Chapter 4
TRP Channels in Visual Transduction

Juan Bacigalupo, Ricardo Delgado, Yorka Muñoz and Peter O'Day

Abstract The original members of the superfamily of transient receptor potential channels are the TRP and TRPL channels underlying *Drosophila* light transduction. Light transduction takes place in the photosensitive microvilli within the photoreceptor cells; it is initiated by photon absorption which leads ultimately to the generation of a depolarizing receptor potential caused by TRP and TRPL channel opening. Channel opening is mediated by a phospholipase C pathway where the membrane second messenger diacylglycerol appears to be the channel activator. TRP is a Ca^{2+} selective channel responsible for nearly 95 % of the net transduction current, whereas TRPL, a poorly-selective Ca^{2+} channel, accounts for the difference. The scaffolding protein INAD forms a complex with TRP and other transduction proteins, offering an extremely fast transduction mechanism. TRP and TRPL are also found in the synaptic terminals of the photoreceptors, where they play a role in presynaptic Ca^{2+} increments during synaptic transmission.

Keywords Light · Transduction · Vision · Retina · Photoreceptor · Lipids · TRP channels

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4.1 Introduction

The ability of animals to see is remarkable. Even the early steps in vision that involve light detection by photoreceptor cells are very complex and not fully understood in invertebrates and vertebrates. Light detection begins with absorption of photons by photopigments embedded in specialized membranes, and this event triggers biochemical signaling cascades. The nature of these signaling cascades varies widely across species. The biochemical cascades generate transmembrane neural electrical signals that travel to the photoreceptor cell synapse, where the neural information is relayed to the next level of signaling in the visual pathways. The process by which light is coded into an electrical neural signal is called phototransduction, because light energy is transduced into electrochemical energy.

Photoreceptor cell organization and complexity also varies widely, with photoreceptors organized as functional units of simple multicellular tissues or as components of complex eyes that focus light directly to the transducing regions. Some photoreceptors exist as isolated individual cells only serving to detect changes in illumination, forming part of simple photosensitive multicellular arrangements with different degrees of complexity; others are integrated into highly sophisticated organs, such as the compound eyes of insects and the camera eyes of cephalopods and vertebrates. Depending on the photosensitive structure, the animal can distinguish shadows, shapes, colors and even images of great complexity. Visible light represents a small fraction, 400–700 nm, of the vast spectrum of electromagnetic waves that reach our planet, 10^{-11}–10^{3} cm!

Photoreceptors emerged late in evolution relative to more primitive sensory receptors, such as chemo and mechanotransducing cells. The fact that the number of species boosted dramatically after phototransduction evolved hundreds of million years ago clearly suggests a considerable adaptive value of vision (Arendt 2008; Arendt and Wittbrodt 2001; Lamb 2011).

Photoreceptors are extremely sensitive and remarkably efficient photon detectors. In fact, they can generate discernable electrical responses to single photons and transmit single photon responses to secondary neurons, eventually reaching the central processing regions of the nervous system.

In this chapter we will briefly discuss vertebrate photoreceptors and then shift the focus to *Drosophila melanogaster* photoreceptors, the best characterized of all invertebrate visual cells, which employ TRP channels in phototransduction. Notably, these are the founding members of the extensive TRP channel superfamily, with key roles in a wide variety of sensory transduction mechanisms in invertebrates and vertebrates, such as mechano, thermo, taste, olfactory, pain and osmotransduction.

4.2 Vertebrate Photoreceptors

Vertebrate photoreceptors, rods and cones, are widely conserved in function, morphology and biochemical signaling components across species. They are exquisitely arranged and aligned in outer layers of the retina, and they transmit information

