Optogenetic Modulation of Ion Channels by Photoreceptive Proteins

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(Citation)
Optogenetics:73-88

(Issue Date)
2021-01-06

(Resource Type)
book part

(Version)
Accepted Manuscript

Title: Optogenetic modulation of ion channels by photo-receptive proteins

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Keywords: G protein-coupled receptor, ion channel, membrane protein, opsin, optogenetics, rhodopsin.

Abstract

In this 15 years, researches to control cellular responses by light have flourished dramatically to establish "optogenetics" as a research field. In particular, light-dependent excitation / inhibition of neural cells using channelrhodopsins or other microbial rhodopsins is the most powerful and the most widely used optogenetic technique. New channelrhodopsin-based optogenetic tools having favorable characteristics have been identified from a wide variety of organisms or created through mutagenesis. Despite the great efforts, some neuronal activities are still hard to be manipulated by the channelrhodopsin-based tools, indicating that complementary approaches are needed to make optogenetics more comprehensive. One of the feasible and complementary approaches is optical control of ion channels using photoreceptive proteins other than channelrhodopsins. In particular, animal opsins can modulate various ion channels via light-dependent G protein activation. In this chapter, we summarize how such alternative optogenetic tools work and they will be improved.

Abbreviations

Ca_V; voltage-sensitive Ca^{2+} channel, ChR; channelrhodopsin, GIRK; G protein-gated inwardly rectifying K^+ channel, GPCR; G protein-coupled receptor, PAC; photo-activated adenylyl cyclase.

1. Introduction

Since channelrhodopsins (ChRs) were identified as light-gated cation channels in 2002 - 2003 (Nagel et al. 2002, Nagel et al. 2003) and photo-induced excitation of neural cells through ChR2 was achieved in 2005 (Boyden et al. 2005), optogenetics has dramatically progressed day by day. Many factors contribute to the rapid progress, and one major factor is finding and making of variants of ChRs as well as other microbial rhodopsins having different molecular characteristics such as absorption spectrum, ion selectivity, on/off kinetics, and so on (Yizhar et al. 2011). By using the ChR-based optogenetic tools, researchers can manipulate a wide variety of cellular activities by light. However, some neuronal activities are still hard to be regulated by ChRs, and thus complementary techniques are needed. Actually, many optogenetic tools using other photo-receptive proteins have provided alternative techniques (Paoletti et al. 2019, Wiegert et al. 2017, Yizhar et al. 2011). In this chapter, we introduce these alternative optogenetic tools and discuss their advantages as well as disadvantages. In particular, our main focus is ion channel modulation by animal opsins to regulate activities of neurons and other tissues.

2. Channelrhodopsins: great functionalities and limitations to be overcome

Because molecular characteristics and usefulness of ChRs are thoroughly reviewed in other chapters, we here briefly discuss neuronal activities that ChRs can or cannot modulate.

ChR2 and other cation-selective ChRs are basically non-selective cation channels and preferably conduct small cations (Nagel et al. 2002, Nagel et al. 2003). In particular, proton (H^+) permeability is the highest in some cation-selective ChRs due to its small size and the conduction through hydrogen-bonding networks, but under physiological condition (at neutral pH; $[H^+] \approx 10^{-7}$ M, and at $[Na^+]_{out} \approx 10^{-1}$ M), the ChRs dominantly conduct Na^+ leading to depolarization of the target cells. Also, the high proton permeability in ChR2 was utilized to manipulate intracellular acidification in glial cells (Beppu et al. 2014). On the other hand, Ca^{2+} permeability in ChRs are relatively low (but detectable), and optical control of Ca signalings by ChRs would be difficult. Despite of this difficulty, in some cases, Ca^{2+} inflow was successfully regulated in a light-dependent manner using ChR2 or its derivatives (Asano et al. 2018, Figueiredo et al. 2014, Perea et al. 2014). Of course, efforts to improve ion selectivity of ChRs are in progress (Cho et al. 2019).

Regarding anion-conducting inhibitory ChRs such as GtACR (Govorunova et al. 2015) and iChloC (Wietek et al. 2015), light-induced opening of the channels leads to hyperpolarization by Cl^- inflow or "shunt" effect caused by increase of Cl^- conductance (Wiegert et al. 2017). The inhibitory effect of the anion-conducting ChRs is much more effective than H^+ or Cl^- pumping microbial rhodopsins due to higher ion conductance. On the other hand, in some cells such as premature neurons (Kaila et al. 2014), olfactory sensory neurons (Kaneko et al. 2004), and rod bipolar cells (Satoh et al. 2001), intracellular Cl^- concentration is substantially high (typically more than 20 - 25 mM), and the anion channel

opening induces depolarization (excitation) by Cl^- outflow (Arosio and Ratto 2014). Furthermore, in axons, light activation of anion-conducting ChRs stimulates (not suppresses) presynaptic activities probably due to locally high Cl^- concentration (Mahn et al. 2016, Wiegert et al. 2017). Unlike the case of Cl^-, intracellular K^+ concentration is universally so high that K^+ channel opening should suppress neural activities in any cells via hyperpolarization. Thus, extensive "gene mining" or rational design in order to find/make ChRs selectively conducting K^+ are challenged all over the world. Notably, a mutant of Na^+ pumping rhodopsin (KR2) acts as light-activated K^+-selective channel, but unfortunately, the mutant works only at non-physiological alkaline pH (~10) (Vogt et al. 2019). Additional mutations would be required for making the KR2 mutant functional at neutral pH.

Taken together, a wide variety of ChRs can excite or inhibit neural cells efficiently, but they are not very good at manipulating K^+ or Ca^{2+} currents. Modulation of resident K^+ or Ca^{2+} selective channels is an alternative way to manipulate K^+- or Ca^{2+}- dependent cellular activities. In comparison with ChRs, modulation of ion channels by an animal opsin or another photo-receptive protein is a less direct way to control cellular excitability, and thus the light-induced cellular responses need to be carefully interpreted. On the other hand, careful studies using such indirect tools would make optogenetics more comprehensive. We thus introduce optogenetic tools based on animal opsins or other photo-receptive proteins, and discuss how they can regulate K^+-, Ca^{2+}-, or other channels. In Table x.1, we summarize (potential) optogenetic tools mentioned in this chapter.

