Review

Advances and Perspectives of Light-gated Phosphodiesterases for Optogenetic Applications

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Abstract: Second messengers, cyclic adenosine 3'-5'-monophosphate (cAMP) and cyclic guanosine 3'-5'-monophosphate (cGMP) are playing important roles in many animal cells by regulating intracellular signaling pathways and modulating cell physiology. Environmental cues like temperature, light and chemical compounds can stimulate cell surface receptors and trigger the generation of second messengers and the following regulations. Spread of cAMP and cGMP is further shaped by cyclic nucleotide phosphodiesterases (PDEs) for orchestration of intracellular microdomain signaling. However, localized intracellular cAMP and cGMP signaling requires further investigation. Optogenetic manipulation of cAMP and cGMP offers new opportunities of spatio-temporally precise study of their signaling mechanism. Light-gated nucleotide cyclases are well developed and applied for cAMP/cGMP manipulation. Recently discovered rhodopsin phosphodiesterase gene from protists established new and direct biological connection between light and PDEs. Light-regulated PDEs are under development and of demand to complete the toolkit of cAMP/cGMP manipulation. In this review, we summarize the state of the art, pros and cons of artificial and natural light-regulated PDEs and discuss potential new strategies of developing light-gated PDEs for optogenetic manipulation.

Keywords: cyclic nucleotides; phosphodiesterases (PDEs); optogenetics; cAMP; cGMP

1. Introduction to Cyclic Nucleotides and Signal Transduction

In mammalian cells, first messengers such as light, nitric oxide (NO), hormones can regulate second messenger cyclic nucleotides (cAMP and cGMP) levels, which further affect the vision signaling, muscle contraction, cardiovascular, memory and many other functions [1-4]. cAMP is produced by adenylyl cyclase (AC), with different isoforms mainly expressed in the plasma membrane among cell types. Some of the AC activities are partially dependent on Ca^{2+} concentrations [5, 6]. Soluble adenylyl cyclase (sAC) can be activated by bicarbonate and plays an important role in sperm motility and fertilization capacity [7, 8]. cGMP is also produced by guanylyl cyclases (GC) of both soluble and membrane-anchored isoforms [9]. Seven transmembrane guanylyl cyclases (pGC A-G) exist in mammals and pGC-G was proved as a thermosensory protein [10, 11]. Nitric oxide (NO) synthesized by NO synthase (NOS) can diffuse through cell membranes and activate soluble guanylyl cyclase (sGC) [12].

The production of cAMP or cGMP can thereafter regulate versatile cellular physiologies (Figure 1). Notably, cNMP (e.g. cAMP or cGMP) can participate in several regulatory processes by activating other components like Epac [13], protein kinase A or G (PKA or PKG) and cyclic nucleotide-gated (CNG) channels [14]. For example, the PKA can be released after the binding of cAMP to the regulatory domain and phosphorylate downstream target proteins or can be transported into the

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nucleus to activate cAMP-response element binding protein (CREB) and initiate gene expression [15, 16]. PKA plays further roles in activating other cytosolic components like phosphorylase kinase (PhK) to regulate glycogenolysis by phosphorylation processes [17]. As one of the intracellular cGMP targets, PKG is highly expressed in different tissues or cell types, such as smooth muscle, cardiomyocytes, platelets, endothelial and neuronal cells [18]. PKG can trigger the gene expression related with the cardiovascular system as well as cytosolic vasodilator-stimulated phosphoprotein (VASP) for the induction of apoptosis [19]. Furthermore, PKA and PKG can phosphorylate the N-terminal NHR or UCR modules and regulate PDE3, 4 catalytic activity. cNMP-dependent phosphodiesterases such as PDE2, 5, 6, 10 and 11 have two cGMP PDE/adenylyl cyclase/FhlA (GAF) domains in the N-terminal, which can bind cGMP or cAMP to trigger PDE activity [20]. Diversity of GAF domains offers possibilities to design potential drugs for specific targeting.

