REVIEW
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Research progress on the structure and physiological functions of PKG

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Competing interests
The authors declare no conflicts of interest.

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Abbreviations
PKG, Protein Kinase G; cGMP, cyclic guanosine monophosphate; VASP, vasodilator-stimulated phosphoprotein; KZ, leucine zipper; CNB, cyclic nucleotide-binding domain; ATP, Adenosine Triphosphate; PBC, phosphate-binding box; FSH, follicle-stimulating hormone; LH, luteinizing hormone; JAK, Janus kinase;

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Abstract
Protein Kinase G (PKG) is an important intracellular signal transduction enzyme, and its activity is modulated by cyclic guanosine monophosphate (cGMP). PKG plays a pivotal role in various significant physiological processes, including vascular smooth muscle relaxation, myocardial cell function regulation, neuron growth, and synaptic plasticity, et al. In recent years, the role of PKG in diseases has gradually attracted attention, and the abnormalities in its signaling pathway are closely related to the occurrence and development of cardiovascular and neurological diseases. Although PKG has been widely studied, its complex functions in different physiological systems and potential innovative applications still need to be further explored. This article reviews the purification techniques for PKG, discusses the advantages and disadvantages of different extraction methods, summarizes the structure and activation mechanism of each domain of PKG, and analyzes the physiological functions of PKG in organisms, especially the well-established roles in the cardiovascular system, nervous system, and endocrine system. The emerging therapeutic applications of PKG are also reviewed. In addition, the challenges of this field are proposed at the end.

Keywords: protein kinase G; plasmid expression technology; structural domain; activation mechanism; physiological function

Introduction

Protein kinase G (PKG) is a cyclic nucleotide-dependent protein kinase widely present in eukaryotic cells. As a core molecule that regulates various key physiological processes [1], PKG transfers phosphate groups to serine or tyrosine sites of target proteins by binding to the intracellular second messenger cGMP, thereby regulating its functional state [2]. PKG plays a unique integrative role in cellular signaling pathways, significantly affecting basic physiological activities such as cell proliferation [3, 4], differentiation [5], and migration [6]. Given its diverse and complex functions, PKG has become an important target for studying cell biology and its disease mechanisms.

In recent years, the importance of PKG in disease research has gradually become prominent. Research has shown that abnormal signal transduction pathways of PKG are closely related to various major diseases, including cardiovascular diseases, neurological diseases, and endocrine diseases [7–11]. Despite decades of research, several critical questions remain unanswered regarding PKG's structural regulation, activation dynamics, and functional diversity. One of the major challenges is the variation in PKG functional activity when expressed in different biological systems. Various extraction techniques-including bacterial, insect, and mammalian cell expression systems-yield PKG with differing post-translational modifications and functional properties, yet a systematic comparison of these methods is lacking. Furthermore, while the structural domains of PKG have been extensively characterized, the precise mechanisms governing its activation and domain interplay require further elucidation. Additionally, the emerging roles of PKG in metabolic regulation are not well-integrated into current models of PKG function, leaving gaps in our understanding of its broader physiological relevance. Beyond fundamental research, the application of PKG as a therapeutic target remains underexplored. While its roles in cardiovascular, neurological, and metabolic diseases are well-established, recent discoveries suggest its potential in immune modulation and cancer therapy.

This article reviews the purification techniques for PKG and summarizes its structural characteristics, mechanism of action, and key physiological functions and applications across various systems. By integrating recent advances and identifying future research directions, this review provides a reference for the research and application of PKG and promotes the application research of PKG in disease treatment.

The Extraction of PKG

At the beginning of the 21st century, researchers successfully obtained PKG in different biological systems through the direct extraction method. Sewit et al. removed the fetal lamb from the pregnant ewe, isolated the pulmonary vein of the fetal lamb, purified PKG from it, and demonstrated the inhibitory effect of hypoxia on the cGMP-PKG signaling pathway in the regulation of vasodilation [12]. Chu et al. isolated the bovine pulmonary aorta to obtain PKG to study the basic conformational changes after PKG phosphorylation [13]. Martin et al. utilized a centrifugal technique to isolate platelets from uncoagulated plasma, and PKG was detected in the platelets. It was elucidated that PKG can phosphorylate the vasodilator-stimulated phosphoprotein (VASP), and it is speculated that PKG may have the effect of inhibiting platelet activation [14]. Despite the simplicity and efficiency of the direct extraction method, the majority of the obtained products are crude extracts with low purity and high raw material and consumable costs.