3. Animal opsins as modulator of ion channels via trimeric G proteins

Animal opsin is light-sensitive G protein-coupled receptor (GPCR), and trimeric G proteins can modulate various ion channels including K^+/Ca^{2+} channels directly or indirectly. Thus, animal opsins would be good alternative tools to regulate excitable cells by light, especially by a light-dependent modulation of K^+ or Ca^{2+} current through trimeric G proteins. In this section, we introduce how the opsins work, and discuss their abilities and potentials to modulate ion channels.

3-1. Binding ability and photo-convertibility of chromophore in "vertebrate-type" and "invertebrate-type" opsins

Success of ChR-based optogenetics proved that the chromophore molecule retinal, the aldehyde form of vitamin A (Fig. x.1), is present in mammalian neurons (Deisseroth 2011). ChRs act as fine optogenetic tools in various cells without addition of the retinal, and animal opsins also use retinal as chromophore. So, it is a natural idea to utilize animal opsin as an alternative optogenetic tool, but there are some concerns.

Animal opsin uses different retinal isomers from ChR (and other microbial rhodopsin). In ChRs, all-*trans* to 13-*cis* photoisomerization of the retinal (Fig. x.1) leads channel opening (Hegemann et al. 1991, Lawson et al. 1991). In animal opsins, G protein

activation is triggered by 11-*cis* to all-*trans* isomerization (Figs. x.1 and x.2) (Shichida and Imai 1998, Terakita 2005, Wald 1968). In retina, a specific enzymatic system named "visual cycle" works to provide 11-*cis*-retinal from normal (all-*trans*) retinoids (Wang and Kefalov 2011, Yau and Hardie 2009), but other tissues lack the system. When "Opto-XR" (see section 3-3) was reported to function in the mammalian brain (Airan et al. 2009), researchers of animal opsin were surprised by the fact that exogenous *cis*-retinal is not necessary. This is because the "Opto-XR" is a derivative of vertebrate visual pigment, and the visual pigment can directly bind 11- (or 9-) *cis*-retinal only (Fig. x.2a) (Jager et al. 1996, Tsukamoto and Terakita 2010). One possibility is 9-*cis*-retinal (Fig. x.1) is present in the brain, because tiny amounts of the visual pigment binds to 9-*cis*-retinal in retina of mice lacking an essential enzyme (RPE65) producing 11-*cis*-retinal (Fan et al. 2003, Fan et al. 2005). Current general consensus is substantial amounts of *cis*-retinal endogenously exists at least in the mammalian brain (Deisseroth 2011, Wiegert et al. 2017), it may not be the case in some other tissues.

On the other hand, invertebrate opsins possess different molecular properties from vertebrate visual pigments (Hubbard and St George 1958, Terakita 2005, Terakita 2010, Tsukamoto and Terakita 2010, Yau and Hardie 2009). Regarding direct binding of retinal isomers, some (maybe not all) of invertebrate opsins can bind exogenous all-*trans*-retinal (Koutalos et al. 1989, Tsukamoto et al. 2017, Tsukamoto et al. 2005), and all-*trans*-retinal-bound (active) and 11-*cis*-retinal-bound (inactive) forms are interconvertible by light absorption (Fig. x.2b). The opsins with these properties in invertebrates (and vertebrates) are

called as “bistable” opsins (Koyanagi et al. 2004, Tsukamoto and Terakita 2010). As a typical example of invertebrate (bistable) opsin, we show the data of cOpsin1 from marine ragworm *Platynereis dumerilii* (Fig. x.2, c, d, and e). The opsin shows bidirectional photoreactions upon illumination with different color of light (Fig. x.2c) and possesses direct binding ability for not only 11-*cis*- but also all-*trans*- retinal isomers (Fig. x.2d). Furthermore, the bidirectional photoisomerization of retinal between 11-*cis* and all-*trans* isomers accompanies with reversible structural changes, which was demonstrated by light-induced difference FTIR spectroscopy for *Platynereis* cOpsin1 (Fig. x.2e) and jumping spider Rh1 (Ehrenberg et al. 2019). The light-induced reversible changes in the protein conformation was successfully monitored by the amide I modes (1665, 1650, 1642, and 1631 cm^{-1} in Fig. x.2e, and also see (Ehrenberg et al. 2019)). The properties of invertebrate opsins as the ON-OFF photo-switch for G protein activation (Fig. x.2b) were also confirmed by various techniques (Ashida et al. 2004, Emanuel and Do 2015, Kawano-Yamashita et al. 2015, Spoida et al. 2016, Stavenga and Schwemer 1984, Tsukamoto et al. 2017, Tsukamoto et al. 2005). Therefore, these invertebrate opsins can function where ChRs can work with endogenous all-*trans*-retinal. Interestingly, in vertebrates, some opsins function in various cells other than visual photoreceptor cells, and these “non-visual” opsins show similar retinal binding ability and bidirectional photoreactions to invertebrate opsins rather than to vertebrate visual pigments (Matsuyama et al. 2012, Panda et al. 2005, Yamashita et al. 2010). For clarity, we hereafter describe invertebrate opsin and vertebrate non-visual opsin having bistable properties as “invertebrate-type” opsin, and use

"vertebrate-type" opsin only for vertebrate visual pigments (rod and cone pigments) (see Fig. x.2, a and b).

3-2. Ion channel modulation via $G_{i/o}$-coupled animal opsins

In rod and cone photoreceptor cells, "vertebrate-type" opsins are coupled with transducin (Kefalov 2012, Yau and Hardie 2009), eye-specific $G_{i/o}$-type trimeric G protein, and they can activate $G_{i/o}$ when reconstituted (Kanaho et al. 1984, Terakita et al. 2002). Classically, invertebrates were thought to possess only G_q-coupled opsins, but $G_{i/o}$-coupled "invertebrate-type" opsins have been identified (Arendt et al. 2004, Koyanagi et al. 2013). As optogenetic tools, these $G_{i/o}$-coupled opsins would regulate cellular activities via $G_{i/o}$ activation whereas arrestin signalings could also be driven by them (Spangler and Bruchas 2017).