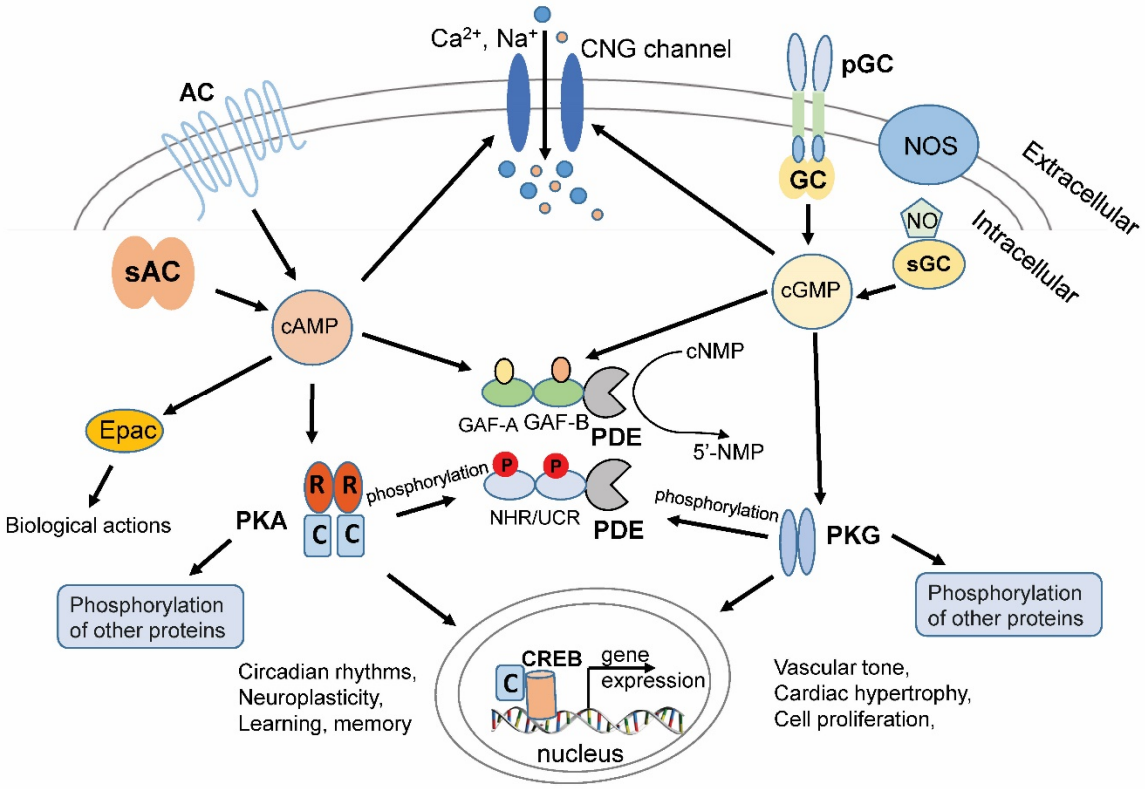

Figure 1. Cyclic nucleotides (cAMP and cGMP) and their typical signal transductions in animal cells. Membrane-integrated adenylyl cyclase (AC) and soluble AC are depicted to produce cAMP. sGC can be activated by NO produced by NO synthase (NOS). Membrane bound guanylate cyclases respond to external signaling molecules. Four main effectors mediated by cAMP and cGMP were depicted. The CNG channels with Ca^{2+}/Na^{+} permeability in plasma membrane can be activated by cAMP or cGMP. In the cytoplasm, various PDEs include GAF-A and GAF-B domains in the N-terminal, which can either bind cGMP (for PDE2, 5, 6 and 11) or cAMP (for PDE10), thus regulating C-terminal catalytical activity.PKA and PKG can further regulate targeted protein phosphorylation and CREB transcription factors for certain gene expression. PKA related gene expression has effects on circadian rhythms, neuroplasticity or learning and memory etc. PKG related gene expression mainly plays roles in the cardiovascular system, such as vascular tone, cardiac hypertrophy, cell proliferation etc. PKA and PKG can also regulate the activity of PDE1, 3-5 and 10 by phosphorylation of the N-terminal modules. Note that PDEs with other different N-terminal modules are not listed in the figure.

In subcellular levels, cAMP or cGMP can be hydrolyzed rapidly due to robust localized PDE activities[21]. The rapid turnover was required to enable physiological consequences. To study the rapid processes that regulate cGMP or cAMP levels in subcellular compartments, spatio-temporally precise methods are needed. It was expected that light-induced PDEs could be designed

and applied in addition to light-gated nucleotide cyclases to regulate cGMP or cAMP levels and dissect the related physiological processes.

2. Therapeutic Regulation of PDEs

In mammalian cells, 11 primary PDE members (PDE1-11) were identified as a superfamily, with over 100 different isoforms due to multiple transcription start sites and alternative splicing. Some of them hydrolyze both cAMP and cGMP (including PDE1-3, 10-11), while others are cAMP (PDE4, 7-8) or cGMP (PDE5A, 6, 9A) specific[22]. They share relatively conserved C-terminal catalytic PDE domains but vary much in N-terminal regulatory modules. This has effects on their individual signaling roles, such as intracellular localization and cell- or tissue-specific expression [23].

Some diseases like pulmonary hypertension and chronic heart failure are caused by the upregulated PDE5 activity and the breakdown of second messenger homeostasis[9]. Therefore, to cure these diseases, the main therapeutic goals focus on PDE5 inhibitors, which mostly bind to the PDE catalytic domain and reduce its substrate affinity [24, 25]. Inhibitors of the cGMP-specific PDE9 were selected for the treatment of diseases such as diabetes or Alzheimer's disease [26, 27]. The highly specific inhibitor for PDE9A was referred to an active Tyr424 site instead of a Phe in all other isoforms [28, 29].

