Cellular Signalling xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Review

G protein-coupled receptor kinases: Past, present and future

Konstantin E. Komolov, Jeffrey L. Benovic*

Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA 19107, United States

ARTICLE INFO

Keywords:
Arrestins
GPCR
GRK
Phosphorylation
Signaling
X-ray crystallography

ABSTRACT

This review is provided in recognition of the extensive contributions of Dr. Robert J. Lefkowitz to the G protein-coupled receptor (GPCR) field and to celebrate his 75th birthday. Since one of the authors trained with Bob in the 80s, we provide a history of work done in the Lefkowitz lab during the 80s that focused on dissecting the mechanisms that regulate GPCR signaling, with a particular emphasis on the GPCR kinases (GRKs). In addition, we highlight structure/function characteristics of GRK interaction with GPCRs as well as a review of two recent reports that provide a molecular model for GRK-GPCR interaction. Finally, we offer our perspective on some future studies that we believe will drive this field.

1. A brief history of GRKs

The history of G protein-coupled receptor kinases (GRKs) really began with the identification of an enzymatic activity in rod membranes that could phosphorylate rhodopsin in a light-dependent manner [1]. This enzyme was called rhodopsin kinase (now GRK1) and it was subsequently purified and found to specifically phosphorylate light-activated rhodopsin [2]. Similar studies in the Lefkowitz lab during the late 70s and early 80s were focused on understanding the mechanisms involved in the loss of responsiveness of β-adrenergic receptor (βAR) signaling following prolonged stimulation with agonist (a process called desensitization). These studies revealed that the βAR underwent a mobility shift on SDS PAGE following agonist treatment [3]. This mobility shift was subsequently shown to be due to phosphorylation of the receptor [4], and additional studies established that at least some of this phosphorylation was due to the cAMP dependent protein kinase (PKA) [5]. In vitro studies demonstrated that PKA could directly phosphorylate the β_2AR to a stoichiometry of 2 mol phosphate/mol receptor and that this phosphorylation attenuated receptor coupling to the heterotrimeric G protein G_s [6]. Thus, these early studies identified a mechanism of feedback regulation that involved phosphorylation of the β_2AR by PKA, the protein kinase activated by the βAR signaling pathway. This feedback regulation of the β_2AR by PKA was termed heterologous desensitization.

While a role for PKA phosphorylation of the β_2AR was evident from these early studies, additional studies in the Lefkowitz lab revealed that the β_2AR could also be phosphorylated in an agonist-dependent manner in S49 lymphoma cell lines that lacked the ability to activate PKA [7]. This observation led to a search for the enzyme that phosphorylated the β_2AR in an agonist-dependent manner and ultimately resulted in the identification of the β-adrenergic receptor kinase or βARK (now called GRK2) [8]. βARK was analogous to rhodopsin kinase, given that both enzymes phosphorylated the active conformation of the receptor, and raised interesting questions about the similarities between phototransduction through rhodopsin and hormonal signaling through the β_2AR [9]. Indeed, subsequent studies revealed that βARK could also phosphorylate light activated rhodopsin while rhodopsin kinase could phosphorylate the agonist-occupied β_2AR [10]. Additional studies suggested that βARK had broad specificity since activation of multiple receptors promoted its translocation from the cytosol to the plasma membrane [11,12]. Moreover, βARK was also able to directly phosphorylate the α2-adrenergic receptor in vitro [13]. βARK was eventually purified [14] and cloned [15] revealing that it is a 689 amino acid serine/threonine protein kinase that specifically phosphorylates the agonist-occupied form of GPCRs such as the β_2AR. Moreover, the cloning studies suggested that βARK is likely a member of a larger family of G protein-coupled receptor kinases [15].

