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MINIREVIEW

New Light on TRP and TRPL

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SUMMARY

Store-operated Ca^{2+} entry, a mode of Ca^{2+} influx activated by depletion of Ca^{2+} from the internal stores, has been detected in a wide variety of cell types and may be the primary mechanism for Ca^{2+} entry in nonexcitable cells. Nevertheless, until recently, no candidate store-operated channel (SOC) had been identified molecularly. Through the serendipity of *Drosophila* genetics, a candidate SOC, referred to as Transient Receptor Potential (TRP), has been identified that is essential for the light-induced cation conductance in photoreceptor cells. A combination of *in vitro* and *in vivo* studies has provided strong evidence that TRP is a bona fide SOC. Moreover, TRP forms a supramolecular complex, proposed to be critical for feedback regulation and/or activation, that includes rhodopsin, phospholipase C, protein kinase C, calmodulin, and the PDZ domain-containing protein, INAD. INAD seems to be a scaffolding protein that links TRP with several of these other proteins in the complex. TRP also complexes with a related channel subunit, TRP-like, to form a heteromultimer with conductance characteristics distinct from those of TRP or TRP-like homomultimers. A family of proteins related to TRP is conserved from *Caenorhabditis elegans* to humans, and recent evidence indicates that at least some of these proteins are SOCs. The human TRP-related proteins may mediate many of the store-operated conductances that have been identified previously in a plethora of human cells.

SOCE Is a Widespread Phenomenon

Ca^{2+} entry is critical for the development and/or physiology of virtually all cells. A diversity of voltage- and ligand-gated ion channels are responsible for Ca^{2+} influx in different types of cells; however, there are similarities in the mechanisms of Ca^{2+} influx among highly divergent cell types, such as excitable versus nonexcitable cells, that are greater than recognized previously. This common Ca^{2+} entry process, SOCE (formerly referred to as capacitative Ca^{2+} entry) (1, 2), has been observed in a plethora of cell types ranging from T lymphocytes, pancreatic acinar cells, hepatocytes, and vascular endothelial cells to *Drosophila* photoreceptor cells (reviewed in Ref. 3). This mode of Ca^{2+} entry may be ubiquitous because it has been detected in most cells that have been specifically examined for SOCE. Moreover, SOCE has been implicated in a variety of processes ranging from mitogenesis in fibroblasts, osteoclast function, and *Drosophila* phototransduction to T cell activation (reviewed in Ref. 3). This latter possibility is strengthened by the intriguing correlation between a form of severe immunodeficiency and a defect in SOCE in T cells (4).

SOCE is activated by depletion of Ca^{2+} from internal Ca^{2+} storage sites. Although the exact location of such pools remains elusive, it seems that portions of the endoplasmic reticulum compose a major component of these stores (reviewed in Ref. 5). Release of Ca^{2+} from the stores results from activation of tyrosine kinase receptors or G protein-coupled receptors, which in turn stimulates PLC-γ. Production of IP_3 by PLC induces a biphasic rise in Ca^{2+}. The initial phase is transient and caused by activation of the IP_3R, a Ca^{2+}-release channel situated in the Ca^{2+} stores. Then, depletion of Ca^{2+} from the internal stores induces a more sustained plasma membrane Ca^{2+} conductance. It may be that not all IP_3-sensitive Ca^{2+} stores are involved in SOCE. Some IP_3-sensitive stores may be involved only in Ca^{2+} release, whereas depletion from other stores may function in SOCE (6). Despite the apparent prevalence and importance of SOCE, the mechanism by which depletion of Ca^{2+} from the

ABBREVIATIONS: SOCE, store-operated calcium entry; ERG, electroretinogram recording; I_{CRAC}, calcium release-activated calcium current; IP_3, inositol-1,3,5-trisphosphate; IP_3R, inositol-1,3,5-trisphosphate receptor; PLC, phospholipase C-β; RH1, rhodopsin; SOC, store-operated channel; TRP, transient receptor potential; TRPL, transient receptor potential-like; TRPR, transient receptor potential-related channel; INAD, inactivation no afterpotential D.

internal stores activates the plasma membrane Ca^{2+} conductance is not understood. Moreover, the identity of SOCs expressed in vertebrate cells has, until recently, been elusive.

Inositol Phospholipid Signaling and Ca^{2+} Stores in *Drosophila* Vision

The founding member of a family of related SOCs has emerged through a genetic approach to the study of vision in the fruitfly *Drosophila melanogaster*. As is the case in vertebrates, *Drosophila* vision is initiated by photoactivation of rhodopsin and interaction with a heterotrimeric G protein. However, in contrast to vertebrate vision, phototransduction in the fruitfly utilizes the inositol phospholipid signaling system. Definitive evidence that this is the case is the observation that null mutations in *norpA*, which encodes the eye-enriched PLC, render the flies completely unresponsive to light (7). Upon activation of phosphoinositide signaling, there is sustained plasma membrane Ca^{2+} and Na^{+} conductance.