in the form of neural signals to higher order cells in more proximal layers. Each cell possesses a clearly distinguishable light transducing region, called the outer segment. This region contains about 2000 internal membrane structures (discs) containing the photopigment rhodopsin, a G-protein coupled membrane receptor, and several other transduction proteins that form the metabotropic signaling cascades that generate and modulate the electrical responses to light. These light responses are mediated by the closing of plasma membrane ion channels, often called transduction channels. These cationic non-selective channels reside exclusively in the outer segment plasma membrane, allowing Ca^{2+} and Na^{+} ions to enter the outer segment. These are the only ion channel types present in the outer segment. Because these channels are open in the dark, they sustain an inward current in the outer segment that keeps the membrane depolarized. This current is counterbalanced by an outward current at the inner segment membrane through K^{+} channels responsible of the receptor potential in most cell types and the membrane potential in darkness is set around −35 mV. As a consequence, there is an extracellular current flow from the inner to the outer segment. Light causes the closure of the transduction channels and the hyperpolarization of the cell due to the reduction of the inward current, shifting the membrane potential towards the K^{+} equilibrium potential, reaching nearly −70 mV if challenged with a saturating bright light (Lou et al. 2008).

These transduction channels are gated by cyclic GMP and belong to the small family of the cyclic nucleotide-gated channels, CNGs. cGMP levels are governed by the balance between cGMP production by guanylate cyclase (GC) and cGMP degradation by phosphodiesterase (PDE) that hydrolyzes cGMP to GMP. This balance maintains stable cGMP levels in the dark within the outer segment. Upon absorbing a photon, rhodopsin couples to a GTP-binding protein (G-protein) that activates the PDE; the resulting drop in cGMP levels leads to closure of the transduction channels (Fig. 4.1).

There is another class of vertebrate photoreceptor in the higher order layers of the retina, called the ipRGCs (intrinsically photosensitive retinal ganglion cells). Phototransduction in these cells appears to involve biochemistry similar to that found in invertebrate photoreceptors, as we explain below.ipRGCs contribute to non-image forming vision, including pupillary responses (Xue et al. 2011) and photoperiodic processes (Provencio et al. 2002). Although the underlying mechanisms of phototransduction in ipRGCs have not been elucidated, there is a growing body of evidence so far consistent with the idea that light responses in ipRGCs are mediated by pathways including melanopsin as photopigment activating a G_q type G-protein, leading ultimately to the opening of TRP channels.

Remarkably, melanopsin initiated phototransduction is found in photoreceptors from the earliest chordate, amphioxus, and evidence suggests the involvement of a PLC-dependent signaling cascade, as in *Drosophila* (see below) (Ferrer et al. 2012; Pulido et al. 2012).