The most major response upon $G_{i/o}$ activation is inhibition of adenylyl cyclase by $G_{i/o}\alpha$ (Taussig et al. 1994). In fact, intracellular cAMP concentrations can be suppressed by light activation of "vertebrate-type" opsins (Koyanagi et al. 2013). Furthermore, "invertebrate-type" opsins can decrease and the increase (reverse) the cAMP levels by stimulation of different color of lights via interconvertible photoreaction between inactive and active forms (Figs. x.2 and x.3) (Kawano-Yamashita et al. 2015, Koyanagi et al. 2013). However, since basal intracellular cAMP levels are usually low (nM order) (Binkowski et al. 2011, Violin et al. 2008), further decrease of the cAMP concentrations by light might little affect activities of target cells.

Rather, a K^+ channel, GIRK (or Kir3) is a more promising target of optogenetics as well as chemogenetics using $G_{i/o}$-coupled receptors (Roth 2016, Wiegert et al. 2017). GIRK is mainly localized in the postsynaptic membrane of various neurons and activated by βγ subunit of $G_{i/o}$ (Luscher and Slesinger 2010, Nagi and Pineyro 2014). Thus, light activation of $G_{i/o}$-coupled opsins expressed in a neuron would cause increase of K^+ outflow, leading to suppression of neuronal excitation (Fig. x.3). In this way, $G_{i/o}$-coupled opsins act as inhibitory optogenetics tools. GIRK activation by GPCR is independent from effector enzymes and diffusible second messengers, and the activation is spatially localized "membrane delimited" process (Nagi and Pineyro 2014, Soejima and Noma 1984). Therefore, animal opsin-induced GIRK activation would be fast and applicable for optical control of subcellular compartments.

"Vertebrate-type" opsins have already been experimentally proved to work well as inhibitory tools in mammalian neurons (Gutierrez et al. 2011, Li et al. 2005). Among "vertebrate-type" opsins, cone pigments possess much faster off kinetics than rod pigments due to rapid inactivation via spontaneous release of all-*trans*-retinal and/or arrestin binding (Kefalov 2012, Shichida and Imai 1998). Also, cone pigments in various vertebrates show a wide variety of absorption spectra (Yokoyama 2008), indicating that researchers find some tools which can be activated by a specific color on demand. Masseck et al. showed that a rod pigment (rhodopsin) can modulate GIRK current with slow off kinetics and two (SWS and LWS) cone pigments induce rapidly attenuated GIRK responses with different color of light (Masseck et al. 2014). The cone pigments-induced GIRK current suppressed action potential

firing in serotonergic neurons of mice (Masseck et al. 2014). In addition, these photo-responses can be repeated many times, indicating sufficient supply of *cis*-retinal.

"Invertebrate-type" opsins also can regulate GIRK activity. A UV-sensitive "invertebrate-type" opsin from marine ragworm can increase GIRK current by UV light and the UV-induced K^+ current is cancelled by visible light in *Xenopus* oocytes (Tsukamoto et al. 2017). Another UV-sensitive bistable opsin, lamprey parapinopsin (Koyanagi et al. 2004), can also induce ON-OFF cycle of GIRK current by UV and visible light in HEK293 cells (Eickelbeck et al. 2019). They have not been tested in neural cells, but would be nice tools that can be turn on and off by different color stimulations.

Interestingly, one study showed that a K^+ channel can be directly activated by an animal opsin itself. Caro et al. reported that a fusion protein of bovine rhodopsin (rod pigment) followed by a K^+ channel Kir6.2 produces significant K^+ current in the dark, and light illumination decreases the current, indicating that the rhodopsin activation suppresses the K^+ channel activity (Caro et al. 2012). Intriguingly, deletion of the G protein interaction site in the rhodopsin moiety has almost no effect on the light-dependent suppression of the channel activity, indicating that the channel regulation is independent form G proteins. This study concluded that in the fusion protein, light-induced conformational changes in rhodopsin directly induces closure in the Kir6.2 moiety. It is not clear if such a direct coupling of opsin and channel activities can occur in other combinations. Currently many crystal or cryo-EM structures of ion channels and GPCRs are available (Cheng 2018, Weis and Kobilka 2018), and

activation processes of channels and opsins are also extensively studied using various biophysical techniques. These structural information with molecular docking simulations could find or predict another ion channel that can be directly regulated by some opsin.

In addition to GIRK, neuronal voltage sensitive Ca^{2+} channels (P/Q- and N- types, Ca_v2) are also modulated by $G_{i/o}$ (Catterall 2000, Dolphin 2003). The Ca_v channels are localized in axon terminal, and negatively regulated by βγ subunit of $G_{i/o}$ leading to suppression of neurotransmitter release. As mentioned in section 2, anion-conducting ChRs could not act as inhibitory tools in axon, but $G_{i/o}$-coupled opsins could function as effective axonal inhibitory tools (Fig. x.3). Actually, a "vertebrate-type" opsin can suppress presynaptic neurotransmitter release in hippocampal neurons upon photo-stimulation (Li et al. 2005). Activation and inactivation of "invertebrate-type" opsins by different color of light could be used as an "OFF-ON" switch for Ca_v2 channels in presynaptic regions.

