Chapter 2

Structure and Function of G-Protein-Coupled Receptor Kinases 1 and 7

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Abstract

The importance of G protein-coupled receptor (GPCR) kinases (GRKs) as regulators of GPCR signaling has been widely recognized. In humans, GRKs constitute a family of seven protein kinases involved in the phosphorylation and desensitization of agonist-activated GPCRs in many physiological processes. The GPCR desensitization process is initiated by GRKs, but involves several subsequent steps including arrestin capping of phosphorylated receptors. High-resolution crystal structures were determined for four members of the GRK family, i.e., GRK1, GRK2, GRK5, and GRK6. This allowed decoding of the molecular basis of GRK activation and interactions with GPCR substrates, as well as the GRK interactions with cellular membranes and inhibitors. Here, we focused on retinal GRKs, or photopigment kinases, rhodopsin kinase (GRK1), and GRK7, in the context of major general advances in the GRK field.

Key words G protein-coupled receptors (GPCRs), Retinal G protein-coupled receptor kinases (GRKs), Visual signal transduction cascade, OGUCHI disease, GRK1–rhodopsin interaction, Structures of retinal GRKs and inhibitors

Abbreviations

CaM	Calmodulin
GPCRs	G protein-coupled receptors
GRKs	G protein-coupled receptor kinases
PDE	Phosphodiesterase
PKA	Protein kinase A
Rho	Rhodopsin
Rho*	Photoactivated rhodopsin

1 Introduction

1.1 G Protein-Coupled Receptors (GPCRs) and Their Kinases

G protein-coupled receptors (GPCRs) are important receptors involved in cell signaling. External signals received by cells, including small molecules, proteins, or light in the case of rhodopsin (Rho), interact with and activate GPCRs and thereby are trans-

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duced intracellularly through a system of proteins composed of G proteins, phosphodiesterases (PDEs), cation channels, and Ca^{2+} sensors, among others. To react on a limited timescale, an activated GRCR needs to be desensitized and returned to its unstimulated state. The desensitization process is typically carried out by a special class of protein kinases called G protein-coupled receptor kinases (GRKs) that preferentially recognize the activated state of their cognate GPCR [1]. GRKs are members of the A, G, and C (AGC) protein kinase family. Although different activated GPCRs initiate different intracellular processes, signal transmission and its consequent termination appear to be similar among them [2]. Pharmacological interventions that regulate GPCR activity are of great interest because GPCRs are involved in virtually all physiological processes. Defects, inhibition, or excessive activity of GRKs are thought to contribute to diseases such as hypertension [3], cardiac [4], Oguchi disease [5], and heart failure [6]. Further attesting to the importance of this class of kinases, the first biannual meeting dedicated to the GRK field was recently held [7].

1.2 Visual Transduction

When light enters the eye, specialized photoreceptor cells in the retina initiate the visual signaling cascade and together with other cell types such as Müller, horizontal, amacrine, bipolar, and ganglion cells transduce this information to the brain (Fig. 1a) [8, 9]. Signal transduction is accomplished by the polarization of photoreceptor cells, which are composed of three major domains: (1) an outer segment which contains the membrane disks where the phototransduction machinery is localized, (2) an inner segment where the protein synthetic apparatus is found, and (3) a synaptic region that connects with other neurons (Fig. 1a). The outer segment is the primary cilium of photoreceptor cells with the soma connected to the synaptic terminal domain. Photoreceptor cells are further categorized as either rods or cones (Fig. 1b), which have notable differences in shape, synaptic connections, and the type of visual photopigments they express.

Rod cells function as the primary visual detector cells under light conditions. Responsible for dim light (scotopic) vision, they are capable of detecting a single photon [10]. Cones are responsible for color (photopic) vision and they are about 100 times less sensitive to light than rods [11]. This decreased sensitivity permits cones to be active and photosensitive throughout the day even under high-intensity light conditions. Another distinguishing feature between rods and cones (Fig. 1b) is the time they need to readapt after light exposure. Rods are quickly desensitized and take about 1 h to recover to their pre-illumination state, whereas the recovery rate for cones is on a minute timescale. This property enables cone cells to quickly adjust to rapidly changing lighting conditions encountered throughout the day [12].