Release of Ca^{2+} from internal stores seems to be essential for *Drosophila* vision, although this issue is controversial. Evidence has been provided that *Drosophila* photoreceptor cells contain Ca^{2+} stores (8) and receptors for both IP_3 (9, 10) and ryanodine (10). The ryanodine receptor is another Ca^{2+}-release channel which is activated through Ca^{2+}-induced Ca^{2+} release. Inhibition of the IP_3R or exhaustion of the ryanodine pools with ryanodine blocks the photoresponse, indicating that Ca^{2+} depletion via both the IP_3- and ryanodine-sensitive stores is required in *Drosophila* vision (11, 12). It has been proposed that release of Ca^{2+} from the IP_3-sensitive stores triggers Ca^{2+}-induced Ca^{2+} release from the ryanodine-sensitive stores, resulting in amplification of the light response (11).

Despite the pharmacological evidence for a role of the IP_3-sensitive stores in *Drosophila* phototransduction, a mutation in a *Drosophila* IP_3R has been reported to be lethal (13, 14) but to have no effect on vision (14). Due to the requirement of the IP_3R for viability, the eye phenotype was analyzed in mosaic flies containing mutant patches in the compound eye of the fly. An additional observation suggesting that Ca^{2+} stores do not function in activation of the light-induced conductance is that thapsigargin, an agent which interferes with the Ca^{2+}-ATPase in the endoplasmic reticulum and causes Ca^{2+} depletion via leak currents (15), does not activate Ca^{2+} influx in photoreceptor cells (8, 16). However, the lack of effect of thapsigargin may be due to insufficient leak current from the stores in *Drosophila* photoreceptor cells. Furthermore, several lines of evidence indicate that the absence of phenotype in the IP_3R mosaics eyes is due to the presence of one or more additional IP_3Rs that remain to be identified: (i) *norpA* flies are blind, demonstrating a crucial role for IP_3 and/or diacylglycerol in activation of the light-induced conductance (7); (ii) application of heparin, an IP_3R blocker, interferes with the photoresponse (12); and (iii) thapsigargin activates the light-sensitive channels *in vitro* (see below). Based on a recent study in a lymphocyte cell line suggesting that there are separate IP_3-sensitive pools which function in Ca^{2+} depletion and influx (6), it is plausible that a similar phenomenon exists in *Drosophila* photoreceptors and that separate IP_3Rs function in each process. Alternatively, there may indeed exist just one *Drosophila* IP_3R, and the lack of phenotype in the genetic mosaics may be the consequence of residual IP_3R protein in the mutant patches.

An alternative possibility is that there are dual mechanisms for activating the light-dependent current, both of which require the NORPA PLC and production of IP_3. The initial light-dependent conductance may be induced by a very rapid mechanism independent of the stores, and the sustained response may involve the internal stores. This latter mechanism involving Ca^{2+} depletion would require diffusion of IP_3 from the rhabdomeres to the cell bodies, Ca^{2+} release, and production of a signal that is sent back to the rhabdomeres, leading to production of the cation conductance. The rhabdomeres, which are the microvillar structures containing most of the components required in *Drosophila* phototransduction, do not seem to contain Ca^{2+} stores. Thus, an involvement of Ca^{2+} depletion in the light response would necessitate signal transmission to and from the cell bodies. However, the light-dependent conductance is activated within a few milliseconds, and this time scale may be too rapid for information to flow between the rhabdomeres and cell bodies. Therefore, it is appealing to propose that production of IP_3 may activate the initial phase of Ca^{2+} and Na^{+} influx by directly binding the light-sensitive channels or binding to a channel-associated protein in the rhabdomeres. The IP_3-sensitive Ca^{2+} stores would be proposed to function in sustaining the cation influx for an extended duration.

Identification of TRP and TRPL

Many mutants affecting *Drosophila* vision have been identified in genetic screens using either ERGs or behavioral assays, such as phototaxis (reviewed in Ref. 17). A locus identified in one such screen, referred to as *trp*, seems to encode a SOC subunit. *trp* mutant flies display normal phototaxis but behave as though blind in the optomotor assay, a test in which wild-type flies orient to visual cues (18). The impaired optomotor response is associated with an ERG phenotype (Fig. 1). The initial response to light is indistinguishable between *trp* and wild-type flies. However, in response to bright light, the sustained component of the ERG quickly decays. No difference between the *trp* and wild-type ERG is detected if dim or moderate light intensity is used as a stimulus.

The basis of this ERG phenotype was greatly clarified upon the advent of technology to perform whole-cell recordings on *Drosophila* photoreceptor cells (19, 20). In wild-type, the channels are ~25–40-fold more permeable to Ca^{2+} than to Na^{+} (20, 21); however, in *trp* photoreceptors, the permeability to Ca^{2+} is reduced ~10-fold, whereas the permeability to Na^{+} is decreased by only ~30% (21). A defect in Ca^{2+} entry is further supported by experiments using Ca^{2+}-sensitive electrodes and Ca^{2+}-indicator dyes in photoreceptor cells (22, 23). These data indicate that the *trp* locus either encodes a Ca^{2+} channel or is required for activation of such a channel. In addition, the observation that the cation conductance is reduced but not eliminated in *trp* flies demonstrates that at least one additional class of channel is still functional in the mutant flies.