The invention and improvement of plasmid expression technology have led to the widespread adoption of the method of obtaining PKG by constructing a plasmid expression system. Escherichia coli, yeast, insect cells, and animal cells are all common host cells for PKG expression. The selection of host cells for transfection is contingent upon the specific experimental requirements. Table 1 provides a comprehensive list of the advantages and disadvantages of obtaining PKG by transfecting different cells.

Escherichia coli is characterized by its ease of cultivation and brief growth cycle, thus rendering it a prevalent choice for the production of PKG through the employment of a plasmid expression system. The phosphorylation modification process of PKG protein is constrained by the inherent limitations of Escherichia coli systems, including its compact genome architecture, incompatibility with heterologous expression systems, deficiency in essential regulatory elements (promoters, terminators), and absence of critical signaling components such as ligands, phosphatases, and cofactors required for complex signal transduction pathways. Escherichia coli's capacity to produce only a single, incompletely folded PKG polypeptide chain hinders the formation of its complete three-dimensional structure, thereby restricting its capacity for complex modifications. As a consequence, PKG expressed by the Escherichia coli expression system often exhibits suboptimal functionality [15]. In comparison with the Escherichia coli expression system, insect cells exhibit more complex genomic architecture and possess relatively complete signaling pathways capable of providing comprehensive cofactors for PKG post-translational modifications. Additionally, their intrinsic cellular environment sustains a stable energy substrate supply, thereby reducing artificial interference with protein functionality under experimental conditions. The PKG obtained by the insect cell expression system exhibits enhanced functionality and meets the requirements of most experimental settings [16]. Sebastian et al. purified PKG using two expression systems, Escherichia coli, and insect cells and found that the PKG expressed by the Escherichia coli expression system only has a simple phosphorylation function and is highly unstable. To obtain a stable and simply functional PKG, a complex gene editing process is required [17]. In contrast, the insect cell expression system circumvents the complex gene editing process, and the cell culture is not difficult. Furthermore, the activity of the obtained PKG is increased by 103 times [16]. Consequently, the insect cell expression system has more advantages and is currently the most popular method for obtaining PKG. In addition to the Escherichia coli and insect cell expression systems, a mammalian cell expression system exists. The mammalian cell expression system demonstrates superior post-translational modification capabilities compared to insect cell systems. Moreover, mRNA templates derived from mammalian cells exhibit higher purity and are devoid of extraneous genetic contaminants, ensuring the functional integrity of expressed PKG proteins remains uncompromised by extraneous genetic interference, thereby yielding more biologically competent PKG. However, the mammalian cell culture process is intricate and demanding, necessitating a stringent experimental environment. The mammalian cell expression system with a sophisticated modification system is reserved for high-precision experiments, such as the analysis of PKG structure [18].

Table 1 Different cells for PKG extraction

Type of cells	Type of PKG	Molecular weight	Temperature for cell culture	Protein purification device	Protein activity	Advantages	Disadvantages	Ref.
Escherichia coli	PKG Iα	75 KDa	18 ℃	HiLoad™26/60Superdex™200 gel filtration liquid chromatography column	427 pmol/min/mg	Fast proliferation, short experimental period	Protein lacking modification and partial function loss	[15]
	PKG Iβ	78 KDa	20 ℃	BioRad Profinia purification system	/	Fast proliferation, short experimental period	Protein lacking modification and partial function loss	[19]

Table 1 Different cells for PKG extraction *(continued)*

Type of cells	Type of PKG	Molecular weight	Temperature for cell culture	Protein purification device	Protein activity	Advantages	Disadvantages	Ref.
Sf9 insect cell	PKG Iα	75 KDa	28 ℃	8- (2- aminoethyl)- amino cAMP agarose column	About 1.3×10^{6} pmol/min/mg	Easy to culture	Limited functions	[16]
Human ovarian cancer cell	PKG Iα PKG Iβ	75 KDa 78 KDa	37 ℃	Polyacrylamide NuPage gel column	About 1.3×10^{4} pmol/min/mg	Accurate expression, capable of complex modification	Difficult to culture cells, strict requirements for the experimental environment	[18]
Rat aortic smooth muscle cell	PKG Iα	78 KDa	37 ℃	/	0.8 pmol/min/mg	Accurate expression, capable of complex modification	Difficult to culture cells, strict requirements for the experimental environment	[20]
Rat vascular smooth muscle cell	PKG Iα	78 KDa	37 ℃	/	/	Accurate expression, capable of complex modification	Difficult to culture cells, strict requirements for the experimental environment	[21]

PKG, Protein Kinase G

The structure of PKG

PKG is comprised of two forms: soluble protein PKG I and membrane protein PKG II [22]. Among them, PKG I includes PKG Iα and PKG Iβ. PKG I and PKG II are expressed by two different genes, prkg1, and prkg2 respectively, and have similar structures, both containing regulatory domains and catalytic domains (Figure 1).

