TRP Channel Proteins and Signal Transduction

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Minke, Baruch, and Boaz Cook. TRP Channel Proteins and Signal Transduction. Physiol Rev 82: 429–472, 2002; 10.1152/physrev.00001.2002.—TRP channel proteins constitute a large and diverse family of proteins that are expressed in many tissues and cell types. This family was designated TRP because of a spontaneously occurring Drosophila mutant lacking TRP that responded to a continuous light with a transient receptor potential (hence TRP). In addition to responses to light, TRPs mediate responses to nerve growth factor, pheromones, olfaction, mechanical, chemical, temperature, pH, osmolality, vasorelaxation of blood vessels, and metabolic stress. Furthermore, mutations in several members of TRP-related channel proteins are responsible for several diseases, such as several tumors and neurodegenerative disorders. TRP-related channel proteins are found in a variety of organisms, tissues, and cell types, including nonexcitable, smooth muscle, and neuronal cells. The large functional diversity of TRPs is also reflected in their diverse permeability to ions, although, in general, they are classified as nonselective cationic channels. The molecular domains that are conserved in all members of the TRP family constitute parts of the transmembrane domains and in most members also the ankyrin-like repeats at the NH\(_2\) terminal of the protein and a “TRP domain” at the COOH terminal, which is a highly conserved 25-amino acid stretch with still unknown function. All of the above features suggest that members of the TRP family are “special assignment” channels, which are recruited to diverse signaling pathways. The channels’ roles and characteristics such as gating mechanism, regulation, and permeability are determined by evolution according to the specific functional requirements.
I. INTRODUCTION

The role of plasma membrane channel proteins is to regulate the flow of different ions between the cell and its environment. Each channel has a typical permeability to different ions, conductance, mechanism of opening and closing (gating), typical agonists and antagonists, voltage dependence, and additional regulatory characteristics. The gating of channels may be direct by voltage or ligand binding, or indirect via cascade of molecular events and production of second messengers that lead to channel opening. A large volume of data and detailed understanding has been accumulated on voltage- and ligand-gated channels. However, channel proteins of the TRP family do not strictly fit to any of the above categories. The activation and regulation mechanisms of TRP channels are largely unknown and highly diverse. Channel activity is affected by several physical parameters such as osmolarity, pH, mechanical force, as well as biochemical interactions with external ligands or cellular proteins.

The structure of TRP channels relates them to the superfamily of voltage-gated channel proteins characterized by six transmembrane segments (S1-S6), a pore region (between S5 and S6), and a voltage sensor, although the voltage sensor in S4 is missing in TRPs. Functionally, they may be categorized as nonselective cation channels, although they demonstrate large diversity in the characteristics of their permeability to ions and some of them are highly selective for Ca\(^{2+}\). TRP channels have been found in many organs, mainly in the brain but also in heart, kidney, testis, lung, liver, spleen, ovaries, intestine, prostate, placenta, uterus, and vascular tissues. They have been found in many cell types, including both neuronal cells, such as sensory, primary afferent neurons and non-neuronal tissues such as vascular endothelial cells, epithelial cells, and smooth muscle cells. Functionally, the most prominent cellular signaling pathways in which TRPs play a role are also mediated via PLC. (for reviews, see Refs. 70, 107, 111, 118, 119, 152, 165).

A. Drosophila Phototransduction Is a Model System for Studying TRP

The archetypal TRP protein was found and extensively studied with relation to Drosophila phototransduction (107, 111, 118, 165). The phototransduction cascade provides a unique and powerful model system to study functions of specific signaling proteins with relevance to many transduction cascades. Studying phototransduction in Drosophila offers many advantages. The small size of the genome, ease of growth, and short generation make Drosophila ideally suited for screening large numbers of mutagenized individuals. Furthermore, the creation of powerful genetic tools such as balancer chromosomes and P-element-mediated germline transformation combined with tissue-specific promoters allow the introduction of cloned genes back into the organism, providing a way to study in vitro mutagenized genes in the living fly (107, 175). In Drosophila it is thus easy to screen for mutants that have physiological or morphological defects (133). Studies of single cells using electrophysiology and microfluorimetry allow characterizing different functional aspects of responses in great detail. The high sensitivity to light allows detection and characterization of responses to activation of single receptor molecules. Moreover, responses to higher light intensities enable the study of fast response kinetics and adaptation. Several important and novel signaling proteins have been identified in Drosophila, and their role was established using the genetic dissection approach (107, 111, 118, 165). TRP is an illuminating example for a novel signaling protein of prime importance whose function has been elucidated due to the powerful genetic tools and functional tests that have been developed in Drosophila. These studies have led to identification and characterization of TRP as a light-sensitive and Ca\(^{2+}\)-permeable channel (60, 126, 140), although the mechanism that leads to channel opening is not yet clear. The studies of Drosophila phototransduction have thus indicated that TRP is permeable to Ca\(^{2+}\), and it is the target of a phosphoinositide cascade, leading to the suggestion that phototransduction in Drosophila might be analogous to the general and widespread process of phosphoinositide-mediated Ca\(^{2+}\) influx (61).

B. Inositol Lipid Signaling Mediates Signal Transduction Cascades

Inositol lipid signaling is one of the most widespread signal transduction cascades, which exists in virtually every eukaryotic cell. This transduction cascade that is mediated via G protein-activated phospholipase C (PLC) produces two second messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (InsP\(_3\)) (14). This cascade begins by activation of a surface membrane receptor, followed by activation of InsP\(_3\) receptors in the endoplasmic reticulum (ER), leading to Ca\(^{2+}\) release, and ends by the opening of surface membrane channels and Ca\(^{2+}\) influx, which activates a large variety of specific and critical cellular functions (Fig. 1). A great deal is known about the initial stages of this ubiquitous cascade; however, the molecular mechanism underlying activation and gating of the channels is elusive (152).

Currently, the surface membrane nonexcitable Ca\(^{2+}\) influx channels are classified into two categories: store-operated channels (SOC) (100, 152) and store-independent channels (70, 152). Both channels require PLC for...
their activation. SOC channels are activated by the reduction in stored Ca$^{2+}$. Store-independent channels include channels that are activated by Ca$^{2+}$ elevation in the cytosol due to Ca$^{2+}$ release and channels that are activated by the DAG branch of the inositol-lipid signaling pathway. In both cases the mechanism of channel activation is unknown (107). The Ca$^{2+}$ release activated current ($I_{\text{CRAC}}$) represents activation of the classical SOC channels (134), and recently a TRP-related channel was suggested as a molecular candidate for $I_{\text{CRAC}}$ channels (215). The paradigm that has been used to demonstrate SOC channel activation rather than store-independent channels is based on a bypass of InsP$_3$ production. Thus depletion of the internal stores by a specific Ca$^{2+}$-ATPase inhibitor, thapsigargin, which is known to deplete InsP$_3$ sensitive stores (83) or by the Ca$^{2+}$ ionophore ionomycin in Ca$^{2+}$-free medium in the presence of strong Ca$^{2+}$ buffers in the cell, activates SOC channels. Accordingly, after store depletion, application of Ca$^{2+}$ to the external medium results in Ca$^{2+}$ influx. The store-independent channels are activated by signaling mechanisms not directly related to the Ca$^{2+}$ stores and, therefore, are not sensitive to thapsigargin or other Ca$^{2+}$-mobilizing agents.

At present, the only candidates for the inositide-mediated Ca$^{2+}$ influx channels, which include both SOC channels and store-independent channels, are proteins of the TRP family. In this review we emphasize the importance of studying TRP functions and properties in native cells and, therefore, first outline the research on TRP and TRP homologs that have been performed on Drosophila photoreceptors. Then we describe the fast-growing literature on a wide variety of TRP homologs that have been recently found in other species. The in vivo properties and possible functions of TRP channels and their
relevance to signal transduction processes are the focus of this review.

II. HISTORICAL BACKGROUND

A. The trp Phenotype

A spontaneously occurring Drosophila mutant was designated transient receptor potential (trp) by Minke et al. (113) because of its unique phenotype. In this mutant the response to prolonged illumination declines to baseline during light (38, 106, 113) (Fig. 2). Drosophila photoreceptors of wild-type and trp mutants have become a preparation of extensive research because of the ability to study TRP within the well-characterized phototransduction cascade in the native tissue (63, 107, 116, 157).

The trp phenotype was originally explained by the hypothesis that some critical factor, which is required for excitation and needs to be constantly replenished during illumination, is in short supply in the mutant and cannot be replenished fast enough due to a mutation in the trp gene. Indeed, several lines of evidence indicate that the decline of the light response in trp mutants is due to exhaustion of excitation. Accordingly, the response to continuous intense light becomes equivalent to a response to dim light and then to darkness during illumination (106, 113).

A correlation between the trp phenotype and exhaustion of cellular Ca$^{2+}$ was provided by exposure of isolated Drosophila ommatidia to Ca$^{2+}$-free medium for a long (1 h) period of time in cells buffered with EGTA to ~50 nM intracellular Ca$^{2+}$. The typical trp phenotype was obtained under such conditions (60). Similar apparent cellular Ca$^{2+}$ deprivation could be obtained in the isolated ommatidia of wild-type (WT) Drosophila during a critical period of development. At this developmental stage (P14), no response to light can be observed unless Ca$^{2+}$ (µM) is applied by the whole cell recording pipette. Interestingly, the light response under such conditions has the typical characteristics of the trp phenotype (65). Presumably under such conditions cellular Ca$^{2+}$ is the limiting factor of excitation as in La$^{3+}$-treated or Ca$^{2+}$-deprived WT cells.

A recent study has proposed that the transient photoreponse of the trp mutants is due to Ca$^{2+}$-dependent inactivation of the TRP-like (TRPL) channels rather than exhaustion of Ca$^{2+}$-dependent excitation (164). According to this model, light-induced influx of Ca$^{2+}$ through the TRPL channels (which remain operative in the trp mutant and have a considerable permeability to Ca$^{2+}$) activates calmodulin (CaM), which in turn binds to CaM binding sites of TRPL (147) and deactivates the channels. In contrast, other experiments revealed that the trp phenotype is exacerbated in Ca$^{2+}$-free medium, thus indicating that the negative feedback hypothesis probably does not underlie the trp phenotype (37). This conclusion was strongly supported by Hardie et al. (67), who found that trp decay and the associated inactivation are correlated with a drastic Ca$^{2+}$-dependent reduction of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) levels in the microvilli, while recovery from inactivation is abolished in mutants of the PIP$_2$ recycling pathway (rdgB and cds).

A turning point in understanding the trp phenotype and the possible role of TRP in phototransduction was brought about by the discovery that lanthanum (La$^{3+}$) mimics the trp phenotype in WT flies (72, 181).

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**FIG. 2.** The trp phenotype. Light-induced currents in response to prolonged intense orange lights were recorded in voltage-clamped photoreceptors of wild type (WT), the trp mutant, and WT treated with La$^{3+}$. A peak response and a plateau characterize the light response of WT. The rapid peak-plateau transition is a manifestation of Ca$^{2+}$-dependent light adaptation. The response of the trp photoreceptor decays close to baseline during light due to exhaustion of excitation. A similar decay of the light response close to baseline is obtained by application of 10 µM La$^{3+}$ to the extracellular medium of WT photoreceptors. The horizontal bar indicates the light monitor. The intensity of the orange light stimulus is 10-fold dimmer in WT than in the trp mutant or WT treated with La$^{3+}$. [From Minke and Selinger (111).]
1. \( \text{La}^{3+} \) mimics the trp phenotype in WT

Application of \( \text{La}^{3+} \) in micromolar concentrations to the extracellular space of several species of WT flies accurately mimics the trp phenotype (Fig. 2) (60, 72, 181). The rate of occurrence of the single photon responses decreases in \( \text{La}^{3+} \)-treated WT flies with increasing intensity of long light stimulation in a similar manner to the effect of light found in the mutant. Furthermore, application of \( \text{La}^{3+} \) to the mutant has virtually no effect (181). The effect of \( \text{La}^{3+} \) is reversible upon removal of \( \text{La}^{3+} \) from the external solution. Because \( \text{La}^{3+} \) is a known nonspecific Ca\( ^{2+} \) channel blocker, it was suggested that exhaustion of excitation in \( \text{La}^{3+} \)-treated WT flies or in the trp mutant, which underlies the trp phenotype, is Ca\( ^{2+} \) dependent. Thus, with the assumption that cellular Ca\( ^{2+} \) is required for sustained excitation, it has been suggested that a TRP-mediated mechanism is responsible for replenishing cellular Ca\( ^{2+} \) fast enough during strong illumination and, therefore, TRP is a Ca\( ^{2+} \) channel/transporter (109, 110).

2. TRP and TRPL are light-activated channels

Whole cell voltage-clamp recordings applied to isolated Drosophila ommatidia (55, 156) revolutionized the field of Drosophila phototransduction and made it possible to examine the hypothesis that TRP is a Ca\( ^{2+} \) channel/transporter (109). The experiments of Hardie and Minke (60) revealed that the fundamental defect in the trp mutant is a change in the ionic selectivity of the light-sensitive conductance. The relative Ca\( ^{2+} \) permeability of the light-sensitive conductance in trp mutant was reduced by a factor of \( \sim 10 \) (60, 151, 158), along with a significant change in the relative permeability to different monovalent ions. The reduced Ca\( ^{2+} \) permeability in the trp mutant has also been corroborated by the demonstration of a reduced light-induced Ca\( ^{2+} \) influx using Ca\( ^{2+} \) indicator dyes (59, 140) or Ca\( ^{2+} \)-selective microelectrodes (139). The effect of the trp mutation on ionic selectivity could be mimicked in WT photoreceptors by bath application of \( \text{La}^{3+} \) in micromolar quantities (60, 181).

The effects of the trp mutation on the permeability properties of the light-sensitive channels and the fact that in null trp mutants a significant response to light is preserved led Hardie and Minke (60, 61) to propose that there are at least two light-sensitive channels. Accordingly, one channel has the permeation properties of the channels remaining in the trp mutant, and a second class has high Ca\( ^{2+} \) permeability, which is absent in trp mutants and blocked by \( \text{La}^{3+} \).

A significant step forward in the study of TRP became possible after the trp gene was cloned and sequenced by Montell and Rubin (121) and described as encoding a novel membrane protein. Subsequent reanalysis of the sequence by Kelly and colleagues (147) revealed significant homologies of the transmembrane domain to voltage-gated Ca\( ^{2+} \) channels, the dihydropyridine receptors, and thus raised the hypothesis that TRP and its homolog, TRPL, may be channel proteins (147). A second putative channel gene, trp-like (trpl), was isolated using a screen for calmodulin binding proteins and was found to show \( \sim 40\% \) overall identity with trp (147; for review, see Refs. 111, 116). This homology suggested that trpl might encode a second class of light-sensitive channel, responsible for the light-induced current recorded in the trp mutant (60, 147). Subsequent isolation of a null mutant of the trpl gene strongly supported the hypothesis that TRPL functions as a second light-sensitive channel (126). Under physiological conditions, the trpl mutation has a relatively small but distinct influence on the light response (92). Importantly, eliminating TRP-dependent current in trpl, either by \( \text{La}^{3+} \) or by examining the double mutant trpl;trp, results in complete abolishment of the response to light. Therefore, TRP and TRPL channels make up all the light-activated channels or are required for their activation (for details, see Refs. 158, 164; for review, see Refs. 107, 111, 116). The isolation of the null trpl mutant allows investigating whether the trp phenotype is a property inherent in the TRPL channels (164). An answer to this question was provided by the creation of the double mutants in which only a small amount of functional TRP channels remain (126, 158). Interestingly, these mutant alleles show the typical trp phenotype (92), indicating that the trp phenotype does not arise only from a specific property unique to the TRPL channel, as previously suggested (164), but also from low level of TRP in the absence of TRPL.

B. Mammalian TRP Homologs

The first report that TRP-related proteins might also be found outside invertebrate photoreceptor came from Petersen et al. (143), who identified partial sequences of TRP homologs from Xenopus oocyte and murine brain cDNA libraries. Shortly thereafter, the full sequence of a human homolog (TRPC1) was reported following homology searches of EST databases (199, 216). Meanwhile, a large number of vertebrate TRP homologs have been cloned and sequenced (see Table 1). The functional role in the native tissues of vertebrate TRP homologs that have high similarity to Drosophila TRP is largely unknown. They show very widespread tissue distribution, but their properties have been mainly inferred from heterologous expression studies. The function of other members of the TRP family with low identity to Drosophila TRP can be hypothesized from their specific tissue distribution and production of knockout mice, which suggests important functions in both neuronal and nonneuronal cells (Table 1).
<table>
<thead>
<tr>
<th>Channel</th>
<th>Number of Amino Acids</th>
<th>Identity to TRP, %</th>
<th>Expression Pattern</th>
<th>Cloned From the Following Species</th>
<th>Reference Nos.</th>
</tr>
</thead>
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<tr>
<td><strong>TRP-homolog channels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>TRP</td>
<td>1,275</td>
<td></td>
<td>Photoreceptors of <em>Drosophila</em>; antenna of <em>Drosophila</em> during development</td>
<td><em>Drosophila, Calliphora</em></td>
<td>32, 79, 80, 94, 121, 126, 148, 164, 170, 187, 188, 214</td>
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<tr>
<td>TRP-L</td>
<td>1,124</td>
<td>39</td>
<td>Photoreceptors of <em>Drosophila</em></td>
<td><em>Drosophila</em></td>
<td>126, 147, 164, 187, 198, 208</td>
</tr>
<tr>
<td>TRP-γ</td>
<td>1,128</td>
<td>54</td>
<td>Photoreceptors of <em>Drosophila</em></td>
<td><em>Drosophila</em></td>
<td>207</td>
</tr>
<tr>
<td>TRPC1</td>
<td>759 (β-Isomorph)</td>
<td>40 (Human)</td>
<td>Brain, heart, ovary, testes (change during development)</td>
<td><em>Xenopus, mouse, rat, bovine, human</em></td>
<td>30, 50, 98, 99, 173, 185, 200, 205, 206, 218, 221</td>
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<td>TRPC2</td>
<td>778 (Xenopus)</td>
<td>25–30 (Mouse)</td>
<td>Bovine: testes, spleen, liver; mouse: testes; rat: vomeronasal organ</td>
<td><em>Bovine, rat, mouse</em></td>
<td>84, 96, 114, 192, 218</td>
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<td>TRPC3</td>
<td>848 (Human)</td>
<td>34</td>
<td>Brain mainly, also in low amount in intestine, lung, prostate, placenta, testis</td>
<td><em>Human, rat, mouse</em></td>
<td>19, 51, 95, 114, 183, 217, 218</td>
</tr>
<tr>
<td>TRPC6</td>
<td>920 (Rat)</td>
<td>37 (Mouse)</td>
<td>Brain, but also in lung, ovary</td>
<td><em>Rat, mouse, rabbit</em></td>
<td>16, 19, 81, 114, 218</td>
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<tr>
<td>TRPC7</td>
<td>862 (Mouse)</td>
<td>33 (Mouse)</td>
<td>Heart, lung, eye, but also in lower amount in brain, spleen, testes</td>
<td><em>Mouse</em></td>
<td>131</td>
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<tr>
<td>TRPC4</td>
<td>974 (Mouse)</td>
<td>41 (Rabbit)</td>
<td>Brain, but also in adrenal, heart, lung, spleen, kidney, testis, uterus, aorta</td>
<td><em>Bovine, rat, mouse</em></td>
<td>122, 143, 144</td>
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<tr>
<td>TRPC5</td>
<td>975 (Mouse)</td>
<td>36 (Mouse)</td>
<td>Brain, olfactory bulb</td>
<td><em>Rabbit, mouse</em></td>
<td>132, 145</td>
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<td>OSM-9</td>
<td>937</td>
<td></td>
<td>Offactory, mechanosensory and osmosensory neurons</td>
<td><em>C. elegans</em></td>
<td>36</td>
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<tr>
<td>VR1 (TRPV1)</td>
<td>828 (Rat)</td>
<td>23 to OSM-9</td>
<td>Dorsal root ganglia, trigeminal nerve</td>
<td><em>Human, mouse, rat</em></td>
<td>25, 27</td>
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<td>VRL-1</td>
<td>761 (Rat)</td>
<td>78 to VR1</td>
<td>Spinal cord, spleen, lung, brain, intestine, vas deferens</td>
<td><em>Rat, human, mouse</em></td>
<td>26</td>
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<tr>
<td>GRC (TRPV2)</td>
<td>756</td>
<td>80 to VRL-1</td>
<td>Spleen, lung, brain</td>
<td><em>Mouse</em></td>
<td>86</td>
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<td>OTRPC4 (TRPV4)</td>
<td>871 (Mouse)</td>
<td>41 to VR1</td>
<td>Kidney, heart, liver</td>
<td><em>Mouse</em></td>
<td>177</td>
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<td>ECAc (ECaCl)</td>
<td>730 (Rabbit)</td>
<td>30 to VRL-1</td>
<td>Intestine, kidney, placenta</td>
<td><em>Rabbit</em></td>
<td>73</td>
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<tr>
<td>CaT2 (TRPV5)</td>
<td>723</td>
<td>84 to ECaC</td>
<td>Kidney, heart</td>
<td><em>Rat</em></td>
<td>137</td>
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<tr>
<td>CaT1 (ECaC2)</td>
<td>727 (Rat)</td>
<td>73 to CaT1</td>
<td>Intestine, brain, thymus, adrenal gland</td>
<td><em>Rat</em></td>
<td>138</td>
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<tr>
<td>CaT-L (TRPV5)</td>
<td>725</td>
<td>90 to CaT1</td>
<td>Placenta, exocrine pancreas, salivary gland, prostate cancer</td>
<td><em>Human</em></td>
<td>202</td>
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<td>Polycystin-2 (TRPP)</td>
<td>968 (Human)</td>
<td>23 to CaT1 within amino acids 596–877</td>
<td>Kidney</td>
<td><em>Human</em></td>
<td>54</td>
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<td>PCL (TRPP)</td>
<td>605</td>
<td>50 to polycystin-2</td>
<td>Kidney</td>
<td><em>Mouse</em></td>
<td>11, 31</td>
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<td>Mucolipin-1 (TRPML)</td>
<td>580 (Human)</td>
<td>38 to TRP within amino acids 331–521</td>
<td>Ubiquitous</td>
<td><em>Human</em></td>
<td>10, 12, 179</td>
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<td>NOMPC (TRPN)</td>
<td>1,619</td>
<td>~20% to TRPC channels over ~400 amino acid residues of TMD</td>
<td><em>Drosophila bristles</em></td>
<td><em>Drosophila</em></td>
<td>196</td>
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<tr>
<td>LTRPC7 (TRP.PLIK) (TRPM7)</td>
<td>1,862</td>
<td>~20 to TRP over ~325 amino acid residue region including, TMD, TRP domain</td>
<td>Brain, hematopoietic cells</td>
<td><em>Mouse</em></td>
<td>123, 159</td>
</tr>
<tr>
<td>LTRPC2 (TRP.M2)</td>
<td>1,503</td>
<td>~20 to TRP over ~325 amino acid residues</td>
<td>Brain, bone marrow, spleen, heart, leukocytes, liver, lung, kidney, prostate, testis, skeletal muscle, ubiquitous</td>
<td><em>Human</em></td>
<td>141, 160</td>
</tr>
</tbody>
</table>
III. MOLECULAR ANALYSIS OF THE TRP FAMILY

A. Molecular Structure and Localization of Drosophila TRP and TRPL

The trp gene encodes a 1,275-amino acid membrane protein (121). Expression of trp genomic DNA in a trp mutant (trp<sup>CM</sup>) background by germ-line transformation rescued the mutant phenotype (120), indicating that the gene is responsible for the trp mutant phenotype. The molecular structure of the TRP proteins as depicted in Figure 3 shows multiple domains that are likely to play important roles in cellular functions.