During the course of these studies, another protein that contributes to receptor desensitization was identified. This protein was initially identified in the visual system and was termed S-antigen or 48 kDa

Abbreviations: αN-helix, N-terminal α-helical domain; α_{2A}AR, α_{2A}-adrenergic receptor; AST, active site tether; βAR, β-adrenergic receptor; β_2AR, β_2-adrenergic receptor; βARK, β-adrenergic receptor kinase; CLBD, C-terminal lipid binding domain; EM, electron microscopy; GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; GST, glutathione-S-transferase; HDX-MS, hydrogen deuterium exchange mass spectrometry; ICL, intracellular loop; MD, molecular dynamics; mGluR1, metabotropic glutamate receptor 1; NLBD, N-terminal lipid binding domain; PG, phosphatidylglycerol; PH, pleckstrin homology; PKA, cAMP dependent protein kinase; RH, regulator of G protein signaling homology; TM, transmembrane

* Corresponding author.
E-mail address: jeffrey.benovic@jefferson.edu (J.L. Benovic).

http://dx.doi.org/10.1016/j.cellsig.2017.07.004
Received 7 July 2017; Accepted 10 July 2017

protein and later named arrestin by Herman Kühn [16,17]. Arrestin had the interesting property of binding to light activated rhodopsin that had been phosphorylated by rhodopsin kinase and was found to quench phototransduction [16]. Studies in the Lefkowitz lab identified a similar role for an arrestin in desensitizing β_2AR signaling in a βARK-dependent manner [18]. These efforts ultimately led to the identification of a non-visual arrestin termed β-arrestin that specifically binds to βARK-phosphorylated β_2AR to inhibit receptor interaction with G_s [19]. Thus, these early studies revealed that GRKs play a central role in promoting arrestin binding to agonist-activated GPCRs to turn off receptor activation of heterotrimeric G proteins, a process termed homologous desensitization.

Once βARK was cloned, additional efforts led to the cloning of βARK2 (now called GRK3) [20], rhodopsin kinase [21], IT11 (now called GRK4) [22], GRK5 [23], GRK6 [24] and GRK7 [25,26]. The seven mammalian GRKs contribute to the phosphorylation and regulation of hundreds of G protein-coupled receptors (GPCRs). While GRKs have been extensively reviewed [27–33], here we focus on our current understanding of how GRKs interact with activated GPCRs.

2. Structure/function analysis of GRK-GPCR interaction

2.1. GRK structure

GRKs are serine/threonine protein kinases most related to the AGC kinase subfamily. GRKs have a modular structure with a central catalytic domain that sits within a regulator of G protein signaling homology (RH) domain [34,35] that is bracketed by a short N-terminal α-helical domain (αN-helix) and a variable C-terminal lipid-binding region [36] (Figs. 1 and 2). This basic structure is conserved in all GRKs going back to unicellular eukaryotes and non-metazoan opisthokonts [37]. The C-terminal region mediates membrane localization via prenylation (in GRK1 and 7), palmitoylation (in GRK4 and 6), or direct lipid binding either via a pleckstrin homology (PH) domain (in GRK2 and 3) or a polybasic/hydrophobic domain (in GRK5).

To date, all mammalian GRKs except for GRK3 and GRK7 have been crystallized. The first published structure was of GRK2 in complex with Gβγ from the Tesmer laboratory [38]. This structure provided important insight including the observation that the kinase domain is inserted into the RH domain and that contacts between the RH and kinase domains help to maintain the kinase in an inactive, open conformation. The X-ray crystal structure of GRK6 revealed a similar architecture with the RH domain making extensive contacts with the kinase domain, which remains in an open conformation even with a bound ATP analog [39]. The RH domain also forms an extensive dimer interface in GRK6 and while it is unclear whether this has a physiological role, there is evidence that a similar interface in GRK5 plays a role in membrane localization [40]. Interestingly, GRK6 was also crystallized in a more active conformation with a partially closed kinase domain and an extended αN-helix that bridged the kinase domain [41]. The authors proposed that this structure provides potential insight into a conformation similar to GRK bound to a receptor. GRK1 was crystallized next and found to homodimerize using a conserved interface within the RH domain [42]. GRK1 also crystallized in several conformations including some that revealed the C-terminal extension of the kinase domain and one where the αN-helix was observed. Based on the