3-3. Ion channel modulation via G_s-coupled opsins and Opto-XRs

Since vertebrate visual pigments are $G_{i/o}$-coupled, other G protein subtypes including G_s cannot be modulated by intact them. However, G_s-coupled opsins have been identified from Cnidarians such as jellyfish (Koyanagi et al. 2008, Kozmik et al. 2008, Suga et al. 2008). In mammalian cultured cells expressing a jellyfish opsin, and photo-stimulation induces elevation of intracellular cAMP concentrations via adenylyl cyclase activation by $G_s\alpha$ (Bailes et al. 2017) (Fig. x.4). Alternatively, chimeric receptors named "Opto-XRs" of vertebrate

visual pigments and other GPCRs have overcome this limitation (Airan et al. 2009, Spangler and Bruchas 2017, Tichy et al. 2019). In this section, we describe architecture and functionality of Opto-XRs and how G_s-coupled opsins and Opto-XRs can modulate ion channels.

Even before the optogenetics era, it had been reported that G protein coupling specificity of animal opsins as well as other GPCRs can be changed by replacement of cytoplasmic regions that interact with G proteins (Kim et al. 2005, Liu et al. 1995, Terakita et al. 2002, Yamashita et al. 2000). "Opto-XRs" utilize this insight. In an Opto-XR, an animal opsin (typically vertebrate visual pigment) is an "acceptor", and an "XR" (target GPCR) is a "donor" providing cytoplasmic loops and C-terminus. In the constructed "Opto-XR", extracellular and transmembrane regions are from the "acceptor" opsin and G protein interaction sites are from the "donor" GPCR (Fig. x.5) (Airan et al. 2009, Spangler and Bruchas 2017, Tichy et al. 2019). The Opto-XR can activate G proteins (and arrestins) that are coupled to the "donor" receptor in a light-dependent manner. The most widely used tool based on this approach is the Opto-β_2AR, in which cytoplasmic regions of G_s-coupled β_2-adrenergic receptor are introduced into $G_{i/o}$-coupled bovine (or other vertebrate) rhodopsin (rod opsin) (Airan et al. 2009, Siuda et al. 2015b). The Opto-β_2AR can drive G_s-dependent pathways, and successfully modulated activity of neural cells and animal behaviors by light stimulation (Airan et al. 2009, Siuda et al. 2015b), although it is less clear how the tool regulates neuronal activities.

Even $G_{i/o}$-coupled receptors are used as "donor" for Opto-XR (Siuda et al. 2015a).

This is probably because replacement of $G_{i/o}$ interaction sites would improve fine tuning of G protein coupling specificity (e.g. G_i vs G_o, G_{i1} vs G_{i2}, etc.), interactions with arrestins, and trafficking in target cells. However, conformational changes upon activation are somewhat different between GPCRs coupled to different G protein subtypes (Kang et al. 2018, Kato et al. 2019, Koehl et al. 2018, Rasmussen et al. 2011). Furthermore, there are some differences in the conformational changes even among $G_{i/o}$-coupled "vertebrate-type" and "invertebrate-type" opsins (Tsukamoto et al. 2009). Taken together, signaling characteristics of Opto-XRs may be somewhat different from those of the "donor" receptors (XRs), although basic properties in G protein activation can be successfully transferred into the "acceptor" opsins.

Regarding ion channel modulation by G_s-coupled opsins and Opto-XRs, CNG and HCN channels can be positively regulated by binding of cyclic nucleotides produced by adenylyl cyclase activated by $G_s\alpha$. Since CNG and HCN channels are non (or less) selective cation channels, opening of these channels by G_s-coupled opsins and Opto-XRs would cause depolarization of the target cells in a light-dependent manner (Fig. x.4).

Another attractive target of the G_s-coupled opsin-based optogenetic tools is cardiac voltage-sensitive Ca^{2+} channel (L-type, Ca_v1). In cardiomyocytes, adrenergic signals activate β-adrenergic receptors leading to increase of beating rate via potentiation of cardiac Ca_v channels (Catterall 2000, Hagiwara et al. 1988). The signaling pathway between the receptor activation and the Ca_v potentiation is activation of adenylyl cyclase by $G_s\alpha$ leading to elevation of cAMP levels which in turn stimulates the protein kinase A to phosphorylate the Ca_v channels

(Fig. x.4). Thus, the G_s-coupled optogenetic tools are expected to regulate heartbeat in a light-dependent manner. Recently, a G_s-coupled jellyfish opsin was introduced into cardiomyocytes, and photo-stimulation successfully increased intracellular cAMP levels and contradiction rate in isolated cardiomyocytes (Makowka et al. 2019). Furthermore, activation of the jellyfish opsin can increases beating rate of explanted heart at the level comparable to stimulation of an adrenergic agonist (Makowka et al. 2019). This study beautifully proved usefulness of the G_s-coupled opsin as a powerful optogenetic tool.

3-4. Ion channel modulation via G_q-coupled opsins

"Opto-XR" approach can be applied to G_q-coupled receptors: introduction of cytoplasmic regions of G_q-coupled GPCRs enables "acceptor" opsin to interact with G_q (Airan et al. 2009, Morri et al. 2018). It is known that opsins in eyes of invertebrates such as molluscs and insects are G_q-coupled (Koyanagi and Terakita 2008, Terakita 2005). Furthermore, even many vertebrates including mammals possess G_q-coupled opsins named as melanopsin (or Opn4), which functions in some of retinal ganglion cells to regulate circadian rhythms and pupil constriction (Do and Yau 2010, Do 2019). Mammalian melanopsins can be activated by blue light and inactivated by green light (Emanuel and Do 2015, Matsuyama et al. 2012, Spoida et al. 2016), and in the classification of this chapter, mammalian melanopsin is regarded as "invertebrate-type" opsin.

As optogenetic tools, G_q-coupled Opto-XRs and opsins are notable because G_q

activation leads to opening of Ca^{2+} channels. The major pathway in this process is that $G_q\alpha$ activates phospholipase C (PLC) to produce signaling molecules (typically, IP_3 and diacylglycerol) that activate several Ca^{2+} channels, such as IP_3 receptor and TRP channels (Fig. x.6). Actually, Opto-α_1AR, in which G_q-coupled α_{1a}-aderenergic receptor is a "donor", has already been proved to activate some neurons in a light-dependent manner (Airan et al. 2009), and mammalian melanopsins also have been used as optogenetic tools to drive G_q-dependent signaling pathways.