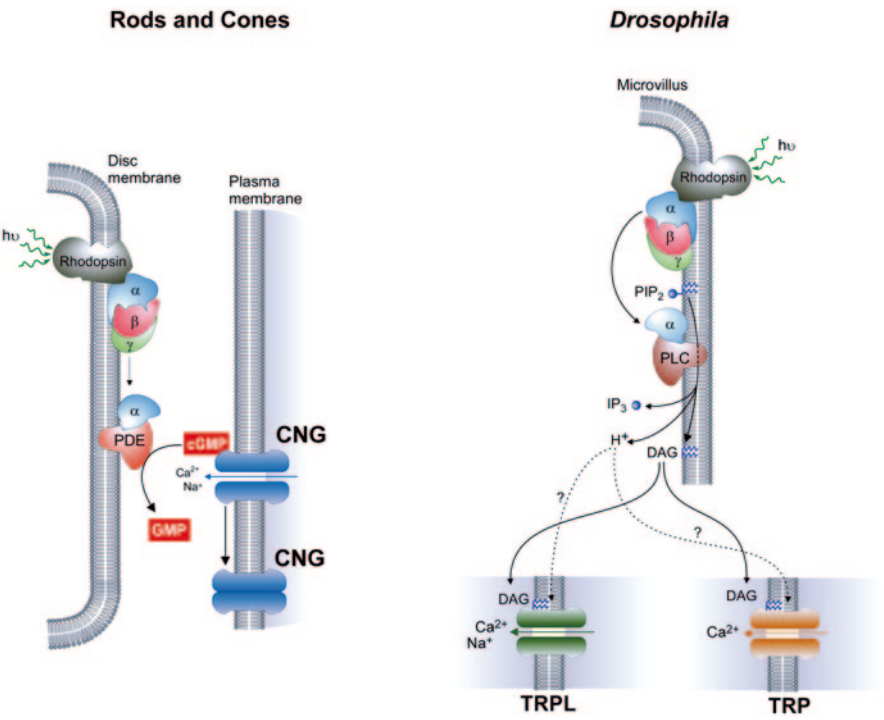

Fig. 4.1 Light transduction in vertebrate and *Drosophila* photoreceptors. *Left*. In vertebrates (*rods and cones*), when a photon hits rhodopsin in the disc membrane it undergoes a photoisomerization and couples to a heterotrimeric G-protein ("transducin"), whose Gα subunit is released and binds to phosphodiesterase (PDE), activating this enzyme. PDE hydrolyses cGMP, the ligand that opens a cationic non-selective cyclic-nucleotide-gated channel (CNG) in the plasma membrane, as a consequence of which the channel closes, generating a hyperpolarizing receptor potential. *Right*. In the microvilli of *Drosophila* photoreceptors, photoactivated rhodopsin couples to a G-protein which activates the enzyme phospholipase C. This enzyme cleaves the membrane phospholipid phosphatidylinositol bisphosphate (PIP_2), generating inositol trisphosphate IP_3, diacylglycerol (DAG) and a proton. DAG somehow opens the TRP and TRPL channels, giving rise to a depolarizing receptor potential. Protons may also contribute to channel opening

4.3 *Drosophila* Photoreceptors

Invertebrate photoreceptors are very different from rods and cones in many ways, biochemically, physiologically and morphologically. There is also great variation among invertebrate photoreceptors, but their photosensitive organelles generally consist of microvilli instead of discs and, with few exceptions, most invertebrate photoreceptors, such as crustaceans, molluscs, arachnids and insects, respond to light with depolarizing receptor potentials resulting from opening of transduction channels, but involving a diversity of signaling cascades.

Drosophila melanogaster photoreceptors have been the most extensively studied among invertebrates because they are most experimentally tractable for biochemical, genetic, molecular, physiological and morphological approaches. A diversity of genetic tools and mutants defective in phototransduction have been developed providing an incomparable advantage over the use of other animal species. These tools, in combination with the other techniques, have been effectively utilized for unraveling phototransduction to a remarkable degree.

Studies of *Drosophila* vision led to the discovery of the TRP class of channels, (Montell and Rubin 1989; Cosens and Manning 1969; Hardie and Minke 1992) which has proven to be a central and widespread theme in neural signaling in biology, with particular relevance for many modalities of sensory transduction. Here we review the properties of the two *Drosophila* light-dependent channels, the transient receptor potential (TRP) and the transient receptor potential-like (TRPL), in the context of phototransduction.

The fruit fly compound eye contains some 800 units known as ommatidia, each possessing eight photoreceptors, or retinula cells. Each photoreceptor has a slender cell body (40 μm × 2 μm), with a clearly distinguishable longitudinal narrow ribbon-like organelle called the rhabdomere, which consists of roughly 30,000 highly packed photosensitive microvilli. The outer six cells of each ommatidium are positioned circularly beside one another, with two cells positioned centrally; all eight rhabdomeres face each other, oriented towards the inner core matrix of the ommatidium (Fig. 4.2). The axons of the outer six photoreceptors project to the lamina while the axons of the other two central photoreceptors project to the medulla, where they synapse.

Within the microvilli, the phototransduction "machinery" is tightly organized in scaffolded macromolecular assemblies, variously called transducisomes or signaplexes (Tsunoda et al. 1997). The scaffolding protein, INAD, encoded by the *inaD* gene, binds key transduction proteins: TRP, PLC, the regulatory enzyme protein kinase C (PKC) and the myosin NINAC, which anchors the transducing complex to the actin cytoskeleton (Hicks et al. 1996; Wes et al. 1999). INAD scaffolding molecules also bind one another, presumably forming a large supercomplex in the microvillus. Normally, one photon induces only one of these events, termed quantum bump and. Under dim light, it is possible to distinguish the individual quantum bumps, which present obvious differences in size and shape. Using a fly deficient in the regulatory Ca^{2+}-binding protein calmodulin, which is necessary for the deactivation of photoactivated rhodopsin, it was observed that a single photon hit triggered multiple repetitive unitary responses (Scott and Zucker 1998). Remarkably, the size and shape of the repetitive events were almost identical, in contrast to those observed in wild type photoreceptors. This result suggests that in the wild type cell every quantum bump originated in a different microvillus, whereas in the mutant the bumps were generated in the only photoactivated organelle where the unitary delivered photon hit. The fact that the single photon response in the mutant consisted of a sequence of individual bumps rather than a continuous depolarization occurred in response to a single photon, is thought to reflect a refractory period following each bump during which the supply of PIP_2, exhausted during the response,