1. Ankyrin repeats may regulate TRP activity

At the NH<sub>2</sub> terminal of TRP there are three or four ankyrin repeats (147). Ankyrin repeats are 33-amino acid
residue motifs that mediate specific protein-protein interactions with a diverse repertoire of macromolecular targets (166). Ankyrin has been shown to inhibit InsP3 and ryanodine receptor-mediated Ca\(^{2+}\) release from the internal stores (21, 22). Ankyrin also provides a mechanism for linking membrane proteins to the cytoskeleton and plays a role in subunit interactions of proteins with two or more subunits (105). If TRP is a subunit of an oligomeric channel, the ankyrin repeat may play a role in subunit interactions or in TRP localization. Importantly, the ankyrin repeat is highly conserved throughout evolution in most members of the TRP family, strongly suggesting that it plays an essential, although still unknown, role in TRP function.

2. TRP displays transmembrane region typical for voltage-gated channels

The most prominent structure of TRP is the transmembrane segments S1-S6 and the pore region loop between transmembrane segments S5 and S6, which is typical of voltage-gated channels. The identification of this region was the first molecular confirmation of the hypothesis that TRP may function as a channel (147). Furthermore, parts of this sequence are the most conserved region of TRP in all members of this family (see below).

3. The TRP domain

A highly conserved region of TRP is found COOH terminal to S6 and includes 25 amino acids, six of which (Glu-Trp-Lys-Phe-Ala Arg) are referred to as the TRP box because of their invariant sequence. The function of this unique region is still unknown (119).

4. TRP contains a CaM binding domain

TRP contains a putative CaM binding domain somewhere in the region of the COOH terminal (residues 628–977) (147). A more defined fragment of the TRP sequence has been reported to bind CaM in a Ca\(^{2+}\)-dependent manner (32). Sequence analysis suggests that the most likely CaM binding site in this region would be residues 705–725, which constitute the equivalent location to the CaM binding site in TRPL, even though there is no amino acid identity in this region between TRP and TRPL (see below). Drosophila photoreceptors contain an abundant amount of CaM and have specific proteins (NINAC) that stock-pile large amounts of CaM in the cell (149), yet the function of CaM in Drosophila phototransduction seems to be diverse and not entirely clear (6, 164).

5. TRP contains a PEST region but has a slow turnover rate

The COOH-terminal region near the putative CaM binding domain contains a PEST sequence, which is a signal for protein degradation by the calcium-dependent protease calpain, typical of CaM binding proteins. Thus it has been expected that TRP will show a fast turnover rate. In contrast to this expectation, recent studies that measured the turnover of TRP in flies between 1.5 and 9.5 days old revealed only 25% turnover of TRP during that time, indicating a slow turnover of TRP (94).

6. TRP has special structural features at the COOH terminus

At the COOH-terminal region there is also a proline-rich sequence in which the dipeptide KP is repeated 27 times (KP, in Fig. 3). Near the end of the COOH terminal, TRP contains the following amino acid sequence repeated in tandem nine times: D K D K P G/A D. This \(8 \times 9\) repeats region is likely to be important for protein-protein interactions. This region is unique to TRP and has not been found in any other member of the TRP family.

At the end of the COOH terminal there is a binding domain for the PDZ scaffold protein INAD (169, 187, 208) (see sect. vi and Fig. 3).

7. TRPL is a second light-activated channel with CaM binding domains

The TRP homolog protein TRP-like (TRPL) was isolated using a screen to detect CaM binding proteins and shared an overall 40% identity with TRP with much greater similarity (70%) in the putative transmembrane regions. Two regions that contain a typical CaM binding site were found in the trpl-encoded protein (Fig. 3A). A region homologous to the first putative CaM binding site in TRPL is not present in TRP. One putative CaM binding regions is capable of forming amphiphilic helices, which is typical for CaM bindings sites (147, 169, 187). Localization of the two CaM binding sites (designated CBS1 and CBS2) was determined by CaM overlays on fusion protein fragments (198). CBS1 is localized within residues 710–728, while CBS2 lies within residues 865–895, where there is no predicted amphiphilic helix. CBS2 is also unconventional in binding CaM in the absence of Ca\(^{2+}\) (198). The COOH termini of TRP and TRPL show very little homology (17%); nevertheless, both proteins show a large number of proline residues and thus contain a proline-rich domain at the COOH terminus part.

8. Are the light-activated channels homomers or heteromultimers?

Both TRP and TRPL have weak but significant homologies to a known channel subunit of the vertebrate voltage-gated Ca\(^{2+}\) channel, the dihydropyridine receptor. However, TRP and TRPL contain only one of the four channel motifs with the six putative transmembrane domains S1-S6. By analogy with voltage-gated K\(^{+}\) channels
and the cyclic nucleotide-gated channels, both TRP and TRPL represent subunits of putative tetrameric channels. Because null trp and trpl mutants both respond to light, each can function without the other. However, heterologous coexpression studies and coinmunoprecipitation experiments led to the suggestion that in WT flies the light-dependent conductance was mediated by at least three types of channels, two of which were TRP homomultimers and TRP-TRPL heteromultimer (209). Biophysical measurements of WT, trp, and trpl mutants questioned this conclusion, but these experiments did not exclude the possibility that heteromultimeric TRP-TRPL channels are important for specific, still uninvestigated features of the response to light (158).

A recent study has revealed an additional channel subunit called TRPγ (207), which is highly enriched in the photoreceptor cells. The NH2-terminal domain of TRPγ dominantly suppressed the TRPL-dependent transient receptor potential on a trp mutant background, suggesting that TRPL-TRPγ heteromultimers contribute to the photoreponse. Furthermore, TRPL and TRPγ coinmunoprecipitate, suggesting that a physical interaction between these proteins forms a heteromeric channel (207). Further studies will be required to unequivocally determine the subunit composition of the light-sensitive channels.

9. TRP homologs of the blowfly Calliphora and squid

The trp gene of the closely related blowfly Calliphora (79) and a more distantly related squid, Loligo (115), have been cloned and sequenced. The Calliphora sequence shows 77% sequence identity with Drosophila TRP, indicating that it is the ortholog of TRP rather than TRPL. The greatest difference is in the COOH terminal in which the KP proline-rich sequence in Calliphora is somewhat shorter (79). The squid sequence is more distantly related, showing only 46% identity to TRP and TRPL, and thus is equally similar to both proteins. As in TRP and TRPL, squid TRP and Calliphora TRP have CaM binding domains on the COOH terminus, multiple ankyrin repeats are found in the NH2 termini, which are common feature of all TRP homologs, including those in vertebrates (see below).

10. TRP and TRPL are localized to the rhabdomere

Immunogold labeling indicates that the putative light-sensitive channels TRP and TRPL are localized to the microvillar membrane (187). One immunofluorescence study (148) indicates that TRP is particularly localized to the base of the microvilli in contrast to other studies (32, 187, 214). The reason for these differences in immunofluorescence localization of TRP is not clear. In addition, TRP is found in photoreceptor axons up to the lamina and medulla regions in the brain (148). Functional TRP is found in the antenna of the developing but not in the mature olfactory system of Drosophila (176). The possible localization of TRP to the base of the microvilli in juxtaposition to the putative InsP3-sensitive Ca2+ stores bears important implications on the possible gating mechanism of TRP (see below). It should be noted that TRP is an abundant protein with amounts equal to that of PLC (i.e., ~107 copies per cell, Ref. 79). This is unusual for channel proteins that are usually expressed in low amounts. Because the maximal light-induced current (LIC) in response to very intense light is ~20 nA (at ~60 mV holding potential) and single-channel conductance of TRP is ~4 pS, it follows that ~7 × 104 channels are sufficient to account for the maximal LIC. Accordingly, a small fraction (i.e., ~1%) of TRP channels is sufficient to mediate the maximal LIC measured during very intense light. A possible reason for the unusually high levels of TRP is the second function of this protein as an anchor that localizes signaling complexes to the microvillar membrane (see sect. vi). A support for the notion that the large amount of TRP is not required for its function as a channel stems from experiments in which TRP was dissociated genetically from the INAD scaffold protein in the inadD215 mutant. In inadD215 mutants there is a large reduction of TRP in the microvilli, with minor effects on the response to light that manifests in somewhat slow response termination (32). Similarly, transgenic Drosophila trpΔ1272, in which the binding site of TRP with INAD was deleted, shows a gross mislocalization of TRP in young flies. However, there was no effect on the response to light (94, 169). These data implicate that a large portion of TRP proteins are not necessary for production of the response to light and thus may have additional long-term roles (see below).

The localization of TRPL has not been extensively studied. Nevertheless, TRPL was reported to localize to the rhabdomere of Drosophila (126). Interestingly, TRP is 10-fold more abundant than TRPL (52, 209), and according to Hardie and colleagues (158), TRP and TRPL contribute about equally to the LIC under certain conditions.

B. The trp Gene Is Conserved Throughout Evolution

By searching Caenorhabditis elegans genome database using the sequence that encodes the sixth putative transmembrane segment (S6) and part of the adjacent COOH-terminal cytoplasmic domain of Drosophila TRP, Harteneck et al. (70) identified 13 C. elegans TRP members. These were divided into three groups: short, long, and osm. The “short” group has a considerable similarity to Drosophila TRP and TRPL and therefore will be referred to in this review as the “TRP-homolog” group or TRPC. This group was recently designated classical TRPs by Montell to indicate the very significant similarity of the
mammalian members to the original *Drosophila* member of this group (119). Full-length murine homologs, which constitute most of the known mammalian members of the TRP-homolog group have been cloned and characterized by Birnbaumer and colleagues (218) and Schultz and colleagues (70). Recently, Montell (119) has proposed to classify the nonclassical TRP channels into five subfamilies, which include the members of the “long” and “osm” groups. These five subfamilies are distantly related to the *Drosophila* TRP and TRPL and are referred to as the “TRP-related” group in this review.

Based on the released *Drosophila* genomic sequence, which accounts for ~95% of the euchromatic sequences, a total of 14 members of the TRP family have been reported (2). However, it appears that only TRP, TRPL, and TRPβ belong to the TRP-homolog group while the rest are TRP-related genes (207).

C. “TRP-Homolog” Group

On the basis of similarity to *Drosophila* TRP and TRPL sequences, new TRP isoforms have been cloned using database searches of expressed sequence tags (EST), RT-PCR, or expression-cloning strategies. Seven major groups termed TRPC1–7 have been cloned and sequenced (Table 1; for review, see Ref. 152). The TRPC homologs have been cloned from human, mouse, rat, rabbit, bovine, and *Xenopus*. Five characteristic features of the *Drosophila* TRP and TRPL proteins have been found to be common to most members of the TRP-homolog group. 1) The predicted topology of six (but see Ref. 203) transmembrane segments (S1–S6) includes the typical pore region loop between transmembrane regions S5 and S6. 2) The charged residues in the putative S4 helix, which usually underlies voltage gating, are replaced by noncharged residues. 3) Three or four ankyrin repeats are found at the NH₂ terminal. 4) A proline-rich sequence is found in the COOH-terminal domain (70). 5) The TRP domain exists in all members of this group (119).

Birnbaumer and colleagues and Schultz and colleagues (16, 70, 218) have classified the vertebrate members of the TRP-homologs group into four subgroups according to their primary amino acid sequence (see Fig. 4). Type 1 includes all isoforms of TRPC1. Type 2 includes the TRPC2 homologs, which have the lowest similarity to the other groups. Type 3 includes TRPC3, TRPC6, and TRPC7 channel proteins. Type 4 includes TRPC4 and TRPC5 channels and has a higher similarity to the TRPC1 group relative to the other groups.

1. TRPC1

TRPC1 was the first mammalian homolog of TRP that was cloned independently by two laboratories using EST database of human fetal brain cDNA library. It was initially called TRPC1 (199) or hTRP-1 (216). This TRP isoform is the shortest of all members of the TRP-homolog group (but see TRPC2 pseudogene, below). It has 38–40% identity and 58–62% sequence similarity to *Drosophila* TRP, TRPL, and the *C. elegans* isoform of TRP, which was identified during sequencing of chromosome III of *C. elegans*. This homology does not allow determination if TRPC1 is more similar to *Drosophila* TRP or to TRPL. In addition to three putative ankyrin repeats, the NH₂ terminal was also predicted to have a coiled coil structure at amino acids 256–300. Ninety amino acids at the COOH terminal of the human TRPC1 showed significant homology to dystrophin (199) (Fig. 3).

A spliced variant of TRPC1 (called TRPC1A) was cloned independently (221). This variant and TRPC1 were expressed in Chinese hamster ovary (CHO; Ref. 221) cells, in Sf9 (173, 221) cells, where they were studied functionally (see below), and in *Xenopus* oocytes (185). TRPC1

![FIG. 4. Pairwise similarity phylogenetic tree of the TRP family. Two distinct subgroups can be identified by their phylogenetic relationship. The top group (except for *Drosophila* TRP and TRPL) shows murine members of the “TRP homolog” (TRPC) group. The bottom group includes several members of the “TRP-related” group. The scale represents evolutionary distance calculated by Chustal analysis. [Modified from Strotmann et al. (177).]](http://physrev.physiology.org/10.1.220.33.2)
channels have also been found in other mammalian species: mouse, bovine, and rat (for review, see Ref. 152). Recently, a *Xenopus* isofrom of TRPC1 was cloned (called Xtrp). The amino acid sequence of Xtrp was 82% identical to the mouse TRPC1, 81% identical to bovine TRPC1, and 84% identical to rat TRPC1 (18). The cloning of a *Xenopus* isofrom of TRPC1 and its localization to the oocyte surface membrane is important, since the native inositol lipid signaling of *Xenopus* oocyte has been an important model system to investigate SOCs (142).

The expression pattern of TRPC1 made by several groups (30, 50, 173, 199, 216) shows that TRPC1 is most abundantly expressed in the brain, heart, testes, ovary, bovine aortic endothelial cells, and salivary glands and is barely detectable in the liver or adrenal gland. Interestingly, the rat ortholog (941 amino acid long with 52% identity to TRP) changes its expression pattern during brain development (50), suggesting that it has a role in developmental signaling. Injection of antisense oligodeoxyribonucleotide for mammalian TRPC1 inhibited the native SOC response of the *Xenopus* oocytes (185); however, the expression pattern of TRPC1 and its physiological properties suggest that it is not *I_{CRAC}* that was described in blood cells. Since a variety of SOC channels have been described and some of them are TRPs (46, 142), it is possible that specific TRP homologs mediate *I_{CRAC}* or other SOCs with still unidentified molecular identity. Support for this possibility was found in the report of Birnbaumer and colleagues (218) on carbachol-stimulated store-operated 

Ca\(^{2+}\) influx seen in murine Ltk\(^{-}\) cells expressing the muscarinic M5 receptor. These cells show loss of Ca\(^{2+}\) influx upon coexpression of a mixture of murine TRPC1–6 fragments in the antisense orientation. Several additional studies have reported that a reduction of TRPC1 expression using antisense approach led to inhibition of endogenous SOCs (98). Similar results were obtained in studies of TRPC3 (205) and TRPC4 (146) (for review, see Ref. 46).

### 2. TRPC2

The human TRPC2 (also designated Trp2), which was the first cloned TRPC2 gene (199), is probably a pseudogene since several independent ESTs show mutations introducing early stop codons. A bovine homolog of this pseudogene, which is mainly expressed in the testes, spleen, and liver, was cloned and sequenced (203). Later, a full-length 1,172-amino acid TRPC2 isoform from mouse (192) and a spliced TRPC2 isoform from bovine (GenBank) were cloned. Thus TRPC2 is the longest of all vertebrate TRP-homolog group, having a longer cytoplasmic NH\(_2\)-terminal domain. In contrast to the TRPC1, TRPC2 is tissue specific and in the mouse it was found only in the testes and vomeronasal organ (VNO). In contrast to other TRPC proteins of this group, each having several consensus glycosylation sites within the putative transmembrane domain of the protein, TRPC2 has only one glycosylation site located in the putative pore region. All other sites are localized to the cytosolic NH\(_2\)-terminal. TRPC2 shows only 25–30% identity to *Drosophila* TRP, and it is closer to the TRP-related group than any other TRP member of the TRP-homolog group (see Table 1 and Fig. 4). This is also reflected in the existence of a segment in the NH\(_2\)-terminal domain (amino acid 155 to amino acid 238) of the type 2 polycystic kidney disease gene (*PKD2*). *PKD2* gene product is categorized as TRP-related channel (see below). This sequence is unique to TRPC2 and is absent in the other members of the TRP-homolog group. The function of the PKD sequence of TRPC2 is unknown (192).

A rat ortholog of the mouse and bovine TRPC2 genes was cloned by Liman et al. (96). Importantly, the rat ortholog is highly localized and heavily expressed in the VNO. This organ plays a key role in the detection of pheromones, which are chemicals released by other rats, and elicits stereotyped sexual behavior (96). Turning TRPC2 gene into a pseudogene in humans fits well with the hypothesis that the VNO is no longer functional in apes.

Recent studies revealed that InsP\(_3\) is a second messenger of VNO receptor neurons of snake (184). This fact fits with TRPC2 as a channel, which is activated by the inositol-lipid signaling cascade.

A TRPC2 isoform is highly enriched in the sperm and seems to have an important function in the fertilization of mice (see below and Ref. 84).

### 3. TRPC3, TRPC6, and TRPC7

This group, especially the TRPC3 homologs, has been thoroughly studied by a variety of functional tests and, therefore, significantly contributes to our understanding of TRP function and gating mechanism (see below). The molecular structure of TRPC3 shows the characteristic five features of the TRP family listed above. Human, mouse, and rat orthologs of TRPC3 and TRPC6 have been cloned as well as a mouse ortholog of TRPC7.

TRPC3 homologs are predominantly expressed in the brain and at much lower levels also in ovary, colon, small intestine, lung, prostate, placenta, and testes (51, 114, 218). TRPC6 expression is highest in the brain, but it is also expressed in the lungs and ovaries. Interestingly, the development of tumors is associated with downregulation of TRPC6 isoform in a mouse model for an autocrine tumor (24).