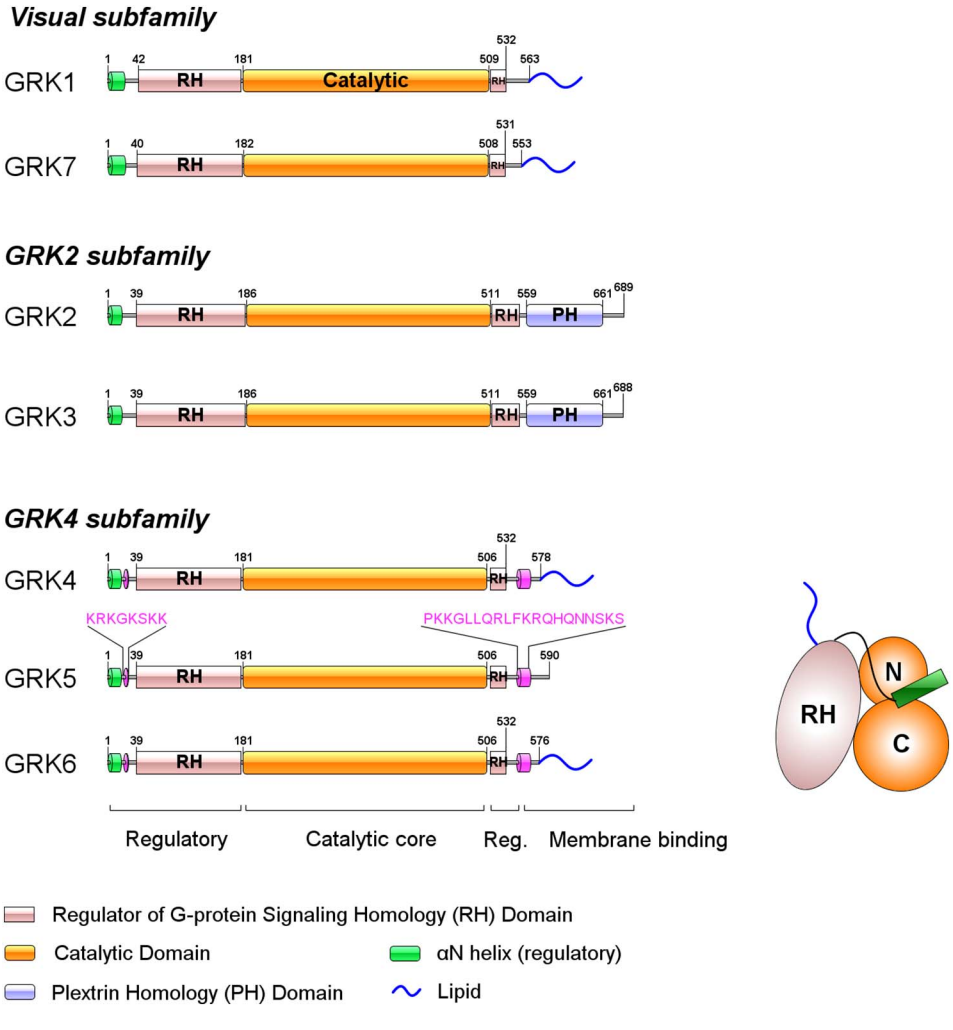

Fig. 1. General architecture of GRKs. GRKs are divided into 3 subfamilies based on sequence homology and are composed of two main domains, regulator of G protein signaling homology (RH) and catalytic domains. αN-helix comprising the first ~20 residues plays a regulatory role by bridging the N- and C-lobes of catalytic domain. The C-terminal fragment mediates membrane localization of GRKs. GRK4 subfamily includes two polybasic regions at N- and C-termini, and GRK5 relies on these regions to interact with negatively-charged phospholipids. GRK2 and GRK3 have a PH domain that interacts with acidic phospholipids and Gβγ subunits.