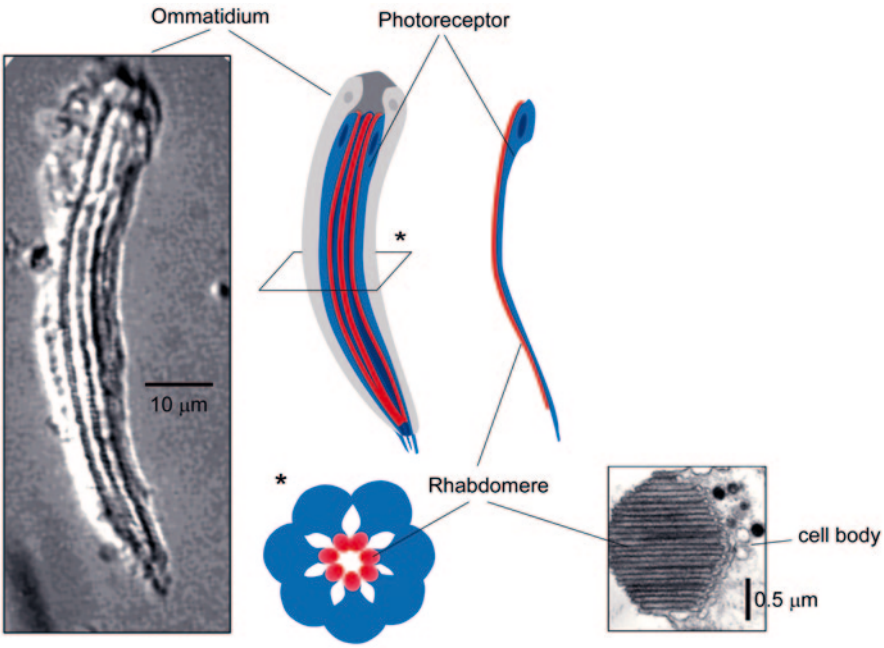

Fig. 4.2 *Drosophila* photoreceptor. Photograph of a dissociated ommatidium (*left*). Schematic representation of an ommatidium depicting the cell bodies of the individual photoreceptors forming part of it and their rhabdomeres (*center*). Individual photoreceptor (*center, right*). Cross section of an ommatidium illustrating the arrangement of the photoreceptors (*center, bottom*); the rhabdomeres of each cell is oriented to the core of the ommatidium. Electron micrograph of the cross section of a rhabdomere, where the highly packed microvilli are appreciated (*right, bottom*) (modified from Hardie 2014)

needed to recover in the microvillus. It is implied that every unitary event would recruit the whole transduction machinery of the respective microvillus, consistent with an all-or-none response. It is concluded that rhabdomeral microvilli are the structural and functional units of phototransduction and contain the proteins responsible for light absorption, signaling cascade, channel opening, and response modulation (Scott and Zuker 1998).

Interestingly, in addition to their rhabdomeral location, TRP and TRPL are also found in photoreceptor axonal terminals in the lamina and the medulla neuropiles of *Drosophila*. Astorga et al. (2012) reported that both channels coexist with the voltage-dependent Ca^{2+} channel, called cacophony (Cac), in those cellular regions and they contribute to the transient increase in Ca^{2+} required for neurotransmitter release, reinforcing the Ca^{2+} influx through Cac. In this synapse, TRP and TRPL work as store-operated channels, but their opening appears to be also mediated by PLC as in the rhabdomere (see below), although opening of these channels is not triggered by rhodopsin as this receptor protein is absent at the synapse. There is additionally IP_3 -dependent release of Ca^{2+} from the endoplasmic reticulum. The contributions from all these Ca^{2+} sources allows a massive Ca^{2+} elevation that is crucial for the

particularly fast synaptic transmission from the photoreceptors that follows the fast kinetics of phototransduction. Indeed, this synapse is of the ribbon type, characteristically associated to rapid transduction events, also occurring in vestibular hair cells (Lenzi et al. 2002).

