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The Retinal cGMP Phosphodiesterase γ -Subunit — A Chameleon

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Abstract

Intrinsically disordered proteins (IDPs) represent an emerging class of proteins (or domains) that are characterized by a lack of ordered secondary and tertiary structure. This group of proteins has recently attracted tremendous interest primarily because of a unique feature: they can bind to different targets due to their structural plasticity, and thus fulfill diverse functions. The inhibitory γ -subunit (PDE γ) of retinal PDE6 is an intriguing IDP, of which unique protein properties are being uncovered. PDE γ critically regulates the turn on as well as the turn off of visual signaling through alternate interactions with the PDE6 catalytic core, transducin, and the regulator of G protein signaling RGS9-1. The intrinsic disorder of PDE γ does not compromise, but rather, optimizes its functionality. PDE γ “curls up” when free in solution but “stretches out” when binding with the PDE6 catalytic core. Conformational changes of PDE γ also likely occur in its C-terminal PDE6-binding region upon interacting with transducin during PDE6 activation. Growing evidence shows that PDE γ is also a player in non-phototransduction pathways, suggesting additional protein targets. Thus, PDE γ is highly likely to be adaptive in its structure and function, hence a “chameleon”.

Keywords

PDE γ ; phototransduction; NMR; structure; intrinsically disordered proteins; protein-protein interaction; non-visual function; eye diseases

1. INTRODUCTION

PDE γ , the inhibitory subunit of the retinal cGMP phosphodiesterase (PDE6), is a critical regulator in vertebrate phototransduction — the primary event of vision. The phototransduction pathway in the rod photoreceptor often serves as a prototypical system for understanding numerous G protein-coupled signal transductions, which are involved in many physiological events (for reviews, see [1–4]). Rhodopsin had been the sole atomic structure model [5] for the G protein-coupled receptor field, prior to the recently reported crystal structure of β_2 -adrenergic receptor [6]. Visual signals are transduced through a cascade of protein-protein interactions in which PDE6 is the central effector enzyme (for reviews, see [4, 7–9]) (Fig. 1). The Rod PDE6 is composed of two large homologous catalytic subunits (PDE $\alpha\beta$, ~100 kDa each) and two small (~10 kDa) identical PDE γ inhibitory subunits (Fig. 2), with a possible exception of $\beta\beta$ catalytic homodimer in the chicken rod [10]. Each of the α and β catalytic subunits contains the catalytic domain that performs cGMP hydrolysis, and two tandem regulatory GAF [11] domains which host non-catalytic binding sites for cGMP. The PDE γ interactions with the PDE $\alpha\beta$ catalytic and GAF

domains, as well as with other key players in the visual cascade tightly control the lifetime of activated PDE6, thus regulating the duration of photoresponse. A limiting step for elucidation of these interactions has been the lack of atomic structure of PDE6. The solution structure of PDE γ , an intrinsically disordered protein (IDP) [12, 13], has been recently resolved through NMR spectroscopy, providing a new scope for understanding the PDE γ -mediated regulations [14]. In this review, we will portray PDE γ from the perspective of an IDP based on recent discoveries. Non-visual functions of PDE γ will also be reviewed to further illustrate its unique IDP properties.

2. PDE γ FUNCTIONS IN PHOTOTRANSDUCTION

2.1. PDE γ as the PDE6 Inhibitor in the Dark-Adapted State

PDE6 is an enzyme ideally suited for phototransduction. The PDE6 catalytic core in the rod (PDE $\alpha\beta$) has a very high catalytic efficiency of $4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ that approaches a diffusion-controlled limit, ensuring maximum signal amplification [4, 7]. Remarkably, in dark-adapted rods, the highly efficient PDE6 can be effectively suppressed by PDE γ , by binding to PDE $\alpha\beta$ with an exceptionally high affinity ($K_D \leq 3 \text{ pM}$) [7, 15, 16]. A very low visual background can thus be maintained. It is well-established that the tight control of PDE6 activity is achieved through coordinated interactions of two distinct PDE γ domains with PDE $\alpha\beta$, the hydrophobic C-terminal domain (residues 62–87) and the central domain (19–49) containing a polycationic region (24–45) [15, 17–26] (see Fig. 3). A full-spectrum interaction interface between PDE γ and PDE $\alpha\beta$ has been mapped [15, 17, 18]. Compelling evidence from biochemical studies [19–22, 25–30] and the recently reported NMR data [14] indicate that the last C-terminal 14 amino acids (74–87) are involved in the inhibitory interactions with the PDE $\alpha\beta$ catalytic domain. In particular, the last four residues (herein termed C-terminus) are believed to directly block the entry of cGMP for hydrolysis at the catalytic pocket [19–22, 27, 28]. A prominent hydrophobicity of the C-terminus is critical for PDE6 inhibition, since hydrophobic photoprobes replacing the last two or four residues mimicked the C-terminus for inhibiting PDE $\alpha\beta$ [20, 21, 31]. The PDE γ -interacting region in the cone PDE6 catalytic domain has been mapped to residues 750–784, and this interaction is mediated primarily by three hydrophobic residues: M758, F777, and F781 at the entry to the catalytic pocket [17, 20, 27, 28]. The central polycationic domain of PDE γ , however, does not directly contribute to the inhibition of PDE6. Instead, this region provides most of the binding strength to PDE $\alpha\beta$, most likely through its interaction with the PDE $\alpha\beta$ GAF domain [15, 17, 18, 32, 33]. Synergistic binding of cGMP together with the PDE γ polycationic region to the PDE6 GAF domain has been suggested to be important for the strong association between PDE γ and the PDE $\alpha\beta$ heterodimer (discussed in reviews [7, 34]).

2.2. Activation of PDE6 by the PDE γ : Transducin Interaction in the Signaling State

Given the low background noise in the dark which is maintained through the tight PDE γ :PDE $\alpha\beta$ interaction (probably also by the glutamic acid-rich protein-2 [35]), vision can be initiated by a single photon in the rod [4]. The energy of a single photon is translated into a conformational change of rhodopsin, which in turn catalyzes the exchange of GTP for GDP bound in the α -subunit of transducin ($G\alpha_t$) and dissociation of $G\alpha_t$ from the $\beta\gamma$ heterodimer due to its altered conformation [1, 36–43] (Fig. 1). The GTP-binding conformation enables $G\alpha_t$ to interact with the PDE γ C-terminus [29, 30, 44–55] and remove its inhibitory constraint from the catalytic pocket of PDE $\alpha\beta$ [19–22, 28, 45, 56]. PDE6 is thus turned on, initiating the signaling state of phototransduction. Through these steps the signal of one photon is amplified to a change of some 10^4 – 10^5 cGMP molecules [57]. A rapid drop of cGMP levels leads to closure of cGMP-gated cation channels, which hyperpolarizes the plasma membrane and triggers a sequence of synaptic neurotransmission that relays signals to the visual cortex. Following activation of PDE6, the GTPase

accelerating protein (GAP) complex is formed to terminate the visual signal, hereby termed the transition state [45]. The conversion of GTP to GDP by the $G\alpha_t$ GTPase resumes its inactive conformation, which releases PDE γ for re-inhibition of PDE6. The rapid turnoff primes the signaling molecules for another round of photoresponse, which is essential for the high temporal resolution of vision. Concomitant with the PDE6 inactivation, declined Ca^{2+} concentrations results in binding of GCAP (guanylate cyclase activating protein) to GC (guanylate cyclase), which then becomes active in producing cGMP and eventually restores the cytoplasmic cGMP concentration to the dark-state level (further reviewed in [58–60]).