The other therapeutic direction would be to uncover and leverage the regulating mechanisms of the variable N-terminal regions. The N-terminal modules of certain mammalian PDEs have different effects such as heterologous protein-protein interactions, the interactions within PDEs and cyclic nucleotide binding [30, 31]. For instance, PDE2, 5, 6, 10 and 11 have N-terminal GAF domains. The cyclic nucleotides binding to the GAF domains can change the overall conformation and regulate the PDE activities [20]. Whereas the phosphorylation in the N-terminal regulatory regions of PDE3 and PDE4 can dramatically impact their activity and cooperation with other proteins in signalosomes [22]. Notably, some diseases are caused by abnormal cAMP increase. For example, the autosomal dominant polycystic kidney disease (ADPKD) with elevated cAMP levels promotes cyst formation leads to renal failure. One allosteric activator compound of PDE4 can lower the cAMP levels and limit the cAMP-mediated signaling pathways. This dramatically inhibits the cyst formation [32]. Manipulating the N-terminal regulatory modules or upstream interventions could give new insights into regulating mechanism of PDE catalytic activity.

The inhibitors, activators and modulators of PDEs have been successfully applied in both basic researches and clinical trials. However, chemical drugs may encounter non-selective and crosstalk problems, especially when targeting against the conserved catalytic domains. Due to the free diffusion effect, it is challenging to restrict the chemicals to specific subcellular regions or to certain cells in a tissue. In addition, chemical approaches also suffer poor temporal resolution and lack reversibility. On the contrary, optogenetic manipulation of cNMP provides reversible control with unprecedented spatio-temporal precision. Light-gated production of cNMP has been well established. However, light-regulated PDEs are under development to complete the toolkit for optogenetic cNMP manipulation.

3. Light-regulated PDEs

3.1. Indirect light regulation of PDE activity in visual phototransduction

In the retina of vertebrate eyes, rod and cone cells can respond to different light wavelengths and intensities and transfer visual information to neural signals. Type II vertebrate rhodopsins, as a member of G protein-coupled receptor (GPCR) superfamily A, play key roles in regulating visual systems in the disc membrane of rod and cone cells. Photon absorption by rhodopsin initiates the visual signaling cascade (Figure 2). The active form of rhodopsin binds to G protein, causing it to dissociate from the bound GDP and bind GTP. The GTP-bound Gα subunit dissociates from Gβγ subunits and becomes active [33]. PDE6 is anchored in the photoreceptor outer segment membranes. It will be activated by the GTP-bound Gα protein, thus decrease the cGMP concentration and down-

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regulate CNG channel activities in the plasma membrane. The following hyperpolarization of the membrane potential in photoreceptor cells enables neurotransmitters to release to different cells in downstream and trigger neuronal signaling in the brain [34-36]. In the dark, the inactive form of rhodopsin leaves the GDP-bound Gα protein in an inhibited form, which inhibits the PDE6 and reduces its cGMP hydrolysis activity. Furthermore, GC is activated by guanylate cyclase activating proteins (GCAP) at low concentrations of Ca^{2+}. This will restore cGMP levels in the cytoplasm and re-open CNG channels in the dark.

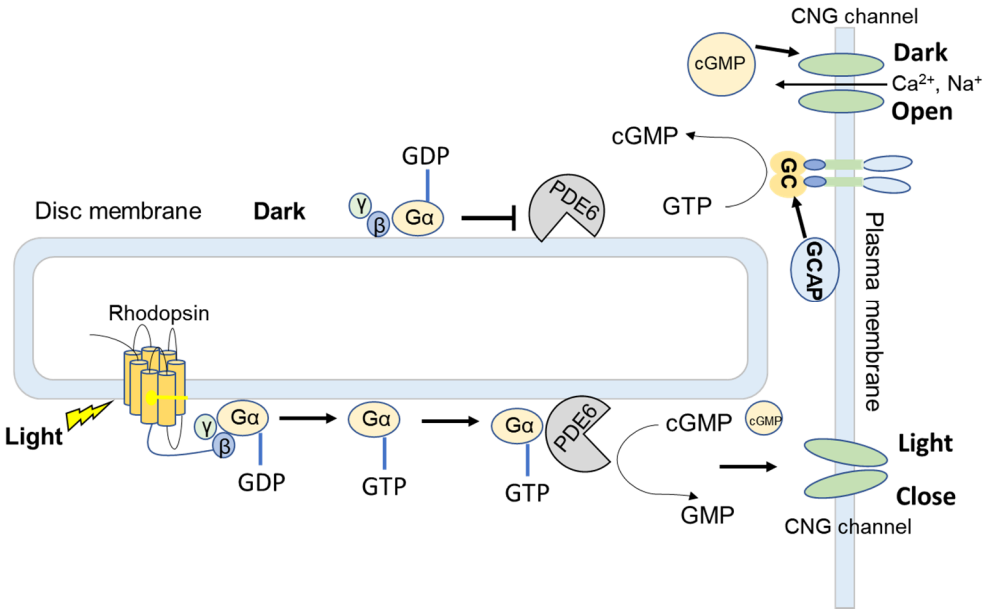

Figure 2. PDE6 is involved in phototransduction of vertebrate visual system. The disc membrane and plasma membrane depicted here are localized in photoreceptor cells of retina. Key proteins related to phototransduction cascades were shown in the membranes. The photosensor rhodopsin is integrated in the disc membrane, where G proteins (Gα, β, γ) and PDE6 are attached. In plasma membrane, CNG channels are permeable to Ca^{2+} and Na^{+} and stay in an open or close state depending on dark or light conditions. GC and GCAP are shown in plasma membrane to maintain cGMP levels in the cytoplasm.