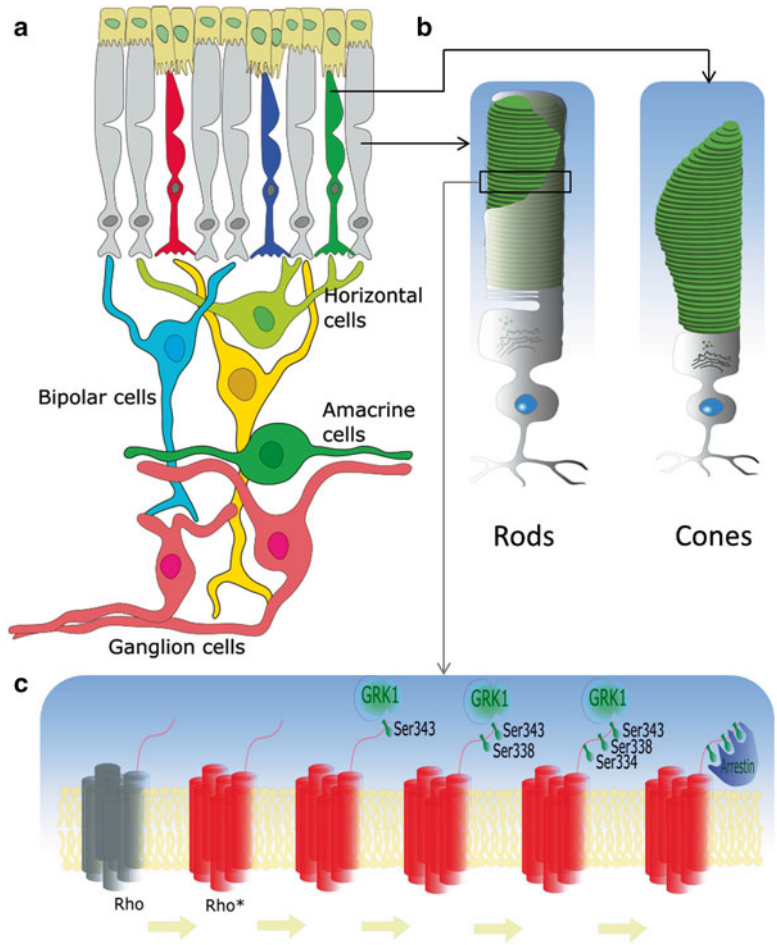

Fig. 1 Rod and cone photoreceptor cells. In the vertebrate system, rods specifically express GRK1 whereas cones express both GRK1 and GRK7. Panel **a** shows the layered organization of the retina with the major cell types involved in visual signal processing. Rods are shown in *grey*, and the three types of cones are colored in *red*, *blue*, and *green*, respectively. Following are the horizontal cells (*lime*), bipolar cells (*cyan* and *yellow*), amacrine cells (*green*), and ganglion cells (*orange*). Panel **b** shows a detailed view of the major components of the two types of photoreceptor cells: rods and cones. Rods possess a plasma membrane that covers the rod disks. Major differences between rods and cones include their shapes, and the cell specific set of gene products that affects responses under different light intensities. Panel **c** shows a simplified view of the Rho* activation and inactivation processes delineating the phosphorylation of residues catalyzed by GRK1 on Rho* and the subsequent binding and capping by arrestin

2 GRKs in Visual Transduction

2.1 The Role of GRKs in the Visual Signal Transduction Cascade

Vision is initiated when a photon of light interacts with the photosensitive chromophore of Rho and cone visual pigments [13]. The molecular mechanism of this event involves a series of sequential steps similar in both rod and cone photoreceptor cells that have been well characterized and reviewed elsewhere [14]. In

addition, enzymes that contribute to signal transduction, Rho/cone pigment desensitization, and chromophore regeneration also have been extensively studied and summarized [15, 16]. Following activation of Rho (Rho*) and its interaction with its cognate G protein, G_t (transducin), visual cascade deactivation is achieved by multiple mechanisms. Rho* is inactivated by phosphorylation catalyzed by GRK1, and G_t is inactivated through its intrinsic GTPase activity. Phosphorylation of Rho* was found to be mainly located on its C-terminal domain with three added phosphates representing the optimal number [17] (Fig. 1c). The main effect of this phosphorylation is an increased interaction with arrestin that results in a diminished interaction of phosphorylated Rho* with G_t [18]. The interaction with G_t is further impaired by the subsequent binding of arrestin to phosphorylated Rho* [19, 20] resulting in inhibition of the signal transduction process [17].