A suggestion that *trp* might encode an ion channel was obtained through molecular identification of the gene (24). *trp* encodes a photoreceptor cell-specific 1275-amino acid protein with multiple transmembrane domains, most likely six

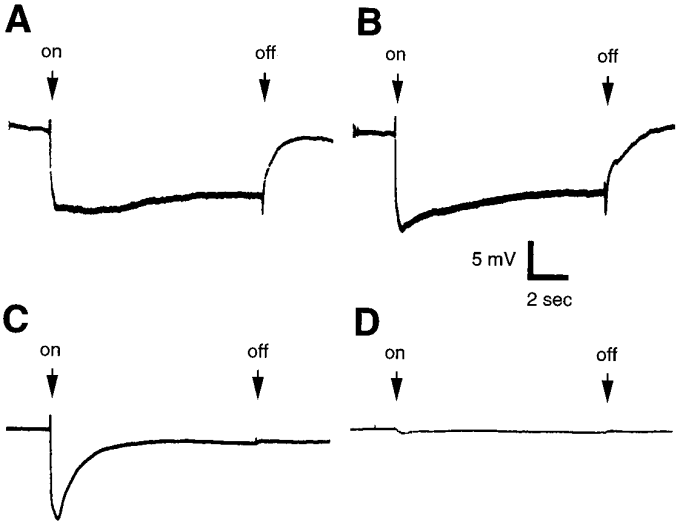

Fig. 1. The *trp* ERG phenotype. Wild-type (A and B) and trp^{CM} (C and D) flies were exposed to a white light. The flies were dark adapted for either 2 min (A and C) or 5 sec (B and D). The initiation (*on*) and cessation (*off*) of the light stimulus, a signal amplitude, and time scale are indicated. [Figure reprinted with permission from Montell, C., K. Jones, E. Hafen, and G. Rubin. Rescue of the *Drosophila* phototransduction mutation *trp* by germline transformation. *Science (Washington D. C.)* **230:**1040–1043 (1985). © 1997 American Association for the Advancement of Science.]

(Fig. 2) (25, 26); this predicted structure is reminiscent of the superfamily of voltage- and second messenger-gated ion channels (27). In addition, analysis of the deduced amino acid sequence suggested that if TRP is a channel, it is not voltage gated because the fourth putative transmembrane segment lacks the positively charged residues believed to form the voltage sensor in voltage-gated ion channels (28).

In a screen for calmodulin binding proteins expressed in the *Drosophila* head, an 1124-residue protein was identified, referred to as TRPL, that is ~40% identical to TRP over the amino-terminal ~700 amino acids but differs significantly from TRP over the carboxyl-terminal ~400 residues (Fig. 2) (29). The identification of TRPL raised the possibility that it is a second light-dependent ion channel, perhaps responsible for the remaining conductance in a *trp* mutant background. This explanation seems to be an oversimplification because *trpl* mutant flies have become available that are reported to elicit a light response indistinguishable from that of wild-type, yet *trpl; trp* double mutants are nearly completely unresponsive to light (30). A possible explanation for this conundrum is discussed below.

TRP and TRPL Form Channels but Only TRP Is Store Operated

Consistent with the analysis of the deduced amino acid sequence, expression of TRP *in vitro*, such as in Sf9 or 293T cells, results in a Ca^{2+} conductance that is not voltage dependent. However, a cation conductance is induced upon depletion of Ca^{2+} from the internal stores with thapsigargin (Fig. 3) (31, 32). The observation that TRP is activated *in vitro* by depletion of Ca^{2+} from the internal stores strongly indicates that it is a bona fide SOC. TRP seems to function as a multimer, similar to other members of the superfamily of voltage- and second messenger-gated ion channels (reviewed in Ref. 27), because the TRP-dependent conductance is almost completely inhibited by a dominant negative form of TRP (32). Such derivatives consist of a substitution of the putative "pore-loop" domain, between transmembrane segments 5 and 6, with the corresponding region from the Shaker B voltage-gated K^+ channel.

The ion selectivity and pharmacology of TRP expressed *in vitro* have parallels with *in vivo* currents in *Drosophila* photoreceptor cells. The relative permeability of Ca^{2+} is higher than that of Na^+ both *in vivo* (~25–40:1) (20, 21) and *in vitro* (~10:1) (32). The TRP channel is also less permeable to Mg^{2+} than to Ca^{2+} in both photoreceptor and tissue culture cells (21, 31). Thus, the TRP conductance has selectivity for Ca^{2+} but is not nearly as selective as one of the best characterized store-operated conductances in vertebrate cells, I_{CRAC} (33–35). However, a wide variety of vertebrate store-operated conductances have been described. Some are Ca^{2+} selective but display higher conductances than I_{CRAC} (36); others are similar to TRP in their relative Ca^{2+}-to-Na^+ permeability (37); and others are nonselective (38). Thus, Ca^{2+} selectivity is not a prerequisite for a store-operated conductance.