TRPC7 from mouse turned out to be very similar (81% identity and 89% similarity) to TRPC3 and also to TRPC6 (75% identity and 84% similarity) while only 33% identical (53% similar) to *Drosophila* TRP and TRPL.
TRPC7 is mainly expressed in the heart, lung, and eye and at a lower level in the brain, spleen, and testes (131).

4. TRPC4 and TRPC5

TRPC4 and TRPC5 channels are similar in structure to TRPC1. Therefore, several investigators consider these three channels types as one group (15, 152), which also share similarity in function (see below). Petersen et al. (143) first cloned a partial sequence of the mouse TRPC4 isoform. Then, full-length mouse bovine and rat orthologs were described (122, 144). Another TRP homolog with high similarity to TRPC4 was cloned and designated TRPC5 (132, 145). The rabbit and mouse orthologs of TRPC5 show 69% sequence identity to bovine TRPC4 and 41% identity to Drosophila TRP. The expression patterns of TRPC4 and TRPC5 are very different. TRPC5 mRNA is expressed predominantly in the brain (145), while TRPC4 is expressed in the brain but also in the adrenal gland and at a much lower level in the heart, lung, liver, spleen, kidney, testes, thymus, aorta, and uterus (132). Of special interest are TRPC4 isoforms, which are expressed in vascular endothelial cells of various species (mouse, human, and bovine) (49; see sect. vD).

D. “TRP-Related” Group

In contrast to members of the TRP-homolog group that were discovered due to sequence homology to Drosophila TRP, the various members of the “TRP-related” group were found following studies aiming to explore specific sensory transduction pathways or specific genetic diseases. These pathways include olfaction and osmosensitivity in C. elegans (36), defects in mechanosensory transduction in C. elegans and Drosophila (196), defects in pain mechanism in primary afferents of dorsal root ganglia (27), $Ca^{2+}$ transport in $Ca^{2+}$ transporting epithelial cells (73), polycystic kidney disease in cells expressing the polycystin genes PKD (31), tumor suppression in the skin (reviewed in Ref. 119), and mucolipidosis type IV in cells expressing defective mucolipin (10, 179). The different approaches resulted in a wealth of functional data about TRP-related members, in contrast to most mammalian members of the TRP-homolog group, whose functions in most of the native tissues are largely unknown (but see below). In contrast to mammals, none of the five C. elegans members of the TRP-related group or other four C. elegans members of the TRP-homolog subfamily has been characterized at the cellular level, neither in the native cells (but see CUP5, below) nor in heterologous systems.

The TRP-related group (also referred to as nonclassical TRP by Montell) has been subdivided into five subfamilies by Montell (119). All members of this group share significant homology to Drosophila TRP in the transmembrane segments. However, the degree of structural similarity to TRP varies considerably among the various subfamilies. The TRPV subfamily is the closest to Drosophila TRP (see below). Some of these subfamilies lack the ankyrin repeats or the TRP domain. Nevertheless, they share some similar structural features and some of them also share similar unusual functional features such as permeability to Mg$^{2+}$ and sensitivity to metabolic stress (see below).

1. TRPV subfamily

This subfamily has all the structural features of the TRP-homolog group and is more similar to this group than any other subfamily of the TRP-related group (see Fig. 4). The sequence identities to Drosophila TRP are primarily limited to homology within ~50 amino acid residues at the COOH-terminal ends of S1-S6, the adjacent COOH-terminal region, and conservation of the TRP domain (119). A gene that functions in a subtype of olfactory neurons of C. elegans and designated osm-9 (36) was the first member of this subfamily.

A) THE C. ELEGANS OSM-9. Six recessive alleles of osm-9 have been identified on the basis of defective response to odors, high osmotic strength, or light touch to the nose (36). A fusion portion between osm-9 and green fluorescent protein (GFP) revealed that OSM-9 is expressed in a subset of chemo-sensory, mechanosensory, and osmosensitive neurons. There is no overlap in expression pattern between osm-9 and the C. elegans TRP (Z C21.2), which belongs to the TRP-homolog group (199) and is expressed in motor neurons, interneurons, vulval muscles, and intestinal smooth muscles (36). Similarly, it appears that there is no overlap in expression pattern between the C. elegans nociceptor and the above TRPs of C. elegans (196).

Osm-9 encodes a predicted protein of 937 amino acid. As in the other TRP-related members, the similarity of OSM-9 and the TRP-homolog group (including Drosophila TRP and TRPL) is very significant in the vicinity of S6 and the putative pore region. The osm-9 homolog also contains ankyrin motifs in their NH$_2$ terminus. These ankyrin motifs do not show higher similarity to those of TRP than to the ankyrin consensus motif. One of the characteristic features of the TRP-homolog group, the proline-rich motif at the COOH terminus, is missing in osm-9 and other members of the TRP-related group.

B) THE MAMMALIAN VANILLOID RECEPTOR 1, VR1, AND ITS HOMOLOG VRL-1. The VR1 member of the TRP-related group was cloned following a search for pain (noxious)-initiated receptor in the peripheral terminals of subgroup of sensory neurons of the dorsal root ganglia. Julius and colleagues (27) used a functional screening strategy based on capsaicin-induced Ca$^{2+}$ influx to transfected HEK 293 cells. Capsaicin belongs to chemical group called vanilloids that are known to induce noxious stimuli. The receptor was
discovered using the above screening library and was therefore named vanilloid receptor 1 (VR1; also designated TRPV1). The cloned VR1 cDNA encodes a protein of 828 amino acid with the six putative transmembrane segments S1-S6. It also has a typical short hydrophobic region between S5 and S6 assumed to form the pore region. Three putative ankyrin repeats are found in the NH2 terminus (27) and a TRP domain near the COOH terminus (119). VR1 is expressed in a subset of sensory ganglion cells of the dorsal root ganglia (DRG) and trigeminal ganglia. When tested in rat DRG, ~40% of all neurons express VR1, predominantly in a population with small cell bodies. VR1 is only 23% identical to C. elegans osm-9 (Table 1).

A structurally related gene to VR1 designated VRL-1 (vanilloid receptor-like protein) was cloned after a search of the GenBank database. Human and mouse orthologs of VRL-1 have been found in EST databases with 78% identity and 86% similarity to one another and 49% identity and 66% similarity to rat VR1 (26). VRL1 (also designated TRPV2) is only 24% identical to the C. elegans osm-9. Interestingly, heterologously expressed VRL-1 is not activated by vanilloids or low pH (like VR1) but only by heat. VRL-1 is expressed in only 16% of all DRG cells of rat, in cells of medium or large diameter, while a similar pattern of expression was found in trigeminal ganglia. VRL-1 is also expressed in tissues other than DRG and spinal cord including lung, spleen, intestine, and brain where it is probably activated by stimuli other than noxious heat (26).

**C. Variations of VR1 Have Been Reported That Differ Due to Alternative mRNA Splicing.** Other members of the TRPV subfamily have been reported to have highly similar structure but distinct mode of activation. An interesting mouse member of this subfamily, which has ~80% sequence identity to rat VRL-1, was designated “growth factor-regulated channel” (GRC). GRC translocates between the ER and the plasma membrane upon stimulation of cells by insulin-like growth factor I (IGF-I). The translocation of endogenous GRC was demonstrated in MIN6 insulinoma cells and in heterologous system in CHO cells upon co-expression with GFP (86). Electrophysiological and fluorimetric measurements showed that IGF-I augmented calcium entry through translocation of GRC to the plasma membrane in CHO cells. However, since the CHO cells were marked by GFP, which is toxic to cells, it was not excluded that the observed currents and Ca2+ influx did not arise from activation of endogenous channels, which are sensitive to stress.

**D. Epithelial Calcium Channels.** Epithelial calcium channel (ECaC; also designated ECaC1, CaT2, or TRPV5) (73) and calcium transporter 1 (CaT1, also ECaC2 or TRPV6) (119, 138) were cloned from rabbit kidney and rat duodenum, respectively. These are members of the TRPV subfamily found in 1,25-dihydroxyvitamin D₃-responsive epithelial kidney cells (ECaC1) (73) and in Ca²⁺-transporting cells of the small intestine (CaT1) (138). ECaC1 is a putative apical Ca²⁺ channel in epithelia. In kidney it is abundantly present in the apical membrane of Ca²⁺-transporting cells and it colocalized with 1,25-dihydroxyvitamin D₃-dependent calbindin-D₂₈k. The ECaC1 cDNA encodes a 730-amino acid protein and shows ~30% homology to the VR1 channel. It has all the molecular features of the TRP-related group including the S1-S6 transmembrane segments with the putative pore region between S5 and S6, three ankyrin binding repeats, and two N-linked glycosylation sites in S1-S6 region and a TRP domain. The protein is predominantly expressed in the kidney, small intestine, and placenta (Table 1).

The cDNA of the calcium transport protein, CaT1, encodes a protein of 727 amino acid residues which has all the structural features of ECaC1 except that four ankyrin repeats have been identified in the NH2 terminus and the N-glycosylation sites are missing. Nevertheless, CaT1 shows 75% amino acid sequence identity to ECaC of rabbit (138). Functional studies on CaT1 have suggested that CaT1 is a promising candidate of the I_{CRAC} channel (see below and Ref. 215). CaT1 shows 34 and 27% identities to VR1 and osm-9, respectively. CaT1 is mainly distributed in the small intestine of rat, but it was also found in small amounts in brain, thymus, and adrenal gland. However, it was not found in the kidney, in striking difference to ECaC1 (138). This difference in expression pattern is surprising in light of the high homology between the two genes that normally indicates a common functional role. By screening a rat kidney cortex library with CaT1 probe, a cDNA encoding additional channel designated CaT2 was isolated (137). CaT2 has 84 and 73% amino acid identities to ECaC1 and CaT1, respectively (137). Unlike ECaC1, CaT2 is kidney specific in rat. A new member of this subfamily designated CaT-like (CaT-L) has been recently isolated from human. CaT-L is abundantly expressed in the placenta, pancreatic acinar cells, and salivary glands. Interestingly, the CaT-L transcripts are undetectable in benign prostate tissue but are present at high level in locally advanced prostate cancer, metastatic lesions, and recurrent androgen-insensitive prostate adenocarcinoma, suggesting a possible link between Ca²⁺ signaling and prostate cancer progression (202).

**E. The Putative Osmoreceptor OTRPC4.** OTRPC4 (now designated TRPV3) has structural similarity to VR1, osm-9, and ECaC, and it is expressed in kidney, heart, and liver (177). The protein is composed of 871 amino acid residues. The hydrophobic profile predicts the typical six transmembrane segments S1-S6, pore-forming loop between S5 and S6, and three ankyrin repeats at the NH2-terminal. Like other members of this group, OTRPC4 lacks the proline-rich motif at the COOH terminal. Sequence alignment with VR1, VRL-1, and ECaC channels reveal 41, 39, and 23% overall similarity, respectively. In
situ hybridization analysis revealed high levels of OTRPC4 transcripts in the inner cortex of the kidney, while higher resolution microscopy indicated a high density in the distal convoluted tubule. This specific localization and functional tests in heterologous system strongly suggest that OTRPC4 is involved in cellular osmoreception (177).

2. TRPN subfamily

This subfamily is distantly related to Drosophila TRP, it does not contain the TRP domain, it is characterized by a large number of ankyrin repeats, and so far it has been found only in invertebrate species.

A) The No Mechanoreceptor Potential C Channel, NOMPC. NOMPC was identified following a screen for Drosophila mutants with behavioral defects in coordinated movement. The mechanosensory organs of mutants showing the behavioral defects were examined electrophysiologically, leading to the discovery of the nompC gene. This mutant displays a large loss of mechanoreceptor current relative to control flies. Cloning of the nompC gene was carried out after mapping and rescue of the nompC mutant. NOMPC cDNA encodes a putative channel protein of unusual structure composed of 1,619 amino acid residues. The 1,150 NH2-terminal amino acid residues consist of 29 ankyrin repeats. The remaining 469 amino acid residues share a significant similarity to the TRP family with S1-S6 transmembrane segments and putative pore region between S5 and S6. Pairwise comparison between NOMPC and the various members of the TRPC family in the channel domain reveals ~20% identity and ~40% similarity. Thus NOMPC is a distant member of the TRP-homolog subfamily. NOMPC is expressed in the mechanosensory organs of Drosophila in agreement with its function as a mechanosensory sensitive channel (196). A C. elegans homolog to NOMPC was identified and revealed high expression in mechanosensitive neurons of the nematode (196). Because no human ortholog of NOMPC has been reported, it would be interesting to see in the future whether the ability to respond to mechanical stimuli is a general feature of TRPs or this activity is specific for invertebrate mechanotransduction.

3. TRPP subfamily

This subfamily is also distantly related to Drosophila TRP, because it neither has the ankyrin repeats domain nor the TRP domain (in the mammalian members) (119). The founding member of this subfamily was discovered in many cases of polycystic kidney disease (PKD, Ref. 205). TRPP channels share ~25% amino acid identity with two members of the TRP-homolog group TRPC3 and TRPC6 in the S5-S6 transmembrane segments and in the pore region (119).

A) Polycystin. Polycystin-1 (encoded by PKDI) and polycystin-2 (encoded by PKD2) are membrane proteins that are defective in human autosomal dominant polycystic kidney disease (204). Members of this subfamily are conserved through evolution from C. elegans to human. Studies in C. elegans reveal that homologs of the polycystins act together in signal transduction pathway in sensory neurons (11). Structural analysis of the amino acid sequence predicts that the human polycystin-2 protein, which is composed of 968 amino acids, has putative six transmembrane domains S1-S6 with putative pore-forming loop between S5 and S6 segments, typical of the TRP-related group. However, the ankyrin repeats typical for the TRP family proteins are missing in the polycystin proteins (31). Although PKD-1 and PKD-2 gene products have structural similarities, only polycystin-2 seems to be a cationic channel while polycystin-1 seems to be a larger structural protein (of 4,572 amino acids in human) that interacts directly with polycystin-2 (11; see also Ref. 204). An interaction between polycystin-1 and polycystin-2 was revealed by coexpression of the two channel proteins in CHO cells. This interaction appears to be crucial for polycystin-2 function as a channel because only in the presence of polycystin-1, polycystin-2 translocates to the plasma membrane and function as a channel (54). Thus neither polycystin-1 nor polycystin-2 alone is capable of producing currents. Moreover, disease-associated mutant forms of either polycystin protein that are incapable of heterodimerization do not result in new channel activity (54). In C. elegans, there is a homolog of PKD-1 called LOV-1 that is composed of 3,125 amino acids and a PKD-2 homolog with an overall identity of 27% to the human PKD-2 (11). Vertebrate polycystins have the Ca2+ binding motif, EF hand, and a coiled coil domain near the COOH terminal. Heterologous expression of a human polycystin-like (PCL) protein, which shares 50% identity and 71% homology to PKD-2 in Xenopus oocytes, confirms that PCL forms a nonselective cation channel that can be activated by Ca2+ (31) (see sect. vD2). PKD2 has modest similarity to the Ca2+ transporting channel, CaT1, in the region between amino acid residues 596–687 of CaT1, where 23% identity between these proteins has been found (138).

4. TRPM subfamily

This subfamily is also distantly related to the TRP-homolog group. The TRPM subfamily is conserved through evolution and exists in C. elegans (CED-11, GON-2) and Drosophila. Members of this subfamily are sharing ~20% amino acid identity to Drosophila TRP, over ~325 residue region that includes S2-S6 transmembrane segments and the TRP domain. This subfamily also lacks the ankyrin repeats (119). Originally this subfamily was designated long TRP (LTRPC) (70) because of the
unusually long NH$_2$-terminal region of ~750 amino acid residues relative to the TRP-homolog group. The total length of this subfamily is 1,000–2,000 amino acids, and it varies considerably because of large diversity of the COOH-terminal domain. Members of the TRPM subfamily have a potential role in cell cycle regulation and in regulation of Ca$^{2+}$ influx in immunocytes (160). Thus C. elegans members of TRPM function in programmed cell death (CED-11) and mitotic cell division (GON-2; reviewed in Ref. 119). Importantly, the melastatin member of this subfamily (TRPM1) has been related to skin cancer as a putative tumor suppressor protein (for review, see Ref. 119). An interesting mode of regulating TRPM gating was recently described for another member of the TRPM subfamily, MLSN (210). It was found that an alternatively spliced short form of MLSN (MLSN-S) interacts directly with and suppressed Ca$^{2+}$ entry of full-length MLSN (MLSN-L) in HEK293 cells. This suppression appears to result from inhibition of translocation of MLSN-L to the plasma membrane (210).

A very interesting member of this subfamily is the bifunctional channel protein designated phospholipase C (PLC)-interacting kinase (TRP-PLIK or TRPM7), which constitutes the first example of a channel with putative kinase activity. This protein has an NH$_2$-terminal domain >50% identical over 1,250 amino acids residues to that of TRPM1 fused to COOH terminal with protein kinase domain (123, 159). Another member of this subfamily now designated TRPM2 (or LTRPC2) also seems to be bifunctional, and in addition to the channel-forming domain, which is similar to the channel domains of other members of this subfamily, there is a NUDT9 homology domain which is similar to the channel domains of other members of the TRP family, two proline-rich regions were identified near the NH$_2$ terminal. A leucine zipper motif is located at S2, a nuclear localization motif at amino acids 43–60, and a serin lipase motive. In the region that shows similarity to TRP there is 38% identity, whereas in the pore region there is 58% identity (179). Mucolipin-1 has no ankyrin repeats or TRP domain. Unlike other subfamilies, no functional analysis of mucolipin is available, and it is still not clear if it functions as a channel protein. Members of the TRPML subfamily are conserved in C. elegans (40% amino acid identity) and Drosophila (44% identity, Ref. 48). A recent study on a C. elegans homolog of mucolipin-1 designated CUP-5 showed that a loss-of-function mutation in CUP-5 resulted in an enhanced rate of fluid-phase markers, decreased degradation of endocytosed protein, and accumulation of large vacuoles (48). Overexpression of the normal CUP-5 caused the opposite effects, thus indicating that CUP-5 is a promising model system for studying conserved aspects of structure and function of mucolipin-1 and mucolipidosis type IV (48).

6. Summary

The TRP family of channel proteins reveals a high structural diversity. This family can be divided on the basis of primary amino acid sequence into six subfamilies. The founding member of this family, the Drosophila TRP, shares structure homology with all subfamilies mainly at the region of the ion pore, strongly suggesting that the fundamental function of all members of this family is to constitute cation channels. Some members without clear pore region may function as regulators of the channels or they may be responsible for channel translocation into the plasma membrane. Additional structural features that are common to most, but not all, subfamilies are the ankyrin repeats at the NH$_2$ terminal and the TRP domain at the COOH-terminal side of the transmembrane domain. Because ankyrin repeats are important regions for protein-
protein interactions, such interactions seem to be fundamental for TRP function, although the actual function of these regions in TRP is still unknown. More mysterious is the function of the TRP domain, which characterizes most members of the TRP family. In addition, one subfamily (TRPM) includes proteins with dual function of channel and enzyme, a property unique to the TRP family. In general, the diverse structural features of members of the TRP family strongly suggest that members of this family are involved in a wide range of cellular functions.

IV. FUNCTIONAL ANALYSIS IN THE NATIVE TISSUE

The discovery of vertebrate TRP channels in a large variety of cells and tissues each having a putative specific function suggests that the channels of the TRP family also have diverse and elaborate gating mechanisms that largely depend on their cellular environment. This fact makes the few cases in which channel proteins have been analyzed in the native cellular environment highly valuable. Genetic manipulations allow conducting studies of structure-function relationship of channel proteins while the other cellular components are intact. Such studies in the native tissue in conjunction with studies in heterologous systems provide powerful tools for studying the function of channel proteins. Nevertheless, due to technical difficulties, heterologous expression is still the main technical approach of native TRPs were carried out suggested that some of the TRP homologs contribute to SOC (98, 146, 160, 205, 218). The native Xenopus TRPC1 isoform has been suggested to contribute to SOC activity of the oocytes, since knockout of TRPC1 isoform reduces SOC activity (18).

A. Drosophila Light-Sensitive Channels TRP and TRPL

1. TRP has a small single-channel conductance

The subcellular localization of TRP and TRPL to the microvilli imposes a considerable difficulty for access with patch electrodes. Accordingly, only very recently it has become possible to record single-channel activities in patches of Drosophila microvilli (53), as previously reported for Limulus (8) and mollusk photoreceptors (125). The light-sensitive conductance in Drosophila has been studied mainly under whole cell voltage-clamp conditions using the dissociated ommatidial preparation (55, 156).

The newly developed preparation of rhabdomeral membranes from Drosophila photoreceptors has been used only under the restrictive conditions of excised patches in the absence of Ca²⁺. Under these conditions the channels are constitutively open with a wide range of conductance amplitudes. Conductance events as large as 144 pS seem to arise from coordinated gating of many small individual events of 4 pS. This large conductance seems to arise from openings of TRP and not TRPL channels, since they were blocked by 10 μM La³⁺ and were completely absent in a null trp mutant (53). It is not clear if the coordinate openings represent a physiological phenomenon, since quantum bumps, which represent coordinate openings of many channels, are not observed under conditions of constitutive activity of the light-sensitive channels during whole cell recordings (62). It is also not clear why the activity of TRPL channels, which is observed under whole cell recordings in the trp mutant (62), is absent in the dissociated rhabdomeral preparation.