2.3. PDE γ as the GAP Enhancer in the Transition State

The discovery of an essential role of PDE γ in signal termination was initiated with the observation of the conspicuously slow intrinsic GTP hydrolysis rate of $G\alpha_t$ compared to the subsecond fast recovery of the rod photoresponse [4, 60]. PDE γ was initially thought to be a GTPase activator since addition of PDE γ to reconstituted photoreceptor membranes increased transducin GTPase activity [53]. However, it was later found that another membrane-associated protein was required for GTPase stimulation [61, 62]. It is now clear that this protein is RGS9-1 [63–65] which forms a heterodimer with its obligatory partner G β 5L [66–70], and is anchored to the membrane through R9AP, the RGS9-1 anchoring protein [71, 72]. Thus the photoreceptor GAP complex is comprised of at least five components [60, 70], in which RGS9-1 is the enzyme stimulating the GTPase activity, PDE γ enhances the $G\alpha_t$:RGS9-1 association [73], and R9AP tethers the whole complex to the membrane through its C-terminal transmembrane helix. In addition to its role as a membrane anchor, R9AP has been found to enhance the GAP function of RGS9-1 [72, 74–76]. Physiological analysis of mouse models has established that the expression level of RGS9-1 rate-limits the recovery of rod photoresponse [77].

Therefore, PDE γ critically regulates phototransduction through on and off interactions with its three primary targets: PDE $\alpha\beta$, $G\alpha_t$ and RGS9-1. Structural information is required to understand how these interactions occur and are coordinated. The only atomic structure available thus far that includes PDE γ is the crystal structure of the partial GAP complex, which contains the C-terminal PDE γ peptide (PDE γ_{46-87}), chimera $G\alpha_{t/i1}$, and the RGS domain of RGS9-1 [45]. This structure has clearly visualized the manner by which the PDE γ C-terminal residues 62–87 “zips up” the GAP complex. PDE γ is sandwiched in between the $\alpha 3$ helix and Switch II of $G\alpha_{t/i1}$, and contacts $G\alpha_{t/i1}$ through a stretch of PDE γ residues (V₆₆ICPWE₇₁) and several other residues spread throughout the C-terminal domain. W70 is critical for this interaction because it is locked in intimate contacts with seven $G\alpha_{t/i1}$ residues. The PDE γ -interacting and RGS-interacting residues dovetail in the $G\alpha_{t/i1}$ Switch II region; a hydrophobic interaction between PDE γ V66 and RGS W362 coupled with an electrostatic complementation between PDE γ D52 and RGS R360 further stabilize the transition-state $G\alpha_t$ conformation [45, 78]. A recently reported interaction between PDE γ C-terminal residues and the $G\alpha_t$ N-terminus, which is missing in the crystal structure, has been suggested to also help stabilize the GAP complex [46]. G β 5L and the non-catalytic RGS9-1 domains, which are also missing in the crystal structure [45], confer tight regulation of PDE γ on the RGS domain [69, 79, 80].

A recent study has reported a possible GAP-independent mechanism by which PDE γ directly terminates the photoresponse [81]. It was observed that overexpression of PDE γ accelerated decay of the light response in mouse rods lacking RGS9-1. The interpretation was that, excess free PDE γ could bind to the PDE6 catalytic site from which endogenous PDE γ had been previously displaced by activated transducin, thus bypassing the GAP mechanism to deactivate PDE6 directly [81]. It is unlikely that this process plays a significant role in wild type rods, since there is little or no free PDE γ in the dark-adapted outer segment although prolonged illumination may generate a small population of free

PDE γ [82]. The recent NMR work indicates that free PDE γ adopts functionally relevant transient structures in solution [14]. The loosely folded PDE γ molecule contains residual secondary structures in the C-terminal domain that constitute a three dimensional topology similar to that previously seen in the partial GAP structure [45].

3. SOLUTION STRUCTURE OF INTRINSICALLY DISORDERED PDE γ

As is evident in visual transduction, PDE γ achieves different functions through alternate interactions with multiple signaling targets. Thereby an intriguing question arises, how can this small protein perform such diverse tasks in signal transduction? The answer apparently lies in the fact that PDE γ belongs to an emerging class of intrinsically disordered proteins [12, 13]. These proteins share a common feature of structural plasticity, which allows them to bind to different partners thus fulfilling various functions [83–85].

3.1. Intrinsically Disordered Proteins

The last few years have seen a surge of publications on IDPs, or otherwise termed natively unfolded proteins and intrinsically unstructured proteins *etc.*, which refer to a category of proteins or protein domains that have little or no ordered structure under physiological conditions (for reviews, see [83–90]). IDPs have long been neglected because they defy the classic paradigm of the structure-function relationship of proteins; that is, the function of a protein is determined by its rigid three-dimensional structure [83]. Thanks to recent advances in genomics, proteomics, NMR, and bio-informatics, more sophisticated technologies allow for prediction, identification, or even structural characterization of IDPs at an ever growing pace [83, 89, 91]. Significantly, greater than 30% of eukaryotic proteins are predicted to have intrinsically disordered regions greater than 50 residues [83]. Rather than of negligible importance, IDPs actually participate in critical cellular processes, such as transcriptional regulation, translation, and signal transduction, thus they are often associated with various diseases, ranging from Parkinson's to cancer [83, 92].

This class of proteins (or domains) distinguish themselves by a prominent sequence signature [83]. They often possess a low content of bulky hydrophobic amino acids that normally form the core of a well-structured protein, and a high proportion of polar and charged amino acids, such as Gln, Ser, Pro, Glu, Arg and Lys [83]. The most interesting feature of IDPs is probably their structural adaptivity and functional versatility (herein dubbed, chameleon). Contrary to the traditional view that protein function equates to a rigid three-dimensional structure, intrinsic disorder may confer a unique functional advantage, since a large structural plasticity allows IDPs to readily interact with different targets [83, 84]. How IDPs function through conformational changes raises intriguing questions, not only important for elucidation of signaling mechanisms, but also fundamental to protein science. As such, IDPs have stimulated enormous interest and have become a current relevant topic in biology.

3.2. PDE γ is Intrinsically Disordered Yet Assumes a Relatively Compact Fold in Solution

The phototransduction pathway is one of the most intensively investigated signaling systems, presenting a wealth of knowledge on the signaling proteins. PDE γ , as one of the major players in phototransduction, provides an ideal model for interrogating the tricks by which IDPs assume their physiological functions. The PDE γ sequence contains 8 Lys and 5 Arg residues (Fig. 3), rendering the molecule highly positively charged at neutral pH (pI = 9.52) [12]. This sequence is also rich in Proline, with 10 Prolines among the 87 residues. Consistently, the calculated PDE γ mean hydrophobicity (0.4195) is lower than the “boundary” value of 0.4298, fulfilling the requirement for IDPs [13]. This prediction has been confirmed by various experimental data. Far and near UV circular dichroism spectra of

PDE γ , respectively, revealed a lack of ordered secondary and tertiary structures [13]. Furthermore, the recent NMR studies have provided direct structural characterizations of PDE γ . Both the observed [^1H , ^{15}N]-HSQC (heteronuclear single quantum coherence) [12] and [^1H]- ^{15}N heteronuclear NOEs (nuclear overhauser effects) [14] of free PDE γ showed a structural disorder. In fact, it has been known that purification of PDE γ through SDS-PAGE [93] or reversed phase HPLC [12], both harsh denaturing conditions, does not compromise the PDE γ function for PDE6 inhibition.

Though deemed intrinsically disordered, PDE γ is not a random coil as previously believed [13, 93]. Transient secondary and tertiary structures have been recently identified in the C-terminal domain of PDE γ when it is free in solution [14]. This conclusion is supported by consistent evidence obtained from several NMR determinations, such as hetero-nuclear NOE, ^{15}N R_1 and R_2 relaxation, NOE connectivity, and RDC (residual dipolar coupling). Overall, there appears to be a gradient of structural propensity from the N-terminus to the C-terminal region, with residues 1–21 being most flexible, 22–50 consistent with a random coil, 51–67 exhibiting marginal evidence for helix, and 68–84 mostly structured. The extreme C-terminus (G85-I87), however, is highly disordered [14].