4.4 Phototransduction

Light-activated rhodopsin couples to a heterotrimeric GTP-binding protein of the G_q class. The $G_{q\alpha}$ catalytic subunit, activated by activated rhodopsin, directly activates inositol-specific phospholipase C (PLC), which is bound in the transducisome to the INAD scaffold. PLC cleaves phosphatidylinositol bisphosphate (PIP_2), a low abundance plasma membrane phospholipid of importance in signaling, giving three products, inositol trisphosphate (IP_3), diacylglycerol (DAG), and a proton (Fig. 4.1). The critical requirement for PLC in phototransduction was revealed by the discovery that a null mutation in the gene coding for this enzyme completely abolished the light response (Bloomquist et al. 1988).

It was initially believed that IP_3 released Ca^{2+} from an intracellular reservoir, possibly the subrhabdomeral cisternae, close to the microvilli somehow caused the opening of the transduction channels in the microvilli. However, two lines of evidence rule out IP_3 as a required messenger in phototransduction. First, elevating IP_3 in the photoreceptors artificially in the dark by photoreleasing caged IP_3 did not affect subsequent responses to light; and second, mutants lacking IP_3 receptors nonetheless respond normally to light. This leaves the substrate PIP_2 of PLC and the two other products, DAG and H^+, as possible activators.

The first demonstration that lipids could activate the light-dependent channels was provided by Hardie and collaborators (Chyb et al. 1999). They showed that polyunsaturated fatty acids (PUFAs) added extracellularly to *Drosophila* photoreceptors in darkness generated an inward current with the same current-voltage relation as that activated by light, suggesting that light and PUFAs activated the TRP/TRPL-dependent current. PUFAs are products of the enzymatic activity of DAG lipase, suggesting this enzyme as a key component of the transduction mechanism and therefore present in the microvilli. Nevertheless, immunohistochemical studies that have detected DAG lipase in photoreceptor cell bodies failed to find it in rhabdomeres (Leung et al. 2008). This result, however, must be taken with caution because of the limitation in the resolution of the technique. Several PUFAs were tested by exogenous application to photoreceptors and found to differ in their potency to activate the channels (Chyb et al. 1999). The profile of lipids present in the rhabdomere was unknown at the time, but the presence of ten DAG species with different fatty acid chains, 11 of which are polyunsaturated, namely FUFAs, was recently reported (Muñoz et al. 2013). It was also found that PUFAs not existing endogenously could open TRP channels. Arachidonic acid, which is absent in *Drosophila* rhabdomeres (Muñoz et al. 2013), was able to open the TRP channels. PUFAs exhibit limited solubility in aqueous solutions and partition into the lipid

bilayer, where presumably they induce channel opening. This action could in principle be exerted by directly interacting with the channel proteins or another protein associated with them, or indirectly through transient modifications the structure of the bilayer; however, the mechanism of TRP channel activation is unknown. Subsequent works addressing the role of PUFA in opening the channels have produced conflicting results (Hardie 2014; Muñoz et al. 2013; Delgado et al. 2014).

The other two lipid products of PLC catalytic activity, PIP_2 and DAG, were extensively investigated by several laboratories. PIP_2 was first considered on the basis of study on TRPL ectopically expressed in Sf9 insect cells (Estacion et al. 2001). Adding PLC to inside-out excised membrane patches stimulated TRPL activity, while applying PIP_2 had the opposite effect. These conditions resembled the light and dark situations in the rhabdomere; so, presumably PIP_2 levels are low and channels are open in light, and PIP_2 levels are high and channels are closed in dark. However, no direct evidence regarding the actual PIP_2 levels in the Sf9 cells or of the presumed PLC enzymatic activity in those experiments was reported. Subsequent work on PIP_2 from different laboratories did not give a consistent picture, as they ranged from inhibitory to excitatory action of the lipid, including absence of an effect on TRPL (Lev et al. 2012). A recent report suggested that PIP_2 might lead to channel opening in a combined action with a drop of pH that accompanies its hydrolysis by PLC, although the observations done in photoreceptors and excised patches from S2 cells expressing TRPL in this paper are contradictory (Huang et al. 2010).