The uncertainty as to the physiological significance of the single-channel recordings emphasizes the importance of noise analysis, which has been used to estimate single-channel properties from the so-called “rundown current” (RDC). The RDC represents a spontaneous opening of light-sensitive channels, which develops after several minutes of whole cell recording accompanied by illuminations (62) or under metabolic stress in the dark (4). In the presence of 4 mM external Mg²⁺, TRPL channels have an apparent conductance of ~35 pS and TRP channels ~4 pS. Both channels, however, are blocked by divalent ions including Mg²⁺, and under divalent free conditions, conductance values increase to ~70 pS (TRPL) and ~35 pS (TRP), respectively (64, 68).

Power spectra have also been calculated from current noise, providing information on channel kinetics. Power spectra of TRPL channels are consistent with a channel with two open states with mean open times of 2 and 0.15 ms. Noise analysis data of heterologously expressed TRPL channels in Drosophila S2 cells were indistinguishable from that measured in Drosophila photoreceptors, and the single-channel conductance of the heterologously expressed TRPL channel was directly confirmed by recordings of single channels (68). Power spectra of TRP channels (in the trpl mutant) are characterized by higher frequencies with a calculated time constant of 0.4 ms.

2. TRP channels are highly permeable to Ca²⁺

The light-sensitive channels in invertebrate photoreceptors are essentially nonselective cation channels, and the channels in Drosophila readily permeate a variety of monovalent ions including Na, K, Cs, Li, and even large organic cations such as Tris and TEA (55, 156). Moreover,
the reversal potential of the LIC in WT and the trpl mutant shows a marked dependence on extracellular 
Ca\(^{2+}\), indicating that TRP channels are permeable to Ca\(^{2+}\).

Our knowledge of the biophysical properties of the 
TRP and TRPL channels has been facilitated by 
measurements of the in situ conductance in the trp and 
trpl mutants. Permeability ratios to monovalent and 
divalent ions in WT and trp flies have been calculated 
(60) using the constant current equation and reversal potential data obtained under various ionic conditions. With the 
assumption of Goldman-Hodgkin-Katz (GHK) constant 
field theory, the quantitative dependence implies a relative 
Ca\(^{2+}\) permeability (\(P_{\text{Ca}}/P_{\text{Na}}\)) of \(\sim 40:1\) (55, 60). More recently, 
the permeability ratios for a variety of divalent and 
monovalent ions ratios have been determined under bi-
ionic conditions, where in these experiments Cs\(^+\) was the 
only intracellular cation while the bath contained one of a 
variety of monovalent or divalent cations. It was found 
that TRPL represents a nonselective cation channel (\(P_{\text{Ca}}/P_{\text{Cs}} = 4.3\)) and TRP, a channel highly 
selective for divalent cations (\(P_{\text{Ca}}/P_{\text{Na}} > 100:1\)). The WT values are explained by 
approximately equal summation of TRP and TRPL con-
ductance. On the assumption of independent mobility of 
ions, these permeability data imply that in WT, 45% of the 
LIC at resting potential is mediated by Ca\(^{2+}\), 25% by Mg\(^{2+}\), and 30% by Na\(^{+}\) (using a physiological Ringer solution, Ref. 158).

3. Magnesium conductance and block

_Drosophila_ photoreceptors bathed in divalent free 
medium respond to light in an outward current at mem-
brane potentials equal to the resting potential (i.e., \(-50\) 
mV). Addition of Mg\(^{2+}\) to the bath resulted in light-in-
duced inward current, indicating that Mg\(^{2+}\) permeates the 
light-sensitive channels (60). Consistent with this obser-
vation, calculation of permeability ratios indicated that 
the light-sensitive channels have a relatively high perme-
ability to Mg\(^{2+}\) (60). This highly unusual property of the 
TRP channels was also recently found in one of the TRPM 
channels (123).

TRP and TRPL channels are also blocked by Mg\(^{2+}\) at 
physiological concentrations. This property is common to 
the variety of channels (64). For TRPL channels, the block is 
moderate (maximally \(~3\)-fold with an IC\(_{50}\) of 2–3 mM) 
and has little, if any, dependence on either voltage or 
extracellular Ca\(^{2+}\). In WT flies, where the current is dom-
inated by the TRP channels, the block is much more 
severe and is voltage dependent. The block is relatively 
relieved at both hyperpolarized and depolarized poten-
tials, thereby generating a characteristic inward and out-
ward rectification, while the block is also stronger in the 
absence of extracellular Ca\(^{2+}\). Under these conditions, 
there is a maximum of \(~20\)-fold block at resting potential, 
with an IC\(_{50}\) of 200 \(\mu\)M Mg\(^{2+}\). In the presence of 1.5 mM 
extracellular Ca\(^{2+}\), the maximum block (in 20 mM Mg\(^{2+}\)) 
is \(~10\)-fold. The block is reflected in a reduction in single-
channel conductance, which may be an important factor 
in improving signal-to-noise ratio in a similar manner to 
the conductance of the vertebrate cGMP-gated channels 
(213).

The inward and outward rectification seen in the 
current voltage (I-V) relationship of the WT conductance 
is due in part to the voltage-dependent Mg\(^{2+}\) block. A 
similar I-V function is found in trpl mutants, indicating that 
this is a property of the trp-dependent channels. Under 
divalent free conditions, the I-V relationship shows 
a simple exponential outward rectification. The I-V rela-
tionship of TRPL channels shows outward rectification 
both in the presence and absence of divalent ions, sug-
gesting that it reflects an inherent property of the 
channels (64, 68, 158).

4. Ca\(^{2+}\) influx

Consistent with the electrophysiological determina-
tions, the ability of the light-sensitive channels to perme-
ate Ca\(^{2+}\) has been directly demonstrated by measure-
ments of very large light-induced Ca\(^{2+}\) influx signals. 
Ca\(^{2+}\)-selective microelectrodes were applied in vivo 
(139), and Ca\(^{2+}\) indicator dyes were used in both isolated 
ommatidia of _Drosophila_ (59, 140, 155) and in Calliphora, 
in vivo (128). With the use of Ca\(^{2+}\) indicators of high and 
low affinity (140) or ratiometric indicators (59), it was 
found that during intense lights cellular Ca\(^{2+}\) reaches the 
micromolar range. Ca\(^{2+}\) influx was much reduced in the 
trp mutant (59, 139). The Ca\(^{2+}\) influx measurement in 
_Calliphora_, in vivo, has shown that cellular Ca\(^{2+}\) is reg-
ulated in a graded fashion over a large range of adapting 
light intensities. In dark-adapted photoreceptors, Ca\(^{2+}\) 
can reach a high level of 200 \(\mu\)M in <20 ms (128). More-
ever, Ca\(^{2+}\) imaging demonstrates colocalization of Ca\(^{2+}\) 
influx and extrusion. This colocalization ensures that af-
ter the cessation of light, removal of Ca\(^{2+}\) from the rhab-
domore, where the phototransduction machinery resides, 
is faster than in the cell body. This situation ensures a 
rapid dark adaptation (127).

B. Activation of TRP and TRPL Channels

In _Limulus_ ventral photoreceptors there is strong 
evidence that light induces release of Ca\(^{2+}\) from intracel-
lular stores via InsP\(_{3}\) receptors localized in the submi-
crovillar cisternae (SMC) and that Ca\(^{2+}\) can activate at 
least one class of light-sensitive channel (reviewed in Refs. 44, 124). The main evidence includes direct mea-
asurements of light-induced Ca\(^{2+}\) release coincident with, 
or a few milliseconds before the light-induced current 
(189), the ability of both InsP\(_{3}\) and Ca\(^{2+}\) to excite the
light-sensitive channels (23, 135, 136) and the inhibition of the light response by Ca\(^{2+}\) chelators (171). However, in *Drosophila*, but not in the large flies (41), InsP\(_3\) and Ca\(^{2+}\) failed to excite the photoreceptor cells (57, 153).

Activation of PLC is necessary for *Drosophila* phototransduction since null PLC mutants (17) eliminate the response to light (110). Therefore, extensive biochemical, electrophysiological, and molecular genetic approaches were applied to *Drosophila* photoreceptors to study the link between activation of PLC and the opening of TRP and TRPL channels. However, the signaling steps linking between the products of PLC activation and the opening of the light-sensitive channels in *Drosophila* remain unknown and controversial.

1. Possible roles of Ca\(^{2+}\) in excitation

There is strong evidence that the presence of Ca\(^{2+}\) in the photoreceptor cell of *Drosophila* is necessary for excitation, since prolonged Ca\(^{2+}\) deprivation reversibly eliminates excitation (37, 60). Nevertheless, photolysis of caged Ca\(^{2+}\) (DM-nitrophen) failed to activate the light-sensitive channels in null rhodopsin or PLC mutants, which do not respond to light (57). The released Ca\(^{2+}\) did, however, induce a substantial electrogenic Na\(^+/Ca^{2+}\) exchange current in WT and the blind mutants. In WT flies, there is an inherent problem since the Ca\(^{2+}\) uncaging light also excites the photopigment. Indeed, in WT flies, when the caged Ca\(^{2+}\) was released during the rising phase of the light response, a large facilitation of the LIC was observed while a profound inhibition was revealed when Ca\(^{2+}\) was released during the steady-state phase. Interestingly, in the trp mutant, upon the release of caged Ca\(^{2+}\), inhibition of the LIC was observed during the rising phase and facilitation at the falling phase. Both facilitation and inhibition occurred with a submillisecond latency, suggesting it occurred at the level of the channels themselves and indicating that the released Ca\(^{2+}\) had direct access to the channels.

Alternative slower methods of application have been used to raise cytosolic Ca\(^{2+}\), including perfusion of up to 0.5 mM Ca\(^{2+}\) from the whole cell patch pipette (56) and application of the Ca\(^{2+}\) ionophore ionomycin (58). None of these procedures appears to activate any light-sensitive channels, although they could activate an electrogenic Na\(^+/Ca^{2+}\) exchange current and modulate the response to light itself. These largely negative results do not rule out the possibility that Ca\(^{2+}\) is necessary but not sufficient for excitation. Also, because Ca\(^{2+}\) is known to exert both positive and negative feedback on the response to light, the exogenous application of Ca\(^{2+}\) in the above experiments might not be sufficiently refined to allow separation between these opposing effects (37).

2. Light induces release of Ca\(^{2+}\)

There is strong evidence for a massive light-induced Ca\(^{2+}\) influx, which is associated with the LIC in the presence of normal extracellular Ca\(^{2+}\) (see above). In contrast, in Ca\(^{2+}\) -free medium, most measurements have shown no, or negligible, Ca\(^{2+}\) rise, suggesting there is very little if any light-induced Ca\(^{2+}\) release from internal stores (59, 140, 155). These initial negative results probably arise from the very small dimension of the putative InsP\(_3\)-sensitive Ca\(^{2+}\) stores, the SMC (61). More refined measurements, which took into account the small dimensions of *Drosophila* SMC, indeed show light-induced release of Ca\(^{2+}\) from internal stores that correlated with the ability of the photoreceptors of the trp mutant to respond to light (37). However, recent measurements by Hardie, Colley, and colleagues (153) have shown that this light-induced release of Ca\(^{2+}\) is similar in WT and in a mutant in which the known InsP\(_3\) receptor was removed genetically. These authors thus argue that this release of Ca\(^{2+}\) is not mediated via the InsP\(_3\) receptor, suggesting that this release is from non-InsP\(_3\)-sensitive stores and has no role in phototransduction (153).

3. Effects of Ca\(^{2+}\) chelators

The effects of Ca\(^{2+}\) chelators have been tested by including the chelator in the patch pipette during whole cell recordings of the LIC in *Drosophila* (56). As expected, the slow buffer EGTA (as high as 18 mM) had only weak effects on the light responses in normal Ringer solution (1.5 mM Ca\(^{2+}\)) and had no apparent effect in Ca\(^{2+}\) -free solutions. The fast chelator BAPTA, operating in the microsecond range, was much more potent than EGTA. A high concentration of BAPTA (14 mM) reduced the sensitivity to light by ~5,000-fold in normal Ringer solution. However, in Ca\(^{2+}\) -free solution, the effects of BAPTA were much lower, with only a 2 log unit reduction in sensitivity (14 mM BAPTA), while no effect was observed with concentrations below 3 mM. Therefore, Ca\(^{2+}\) regulates the light-activated channels with fast kinetics, while cellular Ca\(^{2+}\) has to reach a threshold concentration to manifest this effect.

Taken together, the available data suggest that Ca\(^{2+}\) has profound effects on excitation, but the specific roles of Ca\(^{2+}\) are still unknown.

4. Involvement of InsP\(_3\) in phototransduction

Application of InsP\(_3\) by photoactivated caged InsP\(_3\) in the *Drosophila* photoreceptors did not result in activation of the light-sensitive channels (153). However, pipette application to the cell during whole cell recordings of the hydrolysis-resistant GTP analog guanosine 5’-O-(3-thiotriphosphate) (GTP\(_{S}\)), which is expected to have an excitatory effect, also failed to activate the transduction...
cascade. Therefore, there is probably a problem of accessibility of chemicals to the signaling membrane in the preparation of isolated Drosophila ommatidia during whole cell recordings. It is thus possible that these negative results may be attributed to the highly compartmentalized structure of the phototransduction machinery in Drosophila.

An alternative method of application has been successfully attempted in the larger flies, Lucilia and Musca (41, 180). These studies exploited the finding that Lucifer yellow applied extracellularly is taken up by the photoreceptors following intense illumination, possibly via receptor-mediated endocytosis (201). Minke and Stephenson (112) introduced extracellular GTPγS into photoreceptors by application of intense light. The effect of GTPγS in Musca photoreceptors was manifested by a noisy depolarization in the dark, with properties of the response to light. Similarly, extracellular application of InsP3 into Musca or Lucilia retinae accompanied by intense white light resulted in a large increase in baseline noise that persisted in the dark long (>30 min) after the light. Application of 2,3-diphosphoglycerate (DPG), an inhibitor of InsP3 hydrolysis, greatly enhanced the effect of exogenously applied InsP3 and also enhanced and prolonged the physiological response to light, suggesting an endogenous production of InsP3 during illumination. Noise analysis suggested that the InsP3-induced noisy depolarization is similar to the noisy depolarization induced by dim light (41, 180).

Biochemical studies, which strongly support an essential role of PLC, also suggested indirectly that InsP3 is involved in fly phototransduction. Evidence for operation of a light-dependent PLC in fly photoreceptors was obtained from biochemical experiments in membrane preparations of Musca and Drosophila eyes. An eye membrane preparation was developed in which light-dependent phosphoinositide hydrolysis could be studied under defined conditions, allowing the effects of activators and inhibitors to be analyzed. Musca eyes or Drosophila heads containing intact cells were preincubated with [3H]inositol to label the phosphoinositides. The membrane prepared from these cells was used to analyze the hydrolysis of phosphoinositides. The Musca eye membrane preparation responded to illumination by an increase in the accumulation of InsP3 and inositol bisphosphate (InsP2), the products of polyphosphoinositide hydrolysis by PLC. Addition of DPG substantially decreased the accumulation of InsP2 and concurrently increased the accumulation of InsP3, which thus becomes the major product of the light-induced phosphoinositide hydrolysis (41). It is apparent that fly membranes are endowed with the enzymatic system necessary to produce InsP3 and to eliminate it after it has been produced. Both the robust light-dependent preferential production of InsP3 and the efficient turn-off mechanism to stop its action are consistent with an internal messenger role of InsP3 in fly phototransduction (for review, see Refs. 107, 167).

The Drosophila genomic sequence (2) identifies only one InsP3 receptor gene in the Drosophila genome, and mutations in this gene are lethal (1, 194). However, it is possible to generate mutant photoreceptors in mosaic patches by inducing mitotic recombination in heterozygotes. Intracellular recordings from photoreceptors in such mosaic patches revealed no differences from WT, leading the authors to conclude that the InsP3 receptor played no role in phototransduction (1). A more detailed study used mosaic eyes homozygous for a deficiency of the InsP3 receptor of Drosophila, where elimination of InsP3 receptor was confirmed by RT-PCR, Western blot analysis, and immunocytochemistry (153). In this mutant, all aspects of the photoreponse when examined in detail by whole cell recordings were normal. These results indicate that the InsP3 receptor that was eliminated does not participate in phototransduction, or that it is redundant to a function of another protein. The apparent conclusion from these experiments is that the InsP3 branch of the inositol lipid signaling is not involved in phototransduction of Drosophila. However, this conclusion has been recently questioned following the use of a novel antagonist of the InsP3 branch of the inositol lipid signaling.

The membrane-permeant compound 2-aminoethoxydiphenyl borate (2-APB) has proven effective as a probe for assessing the involvement of InsP3 branch of the inositol lipid signaling in intact cells. 2-APB at 75 μM blocks receptor-mediated Ca2+ store emptying in intact HEK293 cells as well as in several other cells tested (100). In broken cells, 2-APB directly blocks InsP3-mediated Ca2+ release from the ER. 2-APB has no effect on InsP3 binding, does not alter InsP3 production through agonist-sensitive PLC, and does not modify the function of ryanodine receptors or voltage-gated Ca2+ channels (100, 102). 2-APB applied to the external medium reversibly and efficiently blocks the endogenous and robust InsP3-mediated signaling pathway of Xenopus oocytes by specifically inhibiting InsP3-induced release of Ca2+ from internal stores. Thus 2-APB seems to operate at a stage after production of InsP3 but before InsP3 action in mediating the rise in cellular Ca2+ (33). All the above features of 2-APB, together with very fast penetration into the signaling region inside the cell, make 2-APB a useful reagent for studies of the involvement of the InsP3 branch in Drosophila phototransduction. Recent studies indeed showed that 2-APB is highly effective at reversibly blocking the response to light of Drosophila flies in a light-dependent manner at a concentration range that coincides with its effectiveness in oocytes (33). Furthermore, 2-APB had no effect on the opening of the TRP and TRPL channels themselves, indicating that it operates upstream of the channels. Accordingly, because 2-APB seems to
block InsP₃-induced Ca²⁺ release in vertebrate cells, it cannot be excluded that Drosophila photoreceptors use the InsP₃ branch of the inositol-lipid signaling pathway for light excitation even if the mechanism and specificity of 2-APB action are not clear. The reconciliation of the apparently conflicting knockout of the InsP₃R and the 2-APB data is likely to shed important new light on the mechanism of activation of TRP and TRPL (33). In addition, the same dilemma may apply to the activation mechanism of vertebrate homologs of the TRPC subfamily, where similar apparently conflicting knockout of InsP₃Rs and the 2-APB data have been recently reported (101). Thus, in the triple InsP₃ receptor (InsP₃R) knockout DT40 cells devoided of all three functional InsP₃Rs, SOC activity was virtually identical to WT cells. However, in both WT and mutant cells, 2-APB blocked SOC activity in an identical manner (101).

5. Are DAG and polyunsaturated fatty acids second messengers of excitation?

The DAG branch of the inositol lipid signaling has not been considered as a route of excitation in invertebrate phototransduction because mutations in an eye-specific PKC (inaC) lead to defects in response termination and adaptation with no apparent effects on activation. This notion has been recently supported by experiments in Limulus showing that application of phorbol esters or the alkaloid indolactam, which are PKC activators, largely suppressed light-induced Ca²⁺ release and excitation at an early stage of the cascade (39). Contrary to these findings, application of phorbol esters and indolactam did excite mollusk (Lima) photoreceptors in the dark. The indolactam-induced excitation was also antagonized by PKC inhibitors (40). Phorbol ester also mimicked the effects of light in rdgA, a retinal degeneration mutant of Drosophila in which degeneration is largely enhanced by illumination (108).

Recently, a possibility has been considered that DAG does not operate solely as activator of PKC but also in a parallel pathway that leads to TRP or TRPL activation, as well as of mammalian TRPC channels (75; see below). DAG is a potential precursor for formation of polyunsaturated fatty acids (PUFAs) via DAG lipase, although the activity of this enzyme has not been demonstrated in Drosophila photoreceptors. Mutations in DAG kinase (rdgA, Fig. 1) that inactivates DAG result in a severe form of light-independent degeneration, although the reason for this is unknown (103, 104). Recently, a possible mechanism has been suggested following the discovery of Hardie and colleagues (35, 154) that in the rdgA mutant TRP and TRPL channels are constitutively open, while elimination of TRP by a mutation (in the double mutant rdgA; trp) partially rescues both the degeneration and the ability to respond to light. The authors suggest that elimination of DAG kinase by the rdgA mutation leads to accumulation of DAG and hence of PUFAs, which constitutively activate the TRP and TRPL channels, leading to a toxic increase in cellular Ca²⁺. Hardie and colleagues further showed that application of PUFAs to isolated Drosophila ommatidia reversibly activate the TRP and TRPL channels as well as recombinant TRPL channels expressed in Drosophila S2 cells. The latter were also rapidly activated by application of PUFAs in inside-out excised patches. Application of lipoxygenase inhibitors at low concentrations induced production of bumplike unitary responses followed by activation of macroscopic currents at higher concentrations. The authors suggest that PUFAs may be messengers that directly mediate activation of TRP and TRPL in Drosophila photoreceptors (35). It should be noted, however, that genes encoding for lipoxygenases have not been found in the Drosophila genome (2).