Among the 11 PDE family members in mammals, only PDE6 is indirectly regulated by light through G-protein coupled receptor in the disc membrane of rod and cone cells [34-36], and no direct light regulation has been reported in animal PDE superfamily. Rather, the N-terminal chemosensor domains act as direct regulator to manage the catalytic activity of the effector domain. Due to the complicated signaling cascade from light to PDE activity, the GPCR-G protein-PDE pathway can not be used as an universal tool for light manipulation of cGMP in different cells.

3.2. Artificial Light-activated PDE (LAPD)

The signal transduction from the sensor domain to the effector domain shares similar conformation-changing mechanism across different signaling receptors. Therefore, replacing the N-terminal chemosensor of PDE by a photosensor module may endow the photosensitivity. Based on this strategy, LAPD was firstly engineered via combining a bacteria photosensor module with the catalytic domain of human PDE2A [37] (Figure 3A). Crystallography and biochemical study revealed that the linker region between GAF-A, B and catalytic domain plays a key role in regulating the activity of PDE2A. The binding of cGMP to GAF-B induces significant movement of the coiled-coil linker between GAF-B and catalytic domain and activates PDE2A allosterically [38].

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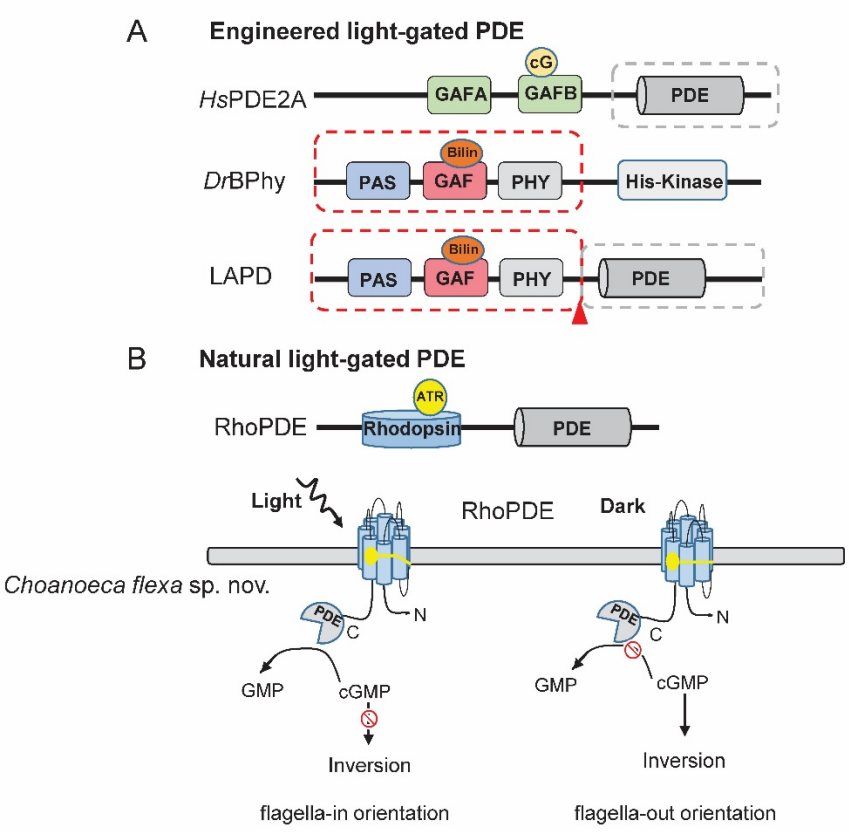

Figure 3. Engineered and natural light-gated PDEs. (A) The schematic model of generating the artificial light-gated PDE, LAPD. The LAPD comprises the light sensor modules from *Dr*BPhy labeled by red dashed box and PDE catalytic domain from *Hs*PDE2A labeled by gray dashed box. The encircled cG indicates cGMP. The chromophore is shown with encircled Bilin (biliverdin). The red triangle indicates the linker connection point. (B) The schematic model of natural light-gated PDE. The chromophore is shown with encircled ATR (all-trans-retinal). In a new discovered protist *Choanoeca flexa* sp.nov, the RhoPDE can be activated by light to hydrolyze cGMP and trigger the inversion to flagella-in orientation. In the dark cGMP levels will be maintained and flagella-out orientation was kept. The lower cartoon in B was modified from [39].