A role for DAG in TRP and TRPL channel opening has also been explored, in cells heterologously expressing TRPL and in inside-out patches from *Drosophila* rhabdomeres and from expression systems. The results, however, varied among the different experimental preparations (Lev et al. 2012). It is noteworthy that the barrier for DAG to flip from the extracellular to the intracellular membrane leaflet, where is normally located, is much larger than for PUFAs, therefore it is not surprising to fail to see a DAG effect on the channels when applied extracellularly, as opposed to PUFAs. Delgado and Bacigalupo (2009) first showed that DAG applied to inside-out excised rhabdomeral membrane patches of the native membrane was a potent activator of TRP and TRPL. PUFAs could activate the channels as well. A later, more detailed investigation also conducted in excised rhabdomeric patches provided strong support for DAG as the physiological activator of the light-dependent channels (Delgado et al. 2014). With this preparation, the biochemical steps in phototransduction could be reconstituted and manipulated. This study focused on TRP, which is responsible for nearly 95% of the transduction current, whereas TRPL only contributes with 5% (Reuss et al. 1997). When rhabdomeral patches were excised in the dark, TRP remained closed. However, when patches were excised under illumination, TRP was found to be constitutively active, behaving as if the transduction biochemistry in the patch was suspended and the molecular constitution of the patch remained locked in the condition existing at excision. Importantly in this preparation, ATP closed the channels and subsequent application of DAG reopened them; subsequent reintroduction of ATP once more closed the channel. This observation suggested that DAG kinase (DGK), an important component

of phototransduction, was retained in the isolated microvilli membranes and that manipulating its enzymatic activity with ATP mimicked the physiological situation, as this enzyme phosphorylates DAG, generating phosphatidic acid. Although DGK was not detected in rhabdomeres by immunostaining (Masai et al. 1997), the physiological evidence suggests that it is present and functions in DAG turnover (Delgado et al. 2014). This ATP effect was suppressed when DGK was inhibited, either pharmacologically or in the mutant deficient in DGK (*rdgA*). These results strongly implicate DAG as endogenous TRP activator. Evidence militated against the notion of PUFAs as channel activators. If PUFAs were to function as channel activators in phototransduction, functional DAG lipase, which generates PUFA from DAG, would be necessary. If this were the case, one would predict that DGL inhibition would affect TRP opening and that PUFA levels would be light dependent. However, pharmacological inhibition of DGL did not affect the opening of TRP by DAG, showing that conversion of DAG into PUFA was not a necessary step (Delgado et al. 2014). Furthermore, ultra high resolution measurements with liquid-chromatography/mass-spectrometry of DAG and PUFAs levels in a membrane fraction enriched in rhabdomere revealed light-dependent increments in DAG, but no change in PUFAs (Delgado et al. 2014). In this work, PIP_2 and protons were also examined as potential channel activators. Application of PIP_2 to constitutively active excised rhabdomeric membranes patches, in which this lipid presumably was at low level as in darkness, did not alter the channel behavior. Insertion of PIP_2 in the membrane was confirmed by a bioassay in patched from rhabdomeres ectopically expressing the PIP_2-sensitive Kir2.1 potassium channel. In contrast, introduction of protons did open the channels from the cytoplasmic side of the excised patches. Nevertheless, the effect of acidification from pH 7.15 to 6.4 proved to be irreversible, suggesting that the effect was non-physiological. A previous study had shown that a similar pH change reversible enhanced S2-expressed TRPL basal activity in excised patches, although this was not consistent with experiments in photoreceptors, where pH decrements with bright light were minimal (Huang et al. 2010).

Aside from *Drosophila*, very few other invertebrate photoreceptors have been studied. Scallops have two separate retinas, one containing microvillar and the other ciliated photoreceptors. Although the molecular nature of their light-dependent channels remains unknown, the transduction cascade in the former seems to involve PLC, as in *Drosophila* and their receptor potential is also depolarizing (Nasi and Gomez 1992), whereas in the latter there is a cGMP cascade that leads to the activation of K^+-selective light-dependent channels (Gomez and Nasi 1994), In the ventral eye of the horshoe crab Limulus, which are also rhabdomeric, the light-dependent channels differ substantially from *Drosophila* and scallop in their relatively large and non-selective cation conductance and ion selectivity; additionally, the underlying signaling mechanism appears to be more complex and is under debate, as there is evidence for the involvement of IP_3-Ca^{2+} and cGMP (Garger et al. 2004; Bacigalupo et al. 1991; Payne and Fein 1986).Summarizing, there is strong evidence indicating that DAG serves as endogenous activator of the light-dependent channels, while PIP_2 and H^+ are not involved directly, and PUFAs do not participate.