Phosphorylation of Rho following light activation was first reported in the 1970s [21–24] and then reviewed in the late 1990s [25, 26]. Rho* phosphorylation correlated with the quenching of cGMP phosphodiesterase (PDE) activity [27], indicating that phosphorylation of Rho* was somehow related to the desensitization process [28]. GRK1 preferentially catalyzed the phosphorylation of Rho* with most of the phosphorylation sites at Ser residues located in the C-terminal tail. Such Ser residues were phosphorylated in the following order: Ser343, Ser338, and Ser334 [29–32] (Fig. 1c). Phosphorylation of Thr residues within the C-terminal region of Rho was recently shown to be involved in facilitating arrestin binding [33]. This phosphorylation desensitization mechanism is prototypical of that of other GPCRs [34]. Dephosphorylation of Rho*, catalyzed by phosphatase 2A (PP2A) [35], showed a similar order of phosphate removal in the Rho recycling pathway [29]. A Rho molecule lacking its C-terminal domain lost its ability to be phosphorylated [30]. The interaction of GRK1 with Rho* is needed for effective phosphorylation.

The mammalian family of GRKs is comprised of 7 members named GRK1 through 7 (Fig. 2). The family was divided into three groups based on their primary sequence homology. The first group contains Rho kinase (GRK1) and cone opsin kinase (GRK7), both expressed in vertebrate retina. GRK1 also was the first kinase characterized in the family [36] (Fig. 2a). The second group is composed of β-adrenergic receptor kinase 1 and 2 (GRK2 and GRK3) with a broad specific tissue expression profile (Fig. 2b). The third group consists of GRK4, GRK5, and GRK6 (Fig. 2c). Members of this group also have no specific tissue expression pattern except GRK4, which has the highest expression in testes.

GRK1 was the first member of the family shown to inhibit Rho's function through phosphorylation and the consequent binding of arrestin [19, 20] as well as the first to be purified and cloned [36]. GRK7 was discovered a decade later in retinas of cone-

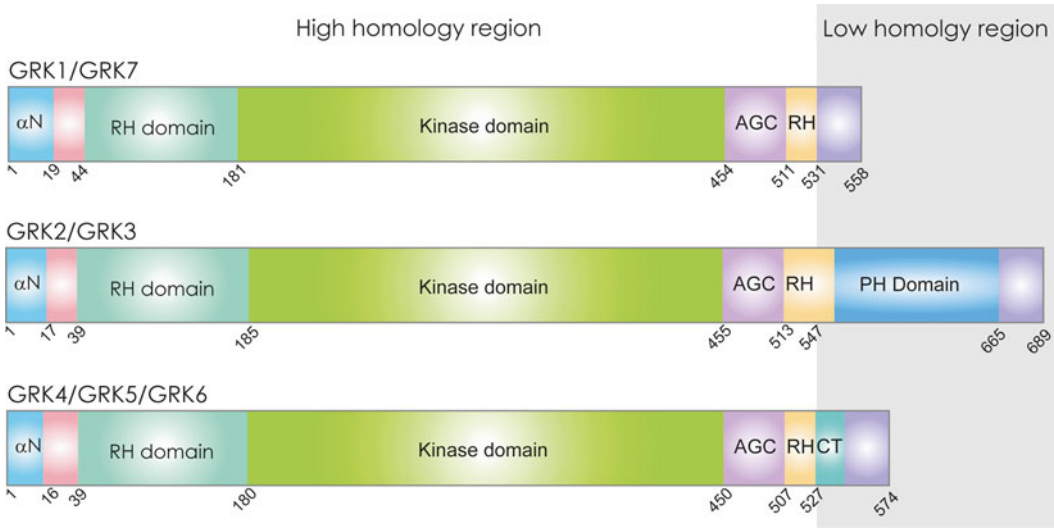

Fig. 2 Major domains and homology of GRK1 and 7 with other GRKs The principal domains of the seven GRK isoforms are shown as follows: α-domain (*blue*) and a connecting region (*red*) to the RH domain (*blue-green*). The AGC kinase domain is shown in magenta, the RH domain in *orange*, the PH domain (specific to GRK2/GRK3) in *dark blue*, and the C-terminal region in *violet*