Despite the higher permeability to Ca^{2+} than Na^+, the TRP-dependent currents measured *in vitro* are larger when Na^+ rather than Ca^{2+} is used as the permeant ion. This phenomenon seems to be due to Ca^{2+}-mediated inactivation of TRP, another feature of TRP observed *in vitro* (32) and in photoreceptor cells (39, 40). Furthermore, inhibition by Ca^{2+} may be a common phenomenon among SOCs because I_{CRAC} is also inactivated by Ca^{2+} (35, 41). In accordance with *in vivo* studies (42, 43), TRP expressed in 293T cells is blocked by Mg^{2+} and La^{3+} (32). The Mg^{2+} block has been proposed to play an important role in determining the gain, kinetics, and signal-to-noise of phototransduction (42).

Despite the similarities in the pharmacology and permeabilities of TRP *in vivo* and *in vitro*, TRP alone does not account for all the characteristics of the light-activated conductance. In contrast to the TRP conductance, the light-activated current is strongly outwardly rectifying (21). Moreover, at least one other channel seems to be present in *Drosophila* photoreceptor cells because the initial response to bright light is similar for TRP and wild-type flies. TRPL would seem to be a plausible candidate for this other channel; however, TRPL mutant flies fail to exhibit a discernible phenotype (30). Furthermore, TRPL expressed *in vitro* is constitutively active (Fig. 3) (32, 44–46), although it seems to be further activated by IP_3 (47). Release of Ca^{2+} from the internal stores does seem to have some effect on TRPL activity because the addition of thapsigargin increases the outwardly rectifying current (Fig. 3) (32). There are reports that Ca^{2+}/calmodulin and/or direct interaction with $G_{\alpha 11}$ functions in TRPL activation (46, 48); however, direct activation of TRPL by G proteins is unlikely to be the main mode of TRPL activation *in vivo* because PLC is required for the response to light (7).

Despite the observation that TRPL is not store operated, conclusive evidence that TRPL is an ion channel has been obtained using single-channel recordings (48, 49). In addition to the constitutive activity, TRPL differs from TRP in its lack of ion selectivity, with respect to Na^+, Ba^{2+}, and Ca^{2+}, and relative insensitivity to inhibition by La^{3+}; however, as is the case with TRP, TRPL is blocked by Mg^{2+} (32, 49).

Interestingly, TRPL can be converted into a SOC by fusing the TRPL amino-terminal and transmembrane domains with

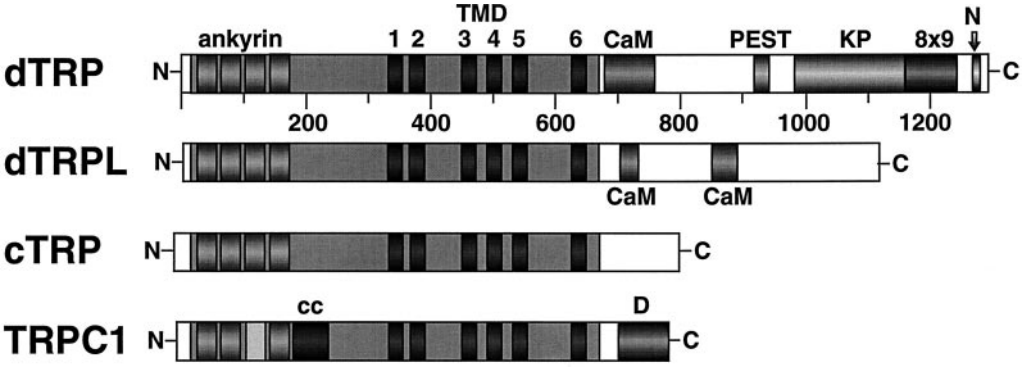

Fig. 2. Domain structures of TRP, TRPL, cTRP, and TRPC1. The putative ankyrin repeats, putative transmembrane domains (*TMD*), calmodulin binding sites (*CaM*), putative PEST degradation signal (*PEST*), region contain multiple repeats of the dipeptide lysine and proline (*KP*), domain containing nine tandem copies of an eight amino acid repeat, D-K-D-K-K-P-G/A-D (8 × 9), and a putative nucleotide binding site (*N*) are indicated. Also indicated in TRPC1 is a region with a high probability of forming a coiled-coiled (*cc*) structure and a segment near the carboxyl terminus with weak homology to dystrophin (*D*). The third putative ankyrin repeat is missing in one of the two isoforms of TRPC1.