Natively G_q-coupled mouse and human melanopsins produce sustained and transient Ca^{2+} current by blue light stimulation, respectively (Spoida et al. 2016), probably because like vertebrate cone pigments (Kefalov et al. 2005), human melanopsin releases the retinal more frequently (Tsukamoto et al. 2015). Mammalian melanopsins have been proved to optically control activities of various types of cells (Beiert et al. 2014, Mederos et al. 2019, Tsunematsu et al. 2013, Ye et al. 2011), but their G protein coupling promiscuousness should be considered. Mammalian (mouse and human) melanopsins are reported to activate not only G_q but also other G protein subtypes. In mammalian cultured cells expressing mammalian melanopsins, light stimulation induces GIRK activation and $G\beta\gamma$ translocation, indicating that the melanopsins can activate $G_{i/o}$-type G protein (Kankanamge et al. 2018, Spoida et al. 2016). Furthermore, a recent study showed that in some population (M2- and M4- types) of melanopsin-expressing retinal ganglion cells, some cyclic nucleotide (probably cAMP) concentration is increased by melanopsin activation leading to opening of HCN channel (Jiang et al. 2018). Although it is

unclear which G protein subtypes are responsible for the melanopsin-dependent elevation of cyclic nucleotide levels, one possibility is that melanopsin can activate G_s. If so, mammalian melanopsin possesses a coupling ability with G_q, $G_{i/o}$ and G_s (Figs. x.3, x.4, and x.6). Anyway, if researchers use mammalian melanopsin in optogenetics, they should consider multiple signaling pathways that the melanopsin can drive. In other words, for selective activation of G_q-dependent pathway, other G_q-coupled opsins or Opto-XRs are better to be used.

3-5. Opto-XRs coupled with G_{12}.

G_{12}-coupled opsins are not identified yet, but like G_s- or G_q- coupled receptors, some Opto-XRs can drive G_{12}-dependent pathways in a light-dependent manner (Morri et al. 2018). Typically, G_{12} activates Rho followed by intracellular MAPK cascades, and some ion channels could be regulated indirectly.

3-6. "OFF-type" animal opsins

Animal opsins activates by 11-*cis* to all-*trans* isomerization of the chromophore retinal (Terakita 2005, Wald 1968), and they selectively or preferentially bind 11-*cis*-retinal (Jager et al. 1996, Tsukamoto et al. 2005) (Fig. x.2). However, an atypical opsin named peropsin possesses higher affinity for all-*trans*-retinal, and upon illumination, the retinal is isomerized to 11-*cis* form (Koyanagi et al. 2002). It is unclear if native peropsin is coupled to G proteins, but peropsin can be a "acceptor" of Opto-XR (Nagata et al. 2018). Interestingly,

the peropsin-based Opto-XRs are inactivated by light stimulation, leading to decrease of G protein activity (Nagata et al. 2018). In addition, Opn5L1, a vertebrate non-visual opsin, also prefers to bind all-*trans*-retinal, and light-absorption causes decrease of G_i activity via all-*trans* to 11-*cis* isomerization followed by formation of an additional covalent bond between the retinal and the extracellular region (Sato et al. 2018).

Taken together, peropsin- or Opn5L1- based Opto-XRs can be used as an "OFF" photo-switch for G protein signalings and G protein-mediate ion channel modulation (Figs. x.3, x.4 and x.6). Again, the ability to directly bind all-*trans*-retinal is advantageous for applying to optogenetics (see section 3-1).

4. Optogenetic tools modulating K^+ / Ca^{2+} channels using other photo-receptive proteins.

In section 3, we summarized animal opsins as optogenetic tools to modulate ion channels, in particular K^+ or Ca^{2+} channels. As already discussed, one advantage of animal opsins as well as ChRs is that the chromophore molecule exists endogenously in native tissues. However, there are other photo-receptive proteins do not need addition of chromophore molecules to function in cells. For example, the blue light sensitive LOV domain is derived from a plant photoreceptor protein phototropin (Christie et al. 1999, Tokutomi et al. 2008), and its chromophore flavin mononucleotide (FMN) is abundant in animal tissues. Hereafter, we briefly introduce several flavin-based optogenetic tools to modulate K^+ or Ca^{2+} channels.

In our knowledge, BLINK1 is the first light-gated K^+ channel, in which the LOV2-Jα photo-switch is fused with a viral K^+ channel Kcv (Cosentino et al. 2015). When BLINK1 is illuminated with blue light, conformational changes occur in the LOV2-Jα photo-switch, the changes induce opening of the Kcv moiety. BLINK1 works well in HEK293T cell lines and can regulate behaviors of zebrafish larvae in a blue-light-dependent manner. On the other hand, the tool is not useful in mammalian neurons very much (Wiegert et al. 2017).

OptoRGK is a LOV domain-based optogenetic tool regulating voltage-sensitive Ca^{2+} channels (Ca_v) (Ma et al. 2018). OptoRGK consists of two components: one component is a membrane-tethered LOV2 domain-containing ssrA protein and another component is a soluble ssrB protein fused with Rem, a negative modulator of Ca_v channels. When the LOV2 domain is irradiated with blue-light, conformational changes enables ssrA protein dimerizes with ssrB, leading to translocation of Rem to the plasma membrane and suppression of the Ca_v channels. The study proved that upon light stimulation, OptoRGK can suppress cardiac Ca_v channel-dependent responses in cell lines (Ma et al. 2018). When OptoRGK is used in neurons, some additional modification may be needed to localize the tools in presynaptic areas where Ca_v channels function.

Photo-activated adenylyl cyclase (PAC) can be used as a modulator of a bacterial cAMP-gated K^+ channel, SthK (Brams et al. 2014). PAC utilizes flavin adenine dinucleotide (FAD) as a chromophore, and FAD is also abundant in animal cells. Recent studies showed that fusion constructs of PAC with SthK function well as light-sensitive K^+ channels (Beck et

al. 2018, Bernal Sierra et al. 2018).