Interestingly, the photosensor module of a bacterial phytochrome from *Pseudomonas aeruginosa* BPhy (*Pa*BPhy) displays remarkable similarities in both overall architecture and coiled-coil linker region with the chemosensor domain of PDE2A [40-42]. Two stable phytochrome intermediates, absorbing red light (Pr) and far red light (Pfr), regulate the activity of the effector domain differently. First trial of fusing *Pa*BPhy light-sensing domain and the *Hs*PDE2A catalytic domain unfortunately fails to yield soluble protein. Substitution of the *Pa*BPhy photosensor module by that from *Deinococcus radiodurans* (*Dr*BPhy) and slightly modulation of the coiled-coil linker region generated the first light-regulated PDE: LAPD (Figure 3A). Upon red-light illumination, LAPD exhibits up to 4-fold and 6-fold catalytic activity increase towards cAMP and cGMP, respectively. Preliminary applications showed that LAPD allowed optical control of cAMP and cGMP levels in CHO cells and zebrafish embryos. In addition, red-light elevated catalytic activity of LAPD can be reverted by far-red light irradiance. However, this photoconversion is not complete [37]. To engineer new LAPDs with improved properties, Stabel et al systematically conducted substitution of either the N-terminal photosensor module or the C terminal PDE effector module and modification of the linker regions. A suite of LAPD variants was engineered. Among the variants, *Dr-Bt*PDE2A exhibits enhanced reversibility of photoactivation as well as highest photodynamic range [43]. Expressing LAPD, *Dr-Bt*PDE2A and several other variants in HEK cells enables regulating cNMP dependent physiological processes such as the gating of CNG channels.

Phytochromes can be regulated by red and far-red light colors which can penetrate deeper into tissues and the bacterial phytochromes (BPhys) use the chromophore biliverdin, which is a natural

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product of heme and universally available among cell types. This makes it applicable without exogenous chromophores.

3.3. Direct Light-gated PDEs (RhoPDEs) from Nature

In fact, a rhodopsisn-phosphodiesterases gene fusion was already found in the genome of a choanoflagellate, *Salpingoeca rosetta* [44]. The protein, named Rh-PDE or RhoPDE (Figure 3B), was expressed in HEK293 cells, and its cGMP and cAMP hydrolysis ability was found to increase 1.4-fold and 1.6-fold respectively with light illumination [45]. The hydrolysis activity of RhoPDE is maximally activated by 492 nm and it is ~10-fold more active towards cGMP than cAMP. However, a following study using purified proteins suggested that light regulation is absent in RhoPDE [46]. Later found that RhoPDE is clearly activated by light with an unusual mechanism: light illumination primarily increased its substrate affinity rather than the maximal turnover [47]. Additionally, we found that the hydrolysis activity for cGMP is ~100 times higher than for cAMP. Both cGMP and cAMP hydrolysis activities can be increased to ~ 5- fold under light illumination at low substrate concentrations [47]. Different from the classical rhodopsins with seven transmembrane helices (TMS), RhoPDE shows 8 TMs topology with cytosolic localization of both N- and C-terminal proved by immunofluorescence microscopy experiments, bimolecular fluorescence complementation (BiFC) experiments [46, 47] and a very recent structure study [48].

Brunet et al. found more new RhoPDEs from different species of choanoflagellates. Four RhoPDE homologs were discovered from *Choanoeca flexa* sp. nov.. In *C. flexa*, they found that the choanoflagellate forms cup-shaped colonies that invert their curvature in response to changing illumination conditions through a rhodopsin-cGMP signaling pathway [39] (Figure 3B). Moreover, from sequenced transcriptomes database, other choanoflagellates species also encode RhoPDEs [49]. Collectively, eight new RhoPDE homologs were identified separately in *Choanoeca flexa* (*C. flexa*1-4), *Microstomoeca roanoka* (*M. roanoka1*), *Acanthoeca spectabilis* (*A. spectabilis1*) and *Choanoeca perplexa* (*C. perplexa*1-2) [39]. All these RhoPDEs are predicted to have similar 8-TM topology like *Sr*RhoPDE. After expression of these RhoPDEs in HEK293 cell, *Cf*Rh-PDE1, *Cf*Rh-PDE4, and *Mr*Rh-PDE exhibited light-enhanced cGMP hydrolysis activity, while *As*Rh-PDE, the one lacking the conserved retinal binding lysine residue, shows constant cAMP-specific PDE activity without light regulation [50].

4. Strategies for Engineering New Light-regulated PDEs

Studies of natural cGMP-related microbial rhodopsin will help to understand deep into the evolution of animal visions. Furthermore, such studies can also provide potential optogenetic tools for precise cNMP manipulation. Optogenetic manipulation of cNMP offers reversible control with unprecedented spatio-temporal precision. Light-regulated cAMP/cGMP-producing cyclases abundantly exist in nature and outperform the conventional chemical cyclase activators in various applications [51-56]. However, current available light-activated PDEs, artificial LAPD and natural RhoPDE, showed similar drawback of relative high dark activity and low light activity to dark activity ratio. Here we provide possible strategies for future engineering new light-gated PDEs.