dominant mammals [37, 38]. The expression pattern of GRK1 and GRK7 was found to be species-dependent [38–41]. All vertebrates express GRK1 in rods whereas in cones some vertebrates express either GRK7 alone or both GRK1 and GRK7 (reviewed in [42]). Mice and rats only express GRK1 in both rods and cones. In carp retina, there are four subtypes of GRK1 and GRK7, i.e., GRK1A-1a and 1A-1b, GRK1Ba and 1Bb, and two subtypes of GRK7-1a and 7-1b [43, 44]. GRK7 was designated as a cone-specific receptor kinase because its expression level was about ten times higher than that of rod-specific GRK1 [45] and its specific activity also was about ten times greater than that of GRK1. Knockdown of GRK7 in larval zebrafish demonstrated that this kinase is essential for cone-specific vision and that its absence resulted in a delayed cone response recovery and dark adaptation [46]. Co-expression of GRK7 in zebrafish rods with endogenously expressed GRK1 caused a considerable decrease in light sensitivity [47].

2.2 Deficiencies and Mutations in GRK1: Oguchi Disease

The gene for GRK1 is localized to chromosome 13, band q34, and encodes a protein composed of seven exons [48] (reviewed in [49]). Deficiencies in either GRK1 [50, 51] or arrestin [52, 53] were shown to cause prolonged insensitivity of rod-controlled vision after light exposure. The disease, termed Oguchi disease, is inherited as an autosomal recessive disorder. First described in Japanese patients, Oguchi disease was attributed to a homozygous

deletion [52] with later reports of heterozygous nonsense mutations in the arrestin gene [53, 54]. Following these initial reports, a homozygous deletion of exon 5 and 3 [55], heterozygous [50, 56], homozygous missense mutations [57, 58], or nonsense mutations [59] in GRK1 were also implicated. Clinically, the disease is characterized by a progressive delayed dark adaptation accompanied by near normal vision in bright light [5].

Differences in expression of GRK1 and GRK7 exist between species. For example, in mice, *Grk1−/−* affects both rod and cone photoreceptors [60, 61], where it was shown to cause severe defects in rod and cone recovery and retinal degeneration [62] (similar to the *arrestin-1−/−* mouse model). In contrast, in humans this effect was shown to be partially ameliorated by the expression of GRK7 in cones [63]. Another mouse model with cone-like photoreceptors (double knockout of GRK1 and the neural retina leucine zipper, *Grk1−/−Nrl−/−*) also developed age-related retinal dystrophy in a light-independent manner, further supporting a role of GRK1 in the maintenance of proper cone health [64]. Though deletion of the *GRK1* gene negatively affected photoreceptor health, GRK1 overexpression failed to protect photoreceptors [65]. These findings in animal models indicate that excessive activity of the phototransduction cascade coupled with exposure to high-intensity light causes photoreceptor cell death [60]. A study that evaluated the effect of variable expression of GRK1 on the kinetics of recovery from dim light stimulation concluded that Rho* inactivation could indeed modulate such recovery [66].

3 GRK1 in Visual Transduction

3.1 Structure of GRK1

X-ray structures provide a fundamental basis for learning how enzymes work at the molecular level, and understanding such mechanisms in the field of vision was greatly enhanced by using this approach (reviewed in [67]). X-ray structures have been determined for several members of the GRK family including: GRK1, GRK2 [68], GRK5 [69, 70], and GRK6 [71–73]. Some of these structures were solved in the presence of inhibitors or ATP. Homologous domains in GRKs are categorized as: the RGS homology (RH) domain, the kinase domain, and the highly homologous N-terminal domain comprised of ~20 amino acids (Fig. 2). X-ray structures do not provide as much information on the N-terminal domain (reviewed in [74]) as the structure of this part is disordered in most of the crystallized GRK structures. The only two structures where the N-terminal region was detected (in one structure of GRK1 and one of GRK6) are distinct, an artifact that could result from differences in crystal packing. Because of this shortcoming, we still lack a definitive molecular view of this domain [75]. The function of GRK1 was suggested to be