A PDE γ tertiary structure in atomic details has been highly desirable for understanding the molecular mechanism of PDE γ functions. A direct structural determination of full-length PDE γ has been hindered, however, by intrinsic disorder which is a common challenge for structural studies of IDPs. As a result, the only structure available for PDE γ was the X-ray structure of the half PDE γ molecule solved in the partial GAP complex [45]. This issue has been recently addressed [14] through an NMR technique termed paramagnetic relaxation enhancement (PRE) [89, 94, 95]. Instead of a random coil, however, a relatively compact fold featuring functionally relevant structural elements, has been revealed by two clusters of PDE γ solution structures (Fig. 4). This tertiary fold is primarily constituted by two autonomous domains, the central polycationic domain and the C-terminal domain, formation of which is largely driven by two kinds of interactions: electrostatic attraction between the central polycationic region and the acidic C-terminal half (Fig. 4), and hydrophobic interactions within the C-terminal domain.

4. FUNCTIONAL RELEVANCE OF THE NATIVE PDE γ STRUCTURE

Functional implications of the PDE γ solution structure have been revealed by a comparison with the crystal structure of PDE γ_{46-87} in the partial GAP complex [45], and by observing the chemical shift changes of PDE γ_{46-87} upon binding to the chimeric PDE5/6 catalytic domain [14].

4.1. Structural Adaptation of PDE γ in the GAP Complex Conforms to a Conformational Selection Mechanism

The highly convergent conformations of the PDE γ C-terminal half (PDE γ_{46-87}) [14] show a striking resemblance to the corresponding crystal structure in the GAP complex [45] (Fig. 5). The crystal structure of PDE γ_{46-87} is formed by three α -helices perpendicular to each other: $\alpha 1$ (residues 62–65), $\alpha 2$ (69–73) and $\alpha 3$ (residues 78–83). The non-helical N-terminal residues 50–61 fold back toward the C-terminus, pulling F50 close to I87. Similarly, the NMR solution structure of the same sequence contains helical propensities that closely match the $\alpha 2$ and $\alpha 3$ helices of the crystal structure, and the N-terminal 50–61 portion also bends toward the C-terminus. The remarkable similarity between the two structures is confirmed by their well-superimposed backbones (Fig. 5). This strongly suggests that the structural transition of PDE γ from the free form to the complexed state follows a conformational selection mechanism [96]; that is, preconfigured transient conformations

suitable for binding to $G\alpha_t$ and RGS9-1 in free PDE γ are actually dynamically populated, and are selected and stabilized in forming the GAP complex [14].

The molecular mechanism underlying disorder-to-order transition of IDPs upon binding to their partners is poorly understood. Currently, the prevailing hypothesis is the binding-coupled folding/induced-fit mechanism, which emphasizes that the folding of an unstructured protein is induced by interaction with its target protein [83, 97, 98]. The PDE γ solution structure, however, provides an interesting example for a different model—the so-called conformational selection mechanism: rather than being completely induced, the functional (folded) conformation pre-exists with many similar ones in a dynamic equilibrium that is shifted toward the functional conformation upon target-binding [14]. Apparently, to some extent induced fitting of the PDE γ tertiary structure could also occur considering that there is only one structure of PDE γ bound in the GAP complex [45] but there are many conformers of free PDE γ in solution [14].

4.2. Conformational Selection May Also Occur During PDE6 Activation

The conformational selection mechanism holds, not only for the interaction between free PDE γ and $G\alpha_t$ but also for *part* of the interaction between PDE6-bound PDE γ and $G\alpha_t$, as suggested by the NMR analysis of the PDE γ interaction with the chimeric PDE5/6 catalytic domain [14] which contained almost the entire PDE γ -interacting region of the PDE6 catalytic domain [99]. Since the peptide PDE γ_{63-87} inhibited the PDE5/6 chimera as potently as the full-length PDE γ , with a K_i value comparable to that for the trypsin-activated bovine PDE6, this PDE5/6 catalytic domain appeared to recapitulate the great majority of the PDE γ :PDE6 contacts that lead to PDE6 inhibition [99]. On the other hand, the NMR solution structure of the PDE γ C-terminal domain mirrors its $G\alpha_t$ -binding conformation, as evidenced by its high similarity to the X-ray structure [45] (Fig. 5). Thus detection of chemical shift changes in PDE γ_{46-87} upon binding to PDE5/6 provides for a unique opportunity to uncover the molecular mechanism of $G\alpha_t$ -mediated PDE6 activation, during which the conformation of the PDE γ C-terminal region is altered from the PDE6-bound state to the $G\alpha_t$ -bound state.

Chemical shift analysis revealed that PDE γ residues 74–87 were involved in the PDE γ_{46-87} :PDE5/6 association but the remainder of the sequence was not [14]. Remarkably, the NMR data showed that the W70 indole group, which is critical for the PDE γ : $G\alpha_t$ interaction [44, 45, 49, 51], remained free in the PDE γ :PDE5/6 complex. This result is in strong agreement with an early observation that the W70A mutation abolished the GAP function of PDE γ but had no effect on PDE6 inhibition [44]. Therefore, the pattern of PDE γ :PDE5/6 interaction determined by NMR has confirmed the notion that the PDE6-inhibiting region of PDE γ is confined to the C-terminal dozen or so residues [19–22, 25–30], whereas the major $G\alpha_t$ -binding residues are located in an adjacent yet distinct region [19, 22, 31, 44, 45]. Moreover, lack of chemical shift perturbations in the 46–73 sequence of PDE γ_{46-87} upon association with PDE5/6 implies that the structural propensities of residues 51–73 observed in free PDE γ are retained in the PDE γ :PDE6 complex. Thus, the combined NMR and X-ray structural information yields new insights into the molecular details of the PDE6 activation process. It is highly likely that the $G\alpha_t$ -bound conformation of the PDE γ 51–73 region is actually preserved in the holoPDE6 complex, providing ready availability for it to bind $G\alpha_t$ during PDE6 activation and subsequent GAP complex formation [14].

It remains unclear, however, what the structural determinant is for the PDE6 inhibition in the PDE γ C-terminal 74–87 residues. Severe NMR signal attenuation in this region resulting from the interaction with PDE5/6 suggests a possible structural change compared to free PDE γ [14]. A plausible hypothesis based on mutagenesis analysis and molecular modeling has been put forward by the Artemyev group [19]. In their model, the PDE6-bound structure

of the PDE γ C-terminal domain is mainly constituted by a helix extension from the $\alpha 3$ helix (78–83) to residue H75. This structure positions I86/I87 to form contacts with F777/F781 in PDE6 and also exposes L76 for interaction with G α_t . PDE6 activation is initiated by a L76:G α_t contact that triggers a conformational change of PDE γ thus twisting I86/I87 away from the catalytic site. In accordance with this hypothesis, the NMR data [14] suggest that upon PDE6 activation, the structural propensities pre-existing in residues 63–73 may serve as a platform to dock G α_t to an L76-contacting position, thus facilitating the G α_t sequestration of the PDE γ C-terminus. Nevertheless, structural changes likely occur in the PDE γ inhibitory region (residues 74–87) upon a transition from PDE6 binding to G α_t binding during the process of PDE6 activation.

4.3. The Loose Fold of Free PDE γ May Provide An Intra-Molecular Switch

As mentioned above, while the C-terminal domain of PDE γ performs its PDE6 inhibition task by blocking the entry of cGMP to the catalytic pocket, the central polycationic region assists this task by tightly binding to the GAF domain [7]. The NMR structure of free PDE γ shows that the central polycationic domain and the C-terminal domain are held together by electrostatic attraction in a loose tertiary fold [14] (Fig. 4). In contrast, a linear extended structure is most likely adopted by PDE γ in the holoPDE6 complex [9, 17, 18, 93, 100] (Fig. 2). Two-fold interpretations can be derived from this comparison. On one hand, a loose fold of free PDE γ makes it possible that the central and C-terminal domains be readily separated and extended upon binding to PDE6. On the other hand, when PDE γ is not in a complex, the transient *intra*-molecular electrostatic interactions between these two domains may provide an internal control switch to protect the functional specificity of PDE γ [14]. Therefore, the structural flexibility of PDE γ is indicative of its chameleon-like features.