4.1 Allosteric Light Regulation

The Light-oxygen-voltage (LOV) domains are found in various protein sensors which response to environmental change in plants and bacteria. The most widely used and best-studied LOV domain comes from the second LOV domain of *Avena sativa* phototropin 1 (*As*LOV2) [57]. It comprises ~125 amino acids with a chromophore-binding pocket for the covalent adduct flavin. Two α helices are flanking in the N- and C-terminal of *As*LOV2, named A'α and Jα helices, respectively [58, 59]. In the dark stage, the C-terminal Jα helix is caged in the LOV core motif, while it can be exposed under blue light illumination [60]. This light-triggered conformational change makes the *As*LOV2 domain suitable for designing light controllable protein in an allosteric manner. For example, Wu et al. fused a GTPase Rac1 with the LOV2 domain, obtaining a photoactivatable Rac1 (PA-Rac1). PA-Rac1 was

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sterically blocked from interacting with its effectors in the dark. Light illumination induces the movement of Jα and subsequently unbinding of Rac1 from LOV2, allowing Rac1 to bind with its effector [61]. Similarly, it is feasible to engineer a light-regulated PDEs by combining PDEs and LOV2 with systematical screening of the proper fusion strategies (Figure 4A). Moreover, in red/far-red range, linking the allosteric transition of the phytochrome to PDE activity regulation has been proved to be very effective in LAPDs engineering [37, 43].

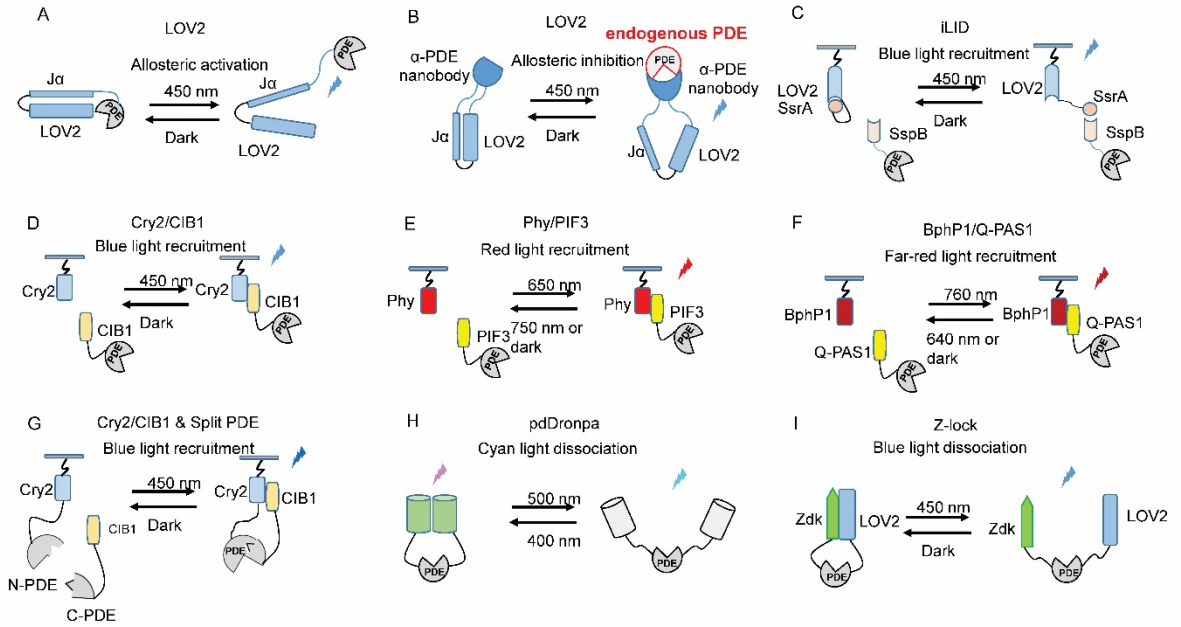

Figure 4. Potential strategies to design new light-regulated PDEs. (A) LOV2 based allosteric activation: tight linkage to the LOV domain can block the function of PDE, while light-induced conformational change in the LOV domain recover its function. (B) LOV2 based allosteric inhibition: LOV2 domain fused at the loop area of anti-PDE single domain antibody (α-PDE nanobody), blue light illumination triggers conformational changes in the nanobody, leading to binding of nanobody to PDE and inhibiting or regulating endogenous PDEs. (C-F) Four light-induced translocation systems: blue light mediated dimerization of iLID (LOV2-SsrA/SspB) and Cry2/CIB1, red light triggered dimerization of Phy/PIF3 and far-red light induced dimerization of BphP1/Q-PAS1. One component (LOV2-SsrA, Cry2, Phy or BphP1) can be tethered to membrane fractions in certain organelles. Targeted PDEs could be fused to the corresponding dimeric partners. This could potentially achieve the recruitment of PDEs to certain subcellular compartment by light illumination. (G) Reconstitution of Split PDE fragments. Two split fragments N-PDE and C-PDE are fused with Cry2 and CIB1 respectively. Blue light can induce the dimerization of Cry2/CIB1 and recover the full PDE hydrolysis activity. (H) pdDronpa system: The catalytic domain of PDE are fused with two pdDronpa proteins in N- and C-terminus or other proper positions. Cyan light induced dissociation free the active site of PDE while violet light triggered association block the active site. (I) Z-lock system: Zdk and LOV2 are fused at the N- and C-terminus or other proper positions of PDE, blocking PDE active site in dark. After blue light illumination, Zdk and LOV unlinked, uncaging the active site.

