membrane sorting or in a late step of endocytosis (*126*). However, a clear understanding of the biochemical basis of the disease may require characterization of mucolipidin in tissue culture systems and in model organisms.

Reductions in the activity of TRP channels and SOCE may be at the heart of other types of neurodegenerative disease. Two studies suggest that reduced SOCE may be an early event leading to Alzheimer's disease (*127*, *128*). Alzheimer's disease is commonly associated with the production of increased levels of a 42-amino-acid cleavage product (Aβ42) of a single-pass membrane protein, the amyloid precursor protein (APP) (*129*). Certain mutations in the presenilins, one of several proteins that participates in the cleavage of APP, lead to the generation of abnormally high concentrations of Aβ42. Cell lines that express these altered forms of the presenilins show lower levels of SOCE (*128*). Furthermore, application of a drug, SKF96365, which inhibits SOCE, results in a rise in production of Aβ42. SOCE is not increased in cells producing elevated levels of Aβ42, due to overexpression of APP (*128*). Thus, a reduction in SOCE may be a cause rather than an effect of increased levels of Aβ42.

An intriguing possibility is that an increase in TRP activity might also account for cell death in the mammalian brain due to metabolic stress caused by ischemia. *Drosophila* TRP and TRPL are constitutively active in vivo under anoxic conditions or as a result of application of mitochondrial uncouplers or depletion of ATP (*130*). Furthermore, mutations that cause constitutive activation of TRP result in neurodegeneration in *Drosophila* photoreceptor cells (*131*). Oxidative stress may also result in activation of mammalian TRPC proteins. Endothelial cells express an oxidant-activated nonselective cation channel that functions as a redox sensor in the vascular endothelium, and a dominant negative form of TRPC3 abolishes the oxidant-induced current (*67*). These experiments suggest that either TRPC3 or a channel capable of heteromultimerizing with TRPC3 contributes to this conductance. On the basis of these studies, oxidative stress in the mammalian brain could potentially result in constitutive activation of TRPC proteins, which in turn could result in cell death due to uncontrolled influx of Ca^{2+}. If such a phenomenon occurs, it is plausible that drugs that inhibit TRPC proteins would offer a new therapy for minimizing the neurodegeneration associated with strokes and other traumas that induce oxidative stress.

Lastly, members of the TRPV subfamily are potential targets for drug therapy. The discovery that at least two TRPV proteins, VR1 and VRL-1, function in pain pathways (*41*, *44*, *122*, *132*) offers the possibility that agents that specifically inhibit such proteins may provide new avenues for pain management.

Activation Mechanisms of the Classical TRPs

All members of the TRPC subfamily are activated through signaling pathways that are coupled to PLC. Despite the high level of relatedness among the TRPC subfamily, there may not be a single unifying mechanism by which stimulation of PLC leads to activation of TRPC channels. Some TRPC channels appear to be activated by DAG or polyunsaturated fatty acids (PUFAs), whereas others seem to require release of Ca^{2+} from internal stores.

Activation of Drosophila *TRP is independent of the IP_3R.* TRP is capable of functioning as a SOC because TRP can be activated in tissue culture systems using drugs such as thapsigargin that cause release of Ca^{2+} from the internal Ca^{2+} stores (*22-24*). Thapsigargin treatment results in Ca^{2+} release because it inhibits the smooth endoplasmic reticulum Ca^{2+}-ATPase that normally counterbalances the constant leak current from the Ca^{2+} stores (*133*, *134*). The residual response to light in *trp* flies was proposed to be due to normal release of Ca^{2+} from the internal stores (*135*). Furthermore, it was suggested that the response was not sustained due to absence of store-operated Ca^{2+} influx (*135*); however, more recent studies described below

show that these assumptions are most likely incorrect.