4.4. Possible Functions of the PDE γ Linker Region

Marginal structural propensity has been found through NMR analysis in the linker region (residues 50–62) that connects the central domain and the C-terminal domain [14] (Fig. 3). No specific function has been attributed to this linker region. Mutations at three charged residues (D52N/D53N/E58Q) reduced PDE γ affinity for PDE $\alpha\beta$, although the mutant could still inhibit PDE6 [24]. It is thus likely that this linker region provides extra binding strength to PDE $\alpha\beta$. A remarkable finding is that a PDE γ construct lacking this linker was not able to inhibit PDE6 [24]. Considering a linear arrangement of the PDE $\alpha\beta$ domains [101, 102], this linker may provide a critical chain length allowing the PDE γ C-terminus (Y84-I87) to reach the PDE $\alpha\beta$ catalytic pocket while the central domain binding to the GAF domain. This scenario is in accordance with the linear extended PDE γ PDE $\alpha\beta$ interaction model [9, 17] (Fig. 2). It has also been shown that a mutation substituting proline (P55L) in this linker sequence reduced PDE γ inhibitory potency [23]. The best explanation may be that this proline residue is needed for correct orientation of the C-terminal and central domains to bind to their respective sites on PDE $\alpha\beta$. Furthermore, this sequence contains several glycine residues that often confer conformational flexibility (Fig. 3). Interestingly, the linker region is shown to constitute part of the “turn” portion in the NMR structure [14]. Thus the linker region may actually function as a “hinge”, for separation of the oppositely charged central domain and C-terminal domain (Fig. 4), when they bind to the PDE $\alpha\beta$ GAF domain and catalytic domain, respectively.

4.5. Interactions of the Disordered PDE γ Polycationic Region with PDE $\alpha\beta$ and G α_t

Compared to the C-terminal domain (residues 62–87), the manner by which the PDE γ central domain (19–49) interacts with its targets is much less well understood, primarily due to a lack of atomic structural information for the N-terminal half. It is not known if this highly disordered PDE γ central domain [14] adapts to an ordered structure upon binding to a partner. Efficient trypsin cleavage of this polycationic region occurs to PDE γ in the

holoPDE6 [9, 103, 104], suggesting a lack of ordered structure. It remains a mystery as to how the polycationic region achieves a very tight binding with the PDE $\alpha\beta$ GAF domain. A high content of positive charge apparently plays a role, since protamine, polylysine, as well as histone compete with PDE γ for binding to PDE6 [25, 105]. However, a low potency of these functionally irrelevant peptides indicates that other contacts made by hydrogen bonds or hydrophobic interactions may be more important. The NMR data suggest that the extended backbones of residues 1–45 statistically favor the β and polyproline (PPII) conformations [14], which generally benefits a larger inter-action interface between an IDP and its target [87]. It is thus highly likely that multi-site “caterpillar” contacts spread along the extended PDE $\alpha\beta$ interface contribute to the high binding affinity, which is further enhanced by non-catalytic cGMP binding in the GAF domain (as will be discussed later). Future NMR studies using PDE γ peptides corresponding to this central region and the PDE6 GAF domain would be expected to provide critical information on this issue. The successful bacterial expression of the chicken cone PDE6 GAF domain is an encouraging progress to this end [10].

It is well documented that the PDE γ central domain also interacts with $G\alpha_t$ [22, 26, 29–31, 55, 106], but the significance of this observation is unclear. Studies showed that the interaction of this domain with $G\alpha_t$ was not GTP-dependent [30, 106]. The interaction site has been mapped to the $\alpha 3$ - $\beta 5$ region [30] (or probably also $\alpha 4$ - $\beta 6$) [107] on $G\alpha_t$, which does not undergo conformational change upon GTP binding [36–38]. Moreover, PDE γ has been observed to interact also with GDP-bound $G\alpha_t$ [30, 106–108], albeit with much lower affinity (880 nM) than with the GTP-bound form (12 nM) [30]. Taken together, it is likely that the interaction of the PDE γ central domain with the $G\alpha_t$ $\alpha 3$ - $\beta 5$ region constitutes an important part of the PDE γ : $G\alpha_t$ GDP association, and remains in the PDE γ : $G\alpha_t$ GTP complex. This interaction may initially engage $G\alpha_t$ GTP to assist its binding to the PDE γ C-terminal domain at an early stage of PDE6 activation, and later serves to reinforce the PDE γ : $G\alpha_t$ GTP signaling-state complex.

The fact that the central domain of PDE γ interacts with both PDE $\alpha\beta$ and $G\alpha_t$ raises an interesting and important question — are these two interactions independent or competitive? Overlapping of the $G\alpha_t$ -binding region and the PDE $\alpha\beta$ -binding region in the central domain (Fig. 3) seems to support the view that the $G\alpha_t$:central PDE γ contacts may interfere with the binding of this central domain to PDE $\alpha\beta$. This speculation is confounded, however, by the observation that triple mutations on the C-terminal side of the polycationic region (K41Q/K44Q/K45Q) impaired the PDE γ interaction with $G\alpha_t$ GTP γ S but did not affect its affinity with PDE $\alpha\beta$ [24].

It has been controversial as to whether PDE γ completely dissociates from PDE $\alpha\beta$ during PDE6 activation [29, 51, 54, 81, 109–113]. Current arguments favor a scenario that the PDE γ polycationic region stays bound to PDE $\alpha\beta$ in the early signaling state when the GAF domain is still occupied with cGMP [7]. The simultaneous binding of PDE γ to two targets may be involved in an intermediate $G\alpha_t$:PDE γ : $\alpha\beta$ complex [51, 81, 107, 114, 115]. But is the PDE γ central domain still bound to PDE $\alpha\beta$ in the ensuing transition state? or does it instead “jump” to an interaction with RGS9-1/G $\beta 5$? Alternatively, is there a transient state in which PDE $\alpha\beta$ is still associated with the GAP complex? The answer to these questions likely depends on the state of cGMP occupancy in the non-catalytic site of PDE $\alpha\beta$, and on the state of membrane association of the involved signaling proteins. Early studies showed that in the presence of disc membranes, direct $G\alpha_t$:PDE $\alpha\beta$ interaction occurred, and at least partially accounted for the formation of the intermediate $G\alpha_t$:PDE γ : $\alpha\beta$ complex [110, 111, 114].

The importance of membrane association for optimal functionality of signaling proteins is becoming more appreciated [116–121]. Very likely, in the photoreceptor outer segment, all the essential proteins in the phototransduction cascade are held to the disc membrane and in close proximity to one another, either directly by tethering to the membrane or indirectly through binding to a membrane-engaged protein (Fig. 1). This membrane-assisted protein constellation should maximally benefit efficient molecular collisions. The “bumper cars” type on-and-off protein-protein contacts may be especially important for multi-targeting PDE γ .

The N-terminal tail of PDE γ is highly flexible, as revealed by the NMR study [14]. This part has not been attributed to any significant function in phototransduction. One report suggested that residues 11–18 were involved in stimulation of cGMP binding to the PDE6 GAF domain [122]. However, truncated PDE γ without the N-terminal tail could still fully inhibit PDE6 [24, 25]. It is rather curious that such a “redundant” tail has remained through evolution. It is possible that a weak interaction of this tail with PDE $\alpha\beta$ assists the tight binding of PDE γ with PDE $\alpha\beta$.

5. REGULATION OF THE PDE γ INTERACTIONS WITH ITS PRIMARY TARGETS

In the PDE γ molecule, the C-terminal domain directly undertakes PDE6 inhibition as well as GAP potentiation, thus can be considered as the “functional” domain. As discussed above, the role of the central polycationic region, in contrast, is to provide a second interaction site to enhance the affinity of PDE γ for PDE $\alpha\beta$ or transducin [7] and may be thus considered as a “regulatory” domain. Most of the regulation of PDE γ functions occurs in this “regulatory” region, with the one exception of the zinc-binding site. As is the case for many other visual signaling proteins, PDE γ has been reported to be a zinc-binding protein. The binding site has been suggested to be formed by the C-terminal residues C68, His75 and His79 [13]. PDE $\alpha\beta$ also contains a zinc-binding site in the catalytic pocket, which is essential for the PDE6 activity [123]. Molecular modeling shows that when bound to PDE6, the postulated zinc-binding residues H75 and H79 on PDE γ are in close proximity to the PDE6 zinc finger [27, 28]. Thus, when PDE6 is inhibited by PDE γ , do both partners bind to the same zinc ion? What is the significance of zinc binding to PDE γ for its inhibitory function? Further work is needed to assess the role of zinc in PDE γ functions.