In this chapter, we focus on optogenetic tools that do not need supplement of co-factors to target cells, but there are many useful tools utilize photo-activatable (in many cases, an azobenzene moiety is introduced) exogenous ligand. In particular, a bacterial K^+-selective glutamate receptor mutant can be modulated by a covalently bound photo-activatable agonist (Janovjak et al. 2010), and small compounds named as LOGO and VLOGO can act as photo-sensitive activators to GIRK channels (Barber et al. 2016, Trads et al. 2016). Such techniques are very useful for light-dependent modulation of K^+ / Ca^{2+} channel in tissues where these photo-activatable ligands are successfully delivered. For further discussion, please read many excellent reviews (Berlin and Isacoff 2017, Kienzler and Isacoff 2017, Paoletti et al. 2019).

5. Conclusion

In this chapter, we introduced several optogenetic tools based on photo-receptive proteins other than ChRs, and discussed their functionalities and usefulness. Currently most of optogenetic studies utilize ChRs, and making use of other photo-receptive molecules such as animal opsins, flavin-based photo-receptive proteins, and organic chemicals will further expand usefulness of optogenetics.

Figure legends

Fig. x.1 Chemical structures of the retinal isomers.

When the retinal isomers bind to opsins, the Schiff base linkage is formed between the aldehyde group and a specific lysine residue in the opsin.

Fig. x.2 Binding ability for the retinal isomers and photo-activation scheme of animal opsins.

(a) Retinal binding and activation scheme of "vertebrate-type" opsins. As mentioned in the main text, "vertebrate-type" opsins selectively bind 11- (or 9-) *cis*-retinal and photoactivation is irreversible. (b) Retinal binding and activation scheme of "invertebrate-type" (bistable) opsins. Unlike "vertebrate-type" opsins, "invertebrate-type" opsins can bind all-*trans*-retinal directly, and photoactivation is reversible. (c) – (e) A typical example of "invertebrate-type" opsin (cOpsin1 from marine ragworm *Platynereis dumerilii* (Tsukamoto et al. 2017)). (c) Absorption spectra of the "invertebrate-type" opsin. The opsin is a UV-sensitive opsin (*black line*), and UV irradiation convert it to the activated form (*red line*). The photoreaction is reversed by irradiation of yellow light (*blue line*). (d) Absorption spectra of the "invertebrate-type" opsin directly bound to 11-*cis*-retinal (*black line*) or all-*trans*-retinal (*red line*), indicating that the opsin can bind both 11-*cis*- and all-*trans*- retinals directly. (e) Light-induced difference FTIR spectra of the "invertebrate-type" opsin. The spectrum plotted with a solid line

is calculated by subtracting the spectrum recorded in dark from that recorded after UV irradiation (*+UV/dark*). The spectrum plotted with a gray line is calculated by subtracting the spectrum recorded after UV irradiation from that recorded after successive yellow-light irradiation (*+yellow/+UV*), which shows a mirror image of the first one. The reversible photoreaction is also confirmed by the spectrum collected in the 2nd round of the UV irradiation (*+UV/+yellow*), which is almost identical to the first one. The bands which can be assigned to amide I (1665, 1650, 1642, and 1632 cm^{-1}) and amide II (1548 cm^{-1}) modes of protein are tagged.

Fig. x.3 Typical signaling pathways driven by $G_{i/o}$-coupled opsins and Opto-XRs

The signaling pathways shown in Figs. x.3, x.4, and x.6 would be oppositely modulated in a light-dependent manner when "OFF-type" opsin-based tools are used (see section 3-6).

Fig. x.4 Typical signaling pathways driven by G_s-coupled opsins and Opto-XRs

Fig. x.5 Molecular architecture of chimeric Opto-XRs

In Opto-XRs, animal opsin is an "acceptor" to provide extracellular and transmembrane domains (*blue*) for retinal-binding and photo-reception. Target GPCR is a "donor" to provide intracellular domain (*red*) to interact with and activate specific G proteins. See the main text in more detail.

Fig. x.6 Typical signaling pathways driven by G_q-coupled opsins and Opto-XRs

Acknowledgements

We cordially thank to Hiroe Motomura and Kayo Inaba for sample preparation of *Platynereis* cOpsin1. This work is partly supported by grants from the Japanese Ministry of Education, Culture, Sports, Science, and Technology to H. T. (25840122 and 17K15109) and Y. F. (26708002), JST CREST to Y. F. (Grant Number JPMJCR17N5), and JST PRESTO to H. T. (JPMJPR1787).

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Table x.1 Molecular properties of photo-receptive proteins to modulate K^+ or Ca^{2+} channels

photo-receptive protein	G protein(s)	effector channel	activation	τ_{ON}	deactivation	τ_{OFF}	tested cell	references
animal opsin								
vertebrate rhodopsin	$G_{i/o}$	GIRK	green	sec	light off	sec	HEK293	Li et al., 2005 Masseck et al, 2014
	$G_{i/o}$	Ca_v2	green	sec	light off	sec	HEK293	Li et al., 2005
vertebrate SWS-opsin	$G_{i/o}$	GIRK	blue	sec	light off	sec	HEK293	Masseck et al, 2014
vertebrate LWS-opsin	$G_{i/o}$	GIRK	orange	sec	light off	sec	HEK293	Masseck et al, 2014
Platynereis cOpsin1	$G_{i/o}$	GIRK	UV	sec	yellow	sec	*Xenopus* oocyte	Tsukamoto et al., 2017
Lamprey parapinopsin	$G_{i/o}$	GIRK	UV	<sec	blue	sec	HEK293	Eickelbeck et al., 2019
vertebrate rhodopsin	independent	Kir6.2	white	sec	light off	>min	*Xenopus* oocyte	Caro et al., 2012
jellyfish opsin	G_s	Ca_v1	blue	sec	light off	min	cardiomyocyte	Makowka et al., 2019
human/mouse melanopsin	G_q, $G_{i/o}$, (G_s?)	GIRK	blue	<sec	green	sec	HEK293	Spoida et al., 2016
		HCN2	blue	sec	light off	sec	retinal ganglion cell	Jiang et al., 2018
flavin-based photo-receptive protein								
LOV2 (in BLINK1)	independent	Kcv	blue	sec	light off	min	*Xenopus* oocyte	Cosentino et al., 2015
LOV2 (in OptoRGK)	independent	Ca_v1	blue	sec	light off	min	HEK293	Ma et al., 2018
PAC	independent	SthK	blue	sec	light off	min	*Xenopus* oocyte	Beck et al., 2018
			blue	sec	light off	min	HEK293	Bernal Sierra et al., 2018

Note that experimental conditions such as light intensities are quite different between studies.