Despite the observation that TRP appears to function as a SOC in vitro (*22-24*), the preponderance of evidence indicates that TRP is not activated through a store-operated mechanism in vivo. Introduction of either thapsigargin (*136*, *137*) or IP_3 (*138*) to photoreceptor cells does not activate cation influx. In addition, the *Drosophila* genome encodes a single relative of the mammalian IP_3R (*139*, *140*), and mutations that eliminate this gene have no discernible effect on the photoresponse (*141*, *142*). Although the IP_3R is dispensable for TRP function, it cannot be excluded that TRP is store-operated through a pathway involving another Ca^{2+} release channel. In fact, there exists a second Ca^{2+} release channel, the ryanodine receptor, which is distantly related to the IP_3R (*139*, *143*). However, as is the case for the IP_3R, there is only one ryanodine receptor homolog in *Drosophila*, and mutations in this locus have no impact on phototransduction (*144*). Thus, TRP function is not dependent on either of the known Ca^{2+} release channels.

An alternative proposal is that activation of TRP is coupled to PLC activity through production of DAG, rather than through the generation of IP_3 and subsequent activation of the IP_3R. Consistent with this proposal, PUFAs, which can be derived from DAG, lead to activation of TRP either in vitro or after application to isolated *Drosophila* photoreceptor cells (*145*). In addition, TRP is constitutively active in a mutant, *rdgA*, that disrupts an eye-enriched DAG kinase (*146*). These results were interpreted as additional evidence that PUFAs gate TRP, because elimination of the DAG kinase should, in principle, result in higher levels of PUFAs. However, it has not been demonstrated that the levels of PUFAs are increased in *rdgA*, and it cannot be excluded that the effects of PUFAs on TRP may be indirect. TRP could be activated by PUFAs either as a consequence of nonspecific effects on the plasma membrane or as a result of oxidative stress. Long-chain unsaturated fatty acids have been shown to uncouple mitochondria (*147*, *148*), and anoxic conditions result in activation of TRP (*130*). Thus, the mechanism through which activation of PLC is coupled to activation of TRP remains unresolved.

Given that Ca^{2+} release does not appear to function in *Drosophila* visual transduction, the transient response to light in *trp* mutant flies could be due to rapid Ca^{2+}-dependent inactivation of the remaining influx channels in *trp* mutant photoreceptor cells (*149*). Consistent with this proposal, mutation of one of the calmodulin binding sites in TRPL results in a sustained rather than a transient light response in *trp* mutant flies (*149*). Furthermore, it was reported that the *trp* photoresponse was similar to wild-type cell response in the absence of extracellular Ca^{2+}. However, this latter result has been challenged (*150*).

An intriguing proposal that may account for the transient light respone in *trp* flies is depletion of the substrate for PLC, phosphatidylinositol 4,5 bisphosphate (PIP_2) in *trp* photoreceptors (*151*). Using the inwardly rectifying K^+ channel Kir2.1 as a biosensor, it appears that PIP_2 levels are lower in *trp* than wild-type photoreceptor cells. The decreased levels of PIP_2 are proposed to be a consequence of a requirement for Ca^{2+} influx to down-regulate PLC activity and up-regulate PIP_2 recycling (*151*). However, direct evidence that the PIP_2 levels are reduced in *trp* photoreceptor cells and that this decrease results in the *trp* phenotype remains to be demonstrated.

Heteromultimeric interactions among Drosophila *TRP family members.* Several observations strongly indicate that TRPL and TRPγ function exclusively as subunits of heteromultimeric channels. Expression of either TRPL (*152*) or TRPγ (*26*) in tissue culture cells results in a constitutively active cation conductance, indicating a requirement for interaction with another protein for proper regulation. Furthermore, the three TRP family members interact in vitro in pairwise combinations and co-immunoprecipitate in vivo (*24*, *26*). Binding between TRPL and TRPγ is mediated at least in part by a coiled-coil domain NH_2-terminal to the transmembrane segments in TRPγ (*26*). TRPL and TRPγ are unlikely to form homomultimers in vivo because they have a greater propensity to interact with TRP than with themselves, and TRP is ~10-fold more abundant in vivo. Although TRPL and TRPγ are both constitutively active in vitro, co-assembly of the two proteins results in a PLC-dependent cation conductance (*26*). TRP, in contrast to TRPL and TRPγ, appears to form regulated homomultimers in vivo, in addition to functioning as a subunit of heteromultimeric channels.