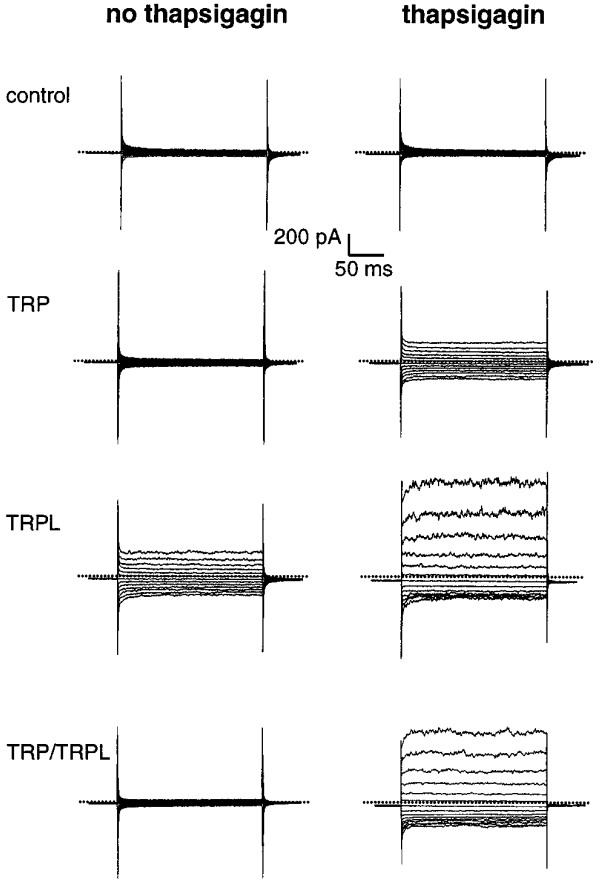

Fig. 3. TRP, TRPL, and TRP/TRPL currents in 293T cells before and after thapsigargin treatment. The cDNA constructs pTRP and pTRPL were transfected either singly or in combination as indicated. Na^+ currents were recorded either before or after depletion of the intracellular Ca^{2+} stores by perfusing the cells for 7–10 min with a Ca^{2+}-free solution containing 1 μM thapsigargin. *Traces*, currents produced using a set of step voltages from −100 to +80 mV in 15-mV increments. The holding potential was −10 mV. *Dotted lines*, 0 pA. [Adapted from Xu, X.-Z. S., H.-S. Li, W. B. Guggino, and C. Montell. Coassembly of TRP and TRPL produces a distinct store-operated conductance. *Cell* **89:** 1155–1164 (1997). © 1997 Cell Press.]

the region of TRP carboxyl-terminal to the transmembrane domains (50). As is the case with the TRPL conductance, the TRPL/TRP current is nonselective. Conversely, a constitutively active conductance with an ion selectivity typical of TRP is produced by joining the amino-terminal and transmembrane regions of TRP with the carboxyl-terminal domain of TRPL (50). Thus, the portion of TRP critical for conferring store-operated activity is the region carboxyl-terminal to the transmembrane domains.

TRP and TRPL Heteromultimerize to Produce a Functional SOC

The observation that TRP and TRPL are expressed in the same cells, *Drosophila* photoreceptor cells, raises the possibility that the two proteins interact directly to form heteromultimeric channels. Consistent with this proposal, TRP and TRPL coimmunoprecipitate from *Drosophila* photoreceptor cells (32). This interaction is most likely direct, rather than through another protein, because TRP and TRPL associate directly *in vitro*. Both the amino-terminal and transmembrane segments contribute to the interaction (32).

Coexpression of TRP and TRPL *in vitro* leads to store-operated currents distinct from currents produced by expression of the individual proteins. In *Xenopus laevis* oocytes, coexpression of both proteins leads to an inward store-operated Mg^{2+} current that was not detected when the individual channels were expressed separately (51). The Mg^{2+} current is different from the light-dependent conductance in that it does not seem to be permeable to Na^+ and is detected mainly at hyperpolarizations more extreme than those that occur *in vivo*. However, introduction of TRP and TRPL into 293T cells produces a store-operated conductance, which exhibits many features of the light-activated current (32). In particular, the TRP/TRPL store-operated current is outwardly rectifying (Fig. 3), more selective for Ca^{2+} than Na^+, and inhibited by either Ca^{2+} or Mg^{2+} but relatively insensitive to La^{3+}. However, the extent of inward rectification produced by expression of TRP/TRPL is more subtle than that of the light-activated conductance. The store-operated activity, ion selectivity, and inhibition by Mg^{2+} are features reminiscent of the TRP-dependent current, whereas the outward rectification and relative insensitivity to La^{3+} are characteristics most similar to those of current generated from TRPL alone. It is possible that TRP/TRPL *in vivo* has some sensitivity to La^{3+} because TRP expressed *in vitro* (32) is inhibited to a lesser extent than *in vivo* (43). Further electrophysiological evidence that TRP and TRPL coassemble is that dominant

negative forms of TRP suppress the TRPL conductance and vice versa.

It has been reported that TRP and TRPL are capable of responding independently to light activation (30), but several observations suggest that this is not the case and that TRPL exists solely as a heteromultimer in *Drosophila* photoreceptor cells (32). First, TRPL expressed *in vitro* is not a SOC but requires TRP for store-operated activity. Second, although TRPL homomultimers form *in vitro*, TRPL binds preferentially to TRP. Third, TRP seems to be ≥10-fold more abundant than TRPL. Thus, two store-operated channels that function in *Drosophila* photoreceptor cells seem to be TRP homomultimers and TRP/TRPL heteromultimers.

In addition to TRP and TRPL, a third TRP-related channel (TRPR) may function in phototransduction. The existence of a third light-activated channel in photoreceptor cells could account for the seemingly conflicting observations that (i) elimination of TRPL has been reported to have no discernible effect and (ii) the photoresponse is dramatically reduced, although not completely eliminated, in *trpl; trp* photoreceptors (30). TRPR may heteromultimerize with either TRP or TRPL; if so, the TRP/TRPL and TRP/TRPR complexes may be functionally redundant. Hence, there is no phenotype in *trpl* photoreceptors. In contrast, TRP/TRPL may not be redundant, with TRPL/TRPR resulting in a significant decrease in the photoresponse in *trp* flies. The small remaining conductance in *trpl; trp* flies may be due to TRPR, which displays little activity as a homomultimer.