regulated by its N-terminal domain. The relevance of the N-terminal region was first described by following its interaction with specific antibodies, which inhibited the phosphorylation of Rho* but failed to prevent phosphorylation of synthetic peptides that had an identical sequence with Rho's C-terminal region [76]. A study that characterized the dynamics of GRK1 under a variety of conditions described a highly flexible molecule in the absence of ATP·Mg^{2+}. The dynamics of GRK1 in the presence of Rho, Rho*, ATP·Mg^{2+} reinforced the concept that conformational changes occur following interaction of GRK1 with either Rho* or ATP·Mg^{2+} [77]. Use of truncated GRK1 with the first 19 residues removed from the N-terminus provided further evidence about the importance of this sequence in the regulation of GRK1's function and interaction with Rho* [67, 76, 78, 79]. Because the interaction with a GPCR is expected to be similar for all GRKs, the same findings would pertain to other GRKs like GRK2 [80]. GRK1 is monomeric in solution, but crystallized in a dimeric form which was suggested to have a role in the function of GRKs. The dimer, also present in the GRK6 structure [72, 73], contained a hydrophobic patch conserved in all but GRK2 and GRK3. The GRK1-(L166K) mutant (a GRK1 with a mutation in the interface region of the dimer structure) crystallized in a novel space group as a monomer. This result is similar to that obtained with another GRK family member, GRK5 [69], which revealed a different conformation for the C-terminal region (amino acid residues 527–541). The structure of the C-terminal region contrasted with other structures reported for GRK1 and GRK6 but was similar to that of the GRK1-(L166K) mutant. In GRK5, however, the C-terminal structures were similar even though they were solved with a different crystal packing [69, 70].

One plausible working model by which GPCRs could activate GRKs is by inducing closure of the kinase domain. This would align the catalytic domain of the large lobe with the ATP-binding region of the small lobe. Conformational changes in GRK1 induced by binding of ATP could also favor closure in the kinase domain as evidenced by hydrogen-deuterium experiments [77] or as noted for other kinases such as protein kinase A (PKA) [81]. More experiments are needed to resolve the molecular details of GRK activation.

3.2 GRK1 Interactions with Membranes

To fully exert their activity on activated GPCRs, GRKs require an interaction with negatively charged phospholipids. Specific details about the interactions of GRK1 and GRK7 with membranes are limited. The GRK interaction site with membranes is proposed to be a relatively flat surface that involves residues near the N-terminal domain, C-terminal tail, and a linker region between the N-terminal and RH domains [78]. Recent structures of GRK5 also provide evidence supporting an interaction with the membrane through its C-terminal region [69, 70]. With the current proposed arrangement, the N-terminal region would be inserted deeply into the

membrane. This insertion is plausible for some GRK members because the N-terminal region contains several hydrophobic residues that would favorably interact with the hydrophobic membrane layer even though this region is not conserved and contains negatively charged residues in certain GRKs [78]. The C-terminal tails of GRKs are the least conserved regions among all GRKs and their involvement in the interaction with the phospholipid bilayer suggests the possibility of different recruiting mechanisms. Recent evidence suggests that in GRK5, interaction with the phospholipid membrane also involves a region from the RH domain (Leu135–Arg169), which appears to be conserved in GRK1 and GRK6 [82].

3.3 GRK1 Interaction with Rho

Because GRK1 competes with G_t to interact with Rho* (or constitutively active mutants of Rho [83]), some of the interaction regions on Rho* are expected to overlap [84]. Interaction of GRK1 with Rho* was described early as involving multiple sites [85]. Cytoplasmic loops I and II of Rho were shown to be needed for the interaction with GRK1 [86], in addition to cytoplasmic loop III previously found relevant for G_t binding [87, 88]. A 1:1 GRK1:Rho* stoichiometry sufficed for efficient phosphorylation of Rho* [89]. Likewise, a 1:1 ratio for the interaction of phosphorylated Rho* with arrestin was also reported [90]. Although a second Rho molecule does not seem strictly required for an interaction with arrestin or for GRKs to achieve full activity, a wealth of information indicates that Rho is dimeric in nature [91–94].

The first N-terminal 30 amino acids of GRK1 are involved in the interaction with Rho* (Fig. 3) [76, 77, 79, 95] together with a region of the C-terminal domain that encompasses residues 457 to 546 [96]. A recent study employing site-directed mutagenesis of the N-terminal region in GRK2 (Asp3-Glu18) evaluated the capability of mutated GRK2 to phosphorylate the β_2-adrenergic receptor. The interaction site with β_2-adrenergic receptor was found to involve the N-terminal domain together with an extension of the kinase domain including residues Gly475 to Ile485. This model of the GRK2-β_2-adrenergic receptor recapitulates that proposed for GRK1-Rho*-Rho [77] (Fig. 4).