6. Constitutive activation of the TRP and TRPL channels in the dark

The ability to open TRP and TRPL channels in the dark may provide some insight on their gating mechanism, since the mechanism of dark openings bypasses at least some of the phototransduction stages that are activated by light.

RdgA is not the only Drosophila mutant in which there is constitutive activation of the light-sensitive channels. Specific mutations in the trp gene also cause constitutive activation of the TRP channel, leading to extremely rapid photoreceptor degeneration in the dark (214, Fig. 5). A new mutant designated TrpP₃65 does not display the transient receptor potential phenotype and is characterized by a near-normal level of the TRP protein and rapid, semi-dominant degeneration of photoreceptors. Despite its unusual phenotypes, TrpP₃65 is a trp allele because a TrpP₃65 transgene induces the mutant phenotype in a wild-type background, and a wild-type trp transgene in a TrpP₃65 background suppresses the mutant phenotype. Moreover, amino acid alterations that could cause the TrpP₃65 phenotype are found in the transmembrane segment region of the mutant channel protein. These mutations are due to the following amino acid substitutions: Pro(500)Thr, His(531)Asn, Phe(550)Ile, and Ser(867)Phe. Whole cell recordings clarified the mechanism underlying the retinal degeneration by showing that the TRP channels of TrpP₃65 are constitutively active, thus presumably allowing a toxic increase in cellular Ca²⁺ (214, Fig. 6).

Constitutive activation of TRP and TRPL was also obtained by metabolic stress, which turned out to be very efficient in reversibly causing channels openings in the dark in vivo. Accordingly, patch-clamp whole cell recordings and measurements of Ca²⁺ concentration by ion-selective microelectrodes in intact eyes of normal and
mutant Drosophila revealed that anoxia rapidly and reversibly depolarizes the photoreceptors and induces Ca\textsuperscript{2+} influx into the photoreceptor cells in the dark (Fig. 7). Furthermore, openings of the light-sensitive channels, which mediate these effects, could be obtained by mitochondria uncouplers or by depletion of ATP in photoreceptor cells, while the effects of illumination and all forms of metabolic stress were additive. Effects similar to those found in WT flies were also found in mutants with strong defects in rhodopsin, G\textsubscript{q} protein, or phospholipase C, thus indicating that the metabolic stress operates at a late stage of the phototransduction cascade (Fig. 8). Genetic elimination of both TRP and TRPL channels prevented the effects of anoxia, mitochondria uncouplers, and depletion of ATP, thus demonstrating that the TRP and TRPL channels are specific targets of metabolic stress. These results suggest that a constitutive ATP-dependent process is required to keep TRP and TRPL channels closed in the dark, a requirement that would make them sensitive to metabolic stress (4). The results also suggest

![Figure 5](image_url)

**FIG. 5.** Electron micrographs of transverse sections through the layer of Drosophila omatidium Trp\textsuperscript{P365}/trp\textsuperscript{CM} raised at 19°C and Trp\textsuperscript{P365}/Trp\textsuperscript{P365}. All samples were obtained from newly eclosed adult flies, and all flies were marked with w. The Trp\textsuperscript{CM} mutant is the original, nearly null, Trp mutant. The electron-dense structures are the rhabdomeres, which are composed of microvilli that contain the signaling molecules. The trp\textsuperscript{CM} mutation eliminates most of the TRP protein. The figure shows that the degree of degeneration in a newly eclosed fly depends on the amount of mutated TRP having the Trp\textsuperscript{P365} mutation. [From Yoon et al. (214).]

![Figure 6](image_url)

**FIG. 6.** Single-cell functional analysis by whole cell recordings of the Trp\textsuperscript{P365} mutant. A: a typical light-induced current (LIC) of a wild-type cell (left trace) in response to an orange stimulus and the absence of any responses in Trp\textsuperscript{P365}/trp\textsuperscript{CM} and Trp\textsuperscript{P365}/Trp\textsuperscript{P365} (middle and right traces). The duration of the orange light stimulus is indicated above each trace. B and C compare families of current traces elicited by series of voltage steps from photoreceptors of wild-type (left column), the light-insensitive cells of Trp\textsuperscript{P365}/trp\textsuperscript{CM} (middle column), and Trp\textsuperscript{P365}/Trp\textsuperscript{P365} homozygotes (right column). For each experiment, a series of voltage steps was applied from a holding potential of −20 mV in 20-mV steps (bottom traces). B: membrane currents were recorded 30 s after establishing the whole cell configuration with physiological concentrations (1.5 mM) of Ca\textsuperscript{2+} in the bath. C: application of 10 mM La\textsuperscript{3+} to the bath suppressed the membrane currents. The figure shows that the trp\textsuperscript{CM} mutation eliminated the response to light due to constitutive activity of the TRP channels that could be blocked by La\textsuperscript{3+}. [From Yoon et al. (214).]
that the effects of PUFAs may be indirect, since these compounds have been shown in many studies to function as efficient uncouplers of mitochondria (7). Suppression of TRP activity is not unique to Drosophila; the NAD-induced activity of LTRPC2 is also suppressed by ATP (see sect. IV D4, Ref. 160).

The constitutive activity of the TRP and TRPL channels may lead to cell death under metabolic stress, not only in Drosophila but also in vertebrate cells, which express TRP and TRPC channels (9, 123). Aortic endothelial cells have been shown to express an oxidant-activated nonselective cation channel that functions as a redox sensor. A dominant negative form of TRPC3 abolished the oxidant-induced current in aortic cells, suggesting that TRPC3 is involved in this conductance (9). Recently it has been suggested that LTRPC7 are reporters of the metabolic status of cells by being sensitive to Mg-ATP (9). The latter finding is in accord with the findings in Drosophila; however, because it is known that the role of TRP channels in the fly is to respond to light stimuli, it is conceivable that some of the reported sensitivities of TRP channels are epiphenomena, while their main role is altogether different.

7. TRP and store-operated Ca
text2+ entry

Because Drosophila TRP is the target of inositol lipid signaling and TRP has a high Ca
text2+ selectivity, Hardie and Minke (63) have proposed that excitation in Drosophila might be analogous to the process of PI-regulated Ca
text2+ influx described in a wide variety of mammalian cell types. Experiments designed to test whether a SOC-like mechanism might operate in Drosophila phototransduction have been largely negative (58). Although both ionomycin and thapsigargin release Ca
text2+ from intracellular compartments (58, 155), store depletion by these agents is not associated with the activation of any currents, nor do these agents block the response to light during several minutes (but thapsigargin does block the LIC after long exposure and confers a trp phenotype on WT flies; Ref. 37). Application of Ca-BAPTA to the cell via the recording pipette, buffering Ca
text2+ at concentrations at a level of \(10^{-5}\) nM, activates an inward current following application of ionomycin (58). However, it is not clear if this effect is due to store depletion or other effects similar to those activated by metabolic stress. The elimination of the InsP3R by mutation without affecting phototransduction has also been used as a strong argument against a role of inositide-mediated store depletion in Drosophila TRP and TRPL activation (1, 153). Thus, although conditions have been found in Drosophila to activate TRP and TRPL channels, which in some studies have been used to demonstrate SOC mechanism in vertebrate cells, it is not clear whether they represent the mechanism operating normally and represent the physiological route of excitation.

C. Role of TRPC3 in Pontine Neurons

At present, TRPC2, TRPC3, TRPC4, and TRPC6 are the TRPC channels that have been studied in detail in the native tissue. A recent study by Montell and colleagues (95) has shown that TRPC3 is highly enriched in neurons of the brain, especially in the forebrain, midbrain, and hindbrain of rat embryo. Because TRPC3 is predominantly expressed in the rat brain during a relatively narrow developmental period before and after birth (95), its function may be related to developmental processes. Im-
Importantly, the temporal and spatial distribution of TRPC3 parallels that of the neurotrophin receptor TrkB. Activation of TrkB by brain-derived nerve growth factor (BDNF) led to production of a PLC-γ-dependent nonselective cation current ($I_{\text{BDNF}}$) in isolated pontine neurons. Evidence that TRPC3 contributes to $I_{\text{BDNF}}$ in vivo is provided by the
following observations: 1) $I_{\text{BNDF}}$ was activated by anti-TRPC3 antibody both at the macroscopic current level and in single-channel recordings. In these experiments BNDF and the antibody acted synergistically. 2) Heterologous coexpression of TrkB receptor and TRPC3 in 293T cells followed by current measurements indicated that $I_{\text{BNDF}}$ was not observed in cells expressing either TrkB or TRPC3 alone. 3) TRPC3 coimmunoprecipitated with TrkB of rat brain, suggesting that the two proteins interact with TrkB and form a protein complex. 4) $I_{\text{BNDF}}$ was blocked by SK&F-96365, a nonspecific inhibitor of TRPC3. Interestingly, the properties of single-channel conductance that was activated by the anti-TRPC3 antibody in pontine neurons showed smaller conductance (i.e., 27 pS, see Table 2) and longer mean open time (20 ms) relative to measurements in heterologously expressed TRPC3 channels (of ~60 pS and <2 ms). In addition, unlike the heterologously expressed TRPC3 channels (100), the native pontine TRPC3 channels could not be activated by the 1-oleoyl-2-acetyl-sn-glycerol (OAG) analog of DAG. Application of InsP$_3$ activated the pontine TRPC3 channels, and inhibition of either PLC by its antagonist U73122 or the InsP$_3$ receptor by xestospongin (100) inhibited the $I_{\text{BNDF}}$ current. These results are consistent with activation of TRPC3 via the conformation coupling hypothesis (see below), not only in heterologous system (89, 100) but also in vivo (95).

D. Control of Ca$^{2+}$ Influx in Vascular Smooth Muscle Cells

There are several studies reporting that TRP homolog channels are involved in the control of vascular smooth muscle contraction and relaxation (for review, see Ref. 163). Thus TRPC1 has been recently reported to be involved in SOC activity of resistance arterioles, arteries, and veins from human mouse or rabbit (206). TRPC1-specific antibody targeted to the outer vestibule of TRPC1 channel identified TRPC1 in native vascular smooth muscles. Interestingly, peptide-specific binding of the antibody selectively blocked SOC activity in these cells, suggesting that TRPC1 underlies SOC activity and hence Ca$^{2+}$ influx to these cells (206).

In perhaps the most detailed study to date, Mori and colleagues (81) provided compelling evidence that TRPC6 is a requisite component of the $\alpha_1$-adrenoreceptor activator (AR) Ca$^{2+}$-permeable nonselective cation channel (NSCC) (reviewed in Ref. 163).

1. TRPC6 is the essential component of $\alpha_1$-AR Ca$^{2+}$-permeable NSCC channel

The $\alpha_1$-AR is distributed widely in the vascular system and played a central role in control of systemic blood pressure via sympathetic innervations. Mori and colleagues (81) have found that heterologous expression of murine TRP6 in HEK293 cells reproduced almost exactly the essential biophysical and pharmacological properties of $\alpha_1$-AR-NSCC, previously identified in smooth muscles of rabbit portal vein. These properties include activation by DAG, S-shaped I-V relationship, high divergent cation permeability, single-channel conductance of 25–30 pS, augmentation by fufanemate, and blockade by Ca$^{2+}$, La$^{3+}$, Gd$^{3+}$, SK&F-96365, and amiloride. The level of TRPC6 mRNA expression was much higher in smooth muscles of both murine and rabbit portal vein than of other TRPC members such as TRPC1 or TRPC3. Furthermore, treatment of primary cultured portal vein myocytes with TRPC6 antisense oligonucleotides resulted in marked inhibition of TRP6 immunoreactivity and selective suppression of $\alpha_1$-AR-activated store depletion-independent cation current and Ba$^{2+}$ influx.

2. TRPC4 mediates vasorelaxation of blood vessels

A relatively small fraction of TRPC4 channels is expressed in vascular endothelial cells. However, TRPC4 of endothelial cells is potentially of high functional importance, since Ca$^{2+}$ influx to these cells, presumably through TRPC4 channels, may contribute to increase in cellular Ca$^{2+}$, which is necessary for synthesis and release of vasoactive compounds (nitric oxide and prostanoids) (49). A strong support for this notion has been recently provided by production of knockout mice designated TRP4$^{-/-}$ (49).

Nilius, Flockerzi, and colleagues (49) have studied primary cultured mouse aortic endothelial cells (MAECs) of normal mice, which express TRPC4, and compared them with similar MAECs of TRP4$^{-/-}$ lacking TRPC4 (but not other TRPC channels, as tested in brain tissue) using a protocol that activates SOC channels. To deplete the InsP$_3$-sensitive Ca$^{2+}$ stores they used either InsP$_3$ applied to the patch-clamp pipette or the Ca$^{2+}$ pump inhibitor tert-butyl-benzohydroquinone (tBHQ). Ca$^{2+}$ store depletion quickly led to a constitutive inward current with inward rectification, which was effectively blocked by La$^{3+}$ or Mg$^{2+}$ (in Ca$^{2+}$-free medium) in a similar manner to the TRP channels of Drosophila. The constitutively active channels revealed a high Ca$^{2+}$ permeability, $P_{Ca}/P_{Na} = 159.7$ (calculated using the constant field theory). Strikingly, the constitutive SOC current was missing in TRP4$^{-/-}$ knockout mice. Furthermore, vasoactive agonists (ACH, ATP) induced an increase of free cellular Ca$^{2+}$, which was markedly reduced in the knockout mice. Importantly, stimulation of endothelial cells of the knockout mice with the vasoactive agonist revealed that vasorelaxation of aorta rings was impaired.

The above experiments constitute the first direct evidence for the physiological relevance in mature cells of mammalian TRPC channels and for SOC in endothelial...
TABLE 2. Functional properties of “TRP-homolog” and “TRP-related” channels

<table>
<thead>
<tr>
<th>Channel</th>
<th>Single-Channel Conductance, pS</th>
<th>Relative Permeability $P_{c_{2}}/P_{Na}$</th>
<th>Mechanism of Activation</th>
<th>Heterologous Expression in the Following Cells</th>
<th>Species Used for Studies in the Native Tissue</th>
<th>Possible Function</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRP</td>
<td>4 (in divalent), 35 (divalent free) in <em>Drosophila</em></td>
<td>110 ($P_{c_{2}}/P_{Na}$ in <em>trp</em> mutant), 40 ($P_{c_{2}}/P_{Na}$ in WT)</td>
<td>PUFAs?; Ca$^{2+}$?</td>
<td>SJ9, 203T, <em>Xenopus</em> oocytes</td>
<td><em>Drosophila</em></td>
<td>Light-activated channel</td>
<td>35, 37, 52, 53, 55, 57–64, 68, 126, 140, 153, 156, 158, 190, 200</td>
</tr>
<tr>
<td>TRPL</td>
<td>35 (in divalent) in <em>Drosophila</em>, 70 (in divalent free) in <em>Drosophila</em></td>
<td>4.3 ($P_{c_{2}}/P_{Na}$ in <em>Drosophila</em> <em>trp</em> mutant, 3 ($P_{c_{2}}/P_{Na}$ in SJ9)</td>
<td>PUFAs?; DAG (in SJ9)?</td>
<td>203T, S2, SJ9, CHO, <em>Xenopus</em> oocytes</td>
<td><em>Drosophila</em></td>
<td>Light-activated channel</td>
<td>35, 37, 52, 66, 68, 69, 76, 77, 90, 91, 129, 130, 174, 186, 198, 210, 211, 219</td>
</tr>
<tr>
<td>TRPγ</td>
<td>16 (from noise analysis)</td>
<td>5</td>
<td>DAG</td>
<td>CHO, HEK, COS</td>
<td>Portal vein myocytes</td>
<td>α₁-AR activated</td>
<td>16, 19, 74, 81, 114</td>
</tr>
<tr>
<td>TRPC1</td>
<td>42 (from noise analysis), 66 (CHO cells), 27 (pontine neurons)</td>
<td>0.4</td>
<td>DAG</td>
<td>CHO</td>
<td>Mouse</td>
<td>Vasorelaxation</td>
<td>49, 144, 161, 183, 185, 197</td>
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<tr>
<td>TRPC4</td>
<td>66, 48</td>
<td>1, 14</td>
<td>SOC? PLC</td>
<td>CHO, HEK-203</td>
<td></td>
<td>132, 145, 161, 178</td>
<td></td>
</tr>
<tr>
<td>TRP-related channels</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>OSM-9</td>
<td>35</td>
<td>3.8 (heat), 9.6 (capsaicin)</td>
<td>PKC, anandamide</td>
<td><em>Xenopus</em> oocytes, HEK293</td>
<td></td>
<td>Osmoreception, olfaction</td>
<td>36</td>
</tr>
<tr>
<td>VR1</td>
<td>107</td>
<td>2.9</td>
<td>High temperature</td>
<td><em>Xenopus</em> oocyte, HEK293</td>
<td></td>
<td>Embryonic dorsal root ganglia from rat</td>
<td>25, 34, 222</td>
</tr>
<tr>
<td>VRL-1</td>
<td>137</td>
<td>6.3</td>
<td>Osmolarity</td>
<td>HEK293</td>
<td></td>
<td>High temperature pain</td>
<td>177</td>
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<tr>
<td>CaT2</td>
<td>130</td>
<td>0.5</td>
<td></td>
<td>CHO</td>
<td>Rabbit kidney</td>
<td>Ca$^{2+}$ transport in epithel</td>
<td>73, 195</td>
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<tr>
<td>CaT1</td>
<td>138, 215</td>
<td>42 in divalent free</td>
<td></td>
<td>CHO</td>
<td>Ca$^{2+}$ absorption in the intestine</td>
<td>137, 138, 215</td>
<td></td>
</tr>
<tr>
<td>PCL</td>
<td>137 (at ±50 mV)</td>
<td>6</td>
<td>Ca$^{2+}$</td>
<td><em>Xenopus</em> oocytes, CHO</td>
<td></td>
<td>Osmosensory transduction</td>
<td>11, 31</td>
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<tr>
<td>Polycystin-2</td>
<td>105</td>
<td>2.8</td>
<td>ADP-ribose reduction in Mg-ATP</td>
<td>CHO, HEK-293</td>
<td></td>
<td>Male mating (LOV-1)</td>
<td>54</td>
</tr>
<tr>
<td>LTRPC2</td>
<td>60</td>
<td>Nonselective</td>
<td></td>
<td>CHO</td>
<td>U937 cells</td>
<td>Channel linked to cellular metabolism</td>
<td>141, 160</td>
</tr>
<tr>
<td>LTRPC7</td>
<td>105</td>
<td>2.8</td>
<td>ADP-ribose reduction in Mg-ATP</td>
<td>CHO, HEK-293</td>
<td></td>
<td>C. elegans; renal tubular cells,</td>
<td>123, 159</td>
</tr>
<tr>
<td>TRP-PLIK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Human cells</td>
<td></td>
</tr>
</tbody>
</table>

CHO, Chinese hamster ovary cells; DAG, diacylglycerol; InsP$_3$, inositol 1,4,5-trisphosphate; PLC, phospholipase C; WT, wild type; PUFA, polyunsaturated fatty acid; PKC, protein kinase C.
cells, which seems to be specific to TRPC4 in these cells (49). It is interesting to note that heterologous expression of mouse TRPC4 and TRPC5 in HEK cells resulted in markedly different results (161). In the heterologous HEK cells, TRPC4 could not be activated by store depletion; it shows relatively large single-channel conductance and much smaller selectivity to Ca\(^{2+}\) relative to Na\(^+\) (see sect. vC2). Further experiments are required to determine if the above differences arise from the different cellular environment of the heterologously expressed TRPC4 channels.

E. TRPC2 Regulates Ca\(^{2+}\) Entry Into Mouse Sperm Triggered by Egg ZP3

An important function of a TRP homolog channel, TRPC2, in the native cell has been recently demonstrated in the fertilization process of the mammalian egg. This multistep process begins by association of the sperm with a glycoprotein constituent of the zona pellucida, ZP3, of the egg leading to release of hydrolytic enzymes from the sperm acrosome and remodeling of the sperm surface. This so-called acrosomal reaction is critical for penetration of the sperm into the egg and involves a G protein and PLC-mediated transduction cascade that eventually leads to activation of SOC channels and sustained increase in intracellular Ca\(^{2+}\). It has been recently shown that TRPC2 is essential for activation of the sustained Ca\(^{2+}\) influx into sperm by ZP3 (84). The essential role of TRPC2 in the acrosomal reaction was demonstrated by inhibition of ZP3-induced Ca\(^{2+}\) influx and acrosomal reaction by anti-TRPC2 antibody (84). The abundant expression of TRPC2 isoform in the sperm is also consistent with the role of TRPC2 in the acrosomal reaction. The same argument is valid for the localization of TRPC2 to the VNO, which suggests that it may function in the specific sensory system of pheromone detection. Because TRPC2 is a pseudo gene in humans, pheromone detection seems to be lost in humans. However, with regard to fertilization, a protein different from TRPC2 should function in the acrosomal reaction of humans.