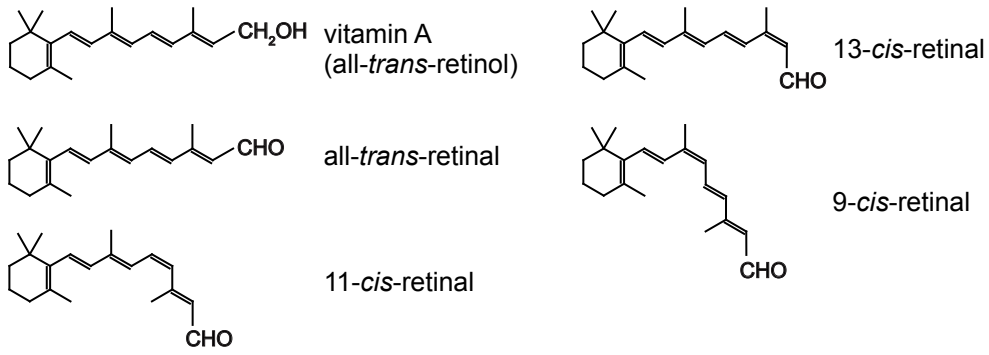

Fig. x.1 Tsukamoto and Furutani

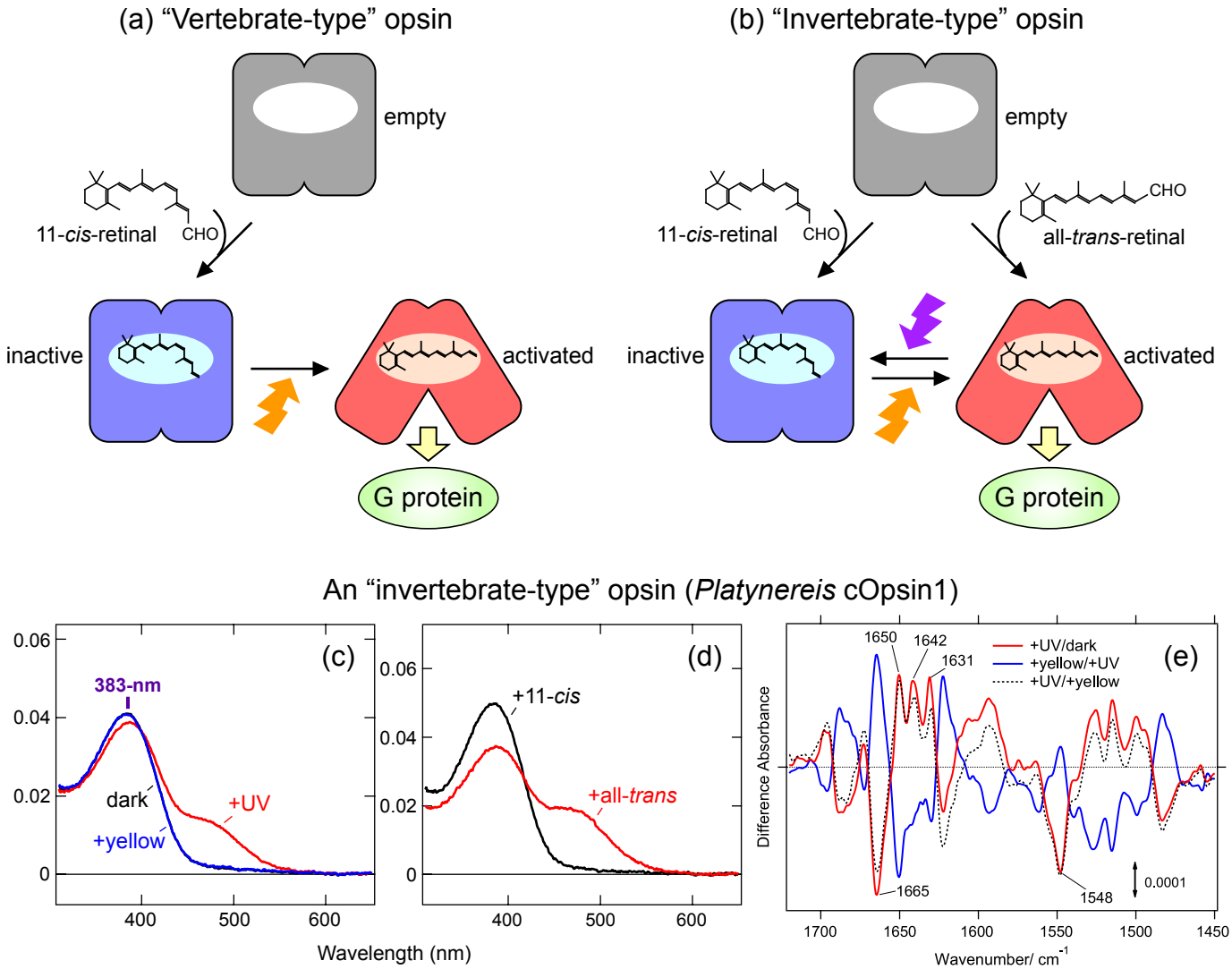

Fig. x.2 Tsukamoto and Furutani

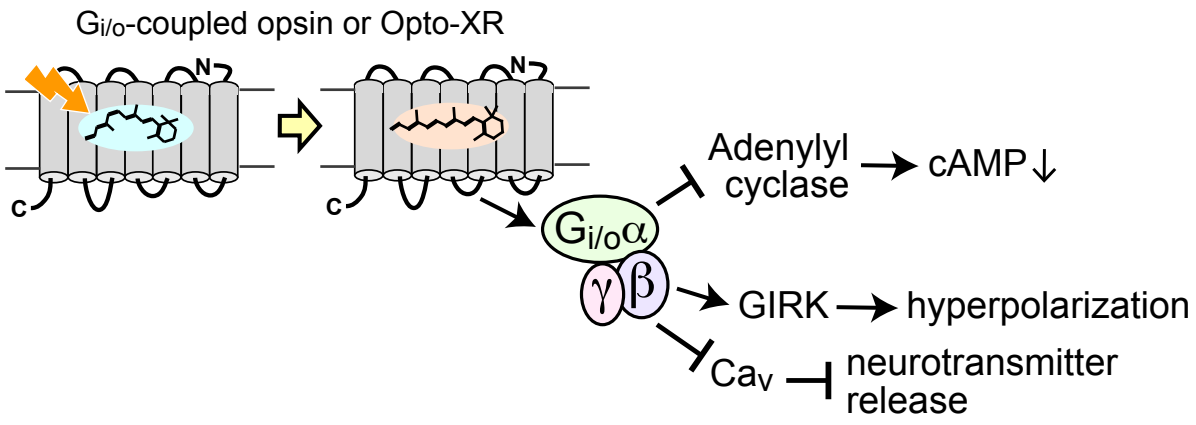

Fig. x.3 Tsukamoto and Furutani