5.1. Positive Cooperativity of the Heterogeneous Binding of PDE γ and Non-Catalytic cGMP

Non-catalytic cGMP binding in the PDE6 GAF domain is probably the most important yet least understood regulation of PDE γ functions in phototransduction. Unlike other GAF-containing PDEs (such as PDE5 and PDE2) [124–127], cGMP occupancy of the PDE6 GAF domain does not directly cause an allosteric effect on the catalytic domain, but indirectly does so through alteration of the PDE γ binding which bridges the GAF domain and the catalytic domain [15, 16, 109, 128]. The GAF cGMP binding couples PDE γ binding in a reciprocal manner, thus regulating the cGMP hydrolysis in the catalytic domain [15, 56, 115, 129]. GAF conformational changes, allosterically induced by cGMP binding, enhance PDE γ binding, and *vice versa* through a mechanism of positive cooperativity; accordingly, dissociation of either partner weakens the binding of the other. In the absence of PDE γ , bovine rod PDE $\alpha\beta$ contains a high affinity site ($K_D = 60$ nM) and a low affinity site (or termed “exchangeable site”) for cGMP ($K_D > 1$ μ M). Binding of the PDE γ polycationic region restores high affinity to the low affinity site [7, 15, 16]. Likewise, there are also two classes of PDE γ binding sites [15, 18, 109, 130, 131] in the GAF domain(s), the occurrence of which depends on the GAF cGMP occupancy [15, 16, 109, 128].

The significance of heterogeneous interactions of PDE γ and cGMP with the rod PDE6 GAF domain can be traced to the unique rod phototransduction that is responsible for night vision. In comparison to the cone-specific day vision, a prominent feature of night vision is the extremely high sensitivity of the one-photon response that requires very low basal activity as well as fast activation/deactivation of PDE6. The tight binding of PDE γ to PDE $\alpha\beta$ maintains very low PDE6 activity but may thus be expected to also hinder rapid activation. This dilemma is elegantly resolved in the rod visual cascade, probably in large part by the cooperative heterogeneous PDE γ and cGMP binding to the GAF domain. In the dark a very high level of cytoplasmic cGMP saturates binding in the GAF domain thus maintaining one class of high-affinity PDE γ binding sites, which is essential for low background noise [15]. Upon photoactivation of PDE6, lowered cGMP levels alter the exchangeable cGMP site to low affinity, which in turn converts one PDE γ binding site (exchangeable site) to lower affinity [15]. This conversion may also assist PDE6 activation by facilitating efficient sequestration of PDE γ by G α_t . Under prolonged light stimulation, the exchangeable PDE γ now probably dissociates from PDE $\alpha\beta$ due to a very low cGMP level and thus becomes available for binding in the GAP complex to achieve rapid signal termination [7]. Recovered cGMP levels through a Ca²⁺ feedback mechanism [60] then restore high affinity to the exchangeable PDE γ binding site, facilitating a rapid re-inhibition of PDE6. The physiological importance of the binding cooperativity of PDE γ and noncatalytic cGMP in regulating the lifetime of activated PDE6 awaits further investigation. Transgenic study [132] on mutations of the PDE6 GAF residues critical for cGMP or PDE γ binding [10, 33, 56] may provide a powerful approach.

5.2. Asymmetric Interactions Between the PDE γ Central Domain and the PDE α /PDE β Subunits

The nature and structural basis for the observed heterogeneous PDE γ /cGMP binding sites in GAF are poorly understood. It appears that this GAF-binding heterogeneity stems from the uniqueness of the PDE $\alpha\beta$ heterodimer in the rod. All the other PDEs are homodimers, including the cone PDE6 catalytic core (PDE $\alpha'\alpha'$) which shares a very high sequence homology with the rod PDE6 [7, 9]. In contrast to the rod PDE6, neither non-catalytic cGMP nor PDE γ binds to the PDE $\alpha'\alpha'$ homodimer heterogeneously [9, 28, 133]. Non-catalytic cGMP binding to the cone PDE6 is much weaker than that to the rod PDE6 [9, 133, 134]; likewise, the affinity of PDE γ for cone PDE6 is lower than that for the rod PDE6 [135]. Notably, the major sequence difference, either between the cone and rod PDE6s, or between PDE α and PDE β , resides in the GAF domain [7]. Thus the contrast between rods and cones highlights a difference between the PDE α and PDE β GAF domains that may account for the heterogeneity of cGMP and PDE γ binding.

Accordingly, it has been recently demonstrated that the interactions of the PDE γ polycationic region with PDE α and PDE β are asymmetric, with the F30 region favoring PDE α and the S40 region favoring PDE β [18]. A new model has thus been proposed to explain the composition of two classes of PDE γ binding sites on the $\alpha\beta$ heterodimer [17] (Fig. 2). This model suggests that the high-affinity PDE γ : $\alpha\beta$ binding site is constituted by the F30 region: α -GAFa and the S40 region: β -GAFb interactions. The low-affinity site is on the complementary side of PDE $\alpha\beta$, formed by the F30 region: β -GAFa and S40 region: α -GAFb interactions. Remarkably, the α and β subunits could indeed be experimentally “stapled” together by two photoprobes placed at the central and C-terminal domains of PDE γ . This evidence supports the notion that in holoPDE6 each of the two PDE γ s binds to both α and β simultaneously in a linear extended structure [17] (Fig. 2).

Therefore, the observed asymmetric PDE γ : $\alpha\beta$ interactions rationalize the uniqueness of the rod $\alpha\beta$ heterodimer. Furthermore, since the heterogeneous GAF binding of PDE γ results from two classes of cGMP binding sites [7], it is highly likely that cGMP binds to the GAF

domains of the α and β subunits also in an asymmetric manner. Interplay of the heterogeneous interactions of cGMP and PDE γ with the different GAF domains of the α and β subunits may confer a strict, yet flexible, control of PDE6 activity during rod photoexcitation, recovery and light/dark adaptation.

The ability of two identical PDE γ s to bind to different GAF domains on PDE α and PDE β is further supported by the flexible nature of the PDE γ structure [14]. Although data from binding kinetics have implied that either the cGMP binding or PDE γ binding leads to allosteric GAF conformational perturbations, no direct structural evidence has thus far been available. An interesting molecular modeling of the PDE6 GAF domain based on the PDE2 GAF structure [127] and mutagenesis using chimeric PDE α' has been recently reported [33]. This model predicts that the cGMP and PDE γ binding sites in GAF α are in close proximity. It can not be excluded, however, that enhanced PDE γ :GAF interaction may be in part achieved by the conformational change of PDE γ that is elicited by the cGMP binding to the GAF domain. Future structural investigation, together with comparative studies of rod and cone PDE6s, should provide important insights into the nature of the allosteric modulation in the GAF domain that is achieved by the heterogeneous binding of cGMP and PDE γ .

5.3. Post-Translational Modifications in the PDE γ Polycationic Region

Post-translational modifications, such as phosphorylation [136] and ADP ribosylation [137] (Fig. 3), have also been implicated to regulate PDE γ functions. The real physiological impact of these modifications, however, remains controversial.