Heteromultimeric interactions may also occur among mammalian TRPC channels. Each of the TRPC proteins has been expressed in tissue culture cells, and in many cases expression of these proteins results in the appearance of constitutive cation influx [for example, see (*39*, *153*, *154-159*)]. The activity of these TRPC proteins is suggestive of the constitutive influx resulting from in vitro expression of either *Drosophila* TRPL or TRPγ. TRPL and TRPγ co-assemble to produce a regulated PLC-operated channel (*26*); thus, it is plausible that TRPC proteins are channel subunits that depend on interactions with other TRPC proteins for regulated activity. Consistent with this proposal, a TRPC3-dependent conductance endogenous to pontine neurons is not constitutive; rather, it is activated through a signaling pathway involving TrkB and PLCγ (*112*). Whether TRPC3 interacts with another TRPC protein in vivo has not been addressed, although TRPC3 does interact in vitro with TRPC1 (*24*). However, in contrast to TRPL-TRPγ heteromultimers, co-expression of TRPC1 and TRPC3 in tissue culture cells generates a larger constitutively active conductance than do either of the individual proteins (*160*). Thus, if TRPC1 and TRPC3 form heteromultimers in vivo, they may include additional subunits to form regulated channels.

TRPC1 also appears to be capable of forming functional heteromultimers with either TRPC4 or TRPC5. TRPC4 and TRPC5 co-immunoprecipitate with TRPC1 from rat brains (*161*). Moreover, co-expression of either TRPC4 or TRPC5 with TRPC1 in tissue culture cells results in the production of nonselective cation conductances distinct from those generated by expression of the individual proteins (*161*). The TRPC1-TRPC4- and TRPC1-TRPC5-dependent conductances are augmented by activation of receptors that engage G_q proteins (G protein family of α subunits that controls PI-specific PLs), but not by release of Ca^{2+} from internal stores. However, constitutive activity occurs in the absence of receptor activation. Thus, as is the case with TRPC1-TRPC3 heteromultimers, it is likely that additional subunits interact with and participate in the regulation of TRPC1-TRPC4 and TRPC1-TRPC5 heteromultimers in vivo. An important challenge will be to identify conductances in the mammalian brain that are mediated by the various TRPC1 heteromultimeric channels.

Activation mechanisms of mammalian TRPC channels. A common feature of the mammalian TRPC channels is that they are activated or augmented in vitro through pathways that engage PLC [for example, see (*31*, *37-39*, *114*, *155-157*)]. All of

the TRPC-dependent conductances are nonselective cation channels, although there are differences in the permeabilities of Ca^{2+} relative to Na^{+} and other cations (*17*). As with the *Drosophila* TRPs, a controversial issue concerns the mechanism through which stimulation of PLC, and production of IP_3 and DAG activates or potentiates TRPC-dependent conductance. Several TRPC proteins, such as TRPC1, -2, -4, and -5, appear to be activated through a store-operated mechanism because application of IP_3 or thapsigargin results in increases in cation influx in tissue culture cells expressing any one of these proteins (*31*, *35*, *36*, *114*, *154*).

The mechanism underlying SOCE is unresolved; however, the prevailing view is that it involves conformational coupling between the IP_3R and the influx channels (*162*). According to this model, there is a direct interaction between the IP_3R, situated in the intracellular Ca^{2+} stores, and the Ca^{2+} influx channels in the plasma membrane. Upon release of Ca^{2+} from the internal stores, there is a change in conformation in the IP_3R that induces a conformational shift in the store-operated channels, resulting in activation of Ca^{2+} influx. In support of the conformation coupling model is the demonstration that manipulations that interfere with access of the endoplasmic reticulum to the plasma membrane preclude SOCE and TRPC3 activation in vitro (*163*, *164*). Activation of some SOCs might involve exocytosis of the channels from intracellular vesicles to the plasma membrane (*165*), because inhibitors of vesicular trafficking block SOCE (*166*). Furthermore, SOCE is prevented by inhibition of a protein, SNAP-25, that is required for the fusion of vesicles with their target membranes (*167*).