Interestingly, mammalian homologs of TRP such as TRPC2, 3, 6 and 7 have been shown to activate by DAG independently of protein kinase C, its most common target (Estacion et al. 2006; Hofmann et al. 1999; Lucas et al. 2003; Okada et al. 1999; Trebak et al. 2003).

4.5 Mechanism of Activation of TRP and TRPL

Although it is well established that lipids related to PLC activity are constituents of the phototransduction cascade and that DAG seems to be the most likely activator of the TRP and TRPL channels, the biophysical basis of the gating of these channels has been scarcely investigated. One obvious possibility is that DAG binds to the channels in one or more specific sites within the membrane. Another alternative is that lipids may lead to channel gating by means of altering the membrane fluidity or curvature, thereby modifying the lipid-channel interaction resulting in a mechanical gating of the channel mechanism, as shown in a mechanotransduction channel (Perozo et al. 2002). A recent report supports this possibility. In a set of elegant experiments Hardie's group (Hardie and Franze 2012) observed a change in photoreceptor length associated with the light response. Notably, the time course of the light-activated current and the photomechanical response measured with atomic force microscopy were similar. The mutant lacking PLC (*norpA*) was unresponsive to light and did not present mechanical responses as well, indicating that the enzymatic activity of this enzyme was crucial. The mechanical response took place even in the mutant devoid of both light-dependent channels (*trp;trpl*), suggesting that it did not involve the channel activity. Furthermore, incorporating the mechanosensitive channel gramicidin in this double mutant restored the ability to generate electrical light responses to these cells, mediated by the truncated phototransduction cascade.

The light response involves a rapid and massive inward current mainly relying on the TRP channel. However, the fact that this channel is normally largely blocked by Ca^{2+} like other Ca^{2+}-selective channels poses a paradox. Remarkably, it was recently shown that this divalent open channel block can be alleviated upon light activating the PLC enzymatic activity (Parnas et al. 2009), suggesting that the light-induced transient changes in the lipid composition of the membrane moiety of the channels would somehow unblock the channels allowing dramatic transient increment in its Ca^{2+} permeability, being rapidly inactivated in a Ca^{2+}-calmodulin dependent fashion.

4.6 Molecular and Biophysical Characteristics of TRP and TRPL

Four protein subunits form the TRP and TRPL light-dependent channels, each possessing six transmembrane domains. In this aspect they resemble K^+ rather than Ca^{2+} channels, which possess a subunit having four identical six transmembrane

domains. The *Drosophila* channels share 40 % homology between one another and also have a significant homology with mammalian TRPC channels, which are members of the same "canonical" TRP subfamily. The subunit composition of the light-dependent channels was recently shown to be strictly homomeric (Katz et al. 2013).

TRP and TRPL differ in their cation-selectivity. TRP is highly Ca^{2+} selective (~ 100 Ca^{2+} : 1 Na^{+}) while TRPL is poorly selective for Ca^{2+} (4 Ca^{2+} : 1 Na^{+}). Around 90 % of the phototransduction current flows through TRP. The relevance of TRPL is still mysterious. Under physiological conditions noise analysis of light-dependent currents recorded from dissociated photoreceptors estimated conductances of 8 for TRP and 35 pS for TRPL (Reuss et al. 1997), whereas they respectively have 58 and 40 pS under low divalent cations conditions.

4.7 Concluding Remarks

TRP channels in visual transduction have been under investigation for quite some time, and many channel properties and roles have been revealed that have been of broad importance. The mechanism underlying TRP gating has been elusive, however. Recent evidence points to DAG as activator of TRP channels in the *Drosophila* retina. This finding will have significance for many signaling systems, beyond phototransduction and it will have even greater significance when the molecular mechanisms by which DAG activates TRP are elucidated.

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