F. The TRP-Related Group

Technical difficulties preclude the analysis of several members of this subfamily, such as VR1L, the C. elegans osm-9, ECaC1, and other channels in their native tissue. An important approach to overcome this difficulty is to produce knockout mice, in which the specific TRP-related channel has been eliminated, and compare specific function, which is mediated via TRP-related channels. Their specific role can thus be studied from the behavior to the cellular level. This approach has been used for TRPC4 (see sect. vD2) and for VR1 channels (see below). In addition, the cellular localization of these channels to specific tissue or sensory neurons allows some predictions on the function of these channels and comparison of these predictions to the actual measured properties in heterologous systems.

I. VR1 channels mediate specific pain

Pain is initiated when peripheral terminals of a subgroup of sensory neurons are activated by noxious chemicals, pH, and mechanical or thermal stimuli. Nociceptors are characterized, in part, by their sensitivity to capsaicin (27). Capsaicin, the active ingredient in hot pepper, is an excitatory neurotoxin that selectively destroys primary afferent nociceptors in vivo and in vitro. Heterologous expression of VR1 in HEK293 cells induced necrotic cell death within several hours of continuous exposure to capsaicin while control cells were not affected (27). These results are reminiscent of the effect of the trpP\(^{396}\) and the rdgA mutations in Drosophila; they produce similar effects in vivo presumably via constitutive activity of the TRP channels (see above). The natural stimulus that activates VR1 channels (also designated TRPV1) seems to be noxious heat, since the temperature-response profile of VR1 matches very closely those reported for heat-evoked pain response in mammals and in cultured neurons. Also, ruthenium red, which blocks VR1 activity in mammalian cells or in oocytes, blocks heat-evoked nociceptive response in the rabbit ear (27). In addition, the heat-evoked currents observed in both VR1-expressing cells and cultured sensory neurons are carried through outwardly rectifying, nonselective cation channels. Reduction in tissue pH, which usually results from infection, inflammation, or ischemia, seems also to be endogenous modulators of VR1. Interestingly, the endogenous cannabinoid receptor agonist anandamide was found to induce vasodilatation by activating vanilloid receptor on perivascular sensory neurons. Heterologous expression of VR1 in HEK293 cells and Xenopus oocytes (222) validates that anandamide is an agonist of this receptor.

An important step toward the establishment of the in vivo function of VR1 has been made by the creation of knockout mice lacking VR1. Sensory neurons from mice lacking VR1 are severely deficient in their response to the noxious stimuli heat (>43°C), protons, or vanilloid compounds but exhibited normal responses to noxious mechanical stimuli (25).

Recently, a functional study of the mechanism underlying activation of VR1 channels in both the native neuronal cells and a heterologous system has been reported (150). Because PKC is a prominent participant in pain signaling (28, 29, 87), the role of PKC in VR1 activation was studied. Application of 12-tetradecanoylphorbol 13-acetate (TPA), which activated endogenous PKC, to Xenopus oocytes expressing VR1 markedly increased the
amplitude of capsaicin, anandamide, or proton-activated currents, while TPA induced a current by itself. Specific PKC inhibitors antagonized the above effects. Excised outside-out membrane patches of VR1 expressing oocytes showed pronounced activation of single channels during application of capsaicin or TPA, with a conductance of ~110 pS. This single-channel conductance is much larger than previously reported for the same channel (i.e., 35 pS) (27) (see Table 2). Replacing ATP with the hydrolysis-resistant analog adenosine 5’-O-(3-thiotriphosphate) (ATPγS) activated the channels in a similar manner to TPA, suggesting that phosphorylation of proteins by PKC underlies channels activation. Importantly, similar single-channel activation by capsaicin, TPA, or the algic peptide bradykinin was observed in patch-clamp cell-attached recordings from sensory neurons, isolated from the dorsal root ganglia of rat embryos. Recently, it has been demonstrated that bradykinin and nerve growth factor (NGF) release VR1 from PI(4,5)P2-mediated inhibition. Accordingly, diminution of plasma membrane PI(4,5)P2 by antibody sequestration of PLC hydrolysis mimics the potentiating effects of bradykinin and NGF (34). Modulation of PI(4,5)P2, which may regulate VR1 function, has been suggested also to regulate TRPL activity in heterologous system (47). Such a mechanism is unlikely to function in vitro, since depletion of PI(4,5)P2 in the trp mutant that has only TRPL channels suppresses, rather than facilitates, TRPL channel activity (67). A modulatory role of anandamide in PKC-VR1 signaling was examined by application of anandamide to excised patches of Xenopus oocytes expressing VR1. Application of anandamide (10 μM) produced a progressive increase in single-channel activity and in the macroscopic current (150). This and the above studies demonstrate again the complex nature of TRP gating in different cells.

2. C. elegans osm-9 may regulate olfaction and osmoregulation

Functional analysis of C. elegans OSM-9 was based on behavioral tests in mutant animals. These tests indicate an osm-9 gene function in a specific subset (AWA) of neurons that mediate olfactory transduction, response to osmotic and nose-touch stimuli. It is also involved in slow adaptation to olfaction stimuli (36). No functional studies at the cellular level are available.

3. The epithelial Ca2+ channels ECaC1, CaT1, CaT2, and OTRPC4

Although the functional properties of ECaC1, CaT1, CaT2, and OTRPC4 (see Fig. 4) have been derived from heterologous expression studies (see below), their rather specific localization to epithelial cells of specific organs points to the possible function in the native tissue as the gate keeper of 1,25-dihydroxyvitamin D3-dependent active transepithelial Ca2+ transporter in the kidney (73, 137) or a highly Ca2+-selective SOC channel in the duodenum (CaT1) (129). OTRPC4, which functions as an osmoreceptor and is expressed predominantly in the distal nephron of the kidney, a region exposed to hypotonic solution, is activated by a decrease in osmolarity, and thus its activation mechanism fits with its localization (177). Their functional expression in heterologous cells supports this conclusion (see below).

G. NOMPC Is a Channel That Mediates Mechanosensory Transduction

The mechanotransduction channel NOMPC belongs to a unique subfamily of the TRP-related subfamilies (196). The evidence that NOMPC mediates mechanosensory transduction came from a highly (10% of control) reduced mechanoreceptor current in mechanosensory neurons located at the mechanoreceptor organ of the Drosophila nompC mutant (196). Mechanosensory neurons reveal a pronounced degree of adaptation to mechanical stimuli. Thus the intensity-response relationship between the amplitude of the mechanoreceptor current and displacement of the hair bristle shows a very steep dependence (range of 10 μm) during constant background displacement. The adaptation is manifested by a shift of the intensity response curve with increasing the intensity of the background displacement. Interestingly, one allele of nompC (nompC4) shows an abnormally rapid adaptation, whereas the amplitude of the mechanoreceptor current is normal. This mutant may prove valuable to study the regulation mechanism of the channel. At present there are no data on the ionic mechanism of the mechanoreceptor current and on properties of the NOMPC channels.

H. Summary

The expression pattern of various members of the TRP family to specific cells and tissues may give us a clue regarding their specific functions, which seem to be diverse. Unfortunately, the absence of selective antagonists and the difficulty in analyzing many members of the TRP family in the native tissue impose a great difficulty in understanding the function of these TRP channels and in resolving their mechanism of activation and properties under physiological conditions. The detailed studies in Drosophila that combine genetic dissection with powerful electrophysiological and single-cell Ca2+-measuring techniques may also provide important clues regarding the activation mechanism of at least the mammalian TRPC channels that have striking structural similarity to Drosophila TRP and TRPL. There is a steadily growing research on mammalian members of the TRP family that
have been conducted in the native tissue. New studies show a crucial role of TRPC2 in the process of acrosomal reaction in sperm, while TRPC3 has a role in developing pontine neurons. Similarly, studies of VR1, TRPC1, TRPC4, and TRPC6 in primary afferent neurons, salivary glands, and vascular smooth muscles have revealed an essential role in nociceptors, in control of fluid secretion in salivary glands (172), and in control of smooth muscle tone, respectively. It thus seems that studies in the native tissue are indispensable. In the following section, recent studies on coexpression of two different TRPC channels, which probably form heteromultimeric channels, are reviewed. The striking differences in channel properties of homo- and heteromultimeric channels described below, the possible sensitivity of TRPs to the cellular environment, and the probable protein-protein interactions in most TRPs emphasize the need to study TRP channels in the native cellular environment.

V. FUNCTIONAL ANALYSIS IN HETEROLOGOUS SYSTEMS

A. Heterologous Expression of trpl, But Not trp, Leads to Channels With Similar Properties of the Native Channel

Expression of cDNA of trpl and possibly of trp in a variety of expression systems has provided important support that the above gene products function as channels. The expression cells include two insect cell lines (Spodoptera SF9 cells and Drosophila S2 cells), mammalian cell lines (HEK293T and CHO cells), and Xenopus oocytes (52, 69, 90, 129, 143, 174, 209). Schilling and colleagues (190) first reported heterologous expression of TRP. These investigators found that expression of trp cDNA in SF9 cells led to the appearance of a novel conductance that could be activated by thapsigargin. However, there is only limited similarity between the properties of the heterologously expressed TRP-dependent conductance and the light-induced conductance of Drosophila. The TRP-dependent currents in the SF9 cells markedly differed from the light-activated current of Drosophila in several main aspects. The I-V relationship is approximately linear in SF9 cells expressing TRP, while showing inward and outward rectification in Drosophila. When the holding voltage is stepped, there is instantaneous current with no time-dependent kinetics in Drosophila. The TRP-dependent current of Drosophila shows positive and negative feedback effects of Ca$^{2+}$ on the LIC, which are completely absent in the SF9 cells. The TRP-dependent conductance in SF9 cells has low permeability to Ba$^{2+}$ and is not blocked by Mg$^{2+}$ in concentrations that have a strong blocking effect in Drosophila (158). Xu et al. (209) functionally expressed TRP and found a novel conductance that could be activated by thapsigargin in HEK293T cells. However, the properties of this current like those of the SF9 cells showed several quantitative discrepancies from the in situ current (e.g., less permeable to Ca$^{2+}$, less sensitive to block by either La$^{3+}$ or Mg$^{2+}$, and linear I-V curve without rectification).

Expression of TRP did show appearance of the TRP protein in the heterologous system in the few cases where it was investigated (52, 174, 209). Nevertheless, in several unpublished studies no effect of transient TRP expression was found in either HEK293 cells (Minke, unpublished observations) or in Drosophila S2 cells. Expression of TRP in Xenopus oocytes has provided inconsistent data, whereas one study showed enhancement of the endogenous Ca$^{2+}$-activated Cl$^{-}$ current in TRP expressing oocytes (143), and another study failed to see any significant effect when TRP was expressed alone without TRPL (52). Taken together, it seems doubtful whether the heterologously expressed TRP reaches the surface membrane and functions as a channel; all reported conductance can be explained by enhancement of endogenous activity of channels of the host cells.

Indeed, the GTP binding protein Rab6, which is required for vesicular trafficking within the Golgi and post-Golgi compartments, turned out to be essential for TRP expression in normal amounts in the native tissue (168). Thus overexpression of mutant Rab6 protein shows reduction in both rhodopsin (Rh1) and TRP proteins. Rab6 is not the only protein required for TRP expression. Pak and colleagues (93) found a novel protein called INAF, which appears to be required for normal level of TRP expression and perhaps also for TRP function in the fly eye. Accordingly, a null mutant of INAF specifically reduced the amount of TRP to a very low level and seems to specifically affect the TRP activity (93).

The situation is very different for heterologous expression of TRPL, in which the appearance of a nonselective cation conductance has been reported after expression in SF9 cells (69, 77), S2 cells (68), HEK293 cells (209), CHO cells (69), and Xenopus oocytes (52, 91). In several cases, a constitutive activity of the TRPL channels was largely enhanced by coexpressed receptors, which activate endogenous G protein-coupled PI pathways. A common feature of the TRPL expression studies is a constitutive spontaneous activity, which increases with time during whole cell recording, although Minke and colleagues (52) and Montell and colleagues (209) reported that this was prevented when coexpressed with a large quantity of TRP. The channel properties of TRPL when expressed in Drosophila S2 cells were compared with the TRPL-dependent light-sensitive conductance as determined in the native cells of trp mutants during whole cell recordings (158). A variety of properties including single-channel conductance and open times, ionic selectivity for...
monovalent and divalent ions, block by Mg$^{2+}$, and $I$-$V$ relationship were found to be indistinguishable between native and heterologously expressed TRPL. The properties of TRPL channels expressed in other expression systems appear similar (90, 130). Thus, in contrast to the apparent failure to heterologously express functional TRP, heterologous expression of TRPL appears to form a conductance similar to that found in the native tissue, except that some factor is required to prevent constitutive activity, which does not occur under normal conditions in vivo. The recent cloning of TRPγ indeed solved this latter issue (207). Thus heterologous expression of a TRPγ-TRPL construct in 293T cells displayed an agonist (ATP)-activated current, which was not observed in control cells. The TRPγ-TRPL expressing cells do not show constitutive activity, and the $I$-$V$ relationships show the outward rectification typical for the TRPL channels in situ, strongly suggesting that TRPγ-TRPL forms a heteromultimer (207).

B. Mechanism of Activation of TRPL

1. Receptor-mediated activation

Coexpression of TRPL and the M5 muscarinic receptor in Sf9 cells using the baculovirus expression system reveals an increase in basal Ba$^{2+}$ influx typical of the constitutive activity of TRPL channels in these cells. Addition of carbachol to these cells produced a large increase in cellular Ca$^{2+}$, which could be blocked by atropine and by gadolinium (Gd$^{3+}$, 0.1–1.0 mM) but was insensitive to La$^{3+}$ (10 μM). Depletion of the Ca$^{2+}$ stores by thapsigargin had no effect, suggesting that TRPL is a store-independent channel (76, 77). Similar results were also obtained by coexpression of TRPL with other hormone receptors in Sf9 cells such as the histamine, thrombin, and thromboxane A$_2$ receptors (129, 130). The typical outwardly rectifying TRPL current was observed after hormonal stimulations following the TRPL expression in these cells.

2. Activation by InsP$_3$

TRPL channels expressed in Sf9 cells were activated by inclusion of InsP$_3$ in the recording pipette (45). Although heparin blocked activation by InsP$_3$, it could only partially counteract activation by a coexpressed bradykinin receptor, suggesting an additional alternative route of excitation. The authors concluded that the effect of InsP$_3$ was not mediated via a rise in Ca$^{2+}$, since it was unaffected by inclusion of 1 mM EGTA in the pipette. The authors suggested that InsP$_3$ might directly gate TRPL via conformational coupling (see below). Zimmer et al. (219) also found an excitatory effect of InsP$_3$, but reported that since this was abolished by prior depletion of stores with thapsigargin it was an indirect action via a rise in Ca$^{2+}$. Schultz and colleagues (129) using Sf9 cells did not find any effect of InsP$_3$, but reported that activated G$_{q/11}$ subunits could activate TRPL channels either when overexpressed or, in a few cases, when applied to inside-out patches. Although they initially suggested that TRPL channels were directly gated by the G protein in similar manner to some K$^+$ channels, more recently they concluded that their findings are best explained by G protein-mediated activation of PLC (70).

3. Effects of Ca$^{2+}$-CaM

TRPL was initially isolated on the basis that the expressed protein binds CaM via two putative CaM binding sites (147). Subsequently, the two CaM binding sites (designated CBS1 and CBS2) have been more accurately identified and studied in vitro and in vivo (32, 164). CBS1 binds CaM in a Ca$^{2+}$-dependent fashion (300–500 nM Ca$^{2+}$). In contrast, CBS2 binds CaM in the absence of Ca$^{2+}$ with dissociation occurring at levels above 5 μM. The authors suggest that dissociation of CaM from CBS2 and Ca$^{2+}$-dependent CaM binding to CBS1 are the mechanism of TRPL activation (198). Similar inhibition of TRPC channels by CaM has been recently demonstrated (182). In general, similar dependence of TRPL activation on CaM binding was obtained by expression of TRPL in CHO cells. However, these experiments showed Ca$^{2+}$ dependence of both CaM binding sites CBS1 and CBS2, although they differ in their affinity to CaM with $K_c$ values of 12.3 nM and 1.7 nM for CBS1 and CBS2, respectively. Both sites bind CaM only in the presence of Ca$^{2+}$, and the CaM antagonist calmidazolium inhibits the Ca$^{2+}$-dependent activation of TRPL current, suggesting modulation of TRP currents by cellular Ca$^{2+}$ via CaM (186). Lan et al. (91) reported that heterologous expression of TRPL in *Xenopus* oocytes leads to an elevated free Ca$^{2+}$, which was increased by low concentrations of CaM and inhibited at high. Zimmer et al. (219) reported that TRPL activity in CHO cells was enhanced by application of Ca$^{2+}$ with an EC$_{50}$ of 500 nM and that this could be reduced by the CaM antagonist calmidazolium.

Schultz and colleagues (130) have obtained different results in Sf9 cells that express TRPL channels together with the histamine receptor. The histamine-induced TRPL-dependent currents revealed reversible inhibition by Ca$^{2+}$ (IC$_{50}$ of 2.3 μM). Consistent with these observations, inside-out patches revealed TRPL channel activity that was reversibly inhibited by Ca$^{2+}$ (10–50 μM) in the cytosolic side. Interestingly, the CaM inhibitor calmidazolium had no inhibitory effect on channel activity, thus suggesting that CaM was not involved in the inhibition (130). Consistent with inhibition of the TRPL channels by Ca$^{2+}$ in Sf9 cells, experiments in the native tissue of *Drosophila* have shown pronounced inhibition of the LIC.
in the *trp* mutant, which has only TRPL channels. TRPL channels appear to be negatively regulated by Ca\(^{2+}\), since in the presence of extracellular Ca\(^{2+}\) the response to light is reduced by approximately threefold (158). Similarly, caged Ca\(^{2+}\) released during the rising phase of the light response inhibited the light response in *trp* mutants (59). To examine if CaM involves TRPL inhibition by Ca\(^{2+}\), Zuker and colleagues (164) generated transgenic flies in which TRP channels were absent and the native TRPL channels were replaced with constructs with CBS1 or CBS2 deleted. Activation by light in these flies was essentially unaltered, whereas there were major defects in response termination.

Hardie and Raghu (66) examined the effect of Ca\(^{2+}\) in S2 cells coexpressing the TRPL channels with a muscarinic receptor. Releasing caged Ca\(^{2+}\) resulted in a pronounced facilitation of the spontaneous TRPL activity. Releasing caged InsP\(_3\) released Ca\(^{2+}\) from internal stores but had at most a small effect on TRPL activity and none at all when BAPTA was included in the pipette solution. In contrast, excitation of TRPL via the coexpressed muscarinic receptor resulted in a rapid excitation of TRPL channels, which was not blocked by prior depletion of stores by InsP\(_3\) or thapsigargin or by inclusion of BAPTA in the pipette. The results suggest that a process requiring G protein and PLC, but not InsP\(_3\), underlies activation of heterologously expressed TRPL channels while channel activity can be modulated by Ca\(^{2+}\)-CaM. Taken together, the bulk of data suggest that in heterologous systems TRPL is constitutively open and that the channel can be negatively and positively regulated by Ca\(^{2+}\)-CaM.

4. Activation by PUFAs and DAG

PUFAs have been shown to activate recombinant TRPL expressed in S2 cells (35). This preparation has an advantage over the photoreceptor cells, because it allows measurements of single-channel activity. Importantly, PUFAs elicited TRPL channel activity in excised patches, suggesting direct activation of the channel by PUFAs. The fact that activation of TRPL by PUFAs was observed in the photoreceptors of the null norpA mutant, which lacks the photoreceptor specific PLC (17) and does not respond to light, was used as a strong argument in favor of direct activation of the channels without a need for PLC or other upstream proteins. Schilling and colleagues (47), who measured the effects of DAG analogs and PUFAs on TRPL expressed in SF9 cells, reported apparently different results. In contrast to the lack of any effect of DAG or DAG analogs on *Drosophila* photoreceptors, the DAG analog 1-stearoyl-2-arachidonyl-glycerol (SAG) (but not OAG) induced Ca\(^{2+}\) influx and elicited single-channel activity in TRPL-expressing SF9 cells. Consistent with the data reported by Hardie and colleagues (35), PUFAs activated both Ca\(^{2+}\) influx in intact SF9 cells expressing TRPL and elicited single-channel activity in excised patches. However, in contrast to previous claims that PLC is not directly involved in single-channel activation of excised patches, single-channel activation was elicited by application of three different PLCs. Furthermore, activation of TRPL channels in SF9 cells by PUFAs was markedly attenuated after inhibition of PLC by the U73122 antagonist (but not by the control compound U73343) (47). The authors concluded that both the hydrolysis of PIP\(_2\) and the generation of DAG are required to rapidly activate TRPL channels after receptor stimulation and that the effect of PUFAs is mediated at least in part via PLC. The reports are, therefore, contradictory, supporting either direct or PLC-mediated activation of TRPL by PUFAs. These results may arise from the multiple effects that PUFAs have while the activation of photoreceptors may arise indirectly from the effects of PUFAs as mitochondria uncouplers causing metabolic stress (4).