3.4 High-Gain Phosphorylation of Rho

At low phosphorylation levels (<1%), as much as 50 mol of phosphate are incorporated per 1 mol of Rho* [97]. Similarly, when Rho was photoactivated at 0.04%, about 700 phosphates were incorporated per Rho* [98]. This effect is known as high-gain phosphorylation and one explanation could be that this "high-gain" reaction causes the phosphorylation of unbleached Rho molecules. High phosphorylation increases with elevated amounts of GRK1 and is quenched by the addition of recoverin and Ca^{2+} ions [65, 98]. High-gain phosphorylation, observed only under low illumination, exists because there are far less Rho* molecules than Rho molecules, and

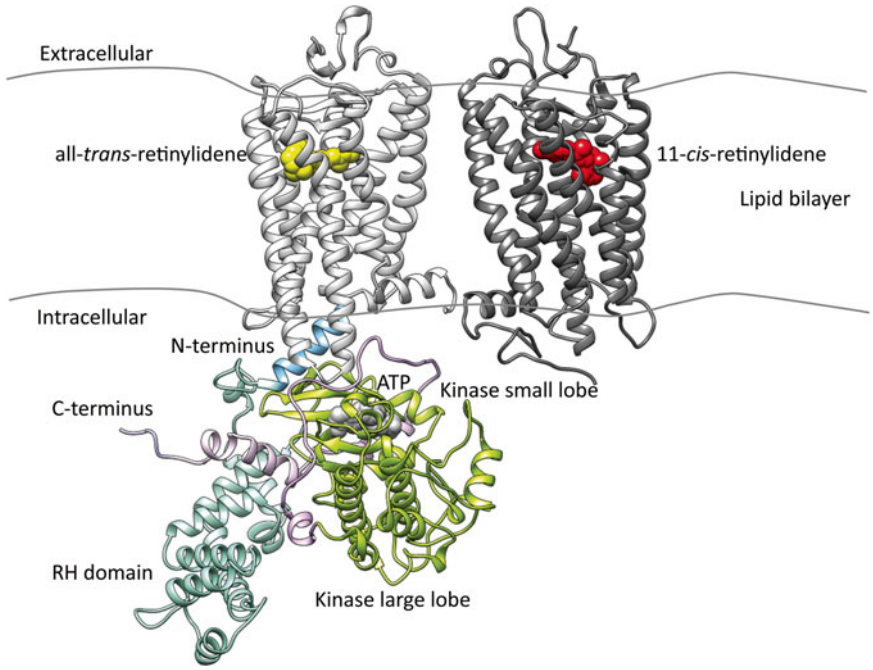

Fig. 3 Interaction of GRK1 with Rho*. The GRK1 molecule is modeled in a closed conformation. The position relative to Rho* was chosen based on the superposition of the N-terminal helix of GRK1 with the C-terminal helix of $G\alpha_s$ as described for the β_2-adrenergic receptor-G_s crystal structure [138]. Labeled GRK1 domains colored according to the code described in Fig. 1 are: the N-terminal region (Ser1-Arg19) in *light blue* and (Gly20-Leu44) in *pink*; the RH domain in *aquamarine* (Pro43 to Glu184 and Trp515 to Arg532); the kinase domain small lobe in *brown* (Asp185 to Asn268), and the large lobe in *green* (Gly269 to Pro514). The C-terminal region of GRK1 is depicted in *gray*. The homology model of the full GRK1 model was constructed using the GRK6·sangivamycin structure as a template [78]. The membrane wherein the Rho*-Rho heterodimer is inserted is depicted by wavy *grey lines*. A theoretical Rho*-Rho heterodimer is modeled in a helix 8-helix 8-dimer orientation. All-*trans*-retinylidene and 11-*cis*-retinylidene are depicted as *yellow* and *red spheres*, respectively. ATP bound to GRK1 is shown as light *grey spheres*. Dark state Rho is shown as a *dark grey cartoon*, whereas the light-activated monomer is in *light grey*

once activated by Rho*, GRK1 can also catalyze the phosphorylation of nearby Rho.