In contrast to some TRPC channels that may be SOCs, other TRPC proteins, notably TRPC6 and 7, are activated in vitro by DAG (*39*, *168*). These results are reminiscent of the report that PUFAs activate *Drosophila* TRP channels (*145*). However, it remains unclear whether DAG and PUFA function directly or indirectly in gating TRP channels. Indirect activation of TRPC proteins by DAG could occur through production of long-chain fatty acids metabolites, which can lead to mitochondrial uncoupling. Metabolic stress induced by mitochodrial uncoupling can activate TRPC proteins (*67*), as is the case with *Drosophila* TRP (*130*).

The findings that some TRPC channels may be store-operated while others may be activated through production of DAG would suggest that different TRPC proteins are gated through distinct mechanisms. However, such a conclusion becomes murky with regard to TRPC3. According to one report, activation of TRPC3 depends on production of DAG (*168*), whereas another study indicates that TRPC3 is store-operated (*31*). Conformational coupling may activate TRPC3 because TRPC3 interacts directly with the type I IP_3R in vitro (*11*). The association between TRPC3 and the IP_3R occurs through two regions in the IP_3R, which are situated between the NH_2-terminal IP_3 binding site and the transmembrane domains, and a small portion of TRPC3 COOH-terminal to the transmembrane domains (*12*, *13*). Additional evidence consistent with the conformational coupling model is that introduction of IP_3 and the IP_3R appeared to restore regulation of TRPC3 by IP_3 in excised patches after the native IP_3R was removed by extensive washing (*11*). Direct interactions between the IP_3R and TRPC channels may be a common phenomenon because TRPC1 can co-immunoprecipitate with the type II IP_3R from human platelets (*14*). Evidence has also been presented that Ca^{2+} release via another Ca^{2+} release channel, the ryanodine receptor, can also lead to activation of TRPC3 (*15*). Distinct TRPC3 channels appeared to be functionally coupled to either the ryanodine receptor or the IP_3R, but not both (*15*).

The disparate observations that TRPC3 may be store-operated in some studies and gated by DAG in others may reflect differences in the cell types used for the expression studies [human embryonic kidney (HEK)- and CHO-derived cell lines, respectively]. Different cell lines may express distinct sets of endogenous proteins that interact with TRPC3 and affect its mode of regulation. Thus, it is critical to characterize the modes of regulation controlling TRPC proteins in vivo. Unfortunately, there is a paucity of such studies because of the difficulties inherent in ascribing native conductances to specific TRPC channels. One native TRPC3-dependent conductance current has been characterized from the brains of neonatal rats and was shown to activate a signaling pathway that involves the neurotrophin BDNF, its receptor TrkB, and PLCγ (*112*). This native conductance, I_{BDNF}, is not activated by DAG and is eliminated by inhibitors of the IP_3R. Thus, at least one endogenous TRPC3 conductance appears to require activity of the IP_3R and is not gated by DAG.

The enigmatic CRAC channel. Neither I_{BDNF} nor any of the TRPC-dependent conductances analyzed in vitro displays the high Ca^{2+} selectivity and other properties of I_{CRAC} (*2*). In principle, I_{CRAC} could be mediated by channels unrelated to TRP, by TRPC heteromultimers, or by heteromultimers consisting of a TRPC protein in combination with a protein weakly related to TRP. Alternatively, a homomultimer consisting of a relative of TRPC proteins might function as a CRAC channel. This latter possibility may be the case, because expression of the TRPV protein CaT1 in tissue culture cells results in the production of a current that displays many of the salient features of I_{CRAC}. These include high Ca^{2+} selectivity, loss of selectivity in the absence of divalent cations, and an activation mechanism that is dependent on depletion of Ca^{2+} stores (*169*). Although expression of CaT1 leads to a current similar to I_{CRAC}, it is unclear whether the CRAC channel is comprised of CaT1 homomultimers or heteromultimers consisting of CaT1 in combination with another subunit expressed in the tissue culture cells.