5. Store-operated Ca\(^{2+}\) entry

Almost all studies have found that TRPL is not activated by store depletion. One study has reported activation of TRPL by thapsigargin (211), but the possibility that the regulation was due to the associated increase in Ca\(^{2+}\) was not rigorously excluded.

In contrast, as mentioned above, heterologously expressed TRP is presumably activated by store depletion.

6. Coexpression of TRP and TRPL channels

Coexpressing *Drosophila* TRP and TRPL in Xenopus oocytes in cRNA ratios of 10:1 reveals a novel inward current showing inward and outward rectification that was activated by Ca\(^{2+}\) store depletion. This current was neither observed in native oocytes nor in oocytes expressing either TRP or TRPL alone by the same amounts of cRNA, implicating a cooperative action of TRP and TRPL in the depletion-activated current (52). Heterologous coexpression of TRP and TRPL in 293T cells produced an outwardly rectifying novel current, which could be activated by store depletion. Immunoprecipitation studies revealed physical interaction between TRP and TRPL as found in the native *Drosophila* retina (209). Evidence that TRP and TRPL form heteromultimeric channels was obtained by suppression of the TRP-dependent current by dominant negative form of TRP, and vice versa. It was also shown that the NH\(_2\) terminal and transmembrane domains contributed to subunit assembly. Because each of the channels by itself can be opened by light, the relevance of coexpression of TRP and TRPL to the native system is still not clear.

C. Heterologous Expression of “TRP-Homolog” Channels

Functional analysis of the TRPC group has been confined mainly to heterologous systems (but see TRPC2,
TRPC3, TRPC4, and TRPC6). Not surprisingly, the activation mechanism and biophysical properties remain unclear and controversial. Thus conflicting results have been reported for the same channel for reasons that remain unclear. A possible explanation may be the use of different expression system and expression levels of the TRPC homolog and possible interaction with endogenous proteins of the expressing cells.

TRPC1, TRPC4, and TRPC5 homologs seem to form one functional group. However, several studies suggest that they are activated by store depletion and therefore consider them as SOCs (15), while other studies show store-independent activation (70).

1. TRPC1

The TRPC1 isoform of human was transiently coexpressed with the M5 muscarinic receptor in COS cells. Activation of the endogenous inositol lipid signaling of these cells through the M5 receptor was obtained by application of carbachol (CCh). The depletion of the Ca^{2+} stores leads to the opening of Ca^{2+}-permeable surface membrane channels. This entry of Ca^{2+} is the manifestation of the endogenous SOC in control cells. In the cotransfected COS cells with TRPC1 and M5 receptor, a significantly larger Ca^{2+} influx relative to control cells was observed (218). Additional studies on a spliced variant of TRPC1 (221) expressed in CHO cells investigated some biophysical properties of the expressed TRPC1 homolog. Transient expression of TRPC1A induced a constitutive inward cationic current in Ca^{2+}-free medium, which was significantly larger than the control. Biophysical analysis of this current revealed a nonselective cation current that was not observed in the control cells. The relative permeability of the expressed channel to cations was P_{Ca}P_{Na}P_{Cs}P_{Ba} = 1:1:7.7:12.3. Inability to resolve single-channel activity was interpreted as an indication of very low single-channel conductance. Thus, according to this study, TRPC4 is a SOC with high permeability to divalent ions (144, 197). Results similar to those obtained in the TRPC4 isoforms were also obtained after expression of TRPC5 in HEK cells (144). The authors concluded that these channels have properties reminiscent of CRAC channels.

Expression of the human TRPC1 isoform in the insect Sf9 cells using the baculovirus expression system (173) provided a different conclusion. Expression of TRPC1 caused an increase in basal cytosolic free Ca^{2+} as well as Ba^{2+} concentration as a function of posttransfection time. The latter indicated activation of an influx pathway. Whole cell recordings indicate that the inward current was nonselective with respect to Ca^{2+}, Ba^{2+}, or Na^{+} and was blocked by La^{3+}. Both Ba^{2+} influx and the cationic current were unaffected by thapsigargin, InsP_{3}, or thapsigargin induced a large inwardly rectifying current that was not observed in the control cells. The relative permeability of the expressed channel to cations was P_{Ca}P_{Na}P_{Cs}P_{Ba} = 14:3:1.5:1.0, showing Ca^{2+} selectivity. La^{3+} and Gd^{3+} and SKF-6365, an inhibitor of nonselective cation and some Cl^{-} channels, inhibited the Ca^{2+} transients. Single-channel activity during patch-clamp recordings at low external Ca^{2+} show single-channel conductance of 47.6 pS. Ca^{2+} store depletion by thapsigargin had no...
The presence of Mg\(^{2+}\) large single-channel conductance of 41 and 63 pS in the U73122. In contrast to the proposed similarity to CRAC, this inhibition of the agonist-induced current by the PLC inhibitor (96) demonstrated by activation with CCh or histamine (after coexpression with the histamine receptor) by application of GTP\(_{\gamma}\)S, which activated single channels, and by inhibition of the agonist-induced current by the PLC inhibitor U73122. In contrast to the proposed similarity to CRAC channels, the studies on mouse TRPC4 and TRPC5 reveal large single-channel conductance of 41 and 63 pS in the presence of Mg\(^{2+}\) and similar conductance to both monovalent and divalent cations. Similarly, single-channel conductance of 47.6 pS was measured in another study of heterologously expressed TRPC5 in HEK293 cells (212). The conductance and the inward and outward rectification of the I-V relation were markedly dependent on Mg\(^{2+}\). In the absence of Mg\(^{2+}\), the rectification was lost, reminiscent of the effect of Mg\(^{2+}\) on Drosophila TRP. Surprisingly, La\(^{3+}\) at concentrations up to 1 mM facilitated TRPC4- and TRPC5-dependent currents (161). The reasons for the marked differences in regulatory and biophysical properties between species and studies in different laboratories remain unknown. Interestingly, the rat ortholog of TRPC4 lacks the entire putative S2 region. A rat ortholog expressed in Xenopus oocytes was functionally characterized as SOC (185). It is still not clear if the missing S2 segment is responsible for this phenomenon. Thus the bulk of the most recent studies on TRPC4 and TRPC5 in heterologous systems suggest that these channels are receptor-activated and store-independent non-specific cation channels, in contrast to studies in native endothelial cells (49). Importantly, DAG or DAG analogs do not activate these channels.

3. TRPC2

Only a few studies of the heterologously expressed mouse TRPC2 channel are available. Nevertheless, the specific expression of rat TRPC2 isofrom to the VNO organ of rat and sperm of mice have already provided a very significant clue as to the functions of TRPC2. Accordingly, TRPC2 may be the target channel of a G protein-coupled receptor that mediates detection of pheromones.

Heterologous expression of mouse TRPC2 in COS cells expressing the muscarinic receptor M6 revealed a typical SOC. The expressed TRPC2 channels were tested by the same paradigm described above for TRPC1 and show a significant enhancement of Ca\(^{2+}\) influx after store depletion by thapsigargin (192). These findings seemingly contradict the expectation of Liman et al. (96) that expect TRPC2 to be store-independent channels, since the VNO organ where TRPC2 is localized is composed of microvilli and has no Ca\(^{2+}\) stores.

4. TRPC3, TRPC6, and TRPC7

The members of this group of TRP channels are closely related in molecular structure and function. The human TRPC3 has been extensively characterized by a number of investigators. It was stably expressed in HEK cells (217) and bovine pulmonary artery endothelial cells (85) and transiently expressed in CHO cells (220). The heterologously expressed TRPC3 channel has been studied by various techniques including whole cell recordings of macroscopic current, excised patch-clamp recordings of single channels, as well as fluorescence Ca\(^{2+}\) measurements using Ca\(^{2+}\) indicators. Essentially all investigators reported similar properties of the TRPC3 channel (193). TRPC3 forms a nonselective cation channel that is activated by a G protein-coupled receptor, G protein, and PLC. La\(^{3+}\), Ge\(^{3+}\), and SK&F-96365 are potent blockers of the channel that has a single-channel conductance of 60–66 pS. The I-V relation shows inward and outward rectification. Most studies also reported that TRPC3 is a store-independent channel and that thapsigargin has no effect. Nevertheless, several investigators have reported some enhancement of the receptor-activated current in TRPC3-expressing cells by thapsigargin treatment (89, 218). A different line of evidence supporting the notion that TRPC3 function as SOC channels was provided by stable transfection of HEK293 cells with a fragment of human TRPC3 in antisense orientation. In the antisense transfected cells, activity of SOC channels monitored by Ba\(^{2+}\) influx after store depletion with thapsigargin was reduced by 32% compared with cells expressing sense TRPC3 (205).

Interestingly, in addition to activation by several agonists via a receptor and in few cases by store depletion, TRPC3 could be activated by DAG or its analogs (74, 100). Different studies have reported conflicting data with regard to the effects of InsP\(_3\). Although Hoffman et al. (74) reported that application of InsP\(_3\) has no effect on human TRPC3 or TRPC6 expressed in CHO cells, other studies on human TRPC3 expressed in either bovine pulmonary artery endothelial cells or in HEK293 cells show clear activation by InsP\(_3\) (89, 145). DAG and its analogs seem to activate the TRPC channels directly and not via the InsP\(_3\)R because application of the InsP\(_3\)R antagonist 2-APB does not affect the activation by DAG analogs (100).
The most interesting studies on TRPC3, which open new avenues toward understanding TRP gating, have described the interactions between TRPC3 and the InsP$_3$R (88, 100). The study by Muallem and colleagues (88) has provided for the first time experimental evidence supporting the conformational coupling hypothesis of inositide-mediated Ca$^{2+}$/CaM entry. This hypothesis proposes that information is transferred through direct coupling between the InsP$_3$R in the ER and a surface membrane Ca$^{2+}$-permeable channel to control Ca$^{2+}$ entry (13, 82). Drosophila TRP has been previously proposed to constitute the surface membrane channel, which interacts with the InsP$_3$R (109), but no experimental evidence has been provided to support this hypothesis. Muallem and colleagues (88), using stably expressed TRPC3 in HEK293 cells, have shown that excised membrane patches containing TRPC3 channels but no InsP$_3$R because of extreme washing can be activated by addition of vesicles containing native or recombinant InsP$_3$R bound to its ligand. The physical interaction of TRP and InsP$_3$R was also demonstrated by coimmunoprecipitation and glutathione-S-transferase (GST)-pulldown experiments and identifies two regions of InsP$_3$R NH$_2$ terminal that interact with one region of the COOH terminal of TRPC3. The interacting peptide regions were overexpressed in HEK293 cells and showed modulation of endogenous Ca$^{2+}$/CaM entry (19). Activation of the InsP$_3$R could be blocked by two known inhibitors, heparin and xestospongin (88). Interestingly, overexpression of the TRPC3 channel induced increased expression of the InsP$_3$R (19, 88). The conformational coupling hypothesis was supported by experiments of Gill and colleagues (100), who compare the dependence of divalent ions entry on the InsP$_3$R in native SOC system and the TRPC3-expressing HEK293 cells. These investigators show that displacement of actin by the phosphatase inhibitor calyculin in HEK293 cells inhibits activation of both native SOC by store depletion and activation of TRPC3 channels by an agonist, presumably by preventing interaction between the InsP$_3$R and TRPC3. Furthermore, inhibition of the InsP$_3$R by xestospongin and by 2-APB blocked both SOC and TRPC3 activities (100). The association with InsP$_3$R has also been reported for TRPC1 and TRPC6 by coimmunoprecipitation (19, 99).

A recent study has shown that interaction of the TRPC channel and the InsP$_3$R is not confined to a few but to all TRPC channels (182). Interestingly, the InsP$_3$R binding domain also interacts with CaM in a Ca$^{2+}$-dependent manner with affinities ranging from 10 nM for TRPC2 to 290 nM for TRPC6. In the presence of Ca$^{2+}$, the TRPC-InsP$_3$R interaction is inhibited by CaM. In addition, other binding sites for CaM and InsP$_3$Rs are present in the α- but not the β-isoform of TRPC4, while TRPC4 is activated strongly by an antagonist of CaM and high (50 μM) concentration of the TRPC binding peptide of the InsP$_3$R in inside-out membrane patches. Thus both the InsP$_3$R and CaM are involved in the control of TRPC gating (182).

A detailed study on TRPC6 heterologously expressed in HEK293 cells has been described above (81). Although TRPC7 has not been studied as extensively as TRPC3, the limited studies show that essentially it has very similar functional properties to those of TRPC3 (20). DAG also activates TRPC7 (131).

5. Coexpression of TRPC1 and TRPC3

The ability of TRPC homologs to coassemble has been established by studies demonstrating that heterologously expressed TRPC1 and TRPC3 can be coimmunoprecipitated (209). Coexpression of human TRPC1 and TRPC3 in HEK cells resulted in formation of channel activity with properties different from those of cells expressing either TRPC1 or TRPC3 alone, which were studied under the same experimental conditions (97). Accordingly, in coexpressing cells, receptor activation by carbachol suppressed Ca$^{2+}$ entry to a level smaller than that observed in TRPC3-expressing cells. Furthermore, OAG-induced Sr$^{2+}$ entry was reduced relative to that measured in cells expressing TRPC3. Importantly, coexpression of TRPC1 together with TRPC3 generated constitutively active conductance that was strongly inhibited by extracellular Ca$^{2+}$. These observations suggest that coassembly of TRPC1 and TRPC3 resulted in formation of heteromeric channel with properties different from the homomultimeric channels with regard to modulation by Ca$^{2+}$ and possibly other characteristics (97). Additional evidence for functional consequences of coexpressing TRPC1 and TRPC3 came from experiments in which HEK cells, which stably expressed human TRPC3, were transiently transfected with human TRPC1 segment in antisense orientation. In these cells, SOC activity as measured by Ba$^{2+}$ influx after store depletion was suppressed by 55% relative to cells coexpressing sense TRPC3 plus sense TRPC1 (205).

6. Coexpression of TRPC1 and TRPC5

The ability of TRPC homologs to coassemble has been also demonstrated in the mouse brain tissue where TRPC1 and TRPC5 were coimmunoprecipitated (178). Coexpression of TRPC1 and TRPC5 in HEK293 cells resulted in novel nonselective cation channel with a voltage dependence similar to NMDA receptor channel (but lacking the Mg$^{2+}$ dependence), but unlike that of any reported TRPC channel including TRPC1 or TRPC5 alone. TRPC1/TRPC5 heteromultimers were activated by G$_{q}$-coupled receptors but not by depletion of intracellular Ca$^{2+}$ stores. Interestingly, single-channel conductance of TRPC1/TRPC5 heteromultimere was approximately eightfold smaller than that of TRPC5 (slope conductance of 5 pS relative to 38 pS) (178).
D. Heterologous Expression of “TRP-Related” Channels

1. VR1 and VRL-1

Heterologous expression of the vanilloid receptor 1 (VR1), the heat-gated ion channel, in HEK293 cells and in *Xenopus* oocytes has indicated that VR1 is a Ca\(^{2+}\)-permeable, nonselective cation channel. Measurements of I-V relations of a capsaicin-induced current show outward rectification with reversal potential of \(-0\) and no significant preference for monovalent cations with \(P_{K}/P_{Na} = 0.94\) and \(P_{Ca}/P_{Na} = 0.85\) (27). The capsaicin-induced current exhibits a notable preference for divalent cations with \(P_{Ca}/P_{Na} = 9.6\) and \(P_{Mg}/P_{Na} = 4.99\) while the permeability sequence is \(Ca^{2+} > Mg^{2+} > Na^{+}\) (see Table 2). The capsaicin-induced inward current is rapid (within \(~200\) ms) inactivated in the presence of external \(Ca^{2+}\) while the inactivation is abolished in \(Ca^{2+}\)-free medium. Capsaicin-induced current was also examined at the single-channel level using excised patches and showed unitary conductance of 76.7 pS at positive potentials and 35.4 pS at negative potentials with \(Na^{+}\) as a sole charge carrier (27). The capsaicin activates the channels when applied on either side of the membrane, most likely because of the hydrophobic nature of the compound. Activation of heterologously expressed VR1 by the endogenous cannabinoind anandamide revealed biophysical properties similar to those measured after activation by capsaicin (222).

VRL-1, which is activated by high temperature but not by capsaicin and pH, has biophysical properties somewhat different from those of VR1. The I-V relation shows both inward and outward rectifications and reduced selectivity to divalent cations, \(P_{Ca}/P_{Na} = 2.94\) and \(P_{Mg}/P_{Na} = 2.40\) (26) (Table 2). Similar properties of I-V relations were obtained in CHO cells expressing the VRL-1 homolog GRC, which has \(-80\%\) amino acids sequence identity with VRL-1 (86).

2. Polycystin-L is a Ca\(^{2+}\)-regulated cation channel

Polycystin-L (PCL) shares significant homology to the polycystin-2 channel (50% identity and 71% similarity). Heterologous expression of PCL in *Xenopus* oocytes has provided strong evidence that this protein is a calcium-modulated nonselective cation channel that is permeable to \(Na^{+}\), \(K^{+}\), and \(Ca^{2+}\); \(P_{Na}/P_{K}/P_{Rb}/P_{Li} = 1.08:0.98:0.97:0.87\). The channel is about five times more permeable to \(Ca^{2+}\) than to \(Na^{+}\) at \(-50\) mV. Single-channel recordings showed that the PCL channels are activated directly by \(Ca^{2+}\) and that the channel conductance is 120–135 pS at 80 mM Ba\(^{2+}\), Sr\(^{2+}\), or \(Ca^{2+}\), with relatively long mean open times. Divalent ions were more permeable than monovalent except for Mg\(^{2+}\) that efficiently blocked the channel at negative holding potentials. A relatively large concentration of La\(^{3+}\) (0.1 mM) inhibited the \(Ca^{2+}\)-activated currents. Store depletion by thapsigargin had no effect (31).

3. Coassembly of polycystin-1 and polycystin-2 produces a nonselective cation channel

Attempts at demonstrating channel activity similar to PCL by in heterologous expression of polycystin-2 have failed. However, the indistinguishable phenotype resulting from mutation in either PKD1 or PKD2 genes suggested that their gene products interact to form a functional unit. Indeed, the COOH termini of polycystin-1 and polycystin-2 interact through their coiled-coil domains. Coexpression of PKD1 and PKD2 in CHO cells generated time-dependent outwardly rectifying current that was \(~20\)-fold larger than in mock transfected cells (54). The expressed channel is a nonselective cation channel (\(P_{Na}: P_{Ca}/P_{NMGD} = 1.1:1.0:0.6\)). The channel is about sixfold more permeable to \(Ca^{2+}\) than to monovalent cations. Extracellular \(Ca^{2+}\) also inhibited the inward current as found in other TRP channels.

4. The \(Ca^{2+}\) transport epithelial channel proteins ECaC, CaT1, CaT2, and CaT-L

Both putative \(Ca^{2+}\) transport channels have been initially heterologously expressed in *Xenopus* oocytes, and ECaC was also expressed in HEK293 cells. Functional data on the ECaC channel includes \(^{45}Ca^{2+}\) uptake, electrophysiology, and fluorimetric measurements. \(^{45}Ca^{2+}\) uptake revealed linear uptake over 2 h, showing increased influx when extracellular \(Ca^{2+}\) was increased up to \(-1\) mM. The \(Ca^{2+}\) influx was inhibited by \(La^{3+} > Cd^{2+} > Mn^{2+}\), whereas \(Mg^{2+}\) had no effect. Permeability to \(Na^{+}\) was negligible in double labeling experiments (in which both \(Ca^{2+}\) and \(Na^{+}\) were labeled), whereas \(Ba^{2+}\) or \(Sr^{2+}\) did not affect the \(Ca^{2+}\) influx. Acidification (to pH 5.9) had a significant inhibitory effect on \(Ca^{2+}\) influx reminiscent of the marked effect of acidosis on calcuresis in vivo. These experiments show that apical \(Ca^{2+}\) influx, possibly via ECaC, which is highly selective for \(Ca^{2+}\), is the rate-limiting step of transepithelial \(Ca^{2+}\) transport (73). Patch-clamp whole cell recordings and fluorimetric measurements of \(Ca^{2+}\) influx in heterologously expressed ECaC in HEK293 cells showed a distinctive \(Ca^{2+}\) permeability and constitutive activity of the ECaC channels with \(P_{Ca}/P_{Na} = 107\) and divalent cation selectivity profile of \(Ca^{2+} > Mn^{2+} > Ba^{2+} = Sr^{2+}\). The I-V relationship showed pronounced inward rectification (195).