Earlier studies showed that phosphorylation at either T22 or T35 drastically increased the PDE γ inhibitory potency for PDE6 but diminished its interaction with G α_t [138, 139]. A novel mechanism was thus suggested whereby PDE γ may be able to terminate phototransduction independent of G α_t GTPase stimulation [140, 141]. This mechanism was later thoroughly re-examined by the Cote group [82]. Their data showed that phosphorylation at T22 or T35 did not increase, but rather, caused a slight decrease in the ability of PDE γ to inhibit the PDE $\alpha\beta$ catalytic activity. Given that only ~10% of the endogenous PDE γ was phosphorylated in response to light activation, the physiological significance of phosphorylation in PDE γ is questionable [82]. Nevertheless, it has been confirmed that the PDE γ interaction with activated transducin is severely disrupted by phosphorylation at either of the two sites [82, 138, 141–143]. Transgenic mice carrying either T22A or T35A, or both of the mutations, have been recently generated [144]. It was found that background light adaptation, which accelerated the decay of responses to brief flashes of the wild type mouse, did not occur in the T35A rods [145]. These studies suggested a possible involvement of PDE γ phosphorylation in modulation of GAP-dependent turnover. The biochemical mechanism remains to be understood.

ADP ribosylation at R33 and R36 on PDE γ has also been observed to abolish PDE γ interaction with G α_t [137, 146]. In addition, N-terminus acetylation of PDE γ has been reported [147]. But the physiological consequence of these modifications in photoreceptor cells is not clear.

A common observation in these studies is that PDE γ is a poor substrate for kinases when bound to PDE $\alpha\beta$, but free PDE γ is readily available for phosphorylation. Therefore, a free PDE γ pool could possibly exist under illumination conditions with an estimated size of ~10% of total PDE γ in the outer segment [82]. Occurrence of an exchangeable PDE γ binding site on PDE $\alpha\beta$ [7] and possible GAP-independent PDE6 deactivation [51, 81] in the light-excited rods also lend clues to a possible free PDE γ population. However, no evidence is available for such a free PDE γ pool in the dark-adapted state. It is interesting to note that,

by analogy to $G\alpha_t$, which undergoes light-stimulated translocation from the outer segment to the inner segment by free diffusion [121, 148–151] (see recent review [152]), free soluble $PDE\gamma$ in the cytosol should also diffuse into the inner segment, or co-translocate in a complex with activated $G\alpha_t$. $PDE\gamma$, however, has not been observed to undergo translocation even under prolonged light stimulation [81, 149].

6. NON-VISUAL $PDE\gamma$ FUNCTIONS

6.1. $PDE\gamma$ as a Regulator in the "Third Eye"

One interesting fact is that all the basic components of a retina-type phototransduction cascade occur in the chicken pineal gland [153–155]. It may not be surprising since the pineal gland and eye are likely evolved from a common ancestral organ [156]. The cone $PDE\alpha'$, cone $PDE\gamma$, rod $PDE\gamma$ and rod $PDE\beta$ (but not $PDE\alpha$) have been identified in the chicken pineal gland. The phototransduction mediated by $PDE6$ in the pineal gland does not result in vision. Rather, this pathway regulates melatonin synthesis which is involved in circadian activities. As a coherent picture, cone $PDE\gamma$ and $PDE\alpha'$ have been recently found in the lizard parietal eye [157, 158], or the "third eye", which is an equivalent organ of the pineal gland in higher vertebrate animals. Likewise, this lizard organ is responsible for global detection of dawn and dusk instead of image-forming vision. A unique antagonism underlying photoresponse in the parietal eye has been discovered, in which the *gusducin*: $PDE\gamma$ interaction activates $PDE6$, leading to the hyperpolarizing blue-light response. The opposing depolarizing green-light response is likely mediated by G_o , which inhibits rather than activates $PDE6$. The biochemical mechanism of $PDE6$ inhibition performed by G_o in the third eye remains to be determined.

6.2. $PDE\gamma$ -Homologous Affinity Adaptor in the Brain

One of the surprising discoveries for $PDE\gamma$ is that it has a functional homolog in the brain, which is the C-terminal domain of the long isoform RGS9 protein, RGS9-2 [159]. The brain-specific RGS9-2 differs from the retina-specific short isoform, RGS9-1, only by its longer C-terminal domain [160]. Interestingly, $PDE\gamma$ -like components have been identified in this domain [159]. Like $PDE\gamma$, the RGS9-2 C-terminal domain consists of an anionic region as well as a cationic region. The anionic region contains 12 residues homologous to $PDE\gamma$. Six of these residues are identical to the $PDE\gamma$ W70 cluster ($V_{66}ICPWE_{71}$), which functions to forge a tight association between $PDE\gamma$ and $G\alpha_t$ and to enhance the $G\alpha_t$:RGS9-1 interaction [45]. The cationic region, although different from that of $PDE\gamma$ in sequence and length, is rich in *ralines* and *arginines*, and also contains SH3 recognition motifs. Intriguingly, functional analysis has indicated that this C-terminal domain mediates the specific recognition and high-affinity interaction between RGS9-2 and its target, $G\alpha_o$. Apparently, the lack of this domain in RGS9-1 is functionally compensated for by $PDE\gamma$. Thus, $PDE\gamma$ and its functional homolog serve as affinity adaptors for the two RGS9 isoforms and their cognate G proteins in two different types of neurons [159].

6.3. $PDE\gamma$ As a Chaperone for $PDE\alpha\beta$

The role of $PDE\gamma$ in normal retinal development also comes as somewhat of a surprise. In the $PDE\gamma$ knockout mouse, which undergoes rapid retinal degeneration, $PDE\alpha\beta$ was expressed at a normal level but was found to be nonfunctional [161]. Apparently, the $PDE\alpha\beta$ heterodimer did not assemble properly in the absence of $PDE\gamma$. Therefore, $PDE\gamma$ appeared to function as a chaperone assisting the $PDE\alpha\beta$ heterodimer to correctly fold and assemble [162]. Curiously however, expression of the C-terminally truncated $PDE\gamma$ (the last 7 amino acids deleted) in the knockout background did not rescue retinal degeneration, but instead generated a phenotype of $PDE\alpha\beta$ down-regulation [163]. This observation is echoed by two other studies showing that transgenic mice expressing constitutively active rod transducin

($G\alpha_{t1}$) [149] or cone transducin ($G\alpha_{t2}$) [164] also caused PDE $\alpha\beta$ down-regulation. In both cases, constant sequestration of the PDE γ C-terminal domain by the constitutive $G\alpha_t$ GTP may have mimicked the effect of the C-terminal deletion, and thus deprived nascent PDE $\alpha\beta$ of the chaperoning assistance of PDE γ . As mentioned earlier, binding of either cGMP or PDE γ causes allosteric changes in the GAF domain [7], and the PDE6 domains fold independently, as evidenced by the electron microscopy structures of PDE6 [101, 102] and the crystal structures of the PDE2 GAF domains [127] and the PDE5 catalytic domain [165]. It is thus reasonable to speculate that exposure of PDE $\alpha\beta$ to proteolysis may be caused by a combination of PDE γ (and/or cGMP)-induced GAF conformational changes and improper folding of the catalytic domain. Alternatively, without the full-length PDE γ to bridge the GAF and catalytic domains, PDE $\alpha\beta$ is unable to assume a correct tertiary structure, and instead adopts a protease-susceptible fold.

To date no heterologous expression of highly active PDE $\alpha\beta$ has been successful [166–169]. Recently, AIPL1 (aryl hydrocarbon receptor interacting protein like-1) has been found to be a chaperone of PDE $\alpha\beta$ [170, 171], yet co-expression of AIPL1 with PDE $\alpha\beta$ did not result in an active heterodimer. It is not yet understood how AIPL1 and PDE γ co-chaperone the PDE $\alpha\beta$ assembly, but it is known that AIPL1 is important for trafficking of the rod PDE6 [171].

Trafficking of visual signaling proteins from the inner segment where they are made, to the outer segment where they function, is emerging as a new frontier [172, 173] (also see reviews [60, 174]). Little is currently known about trafficking of the phototransduction proteins, except for rhodopsin. Based on the knowledge gained from rhodopsin transport [174–178], the transport process of the other proteins has been proposed to follow an intraflagellar transport mechanism [172]. PrBP (prenyl-binding protein), which was initially categorized as the δ subunit of PDE6, has been recently found to be essential for the PDE6 transport [179, 180]. Another recent report [181] provided evidence to the hypothesis that PDE γ co-transport with the membrane-associated PDE $\alpha\beta$ by riding a cargo with guanylate cyclase as the cargo receptor [172]. An interesting question thus arises as to whether the ill-assembled PDE $\alpha\beta$ in the PDE γ -knockout mouse can still transport to the outer segment.