Association of TRPC Channels into Macromolecular Assemblies

The Drosophila *signalplex.* An emerging theme is that members of the TRP superfamily exist in macromolecular assemblies composed of multiple signaling components. The existence of a TRP-containing supramolecular signaling complex (signalplex) was first demonstrated in *Drosophila* photoreceptor cells (*20*). The molecular scaffold for the signalplex is INAD (inactivation-no-afterpotential D), a protein that consists of five ~90-amino-acid protein domains referred to as PDZ (PSD-95, DLG, zonular occludens-1) domains. INAD binds directly to a minimum of seven proteins that function in phototransduction (Fig. 2A). These include TRP (*170*, *171*), TRPL (*172*), PLCβ (*171*, *173*), rhodopsin (*172*, *173*), protein kinase C (PKC) (*172*, *174*), calmodulin (*172*, *173*), and the NINAC (neither-inactivation-nor-afterpotential C) myosin III (*175*). In addition, INAD is capable of forming homomeric interactions (*172*), thus providing the binding capacity to simultaneously nucleate a large array of target proteins.

Of primary importance is the identification of the functions of the signalplex. Because light-dependent cation influx occurs

within milliseconds of activation, it would seem that coupling of the signaling components into a macromolecular assembly would serve to facilitate rapid activation. However, deletion of the INAD binding site in TRP has no effect on the kinetics of activation (*176*). Thus, a direct association of TRP with INAD is not required for the light response. Nevertheless, it has not been excluded that TRP could also associate indirectly with the signalplex and that such an interaction could contribute to activation.

It appears that one role of the signalplex is to retain signaling proteins in the microvillar portion of the photoreceptor cells, the rhabdomeres. In wild-type photoreceptor cells, the proteins that participate in phototransduction are highly enriched in the rhabdomeres (*20*). As such, the rhabdomeres are the functional equivalent of the outer segments in mammalian photoreceptor cells. In *inaD* mutant flies, the localizations of at least three INAD targets, TRP, PKC, and PLC, are severely disrupted (*173*, *177*). INAD appears to function in retention rather