Possible Mechanisms Coupling TRP and TRP/TRPL Activation to Ca^{2+} Depletion

The mechanism by which depletion of Ca^{2+} from the internal stores is coupled to the opening of SOCs is not known; however, the signal does not seem to be Ca^{2+} because SOCE can be activated in the presence of Ca^{2+} chelators (33). Two proposals have received considerable attention (reviewed in Ref. 3), one of which suggests that depletion of Ca^{2+} from the stores activates SOCs through a small soluble factor, referred to as Ca^{2+} influx factor. Such a factor of a molecular weight of <500 was originally isolated from a lymphocyte cell line, Jurkat cells (52), and has been reported to stimulate Ca^{2+} influx in a variety of cell lines (53, 54). However, other studies have cast doubt on the role of such a factor in Ca^{2+} influx (55, 56). Currently, there are no reports addressing the potential role of the Ca^{2+} influx factor in TRP activation, and further studies will be required to either confirm or dismiss a critical role for the Ca^{2+} influx factor in mediating Ca^{2+} influx via other SOCs.

A second proposal is that there is direct coupling between the IP_3R and SOCs (reviewed in Ref. 3). On depletion of Ca^{2+} from the stores, there is a putative conformational change in the IP_3R, which in turns activates the SOC through direct interaction. In support of this proposal, referred to as the conformational coupling hypothesis, it has been reported that TRP is spatially restricted to the base of the rhabdomeral microvilli adjacent to the presumed position of the IP_3-sensitive Ca^{2+} stores (57). However, subsequent studies indicate that TRP is uniformly distributed throughout the rhabdomeral microvilli (58–60), a spatial distribution which would preclude a direct interaction between the vast majority of TRP and the IP_3R. It remains possible, however, that SOCs other than TRP are activated via conformational coupling with the IP_3R.

An appealing modification of the conformational coupling hypothesis is that the putative conformational change in the IP_3R activates TRP indirectly through the actin-based cytoskeleton. The IP_3R could be linked to the cytoskeleton via ankyrin because there are reports indicating that some mammalian IP_3Rs associate with this adapter protein (61, 62). TRP could also be linked to the actin cytoskeleton because the rhabdomeres contain actin filaments (63) and squid TRP seems to associate with the detergent-insoluble cytoskeletal fraction (64). The putative interaction of TRP with the cytoskeleton could be mediated through one or more of the four ~33-amino acid ankyrin repeats located in the amino terminus of TRP. Furthermore, it is noteworthy that other ion channels, such as the *N*-methyl-D-aspartate receptor (65) and the amiloride-sensitive Na^+ channels (66), bind directly to and may be regulated by components of the actin cytoskeleton such as ankyrin and α-actinin. Disruption or stabilization of actin filaments, with specific drugs, has been shown to regulate a variety of ion channels in whole-cell and patch-clamp studies (67–69).

INAD Links TRP to Upstream Signaling Proteins

Drosophila TRP forms a large signaling complex with at least five other proteins that are essential for phototransduction. In additional to binding TRPL, TRP has been shown to associate with the major rhodopsin (RH1) (60), the PLC encoded by *norpA* (59, 60), an eye-specific protein kinase C encoded by the *inaC* locus (59), and calmodulin (60). Thus, most if not all of the phototransduction cascade may be linked into one supramolecular complex (Fig. 4). The interaction between TRP and calmodulin is direct, although the association with NORPA and RH1 is mediated through INAD (60), a protein with five ~90-amino acid PDZ domains (70–72). PDZ domains are protein interaction motifs which bind a variety of ion channels and other signaling proteins (reviewed in Refs. 70 and 73).

TRP binds directly to INAD, and this interaction is disrupted in an allele of *InaD*, $InaD^{P215}$, which contains a point mutation in the third PDZ domain (72). NORPA (60) also binds directly to INAD, although this has not been demonstrated with RH1, INAC, and calmodulin. In $InaD^{P215}$ mutant photoreceptors, TRP no longer associates with either NORPA or RH1, and the spatial distribution of TRP, but not NORPA or RH1, is severely disrupted (60). These data indicate that INAD is a scaffolding protein which serves to link TRP to upstream signaling proteins.