6.4. PDE6 as An Effector in Wnt Signaling in Development

Recent studies have shown that PDE $\alpha\beta$ and the cone transducin ($G\alpha_{t2}$) were involved in a non-canonical Wnt signaling pathway, in which activation of Frizzled2 by Wnt5 led to the $G\alpha_{t2}$ -mediated PDE6 activation, and thus lowered cGMP levels [182, 183]. Blockage of this pathway using PDE6 specific inhibitors caused abnormal zebrafish development. PKG (cGMP-dependent protein kinase), which is down-stream in this pathway, has been found to sense cGMP perturbations, and may mediate a transcriptional regulation that involves the transcription factor NF-AT [184]. It will be interesting to know if the photoreceptor PDE γ :PDE $\alpha\beta$ interaction is “grafted” to this pathway. Consistent evidence comes from higher abundance of PDE γ in the rat embryonic tissues [185], and also from the abnormal photoreceptor development in the PDE γ -knockout mouse [161]. Considering the observation that PDE γ_{1-61} inhibits PKG activity [186], activation of PDE6 could have two synergistic effects on PKG — lowered cGMP and dissociated PDE γ , both of which would suppress PKG kinase activity.

A lack of apparent impairment on overall development in the PDE γ -knockout mouse [161] may argue against a role of PDE γ . However, cone PDE γ , which is expressed in various tissues [187], may substitute for rod PDE γ in the knockout. In this respect, a cone/rod PDE γ double knockout would be desirable for understanding the diverse PDE γ functions. Phylogenetic analysis suggests that the cone PDE6 has evolved from a common ancestral PDE5/6/11 gene, whereas the rod PDE6, which became the specialized PDE $\alpha\beta$ heterodimer,

emerged at a later stage [124, 188]. The evolution of rod PDE γ has followed a similar path. This recent evidence [188] lends further support to the notion that rod PDE γ and PDE $\alpha\beta$ represent the PDE6 versions highly specialized for night vision in rods, while the less specialized cone PDE6 may be more widely distributed in mammalian tissues.

The evolutionary link and homology between PDE5 and PDE6 (reviewed in [124, 189, 190]) raises the question as to whether PDE γ regulates PDE5. It was previously reported that PDE γ -like proteins were detected in guinea pig airway smooth muscle, which appeared to complex with PDE5 [191]. But PDE γ does not interact with purified recombinant PDE5 [56, 99]. In addition, PDE γ has been suggested to be a regulator in the p42/p44 mitogen-activated protein kinase (MAPK) pathway in HEK293 cells [192, 193], and its association with c-Src and Dynamin II could be mediated by a consensus SH3 (Src homology) recognition sequence, P20VTPRKGPP28 (Fig. 3). Two other SH3-containing proteins, formin-binding protein 17 [185] and PACSIN [194], were later shown to interact with PDE γ as well. A possible role of PDE γ in the morphogenesis of photoreceptor cells was thus proposed [194].

6.5. The Multitasking PDE γ is Highly Conserved

As is obvious from the above description, PDE γ is a multitasking protein (Fig. 3). It is more widely distributed than previously thought [154, 158, 185, 187], although a high abundance (~20 μ M) only occurs in the photoreceptor outer segment [8]. The two types of highly specialized photoreceptors, rods and cones, express two isoforms of PDE γ through two different genes (*Pde6g* and *Pde6h*, respectively) [147, 187, 195]. These two isoforms differ in their N-terminal tails, but the remaining sequences are almost identical (Fig. 3). This remainder PDE γ sequence is highly conserved during evolution, with a 100% identity in all mammalian rod PDE γ s reported to date [7], and a greater than 98% similarity even between the bovine rod PDE γ and its counterpart in an ancient vertebrate, sea lamprey [188] (Fig. 3). PDE γ is encoded by three exons: exon2, residues 1–49; exon3, 50–62; exon4, 63–87 [162, 187, 196]. It is interesting to note that these three exons correspond to distinct PDE γ domains (Fig. 3). The transcript of a short cone PDE γ splice variant, which lacks exon3 and exon4, has been detected in mouse lung [187]. Expression and specific functions of PDE γ (or its splice variants) in non-phototransduction tissues [187, 192–194] need to be further determined. Nonetheless these studies raise a possibility that, PDE γ -bridged cross-talk between phototransduction and other pathways may occur in photoreceptors to fine-tune vision under ever-changing illumination conditions.

7. PDE γ AND EYE DISEASES

As mentioned earlier in this review, in the visual signaling cascade the alternate interactions between PDE γ and its targets dictate the dark-adapted inactive state, the light-stimulated signaling state, and the transition state during recovery. Disruption of these interactions causes visual disorders ranging from stationary night blindness to progressive retinal degeneration. Disease-eliciting mutations have been discovered in most of the proteins involved in phototransduction (for reviews, see [197–199]). Some of the genetic defects provide valuable opportunities for studying the PDE γ regulatory mechanisms in phototransduction. It is rather curious, however, that no naturally occurring mutation has been found on PDE γ [162, 198, 200]. Only one case for the cone PDE γ has been reported, in which a mutation in the 5'-untranslated region of the cone PDE γ gene was correlated with a cone dystrophy [201]. The exceptionally high conservation and wide distribution of PDE γ could possibly explain the lack of natural occurrence of PDE γ mutation.

7.1. Retinal Degeneration

Transgenic mice carrying different mutant alleles of the rod PDE γ gene have revealed various defects in visual physiology and retinal morphogenesis [49, 132, 144, 161, 163, 202–204] (specifically reviewed by Farber and Tsang [162]). Knockout [161] (or knockdown [205]) of the rod PDE γ gene leads to rapid retinal degeneration, representing the most aggressive form of retinitis pigmentosa. Degeneration is initiated in the retina outer segment at postnatal day 8, and later extended to the inner segment and other retinal neurons but not the ganglion cells. Rods degenerate first and cones follow, despite the specific knockout of only rod PDE γ . This may indicate some form of cell-cell communication between rods and cones. Retinal degeneration in the PDE γ -knockout mouse could be rescued by transgene of PDE γ carrying the W70A mutation [49]. Introduction of this mutation into the knockout background desensitized the photoresponse and also delayed the recovery, thereby created a new stationary form of nyctalopia.

The molecular mechanism of retinal degeneration in the PDE γ -knockout mouse is not known. A great deal of information has been obtained by analogy to the well-studied rd1 mouse model, which exhibits the same phenotype of cytosolic cGMP accumulation [206, 207]. The rd1 mouse carries a mutation on the PDE β gene that terminates expression of PDE β subunit and thus disables PDE6 [208]. It has been suggested that sustained high levels of cGMP keeps the cGMP-gated channels constitutively open, thus the resultant cyto-toxic levels of Ca²⁺ lead to apoptosis [197]. Given the profound complexity of the photoreceptor cell biology, multiple mechanisms may be involved [209, 210]. A possible effect of PDE γ on the photoreceptor morphogenesis can not be excluded [194].

7.2. Night Blindness and Other Diseases

A Nougaret form of dominant stationary night blindness is known to be caused by the G38D mutation on G α_t [211]. It has been determined that this mutation abolished the interaction of G α_t with PDE γ , leading to impaired PDE6 activation and GTPase stimulation [212]. Transgenic mice carrying this mutation generated a unique phenotype of reduced rod sensitivity, impaired activation, and slowed recovery [213]. An H258N mutation identified on PDE β has been linked to congenital stationary night blindness in a large Danish family [214]. As revealed by a study using chimeric PDE6, disruption of the PDE6:PDE γ interaction by this mutation caused incomplete inhibition of PDE6, thus leading to desensitization of rod photoreceptors [215]. In addition to the photo-transduction-related eye diseases, it has been reported that experimental autoimmune uveoretinitis could be induced by immunization with PDE γ in the Lewis rat [216], and the key responsible residues were found to be W70, E71, F73 and H75. A link between PDE γ and pulmonary hypertension has also been reported [217]. Increased PDE γ expression, dependent on chronic hypoxia, was correlated to hypoxiaindependent increase of p42/p44 MAPK activation.