4 Regulation of GRK1

4.1 Post-translational Modifications

GRK1 from all species studied is post-translationally modified through farnesylation [99], except for chicken GRK1, which has a coding region that can be modified by geranylgeranylation in chicken liver extracts [100]. This modification of GRK1 could help direct the enzyme to the right locations in rods and cones because a non-farnesylated mutant had only a minimal effect on Rho* phosphorylation [101]. Consistent with the idea that lipid modification helps direct GRKs to appropriate compartments within photoreceptor

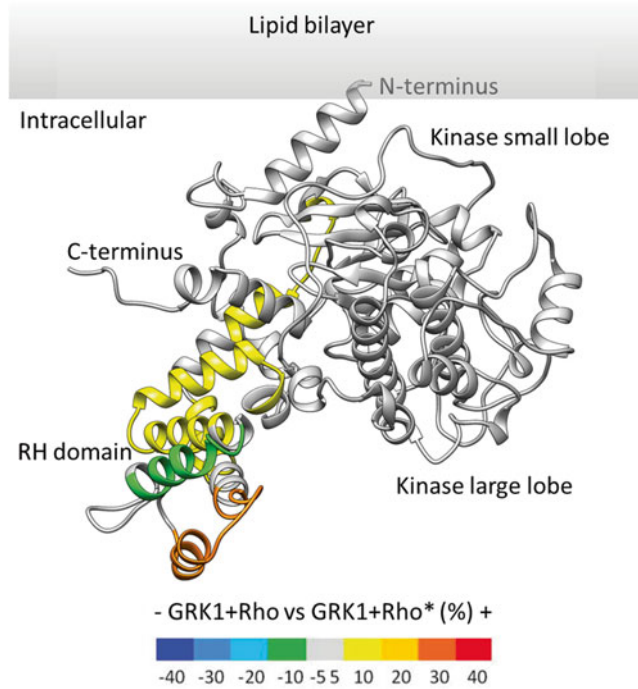

Fig. 4 Conformational changes in GRK1 upon interaction with Rho*. Low-resolution structural information obtained by hydrogen-deuterium exchange coupled to mass spectrometry provides the only information yet available that describes the conformational changes in GRK1 when complexed with Rho* [77]. The differences in normalized deuterium uptakes in the presence of either Rho or Rho* were mapped on the GRK1 molecule in an orientation similar to that described for the GRK1-Rho* model presented in Fig. 3. Color coding, defined as differences between the normalized deuterium uptakes, is shown for differences above the set threshold limit of 10 %. Positive differences (*yellow*, *orange*, and *red*) were assigned to the higher deuterium uptakes found in the case of GRK1 + Rho. Negative differences (*blue*, *cyan, light blue*, and *green*) denote higher uptakes in the GRK1 + Rho* sample

cells, GRK7 has a sequence predicted to be a geranylgeranyl modification site on its C-terminus. Post-translational modifications in GRK1 also involve C-terminal Cys residue α-carboxymethylation. Prenylation (addition of farnesyl or geranylgeranyl in the case of GRK1 and GRK7, respectively) followed by α-carboxymethylation thus could serve as hydrophobic anchors for GRK1 and GRK7 in membranes [102]. Increased GRK1 α-carboxymethylation in dark-adapted rods was found to be regulated by free nucleotides and high Ca^{2+} levels through their effects on recoverin, causing an increased association of GRK1 with recoverin and membranes [103]. As a consequence, increased GRK1 methylation in dark-adapted rods was attributed to increased Ca^{2+} levels. The increase of Ca^{2+} levels then additionally increases the association of GRK1 with both the membranes and recoverin, thereby providing a positive feedback decrease in the nonspecific phosphorylation of inactive Rho molecules.

This mechanism represents another option for increasing the light sensitivity of photoreceptors.

A third set of post-translational modifications of GRK1, other than prenylation and carboxymethylation, involves phosphorylation. Autophosphorylation of GRK1 [104] was found to affect GRK's affinity toward its substrates, both Rho* [105] and recoverin. Phosphorylated sites in GRK1 localized primarily on residues Ser488 and Thr489 [106]. The X-ray structure of GRK1 and further mass spectrometric analysis revealed two extra sites that also were phosphorylated, namely Ser5 and Thr8 [71]. However, GRK1 with mutations at these latter sites showed identical autophosphorylation rates with no obvious effects on ATP binding. This result suggests that these sites could be relevant for other functions of GRK1, such as membrane targeting and/or transport as evidenced by poor expression of some of the mutants [71]. In vitro phosphorylation of GRK1 by PKA at Ser21 and GRK7 at Ser23 and Ser36 in a cAMP-dependent fashion has also been reported [107]. These in vitro experiments were followed by in vivo studies which demonstrated that PKA-catalyzed phosphorylation of GRK1 was high in the dark-adapted state when cAMP levels also were elevated [108].