There may be two functions of the phototransduction signaling complex, one of which may be to facilitate feedback regulation. Compartmentalization of TRP with upstream signaling molecules may be to facilitate feedback regulation by highly localized increases in Ca^{2+} due to the TRP-dependent Ca^{2+} influx. The Ca^{2+} fluxes are likely to be spatially restricted as a consequence of extensive buffering by calmodulin and other Ca^{2+} binding proteins. Release of TRP from the signaling complex, as occurs in $InaD^{P215}$ photoreceptors, would therefore preclude the feedback regulation by the TRP-dependent Ca^{2+} influx. Consistent with the model that one function of TRP may be for Ca^{2+}-dependent feedback regulation are the findings that the activities of proteins associated with INAD, such as NORPA and RH1, may be

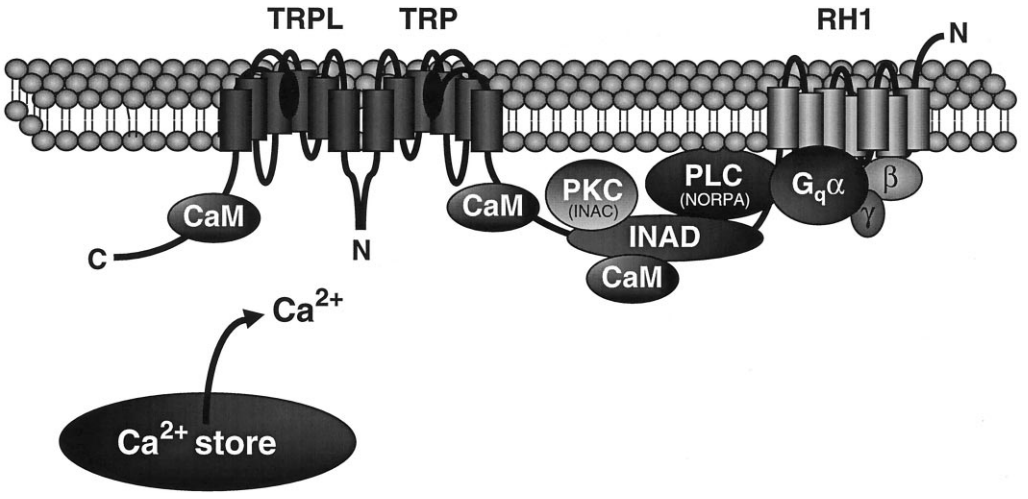

Fig. 4. Model of the signaling complex in *Drosophila* rhabdomeres. TRP binds directly to calmodulin and INAD and indirectly to NORPA, RH1. and INAC via INAD. The DGq trimeric G protein ($G_{q\alpha}$, G_β, and G_γ) is proposed to transiently interact with the complex. [Adapted from Chevesich, J., A. J. Kreuz, and C. Montell. Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. *Neuron* **18**:95–105 (1997). ©1997 Cell Press.]

down-regulated by Ca^{2+} (74, 75). Furthermore, $InaD^{P215}$ has been reported to display a slow deactivation of the light-induced current and increased sensitivity to dim light (71). In the absence of extracellular Ca^{2+}, the $InaD^{P215}$ response is indistinguishable from wild-type (71). Ca^{2+} is also likely to mediate feedback regulation of TRP and TRP/TRPL directly in photoreceptor cells because high Ca^{2+} levels inhibit the light-induced current (20, 39, 76) and both TRP and TRP/TRPL expressed *in vitro* are inactivated by Ca^{2+} (32). The inactivation of these SOCs may be through association with calmodulin, although this has not been demonstrated; however, the issue of whether the INAD signaling complex has a role in feedback regulation is controversial. It has been reported that $InaD^{P215}$ flies seem to display an increase in the mean latency between light stimulation and bump activation rather than a defect in inactivation (77), although it seems plausible that the Ca^{2+}-dependent feedback regulation requires greater light intensities than the single photons that give rise to unitary bumps.

A second potential function of the TRP/INAD supramolecular complex may be in activation. Production of IP_3 in close proximity to the light-sensitive channels could potentially result in rapid activation of the cation influx independent of the Ca^{2+} stores. As discussed above, such a mode of activation may operate during the initial light response, and SOCE may be required for a sustained response to light. Consistent with the proposal that the signaling complex also functions in activation, an *InaD* allele, $InaD^1$, with a stop codon in the second PDZ domain, displays a severely reduced response to light (77). Thus, the signaling complex may be critical for both activation and feedback regulation of *Drosophila* vision.

Proteins Related to TRP Are Conserved Throughout Animal Phylogeny

Proteins related to *Drosophila* TRP and TRPL have been identified in a variety of invertebrates, including a homolog of TRP in the related fly, *Calliphora*, which is ~85% identical to TRP over a amino-terminal segment of ~900 amino acids and ~40% identical to TRPL over the same region (Fig. 2) (59). However, in the carboxyl-terminal end, the homology between *Drosophila* and *Calliphora* TRP falls to <50%. Significant heterogeneity in the carboxyl-terminal region is a recurring theme because TRP-related proteins in the squid (sTRP) (64) and *C. elegans* (cTRP1) (78) contain only ~800 residues, a length ~450 residues shorter at the carboxyl terminus than *Drosophila* TRP. These latter proteins are ~40–45% identical to TRP; however, a much more distantly related protein in *C. elegans* emerged from the Genome Project (cTRP2; accession No. Z66514) which is only ~25% identical to TRP but has an overall predicted topology of six transmembrane domains, similar to other members of the TRP family. The low level of homology in cTRP2 is intriguing because this putative channel may display quite different functional characteristics than *Drosophila* TRP.