A close relative of ECaC designated CaT2 has been recently cloned (137). CaT2 has 84.2 and 73.4% amino acid identity to ECaC and CaT1, respectively. However, unlike ECaC, CaT2 is kidney specific in rat. Heterologous expression of CaT2 in *Xenopus* oocytes produced \(Ca^{2+}\)-activated novel inward current that was also induced by \(Sr^{2+}\).
and Ba$^{2+}$ but not Mg$^{2+}$, whereas Cd$^{2+}$ was a potent inhibitor that also permeated the channel. Antagonists of the L-type voltage-activated Ca$^{2+}$ channels have only little effect even at high concentrations. Thus CaT2 like ECaC seems to participate in Ca$^{2+}$ entry into cells of the distal convoluted tube and connecting segment of the nephron (137).

$^{45}$Ca$^{2+}$ uptake experiments in Xenopus oocytes expressing CaT1 showed properties similar to those described for ECaC channel (138). Electrophysiological studies in oocytes expressing CaT1 channels using two-electrode voltage clamp revealed a large inward current that was initially activated by extracellular application of Ca$^{2+}$ and then rapidly inactivated. The I-V relationship of the current shows inward rectification. Intracellular injection of EGTA reduced the amplitude of the Ca$^{2+}$-activated current and abolished the inactivation phase. The CaT1-expressed channels are permeable to Ca$^{2+}$ but also to Na$^{+}$, K$^{+}$, and Rb$^{+}$ in contrast to the ECaC channel. La$^{3+}$, as well as inorganic ions, which block voltage-gated Ca$^{2+}$ channels (e.g., Ni$^{2+}$, Co$^{2+}$, Cd$^{2+}$), also block the inward current as well as Ca$^{2+}$ influx in CaT1-expressing cells. Like the ECaC channel, the CaT1 channel was also blocked by acidic pH and did not produce current enhancement after store depletion (138).

Additional highly Ca$^{2+}$-selective CaT1-related channel protein has been recently cloned and designated CaT-L (202). Heterologous expression of CaT-L in HEK293 cells resulted in a large inwardly rectifying current, indicating that CaT-L forms a constitutively active ion channel. Detailed electrophysiological tests clearly established that CaT-L has the hallmarks of Ca$^{2+}$-selective channel that can also be partially blocked by Ca$^{2+}$. Measurements of intracellular Ca$^{2+}$ using a fluorescent Ca$^{2+}$ indicator revealed that the constitutive permeability to Ca$^{2+}$ also largely elevated cellular Ca$^{2+}$, thus making CaT-L a suitable channel for Ca$^{2+}$ transport in the endogenous tissue (202).

5. CaT1 manifests pore properties similar to $I_{\text{CRAC}}$

The molecular identity of $I_{\text{CRAC}}$ was an enigma for a long time. Because $I_{\text{CRAC}}$ has been the classical SOC and members of the TRP family have been activated by SOC mechanism, members of the TRP family have been implicated as potential candidates of $I_{\text{CRAC}}$. The Ca$^{2+}$ transport epithelial channel proteins are the only members of the TRP family with the high selectivity to Ca$^{2+}$ characteristic of $I_{\text{CRAC}}$. A recent study by Clapham and colleagues (215) has provided evidence that CaT1 is a promising candidate of $I_{\text{CRAC}}$. Heterologous expression of CaT1 in CHO-K1 cells formed a large inwardly rectifying current when intracellular Ca$^{2+}$ was buffered to 10 mM EGTA in the pipette. The reversal potential of the current was +49 mV as expected from a highly selective Ca$^{2+}$ channel. The relative conductance of the expressed CaT1 was Ca$^{2+}$ > Ba$^{2+}$ > Sr$^{2+}$ > Mn$^{2+}$, with a permeability ratio of $P_{\text{Ca}} / P_{\text{Na}} = 130$, similar to $I_{\text{CRAC}}$. Extracellular Ca$^{2+}$, but not Ba$^{2+}$ or Sr$^{2+}$, inactivated the CaT1 current. CaT1 current also revealed the anomalous mole fraction (the capacity to hold two or more divalent cations in the pore simultaneously) a typical property of a highly Ca$^{2+}$-selective channels. One of the hallmarks of $I_{\text{CRAC}}$ is its increased permeability to monovalent ions in divalent free medium. This property was also found in CaT1-dependent current. The single-channel conductance of $I_{\text{CRAC}}$ has been reported to increase from 0.5 to 40 pS in the absence of divalent ions (134); the CaT1-dependent single-channel conductance to Na$^+$ in divalent free condition was ~42 pS. However, no data in physiological solution were reported. In addition to the above properties, CaT1-dependent current was also similar to $I_{\text{CRAC}}$ in the kinetics of the whole cell current and block by La$^{3+}$. Importantly, the CaT1-dependent current was activated by Ca$^{2+}$ store depletion obtained by application of InsP$_3$ or thapsigargin in the presence of 10 mM EGTA. Thus CaT1 seems to fulfill several criteria associated with a channel producing $I_{\text{CRAC}}$ (215).

6. OTRPC4 confers sensitivity to extracellular osmolarity

Transient transfection of HEK293 or CHO or BBL (rat basophilic leukemia) cells with OTRPC4 resulted in significant elevation of cellular Ca$^{2+}$. Reduction in extracellular osmolarity resulted in a large and reversible increase in cellular Ca$^{2+}$. Conversely, reduction in extracellular osmolarity led to reduction in cellular Ca$^{2+}$. All other members of this subfamily with similar structure (i.e., VR1, VR1-L, and ECaC) do not respond to similar changes in osmolarity. The response to change in osmolarity was rapid (within 30 s). A response to osmolarity is observed at changes of 30 mosM (i.e., ~10% change), whereas Ca$^{2+}$ concentration increases linearly with the increase in osmolarity in a range larger than 100 mosM. Store depletion or activators of VR1, VR1-L, PLC, and mechanical stretch had no effect, whereas La$^{3+}$ (100 μM) and ruthenium red (10 μM) blocked the Ca$^{2+}$ influx. Electrophysiological characterization of the OTRPC4-expressing cells revealed constitutively active channels, with I-V relation showing outward rectification with a zero reversal potential at physiological solutions. Measurements of the ionic selectivity show that OTRPC4 is a nonselective cationic channel with $P_{\text{Ca}} / P_{\text{Na}}$ of ~6. Single-channel recordings confirmed the spontaneous openings of the channels and the I-V relation of the macroscopic current, while the probability of openings depended on the osmolarity with relatively long delay (~70 s) after increase in osmolarity. Regulation of cell volume has been shown to rely on Ca$^{2+}$ influx, and OTRPC4 properties are consistent with these.
its high sensitivity to change in extracellular osmolarity and its specific localization to the region of the kidney where cells are exposed to hypotonic solutions made OTRPC4 a promising candidate for an osmoreceptor channel (177).

7. LTRPC7 (TRP-PLIK or TRPM7) is a MgATP-regulated channel required for cell viability

TRPM7 was cloned independently by Scharenberg and colleagues (123) and by Clapham and colleagues (159) and was characterized as an ion channel with a kinase domain. Its channel properties were characterized by heterologous expression in CHO-K1 cells using patch-clamp recordings. TRPM7-dependent current showed a large outward rectification. The relative permeability to cations was 1.1, 0.97, and 0.34 for K⁺, Na⁺, and Ca²⁺, respectively, and therefore, it was considered as a nonselective cation channel. The TRPM7-dependent current was weakly inhibited by La³⁺ and required a high (2 mM) concentration for a significant block. The slope conductance of single-channel current was 105 pS (159). The effects of mutations that alter the kinase activity on channel function revealed a marked decrease in current, suggesting that the kinase activity is required for channel function. This conclusion was supported by experiments in which current amplitudes in cells dialyzed with an ATP-containing pipette (5 mM NaATP, 1 mM Mg²⁺) initially increased, followed by a slow decrease over several minutes. Currents in cells that were dialyzed with 0 mM ATP were significantly smaller (159).

TRPM7 was also designated LTRPC7 by Scharenberg and colleagues (123). Targeted deletion of LTRPC7 in DT-40 B cells was lethal, indicating that LTRPC7 has a fundamental and nonredundant role in cellular physiology. Heterologous expression of LTRPC7 in HEK-293 cells revealed a large outwardly rectifying current. Substitution of monovalent ions with choline in the bath had no effect, suggesting that the inward current was carried by divalent ions when they are present in the medium. However, in divalent free medium, the channel is highly permeable to monovalent ions. Further studies showed that the pore of the channel had a high affinity for and is permeant to both Ca²⁺ and Mg²⁺, and the channel shows Mg²⁺-dependent anomalous mole fraction behavior. At the same time outward currents are inhibited with increasing Mg²⁺ concentration. The permeability of the channel to Mg²⁺ is reminiscent of a similar property of the Drosophila TRP channel (60, 64). Interestingly, MgATP at millimolar concentrations suppressed channel activity of LTRPC7. It turned out that MgATP acted as a physiological regulator of LTRPC7. This conclusion was supported by application of hydrolysis-resistant ATP analog. Thus Mg-ATPγS (but not Na-ATPγS) effectively and reversibly suppressed LTRPC7-dependent current, suggesting that phosphorylation of the channel in MgATP-dependent manner directly regulates the channel activity. In this respect, LTRPC7 belongs to of Mg-nucleotide-regulated metal ion currents (MagNuM). The author suggested that the effects of NaATP on TRP-PLIK reported by Clapham and colleagues (159) might be explained by a drop in cellular Mg²⁺ and not due to regulation of channel function by the kinase domain.

The studies on LTRPC7 indicate that this channel is an intracellular ligand-gated ion channel whose activation may be linked to cellular energy metabolism through its sensitivity to cytosolic MgATP levels and that it effectively permeates both Ca²⁺ and Mg²⁺. It is thus striking that similar properties characterize the Drosophila TRP channel, which is also permeable (and partially blocked) by Mg²⁺ and shows extreme sensitivity to metabolic stress in vivo in a manner that depletion of ATP opens the TRP channel in the dark while ATP suppresses channel opening (4).

8. LTRPC2 (TRPM2) gating involves ADP-ribose and β-NAD

Heterologous expression of LTRPC2 in HEK293 cells forms a current that was induced by application of 100 μM ADP-ribose (ADPR), showing a linear I-V relationship and reversing at 0 mV, thus suggesting that this is a nonselective cation channel activated by ADPR. No current was formed either by application of NAD⁺, cyclic ADPR, ATP and other nucleotides, or by store depletion (141). The channel was permeable to both monovalent and divalent cations. Excised inside-out patch-clamp recordings revealed that the channel is most likely directly opened by ADPR at concentration of 30 μM or higher showing no desensitization. The slope conductance of the single channel was 60 pS. On the basis of the heterologous expression of LTRPC2, a similar analysis was performed on native U937 cells, which express LTRPC2. Essentially similar properties were found including activation by ADPR (141). These studies show that ADPR can regulate directly the permeability of one member of the TRP family raising the possibility that the NUD9-H domain (see sect. mD) of the channel is involved in channel gating. A more recent study has shown that both ADPR and β-NAD activate the LTRPC2 expressed in monocyte cell lines. Biophysical studies confirm LTRPC2 as Ca²⁺-permeable nonselective cation channel. As mentioned above, ATP strongly suppressed NAD- and ADP-activated LTRPC2 channels (160).

9. Summary

The first TRPs to be studied in heterologous systems are the original Drosophila TRP and TRPL, which are activated in response to light stimuli. Although Drosophila phototransduction is very well characterized, heterol-
ogous expression of TRP and TRPL had limited success. It was virtually impossible to activate TRP, and TRPL was constitutively active spontaneously. Relevance to the native process of phototransduction was only shown unambiguously in the properties of the TRP channels. If researchers had to elucidate the role of TRP and TRPL using heterologous expression experiments, we would still be very far from that goal.

In the new era of genomic advancement, reverse genetics has provided a valuable tool in finding new proteins and using them to elucidate biological processes. However, in contrast to forward genetics where the starting point is a defective function, finding a gene and its expression pattern is far away from finding a functional role in a biological process. Heterologous expression of TRPs has provided a myriad of effectors involving many cellular processes. This variability could result from the characteristics of the TRP channel family; however, some of it most probably results from features of the channels that are not directly related to their main role but become prominent in the foreign cellular environment. The large variability, the sensitivity to cellular conditions, and the lack of a common activator or antagonist make it difficult to study these channels in isolation from their natural cellular environment. Only in conjunction with other strategies will the heterologous expression experiments be able to realize the advantage of allowing analysis of isolated components.

VI. TRP LOCALIZES AND ANCHORS THE TRANSDUCTION SIGNALING COMPLEX OF DROSOPHILA

Recently, it has become apparent that TRP plays an important role in organization of multiprotein signaling complexes. An important step toward understanding Drosophila phototransduction has been the finding that some of the key elements of the phototransduction cascade are incorporated into supramolecular signaling complexes via a scaffold protein, INAD. The INAD protein was discovered because of a Drosophila mutant designated inactivation but no afterpotential D (InaD). The first discovered inaD mutant, InaD<sup>215</sup>, is a dominant mutant isolated by Pak (133) on the basis of an abnormal prolonged depolarizing afterpotential (PDA). The InaD gene was cloned and sequenced by Shieh and Zhu (169) and found to bind TRP by overlay assays and coimmunoprecipitation in the original InaD mutant, InaD<sup>215</sup>, and WT (32). Studies in Calliphora by Paulsen and colleagues (79) have shown that INAD binds not only TRP but also PLC (NORPA) and eye-specific PKC (INAC). The interaction of INAD with NORPA and INAC was confirmed in Drosophila (32, 187). It was further found that inaD is a scaffold protein, which consists of five ~90-amino acid protein interaction motifs called PDZ (PSD95, DLG, ZO1) domains (187). These domains are recognized as protein modules that bind to a diversity of signaling, cell adhesion, and cytoskeletal proteins (5, 42, 162) by specific binding to target sequences typically, although not always, in the final three residues of the COOH terminal. The PDZ domains of INAD bind to the signaling molecules as follows: PDZ1 and PDZ5 bind PLC (170, 187, 191), PDZ2 and PDZ4 bind eye-PKC (3, 187), and PDZ3 binds TRP (32, 169, 187). TRPL appears not to be a member of the complex, since unlike PKC, NORPA, and TRP it remains strictly localized to the microvilli in the inaD1 null mutant (187). However, coimmunoprecipitation studies have indicated that TRP and TRPL interact directly when heterologously expressed in 293T cells as well as in Drosophila head extracts (209). Further studies are required to establish if the observed TRP-TRPL interactions have clear functional consequences in vivo.

Several studies by Montell and colleagues have shown that in addition to PLC, PKC, and TRP other signaling molecules such as CaM, rhodopsin (32), and NINAC (200) bind to the INAD signaling complex. Such binding, however, must be a dynamic process (117). It should be noted, however, that rhodopsin is at least 10-fold more abundant than INAD, and it is an immobile protein. This fact thus suggests that there are two populations of rhodopsin molecules: a minor functional fraction, which is bound to INAD, and a large fraction of nonfunctional unbound rhodopsin molecules. Studies of bump properties in Drosophila are inconsistent with such hypothesis, since every absorbed photon has the same probability of inducing a quantum bump regardless of its site of absorption (71).

Biochemical studies in Calliphora have revealed one important aspect of associating signaling protein together by showing that both INAD and TRP are targets for phosphorylation by the nearby eye PKC (78, 79). The association of TRP into transduction complexes may also be related to increasing speed and efficiency of transduction events as reflected by the immediate vicinity of TRP to its upstream activator, PLC, and its possible regulator, PKC (78, 80).

An important aspect of the formation of the supramolecular complex has been recently established by showing that TRP plays a major role in localizing the entire INAD multimolecular complex. It was found that the association between TRP and INAD is essential for correct localization of the complex in the rhabdomeres as found in other signaling systems (e.g., Ref. 5). This conclusion was arrived at by using Drosophila mutants in which the signaling proteins, which constitute the INAD complex, were removed genetically and by deletions of the specific binding domains, which bind TRP to INAD (94). These experiments show that INAD is correctly localized to the rhabdomeres in inaC mutants (where eye PKC is missing).
and in norpA mutants (where PLC is missing), but severely mislocalized in null trp mutants (94, 188), thus indicating that TRP (but not PLC or PKC) is essential for localization of the signaling complex to the rhabdomere. To demonstrate that specific interaction of INAD with TRP is required for the rhabdomeric localization of the complex, the binding site at the COOH terminal of TRP was removed (94, 188) or three conserved residues in PDZ3 which are expected to disrupt the interaction between PDZ domains and their targets (45) were modified (188). As predicted, both TRP and INAD were mislocalized in these mutants (188). Interestingly, in a null trp mutant, ~25% of the level of rhabdomeric INAD was still present, most likely due to a second site of INAD binding to a still unidentified protein, via PDZ1 (188). The study of the above mutants was also used to show that TRP and INAD do not depend on each other to be targeted to the rhabdomeres; thus INAD-TRP interaction is not required for targeting but for anchoring the signaling complex (94, 188). TRP thus serves as an anchor that localizes the signaling complex to its subcellular site. Because almost all members of the TRP family contain multiple putative protein-protein interaction sites, it is most probable that they have an important functional facet as anchors that localize multiple signal transduction elements. Additional experiments on TRP and INAD further show that INAD has other functions in addition to anchoring the signaling complex. One important function is to preassemble the proteins of the signaling complex (188). Another important function, at least for the case of PLC, is to prevent degradation of the unbound signaling protein by a still unknown mechanism (187).

The mammalian scaffold protein NHERF binds TRPC4, TRPC5 and PLCs. Recent coimmunoprecipitation studies have shown, for the first time, that mammals may organize their TRPC channels in supramolecular complexes similar to the INAD signaling complex. Murine TRPC4 and TRPC5, as well as PLC-β1 and PLC-β2 interact with the first PDZ domain of the Na+/H+ exchanger regulatory factor (NHERF). This interaction was demonstrated in vivo (TRPC4 and PLC-β2), in HEK293 cells expressing TRPC4, and in adult mouse brain by coimmunoprecipitation. Because NHERF has two PDZ domains and it binds also to the cytoskeleton via other molecules, the cytoskeleton seems to be a part of this supramolecular organization (183). Functional studies are required to determine the functional consequences of this organization of signaling and structural proteins.

VII. CONCLUDING REMARKS

Phosphoinositide-mediated signaling is present in almost every eukaryotic cell and plays a central role in many aspects of cell signaling, yet many aspects related to the surface membrane channel, which is the target of this transduction cascade, are elusive. The main unclear aspects are the identity, the mechanism of activation (including gating), and the specific cellular function of the channel. The family of TRP channel proteins constitutes the only surface membrane channels with known molecular identity that are activated by the phosphoinositide cascade. By now, there is sufficient data to indicate that phosphoinositide-mediated channels form a heterogeneous family with diverse properties and functions. Molecular identification as “TRP-homolog” or “TRP-related” of key molecules that mediate specific disease or specific sensory function constitutes an important clue as to the general function of this molecule and strongly suggests that PLC is involved in its activation. With such an important role in signaling, it is not surprising that there is much interest in trying to unravel the mechanism of activation of TRP channels.

Several mechanisms have been proposed to account for activation of TRP channels. The two main mechanisms are 1) store operation, via conformational coupling between TRP and the InsP₃ receptor, and 2) store independent, via activation of PLC that leads to production of second messenger such as DAG or PUFAs. Although both mechanisms have been formulated in very general terms, there are more questions than answers regarding the underlying molecular details, and different investigators have reported conflicting results regarding the basic classification of various TRP channels as store operated or store independent. A possible explanation of this confusion is that the mechanism of activation and regulation of TRP gating involves interactions among several key proteins. Such protein-protein interactions have been reported for voltage- and ligand-gated channels, although the basic mechanism of gating is known for these channels. For TRP channels in which the basic gating mechanism is obscure, the knowledge about other interacting proteins seems to be crucial for understanding TRP activation and gating mechanism. We already know that Drosophila TRP is attached to a supramolecular complex via the scaffold protein INAD, which includes several signaling proteins, and similar mechanisms exist in vertebrate cells (e.g., via the scaffold protein NHERF). There are indications that TRP channels form heteromultimers and that all TRPC channels interact with the InsP₃R and the ryanodine receptors. If such interactions are crucial for TRP activation in the native system, it is expected that overexpression of TRPs in heterologous systems leads to confusing results. This is because of possible absence of crucial interacting signaling proteins or disruption of the normal stoichiometry among the signaling molecules that lead to abnormal and variable signaling complexes according to the expression level of TRP and the specific cellular environment of the host cell. Even heterologous expression of TRPL, which seems to produce channel
activity with properties similar to that observed in the native system, results in constitutive activity of the TRPL channels unlike the situation in the native system.

It is, therefore, important to direct future efforts toward investigating the activation mechanism of the various TRP channels in the native cells. Genetic tools seem to be especially suitable for such an approach. It is not clear if all members of the TRP family share similar mechanism of activation, but the similar structural features of most members and the involvement of PLC in their activation suggest that in general they share a common mechanism of activation, although the details may be different in the various members of this diverse family of channel proteins.

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