4.2 Protein Inhibitors

Recoverin is a Ca^{2+}-binding protein found to inhibit GRK1 function as part of the negative feedback of visual signal transduction [109–112] in both rods [113, 114] and cones [115]. Recoverin's interaction with GRK1 in the presence of Ca^{2+} was localized to the N-terminal region of both GRK1 (residues 1–25) [116, 117] and GRK7 [118], as reviewed in [119]. The interaction site on recoverin involved the C-terminal domain [120] together with a region of residues conserved among other Ca^{2+}-binding sensor proteins, suggesting that the binding site to GRKs would be partially conserved among Ca^{2+}-binding sensor proteins [121, 122]. The inhibitory efficacy of recoverin was also improved if this Ca^{2+}-binding protein was N-myristoylated [123]. These findings were mimicked by S-modulin, a recoverin ortholog in frogs, for both GRK1 and GRK7 [118, 124]. Another Ca^{2+}-binding protein, calmodulin (CaM), also can bind to GRK1 at residues between 150 and 175, although not with the same affinity as recoverin. In GRK5, CaM was shown to have a binding site localized between residues 20–39. In addition to the N-terminal region, the C-terminal region of GRK5 also was implicated in this interaction [125]. In the recently solved X-ray structure of GRK5, the positions of these two domains are in close proximity, suggesting that CaM might bind simultaneously to both [70]. This different binding pattern, as compared with GRK1, is due to the low homology in this region between these two GRKs, which explains the wide range of dissociation constants for CaM among various GRKs [126, 127]. Interactions of GRK1 with these two Ca^{2+}-binding proteins are synergistic, as evidenced by their different binding

sites on GRK1 and the broader range of Ca^{2+}-sensing capabilities when compared to recoverin alone [125, 128]. In carp, visinin (an S-modulin analog in cones) was shown to inhibit GRK7 with a greater potency than GRK1 was inhibited by S-modulin [129]. In addition to the native recoverin/CaM inhibition, antibodies developed initially for purification purposes were also found to inhibit the function of GRK1. This inhibition resulted from interactions with the kinase C-terminal region [130]. Finally, caveolins were shown to inhibit GRK1 activity: peptides derived from caveolin-1 and caveolin-3 had IC_{50} values of 2.7 and 1.8 μM, respectively [131]. Although the exact mechanism by which caveolin inhibits GRK1 remains unknown, a recent study provided evidence for co-localization of caveolin with Rho and GRK1 in vertebrate retina [132].

4.3 Small-Molecule Inhibitors of GRKs

The first small-molecule inhibitors of GRK1 were biochemically identified from a family of nucleoside analogs, the most potent being sangivamycin (K_i = 180 nM) [133, 134] and toyocamycin [134]. Sangivamycin binds to GRK6 with a dissociation constant of 1 μM, about 30-fold less than its analog, adenosine [72]. The structure of GRK6 in the presence of sangivamycin determined at a 2.7 Å resolution revealed that this inhibitor interacted with GRK6's ATP-binding site located between the small and large lobes of the kinase domain in the canonical ATP binding site [72]. X-ray crystal structures of GRK1 were obtained with a variety of inhibitors [135, 136]. Paroxetine, a specific GRK2 inhibitor, was found to bind to GRK1 and GRK5 with 16- and 13-fold lower affinities than to GRK2 [137]. A specific inhibitor of GRK2 and GRK5 denoted as CCG215022 was found to have nanomolar IC_{50} values and selectivity against both GRK1 and PKA. The X-ray structure of the GRK5·CCG215022 complex also was recently solved [70]. The kinase domain in this complex was similar to that of GRK6-sangivamycin with only the C-terminal region adopting a different orientation that could interact with the membrane [70].

5 Future Directions

During the past decade there has been much progress in deciphering the molecular basis of GRKs activation, inhibition, and interactions with their molecular targets, such as activated GPCRs (see review [49] for a 30 years progress report up to 2003). This field has advanced greatly from structural studies done on four members of the GRK family, i.e., GRK1, GRK2, GRK5 and GRK6. This research area would benefit greatly from any structure of an activated GRK. Although much has been accomplished with lower-resolution methods used to characterize the GRK1-Rho* complex, high-resolution structures are still needed to provide a molecular view of the interaction between a GRK and its substrate GPCR.

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