7.3. Potential Therapeutics for PDE γ -Relevant Visual Disorders

Based on the regulatory role of PDE γ and its predominant expression in the photoreceptor outer segment, it may be possible to develop molecular interventions targeting PDE γ for the treatment of visual diseases. For example, bradyopsia, also known as “slow vision”, has recently been found to be caused by genetic defects in RGS9-1 or R9AP [218]. These patients suffer from subnormal acuity, photophobia, and slow adaptation which results in a transient but debilitating blindness. Since excess free PDE γ can produce more rapid recovery of the light response by directly deactivating PDE6 without requiring the GAP complex, “pharmacological” up-regulation of PDE γ may be a candidate therapy for bradyopsia patients lacking the functional GAP complex [81]. In consideration of the diversity of PDE γ targets, however, the effect of PDE γ up-regulation on other signaling systems would also need to be carefully assessed. Another interesting case is the transgenic

mouse expressing the constitutively active $G\alpha_t$ -Q200L, which has been shown to diminish the protein level of PDE $\alpha\beta$ [149]. This would be expected to result in severe retinal degeneration by an analogy to the PDE $\alpha\beta$ down-regulation caused by the transgene of C-terminally truncated PDE γ [163]. But surprisingly, the retinal morphology of the $G\alpha_t$ -Q200L mouse was essentially normal. The reason is most likely the concomitant down-regulation of GC and GCAP observed in the $G\alpha_t$ -Q200L mouse that may have alleviated cGMP accumulation. Thus balanced cGMP metabolism achieved through pharmacological down-regulation of GC could be utilized to rescue retinal degeneration in the PDE γ -knockout mouse and rd1 mouse. In support of this notion, the rhodopsin mutation G90D that produces constitutive stimulation of cGMP hydrolysis can rescue rods from degeneration in the GCAP1 Y99C mutant mouse in which cGMP accumulates [219].

8. SUMMARY

Phototransduction is considered as one of the best understood molecular signaling systems. However, many questions regarding the sophisticated regulation of this pathway remain unanswered, due to incomplete structural information at the atomic level. The NMR solution structure of PDE γ [14], in combination with the knowledge derived from the crystal structure of the C-terminal PDE γ [45], opens up a new dimension for understanding the PDE γ regulatory mechanisms. The electrostatically maintained PDE γ conformation [14] confers an internal switch control for its functional specificity, and this loose fold meanwhile preserves a structural plasticity for interacting with distinct signaling targets. While induced conformational changes may also occur in PDE γ upon binding to its target(s), transducin-interacting secondary and tertiary structural elements preexist in free PDE γ , and are also *partly* preserved and exposed in the PDE6-bound PDE γ , facilitating efficient recognition by transducin during the signaling state and the ensuing transition state. Thus the native PDE γ structure also offers an interesting example of a conformational selection mechanism for the intrinsically disordered proteins.

Therefore, NMR structural determination provides a powerful approach to solve the puzzles of how PDE γ , a molecular chameleon, adapts to its complex signaling environments. The PDE γ :GAF interaction couples the cGMP:GAF binding in a reciprocal manner [7], by mechanisms that are central to visual transduction but yet to be determined. In another important process of phototransduction — the GAP complex assembly, structural information is thus far very limited regarding the PDE γ :RGS9-1 interaction, and not available at all for possible PDE γ :G β 5 contacts [69]. Powered by new NMR techniques and sophisticated biochemical experiments, future studies on these topics promise a comprehensive understanding of the unique chameleonlike properties of PDE γ .

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Fig. (1). $PDE\gamma$ is a central player in phototransduction

The rod photoreceptor cell is illustrated according to Mavlyutov *et al* [220]. In the diagram of phototransduction, the visual signaling proteins are shown associated with the disc membrane, either through transmembrane helices or by fatty acids. The “dashed” $PDE\gamma$ represents its $PDE\alpha\beta$ -bound state prior to $PDE6$ activation. Abbreviations: $G\alpha_t$, $G\beta_t$ and $G\gamma_t$, the α , β and γ subunits of transducin, respectively; $G\beta_5$, the long splice variant of the type 5 G protein β subunit; RGS9-1, the ninth family of regulators of G protein signaling in photoreceptors; R9AP, RGS9-1 anchoring protein; GC, guanylate cyclase; GCAP, GC activating protein. For the definitions of $PDE\alpha\beta$, $PDE\gamma$, and GAF, please refer to the text of this review.

Fig. (2). A recent model of the PDE γ :PDE $\alpha\beta$ interaction

(A). The electron microscopy structure of PDE $\alpha\beta$ [101]. (B). A model of PDE γ :PDE $\alpha\beta$ interaction. Each PDE γ binds both PDE α and PDE β simultaneously in an extended linear structure. The PDE γ shown in the front represents the high-affinity binding with PDE $\alpha\beta$, with the PDE γ F30 region binding α -GAFa and the S40 region binding β -GAFb. The other PDE γ is shown on the opposite side, representing the low-affinity binding. The C-terminal region of PDE γ is shown to bind the PDE $\alpha\beta$ catalytic domain near the “groove”, and the dashed rod (the same for the PDE γ on the reverse side) depicts a general possibility that some component of PDE γ :PDE $\alpha\beta$ interaction may occur between one PDE γ and one catalytic subunit (reproduced from Guo *et al* [17] with permission)

Fig. (3). The multitasking PDE γ

The PDE γ domains are defined according to their distinct roles. The border between the N-terminal tail (residues 1–18) and the central domain (19–49) is set at G18/G19 because the highly conserved PDE γ sequence starts at G19 (see the sequence alignment) [188]. The negatively charged C-terminal domain (62–87) and the linker region (50–61) are shown in red, and the positively charged central domain in blue. Dot: The phosphorylation sites at T22, T35, and T62 [7]. Square: The ADP ribosylation sites at R33 and R36. Triangle: The postulated zinc-binding residues C68, H75, and H79 [13]. As can be seen in the sequence alignment, the sequences from the central domain throughout the C-terminal domain in bovine rod and cone PDE γ s [147, 195], and even in γ 2 of the ancient vertebrate sea lamprey [188], are highly conserved. The grey bars represent identical amino acids. The individual $G\alpha_t$ -interacting residues in the rod PDE γ C-terminal domain have been clearly identified in the crystal structure of the partial GAP complex [45]. The three lysines in the polycationic region, K41, K44 and K45, have been suggested to interact with $G\alpha_t$ but not with PDE $\alpha\beta$ [24]. The high-affinity interaction of rod PDE γ with PDE $\alpha\beta$ is shown to occur mainly in the central domain residues 18–41 [15], with the flanking residues probably providing weak binding strength [24] (depicted by the dotted line). The thinner spindle on the C-terminal side represents the PDE6 inhibitory region which has a lower affinity to PDE $\alpha\beta$ compared to the central domain [15]. The circle refers to the C-terminus that directly blocks the PDE $\alpha\beta$ catalytic pocket [20]

Fig. (4). Conformation ensembles representing the solution structure of native PDE γ
 Shown are the two most populated clusters represented by (a) 56 conformers and (b) 30 conformers from the 100 analyzed structures. The five conformers with lowest energy from each cluster are shown; they are colored from blue at the N-terminus to red at the C-terminus. Residues R24, K45, D52 and E81 are labeled to delineate the boundaries of two oppositely charged segments (reproduced from Song *et al* [14] with permission).

Fig. (5). The conformation of PDE γ favorable for binding G α_t is populated in solution
 (a) The structure of PDE γ (blue ribbon) in complex with G $\alpha_{t/i1}$ and RGS9-1 (surface representation). (b) Superposition of the solution structure of native PDE γ with that in the partial GAP complex (reproduced from Song *et al*[14] with permission).