Regulatory Domain (R Domain) of PKG

The regulatory domain is located at the nitrogen end of PKG, also known as the R domain. This domain contains the nitrogen-terminal leucine zipper (LZ), the cyclic nucleotide-binding domain (CNB), and the isozyme-specific linker (AI) (Figure 1A) [23]. In the absence of activation, PKG I exists as a monomer. Upon activation, a conformational change occurs, resulting in the polymerization of PKG Iα and PKG Iβ into a dimer (Figure 1B) [24]. The dimerization interface of the LZ domain is stabilized by extensive hydrophobic interactions. The dimer consists of two helices that intertwine to form a parallel left-handed coiled coil. The interface covers about 1300 $Å^2$ of surface area, with over eight continuous heptad repeats contributing to its structural stability. Unlike typical leucine zippers, where leucines predominantly occupy position d, the PKG Iβ LZ domain has leucines and isoleucines primarily at position a, while charged or hydrophilic residues (Lys14, Arg21, Tyr49, etc.) occupy position d. These residues form interhelical hydrophobic interactions via a 'knobs-into-holes' packing mechanism, except for Tyr49, whose bulky aromatic ring adopts a unique head-to-tail stacking arrangement to stabilize the dimer. This distinctive packing pattern contributes to the rigidity and specificity of the PKG Iβ dimer structure. The LZ plays a pivotal role in the dimerization process of PKG I due to its disulfide bond, and the LZ can also promote the specific binding of PKG to G-kinase-anchoring protein (GKAP) [25]. The interaction between LZ and GKAP has been demonstrated to facilitate the transmission of signals from major signal pathways, such as calcium ion release [19, 26]. However, the underlying molecular mechanism remains to be fully elucidated. Lee et al. hypothesize that this specificity arises from the charge recognition between amino acids, yet the specific mechanism of action remains to be elucidated.

The CNB domain, which is the binding region of the activator, has been determined to be the critical factor in determining the functionality of PKG. Consequently, numerous researches have been conducted in this field. Schwede and Corbin et al. found that the CNB domain's affinity for cGMP varies [23, 27]. The CNB domain's binding affinity to cGMP is classified into two distinct sites: cGMP-binding site A (CNB-A) and cGMP-binding site B (CNB-B) (Figure 1A) [23]. The observed difference in binding force is attributed to the structural disparities between CNB-A and CNB-B. A detailed analysis of the structures of CNB-A and CNB-B by James C. Campbell et al. revealed that the CNB-A domain is a conserved protein folding structure composed of two secondary structures: an α helix and a β fold. The αA helix located at the nitrogen terminus and eight β folds constitute the binding pocket of the cyclic nucleotide, while the phosphate-binding box (PBC) situated between the sixth and seventh β folds is capable of binding the sugar-phosphate component of the cyclic nucleotide (Figure 1C) [23]. Given the absence of amino acid residues that specifically bind to cGMP in the CNB-A structure, there is an absence of discernible selectivity in the binding to cGMP. CNB-B, on the other hand, contains two helices, αB and αC, three folds, β4, β5, β6, and a PBC binding pocket (Figure 1D) [19]. The Tyr351 on the αC helix possesses an aromatic group that can interact with cGMP through P stacking, thereby providing a capping and stabilizing effect for the binding of PKG to cGMP [28], thus ensuring a more robust binding of cGMP to PKG. R473 on β5, R492, A485, and E483 on PBC form hydrogen bonds with cGMP (Figure 1D) [16, 19, 29]. The hydrogen bonds enhance the binding affinity of cGMP to PKG and induce a conformational change in PKG, thereby providing a structural basis for the activation of PKG. The superposition of multiple interactions renders the binding of CNB-B to cGMP highly specific. While the selectivity of CNB-A and CNB-B to cGMP is largely understood, experimental findings demonstrate that the binding of cGMP to CNB-A and CNB-B is mutually influenced, though the mechanism by which this information is transmitted remains to be elucidated.