Light-induced allosteric regulation could also be applied to target endogenous PDEs. Pioneering work by Dagliyan et al demonstrated that insertion of LOV2 domain to solvent-exposed loops on proteins of interest enables switching the proteins between active and inactive states by light [62]. Inspired by this, Gil et at. engineered a class of opto-nanobodies (OptoNBs) via inserting a modified LOV domain into the selected loop sites of the camelid single domain antibodies (aka. nanobodies) with similar scaffold. The binding of OptoNBs to target proteins can be enhanced or inhibited upon blue light illumination. Expressing these OptoNBs in cell allows reversibly binding to endogenous intracellular targets, modulating signaling pathway activity [63]. Accordingly, inserting the modified LOV variant into proper loop sites of nanobodies against specific PDEs could also generate a suit of

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light-switchable Opto-αPDEs (anti-PDEs). When the binding site of these Opto-αPDEs locates near to the active site of PDEs, Opto-αPDEs could be potentially applied to reversibly inhibit the endogenous PDEs with light application (Figure 4B). Targeting endogenous PDEs would be of great value for the studies of subcellular cAMP/cGMP signaling.

4.2 Light-induced Translocations

Besides allosterically regulation, light-induced changes in protein oligomeric states could also be implemented in engineering light-regulated PDEs. A series of photosensor-derived interaction domains spanning from UV to far red light range provide multiple choices for engineering light-regulated PDEs. In the blue range, a TULIPs (tunable light-inducible dimerization tags) system was developed based on the *As*LOV2 domain and an engineered PDZ domain (ePDZ). A peptide epitope was fused after the Jα helix, which can then interact with the cognate PDZ domain in a light-dependent manner [64]. An updated iLID (improved light inducible dimer) system was established with less cross talk to endogenous signaling pathways than TULIPs. A short bacteria SsrA peptide with only seven residues was embedded in the C-terminal of Jα helix in *As*LOV2 domain. Blue light illuminations exposes SsrA from LOV2 domain, allowing it to bind its natural interaction partner SspB. The engineered iLID system shows over 50-fold changes in the binding affinity after light illumination [65]. Through introducing point mutations in SspB, the binding affinity of iLID system could be further adjusted [66]. Cryptochrome derived Cry2/CIB1 dimerization system offers an alternative approach for blue light mediated control of protein interaction. The two components can dimerize under blue-light illumination at subsecond range while the reversion lasts for minutes [67]. This system can also be activated by two-photon microscopy, enabling *in vivo* application. In the red spectral window, the binding of phytochrome B (PhyB from *Arabidopsis thaliana*) to its natural interaction partner PIF3 is induced by red-light irradiance and reversed under far-red light exposure or dark state [68]. On the contrary, the binding and dissociation between bacterial phytochrome BphP1 and its partner PpsR2 or Q-PAS1 is stimulated by far-red and red light, respectively [69]. Fusing PDE to one component and specific targeting sequence to its binding partner, it enables recruiting PDEs at specific subcellular compartment by light illumination (Figure 4C-3F).

4.3 Light-gated Recovery of Split PDEs

In addition, Cry2/CIB1 system has been used to reconstitute split protein fragments and recover the activity in a light-dependent manner. After fusion of two split Gal4 fragments with Cry2 and CIB1 and co-expression in yeast, the reporter gene expression can be induced by blue light [67]. The Cry2/CIB1 modules was also able to recover a split Cre recombinase to increase DNA recombination efficiency by light [67]. We already found that split fragments of *Hs*PDE2A catalytic domain could recover the cGMP/cAMP hydrolysis activity upon co-expression (unpublished data). Therefore, the Cry2/CIB1 and other light-induced dimerization system could be fused with the split *Hs*PDE2A fragments and recover its hydrolysis activity in a light-dependent pattern at desired subcellular localization when combining with specific targeting strategy (Figure 4G). Other light induced protein-protein interaction systems like iLID and PhyB/PIF3 can also be applied in similar way.