The conservation of TRP in a variety of invertebrates raised the possibility that the TRP family is also conserved in vertebrates and may account for some of the store-operated conductances characterized in vertebrate cells. Consistent with this proposal, a related human protein, TRPC1, has been identified which is widely expressed in many tissues but at the highest levels in the brain, heart, testis, and ovaries (78, 79). TRPC1 is ~40% identical to TRP or TRPL over the ~625 amino acids spanning most of the amino terminus and the putative transmembrane segments. Furthermore, TRPC1 is distinct from the *Drosophila* proteins because it contains a relatively short segment carboxyl-terminal to the transmembrane domains (148 residues) compared with TRP and TRPL (614 and 464 amino acids, respectively; Fig. 2).

In view of the requirement for the extended carboxyl terminus of TRP for store-operated regulation (50), the relatively short carboxyl-terminal segment in TRPC1 suggested that it might not be a SOC. However, functional expression of TRPC1 in COS or Chinese hamster ovary cells results in a store-operated nonselective cation conductance (80, 81). In contrast to these studies, TRPC1 expressed in Sf9 cells produces a constitutively active cation conductance (82), a feature reminiscent of *Drosophila* TRPL. Thus, as is the case with TRPL, TRPC1 may require interaction with another protein to confer regulation by Ca^{2+} depletion. In Sf9 cells, TRPC1 may not be store operated due to the requirement for another TRPC protein that is absent in insect cells but present in mammalian cells. In support of this proposal, TRPC1 forms heteromultimers *in vitro* with TRPC3 (32), a brain-enriched member of the TRP family (80). TRPC3 expressed in COS cells seems to be a SOC (80); however, it has not been analyzed in other cell types, such as Sf9 cells.

The full extent of the vertebrate TRP family remains to be determined, although the minimum size is six because the

partial or complete sequences of six types of TRPC genes have been identified in various animals; these include the full-length sequence for the mouse homologs of human TRPC1 (83) as well as portions of mouse homologs for human TRPC3 and ψTRPC2, the latter of which may be a pseudogene (80). The partial sequence of a fourth mouse TRPC gene, *mtrp4*, has been reported (80) and is a homolog of the full-length bovine clone, bCCE (84), and a TRP-related rat protein, TRP-R (85). These genes, which are collectively referred to here as *TRPC4*, are widely expressed in many tissues, including the brain and retina. Although rat and bovine TRPC4 are highly related, the conservation falls off considerable in certain portions of the carboxyl-terminal tail. Finally, segments of two other mouse sequences, *Mtrp5* and *Mtrp6*, have also been reported (80). Evidence that some of these additional vertebrate TRPC proteins may be SOCs is that expression of antisense constructs inhibit the endogenous SOCE in mouse L cells (80). Furthermore, bovine TRPC4 has been introduced into human embryonic kidney cells and seems to be an inwardly rectifying SOC with somewhat higher permeability for Ca^{2+} than for Na^{+} (P_{Ca}/P_{Na} ~8:1) (84).

Overall, the regions most highly related among the TRPC family members are the ~300 residues amino-terminal to the transmembrane domains, which typically contain three or four ankyrin repeats, followed by the ~50 residues immediately carboxyl-terminal to the sixth transmembrane segment and then the transmembrane domains. The carboxyl-terminal regions of each TRPC protein are quite heterogeneous in sequence. It is noteworthy that rat and bovine TRPC4s have a longer carboxyl-terminal extension (~260–300 amino acids) than either of the other TRPC proteins for which a complete sequence is available (148 and 178 residues for TRPC1 and TRPC3, respectively). This raises the possibility that the TRPC proteins with longer carboxyl-terminal extensions, such as TRPC4, can form homomeric SOCs, whereas TRPC proteins with shorter extensions are constitutively active unless they heteromultimerize with the appropriate TRPC partner.

Remaining Questions and Perspective

A key question on the minds of many researchers studying I_{CRAC} is whether any of the TRPC channels are responsible for the highly Ca^{2+}-selective, low-conductance, store-operated current typical of I_{CRAC}. Although it is clear that there are many types of store-operated cation conductances, none of the invertebrate or vertebrate TRP proteins induce a conductance with the properties of I_{CRAC}. One possibility is that I_{CRAC} is mediated by channels unrelated to TRP. However, an alternative possibility is that a diversity of currents are generated by heteromultimeric interactions between different TRPC proteins and that I_{CRAC} is generated through formation of specific sets of heteromultimers. Regardless of whether I_{CRAC} can be accounted for by any homomultimeric or heteromultimeric TRPC channel, it is of great interest to characterize the biophysical properties of the various conductances produced by these channels. A second important question concerns the mechanism by which depletion of Ca^{2+} from the internal stores activates members of the TRP family. Are there biologically relevant alternative modes for activating TRPC proteins independent of Ca^{2+} depletion from the internal stores? Furthermore, do the vertebrate TRPC proteins form supramolecular signaling complexes, and what is the nature of the proteins in the complex? Equally interesting are questions concerning the biological roles of the TRPC proteins. Are there human diseases, such as severe immunodeficiencies, that are due to perturbations in TRPC proteins? Although there has been considerable recent progress in characterizing the TRP family, many critically important questions clearly remain.

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