Catalytic Domain (C Domain) of PKG

The catalytic domain is located at the carboxyl terminus and covers the ATP binding site, the target protein interaction domain, and the C-terminal residues whose functions are not yet clear (Figure 1A). Similar to the regulatory domain, the catalytic domain is also composed of β-sheets and α-helices, but there is a highly acidic active site between the large and small lobe structures it forms, which can bind Adenosine Triphosphate (ATP) and substrates [13], and transfer the γ-Pi on ATP to the serine, threonine or tyrosine residues of the substrate, promoting the phosphorylation of the substrate [12]. It has been demonstrated that the catalytic domain can indirectly affect the stability of the dimer, proteolysis, and the ability of the CNB domain to bind to cGMP [30]. Kim C. et al. found that the catalytic domain has an impact on the function of the regulatory domain [15], but the specific mechanism remains unclear to date. Currently, the research on the regulatory domain and the catalytic domain has been relatively in-depth, the structures of the two domains have been basically determined, and their functions have also been understood to some extent. However, the interaction mechanisms between the two domains and with other domains are still being explored, especially

since the understanding of the catalytic domain is relatively limited. Future studies will be dedicated to revealing the fine regulatory mechanisms and their synergistic effects between domains to achieve complex biological functions. At the same time, using advanced technical means, such as single-molecule technology and time-resolved spectroscopy, to observe the dynamic behavior and interaction of the regulatory domain and the catalytic domain in real-time, to modify and optimize them for application in drug design and individualized medicine. These directions are precisely the goals that current scientists are striving for.

The activation of PKG

In the absence of cGMP in the environment, the R domain is locked onto the C domain through the connection of the αC helix, thereby maintaining the ground state and preserving its inactivity [31]. This results in an ordered helical state. However, in the presence of cGMP, a sequence of events unfolds. Initially, cGMP binds to PKG, which results in an elongation of the PKG as a whole (Figure 2A) [31]. Concurrently, the PBC binding pocket, due to its open state and the presence of hydrophobic interactions, undergoes a movement that brings it closer to the direction of the β-sheet, in conjunction with the αB helix. This ultimately leads to the encapsulation of cGMP [19]. Consequently, C domain and R domain will interact with each other and release the catalytic domain (Figure 2B) [19]. This process is influenced by the Tyr351 residue of the αC helix in the regulatory domain. A significant number of capping residues, specifically Tyr351, are recruited by guanine, resulting in the rearrangement of the αB and αC helices and the subsequent disorder of the αC helix. This, in turn, is believed to be a response to the binding of cGMP, leading to the release of the catalytic domain and, consequently, the activation of PKG. The precise mechanism by which the catalytic domain is released remains to be elucidated, as does the information transfer process between the catalytic and regulatory domains.

The physiological functions of PKG

PKG has been extensively studied for its regulatory roles in various physiological systems, particularly in the nervous system, cardiovascular system, and endocrine system, where its functions are well established (Figure 3) [7–11]. These applications have led to significant advancements in conditions such as blood pressure regulation [32], neuronal growth and differentiation [5], and fat metabolism [33]. However, emerging research suggests that PKG also plays a critical role in immune modulation and cancer therapy which are still being actively explored. Understanding PKG's involvement in these fields could open new avenues for targeted therapeutic strategies, highlighting its potential as a key regulator in disease intervention.