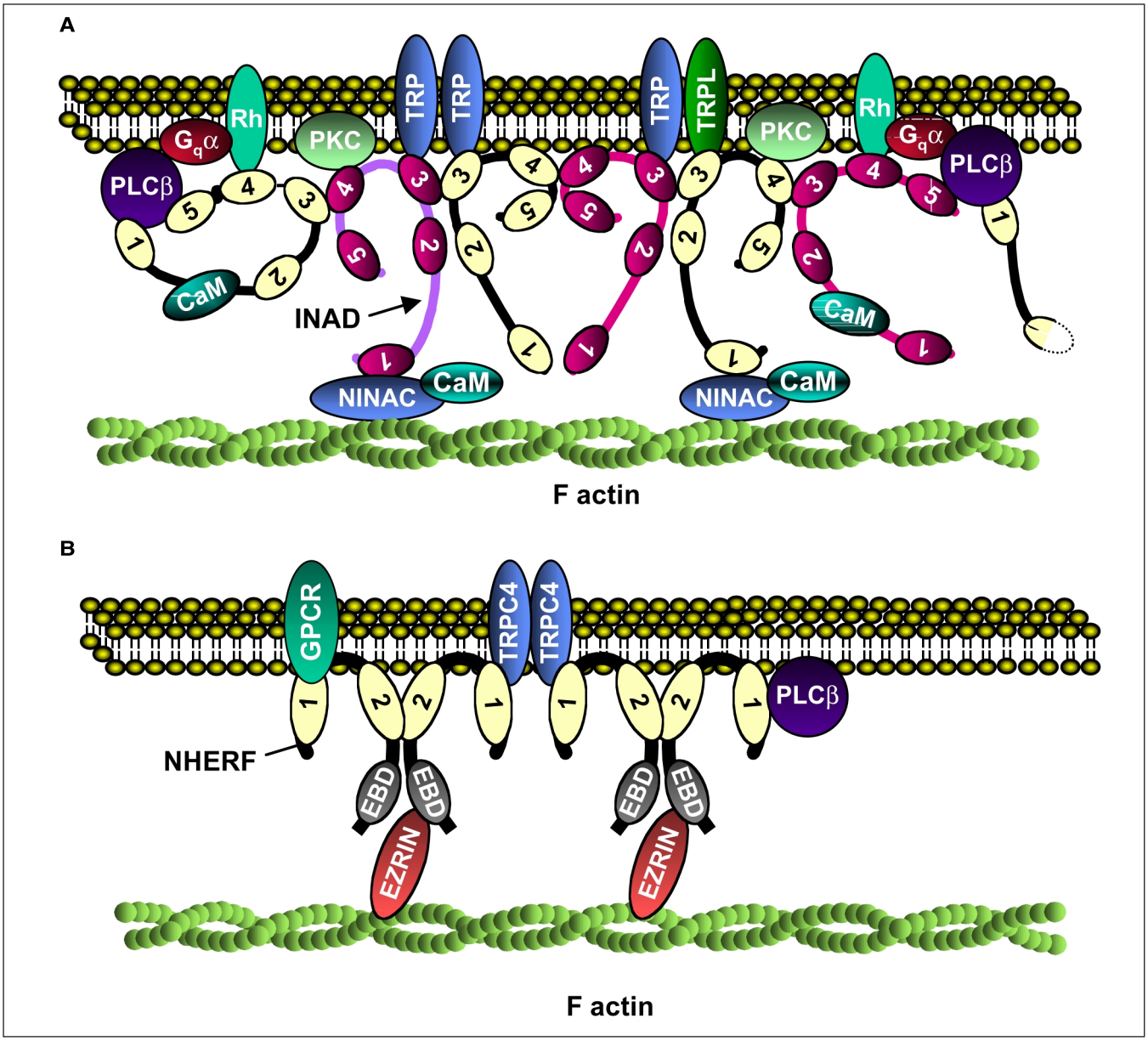

Fig. 2. Classical TRP proteins associate with signaling complexes. (**A**) Model of the *Drosophila* signalplex. INAD is a scaffold protein with five ~90 amino acid PDZ domains that binds directly to TRP, TRPL, PLCβ, PKC, rhodopsin (Rh), the NINAC myosin III, and calmodulin (CaM). The signalplex could be linked to actin filaments through NINAC. INAD is also capable of forming homomultimers. (**B**) Speculative model of the TRPC4 signalplex. TRPC4 has been shown to bind directly to a protein, NHERF (also known as EBP50), containing two PDZ domains and a COOH-terminal domain that is an ERM binding domain (EBD). Although NHERF can bind to at least one G protein-coupled receptor (GPCR) and members of the ERM family, the TRPC4 signalplex, has not been shown to include a GPCR, ezrin, or any other actin binding protein. Thus, these latter interactions are speculative. The complexity of the NHERF signalplex may be increased by homomultimerziation of NHERF and TRPC4. Reprinted, with permission, from the *Annual Review of Cell and Developmental Biology*, Volume 15, copyright 1999, by Annual Reviews (http://www.AnnualReviews.org).

than targeting of these proteins to the rhabdomeres (*176*). In addition, elimination of INAD or the INAD binding sites in TRP, PKC, or PLC results in instability of these INAD binding proteins (*176-178*).

The requirement for the TRP-INAD interaction for retention in the rhabdomeres appears to be reciprocal. Mutation of the INAD binding site in TRP results in mislocalization of INAD and, as a consequence, mislocalization of PLC, PKC, and TRP (*176*). However, elimination of any other known INAD binding protein has no effect on the localization of INAD. Thus, it appears that TRP and INAD form the core complex required for retention of the signalplex in the rhabdomeres.

Decreases in the concentration of signaling proteins in the rhabdomeres, due to disruption of INAD-target protein interactions, have at least two consequences on phototransduction. First, the overall amplitude of photoresponse is reduced (*177*). Second, a reduction in the levels of PLC result in slower response termination (*178*, *179*). This defect may be due to loss of the proper stoichiometry between the PLC and the G protein (*179*). The relative concentrations of these two proteins are critical because the PLC functions as a GTPase-activating protein for the trimeric $G_q\alpha$ subunit (*179*, *180*), in addition to its more recognized phospholipase activity (*181*). A reduction in the levels of PLC results in delayed termination, due to slower inactivation of the G protein. Thus, the signalplex maintains both the proper stoichiometry and absolute concentrations of signaling proteins in the rhabdomeres.