4.4 Light-gated uncaging of PDEs

Additional to these light-regulated two-component systems, fluorescent protein Dronpa with light-dependent changes in oligomerization state could also be applied to engineer light regulated PDE. Zhou et al has shown that fusion of tetrameric Dronpa at both end of Cdc42 or protease caged their functions in the dark, while light-induced Dronpa dissociation allows uncaging and functioning [70]. Moreover, the improved dimeric variants pdDronpa dimerized in violet light and dissociated in cyan light [71]. Fusing two pdDronpa copies at rationally selected positions in the kinase domain generates photo-switchable kinase [71]. Similarly, attaching two pdDronpa at locations flanking the active site of PDE, a single-chain light-switchable PDE could be engineered. Violet light illumination induces formation of intramolecular dimer, thereby caging PDE's activity, whereas cyan light dissociates the dimer and expose the active site for hydrolyzing cNMP (Figure 4H). A similar Z-lock system comprising LOV and Zdk domains could also be adapted for generating reversible, light-

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controlled steric inhibition of the active sites of PDEs (Figure 4I). Previous work has demonstrated that attaching Zdk and LOV2 to the C and N termini of cofilin respectively could effectively occlude the cofilin active site in dark. Upon irradiation, the dissociation of Zdk and LOV frees the activate site [72].

Principally, there are plenty of methods to generate light-gated PDEs for optogenetic applications. However, all these conceptions may require tremendous trials and intensive optimizations to obtain an ideal light-gated PDE for robust applications.

5. Applications of Light-gated PDEs

In parallel to engineering light-regulated PDEs, fully exploiting the potential of those perspective optogenetic tools is of equal importance. The enzymatic activity and photodynamic range of each specific light-regulated PDE must be carefully considered at the very beginning of applications. Unlike the light-gated ion channels or pumps with no detectable dark activity, existing light-regulated enzymes often show considerable dark activity. These might lead to changes of cNMP level already in the dark, which is often cell type and expression level dependent. Developing tightly light-regulated PDEs is the first step to improve this. Real applications with existing light-gated PDEs can be improved by selecting the tools with proper photodynamic range and manipulating the expression level in targeted cells.

Increasing evidences suggest that the cNMP regulated events were precisely controlled in distinct subcellular confinement through recruiting isoform-specific PDEs into the specialized 'signalosomes' [73-76]. The superior spatio-temporal precision and tunable activity affords researcher to study the cell signaling at unprecedented detail, even at quantitative manner [77]. For example, when regulating the activity of CNG channel or mimic the functions of plasma membrane localized endogenous PDEs, membrane-integral RhoPDEs could be good choices. Employing LAPD enables regulating the cytosol cNMP level. Moreover, light-induced dimerization system allows recruiting PDEs to intended compartment when one part of the dimer is fused with specific targeting module (e.g. mitochondria, Endoplasmic Reticulum or nucleus etc.). Rost et al have detailed summarized a number of general principles and specific motif information for subcellular targeting of photosensitive proteins [78]. It should be noted that simply fusion of the targeting motif may not always successfully bring the actuator into expected subcellular localization. Moreover, fusing the targeting motif may also alter the properties of PDE itself *per se*. Accordingly, it is crucial to select a proper linker between the targeting motif and the client protein.

In addition, a palette of available spectrum separated light-induced dimerization systems allows simultaneous control of discrete cell signaling by different light. The red light regulated BphP1/Q-PAS1 and blue light regulated LOV system has been applied to tridirectionally translocate protein between the cytoplasm, nucleus and plasma membrane [69]. Applying BphP1 and LOV derived light-regulated PDEs would also be possible to achieve dual-color control of cNMP at specific confinement of the cell.

More interestingly, co-application of spectrum separable light-regulated cyclase and light-regulated PDEs would enable bi-directional control of the level of cNMP, either at close or discrete locations. In addition, numerous genetically encoded cAMP and cGMP sensors confer optical visualization of the distribution and dynamics of cAMP and cGMP [79, 80]. The combination of spectrum compatible light-gated cyclases, PDEs with fluorescent biosensors [81] holds great promise to simultaneously manipulate and map the dynamics of cNMP signaling in live cells in superior precise and quantitative manner.

6. Conclusions

cAMP and cGMP play essential roles in cell division, differentiation, growth, and death. Cyclic nucleotide phosphodiesterases are widely distributed in the animal kingdom, hydrolyzing the ubiquitous second messengers cGMP and/or cAMP. Therefore, PDE enzymes are crucial to

manipulate concentrations of both second messengers to maintain normal responses, thus being regarded as important therapeutic targets. Different regulatory modules in the N-termini of most PDEs function diversely to regulate PDE activities through ligand binding, oligomerization and kinase recognition/phosphorylation. Indirect regulation of PDE activities has been well studied in animal vision systems. Direct-coupled light regulation of PDE inside one protein has also been discovered recently in some protists, the ancestor of animals. Further studies into nature rhodopsin phosphodiesterases will help to elucidate the vision evolution.

Optogenetic strategies for regulating PDE activities could give new insights to regulate cNMPs accurately in cellular microdomains. Artificial light-gated PDEs has been developed in a deliberate way in addition to the well-established light-gated nucleotide cyclases. However, further developments of more superior Opto-PDEs with tighter light regulations are of demand. Furthermore, optogenetic inhibiting or targeting endogenous PDEs will be of great value to basic researches and have therapeutic importance. Many newly developed optogenetic systems can be applied for light manipulation of PDEs. However, intensive efforts are needed to advance this field.

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