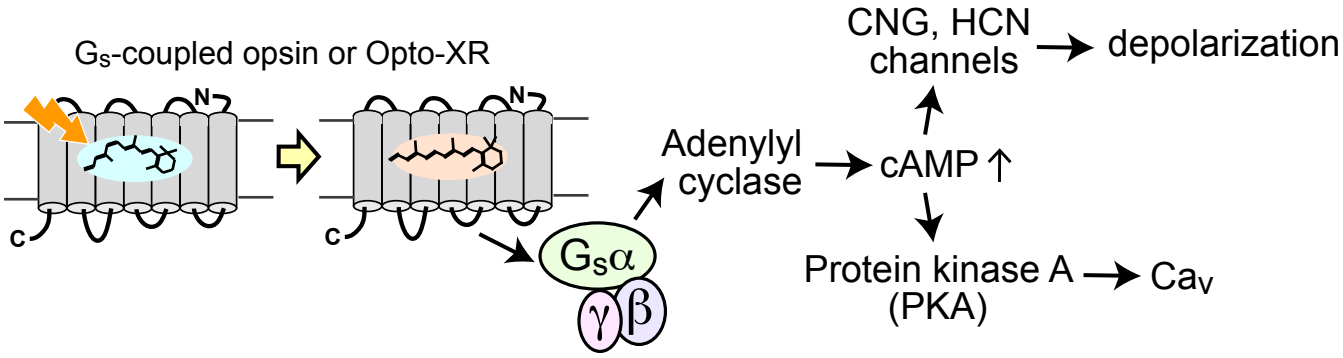

Fig. x.4 Tsukamoto and Furutani

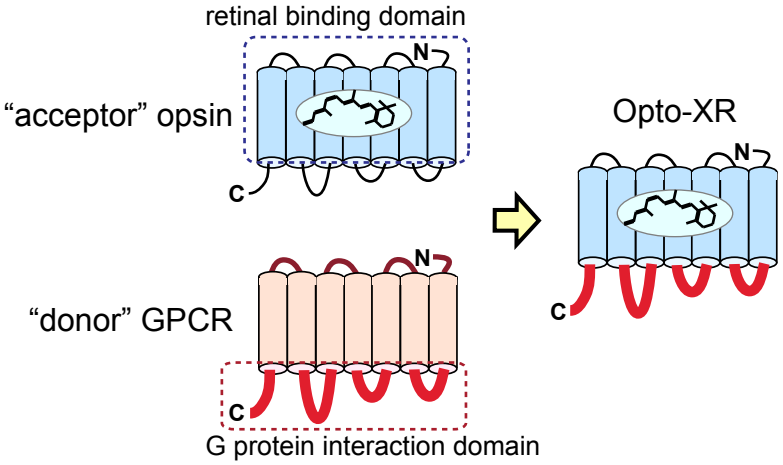

Fig. x.5 Tsukamoto and Furutani

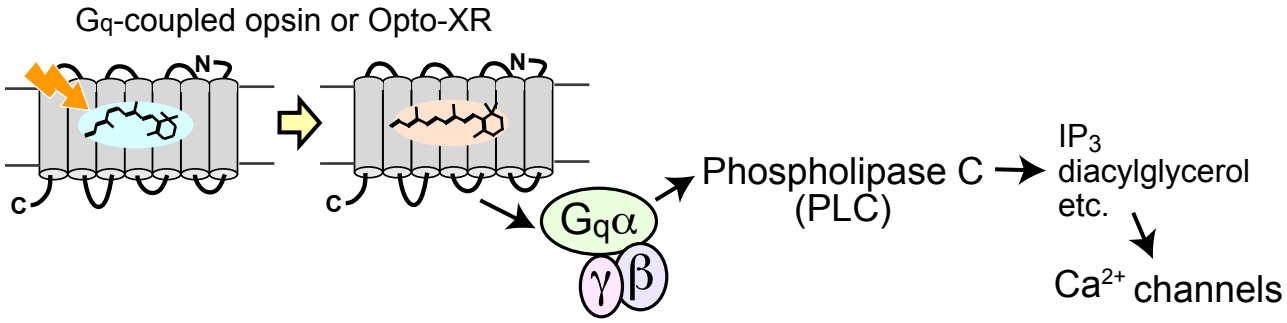

Fig. x.6 Tsukamoto and Furutani