A key question is whether the association of any target protein with INAD functions directly in the photoresponse, independent of any requirement for retention or protein stability. Disruption of the INAD binding site in PKC decreases the rate of termination of the photoresponse (*182*), though this effect may be due to mislocalization of PKC. However, interaction of INAD with NINAC has a direct role in signaling. Mutation of the INAD binding site in NINAC has no impact on its expression or rhabdomeral localization, but causes a profound delay in termination (*175*). The basis for the requirement for the NINAC/INAD interaction for response termination is not known, although the observations that NINAC binds actin and that INAD associates with both NINAC and TRP raise the possibility that actin or myosin force generation functions in turning off the light-sensitive cation channels.

Organization of mammalian TRPC proteins into supramolecular complexes. Mammalian TRPC proteins also appear to be organized into macromolecular assemblies. For example, TRPC3 is activated through a pathway initiated by TrkB, and TRPC3 immunoprecipitates with the BDNF receptor from rat brains (*112*). This interaction is most likely indirect, although the molecular link between these two proteins has not been identified.

TRPC1 may also associate with a multicomponent complex and do so in a subset of lipid rafts referred to as caveolae. Lipid rafts are glycosphingolipid- and cholesterol-enriched membrane microdomains that appear to concentrate certain transmembrane proteins and proteins with glycosylphosphatidylinositol anchors or hydrophobic modifications (*183-186*). Caveolae are invaginations in the plasma membrane that form through coalescence of lipid rafts. These latter specialized portions of the membrane may have particular importance in Ca^{2+} signaling because they are enriched with a variety of proteins that participate in Ca^{2+} regulation, and may be sites for Ca^{2+} entry and sequestration (*187*). Caveolin, a transmembrane cholesterol-binding protein that is concentrated in caveolae (*188-190*), may be a scaffolding protein that nucleates signaling complexes [reviewed in (*191*)]. TRPC1 appears to be localized to caveolin-containing lipid rafts and co-immunoprecipitates with caveolin, the IP_3R, and $G_q\alpha$ from a salivary gland cell line (*192*). Furthermore, thapsigargin-induced Ca^{2+} influx is disrupted in this cell line upon depletion of cholesterol from the plasma membrane. Because cholesterol depletion disrupts lipid raft domains, this suggests that TRPC1 function is dependent on association with caveolae. However, there is no direct evidence that the current was mediated by TRPC1, and it remains to be determined whether TRPC1 binds directly to caveolin.

Recent evidence indicates that TRPC4 and TRPC5 associate with macromolecular complexes that bear similarities to the *Drosophila* signalplex (Fig. 2B) (*193*). The central protein in these complexes is the Na^+/H^+ exchanger regulatory factor (NHERF, also referred to as EBP50), a protein containing two PDZ domains (*194*, *195*). In addition to TRPC4 and TRPC5, NHERF also binds in vitro to PLCβ (*193*). Moreover, TRPC4, PLC, and NHERF co-immunoprecipitate from the brain cells of mice. PLC and TRPC4 are unlikely to bind to the same NHERF monomer because they both interact through PDZ1. As with INAD, NHERF appears to self-associate, and such homomultimerization could provide NHERF with the capacity to cluster an array of proteins (*193*). The complexity of the NHERF signalplex could be further increased by multimerization of TRPC4 or TRPC5 (Fig. 2B). Other known targets for NHERF include a G protein-coupled receptor (*196*) and members of the ezrin-radixin-moesin (ERM) family (*197*), which could provide a link to the actin cytoskeleton. It remains to be determined whether these latter classes of proteins are complexed with the same NHERF molecules that associate with TRPC4 and PLCβ. If so, then mammalian TRPC proteins may be organized into signaling complexes that resemble the *Drosophila* signalplex. The next challenge will be to determine whether such assemblies contribute to signaling, as well as to the localization and stability of the component proteins, as is the case in *Drosophila* photoreceptor cells.

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