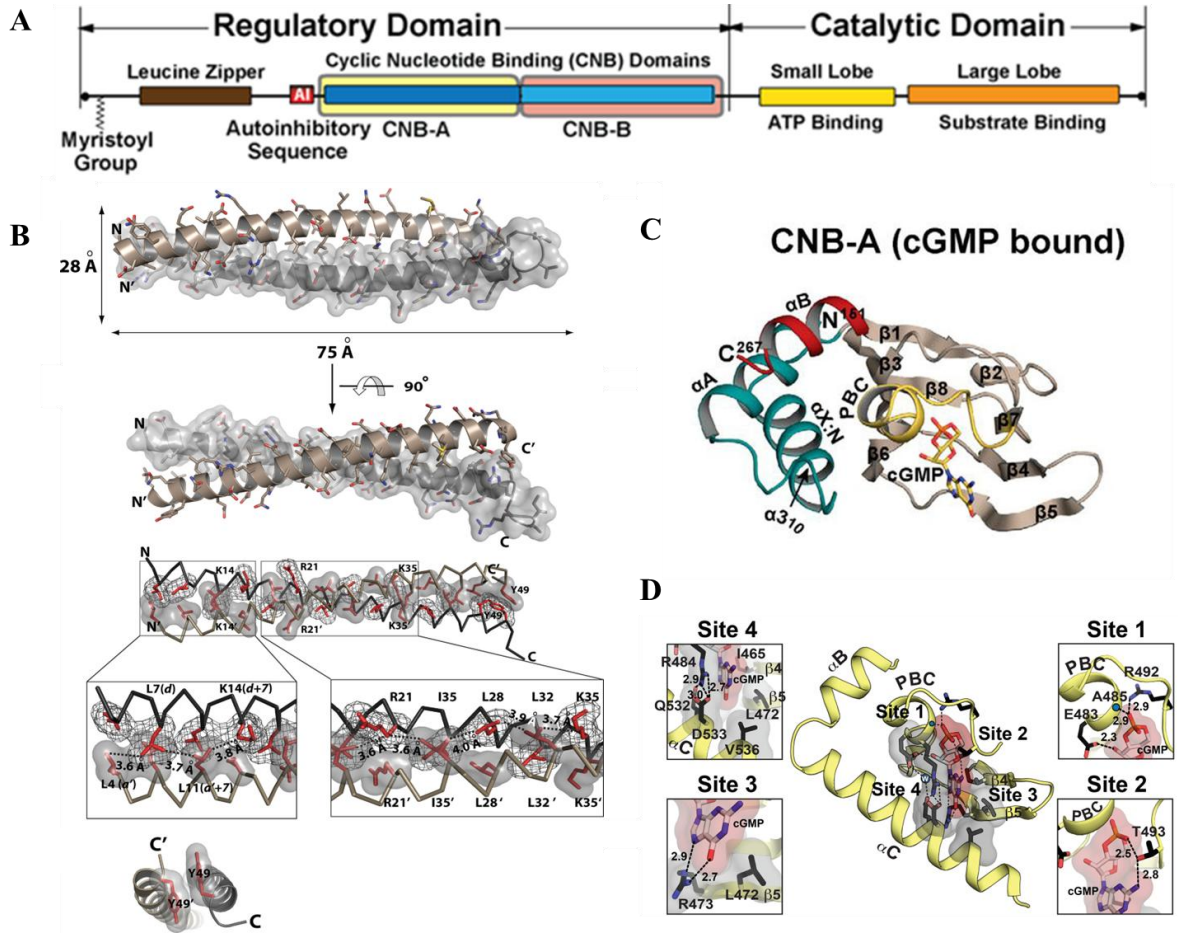

Figure 1 The structure of PKG. (A) PKG I domain sequence diagram. (B)The hydrophobic interactions at the dimer interface of PKG Iβ domain. (C) Schematic diagram of the crystal structure of CNB-A and cGMP binding in PKG I. (D) Schematic diagram of the crystal structure of CNB-B and cGMP binding in PKG I. Reproduced with permission. PKG, Protein Kinase G; cGMP, cyclic guanosine monophosphate; ATP, Adenosine Triphosphate; PBC, phosphate-binding box. Campbell James C, Kim Jeong Joo, Li Kevin Y, et al. Structural Basis of Cyclic Nucleotide Selectivity in cGMP-dependent Protein Kinase II. *J Biol Chem*, 2016; 291(11): 5623–5633. Copyright 2016, Elsevier. Reproduced with permission. Darren E. Casteel, Eric V. Smith-Nguyen, Banumathi Sankaran, et al. A Crystal Structure of the Cyclic GMP-dependent Protein Kinase Iβ Dimerization/Docking Domain Reveals Molecular Details of Isoform-specific Anchoring. *J Biol Chem*, 2010;285(43): 32684–32688. Copyright 2010, Elsevier. Reproduced with permission. Kim JJ, Flueck C, Franz E, Sanabria-Figueroa E, et al. Crystal Structures of the Carboxyl cGMP Binding Domain of the Plasmodium falciparum cGMP-dependent Protein Kinase Reveal a Novel Capping Triad Crucial for Merozoite Egress. *PLoS Pathog*

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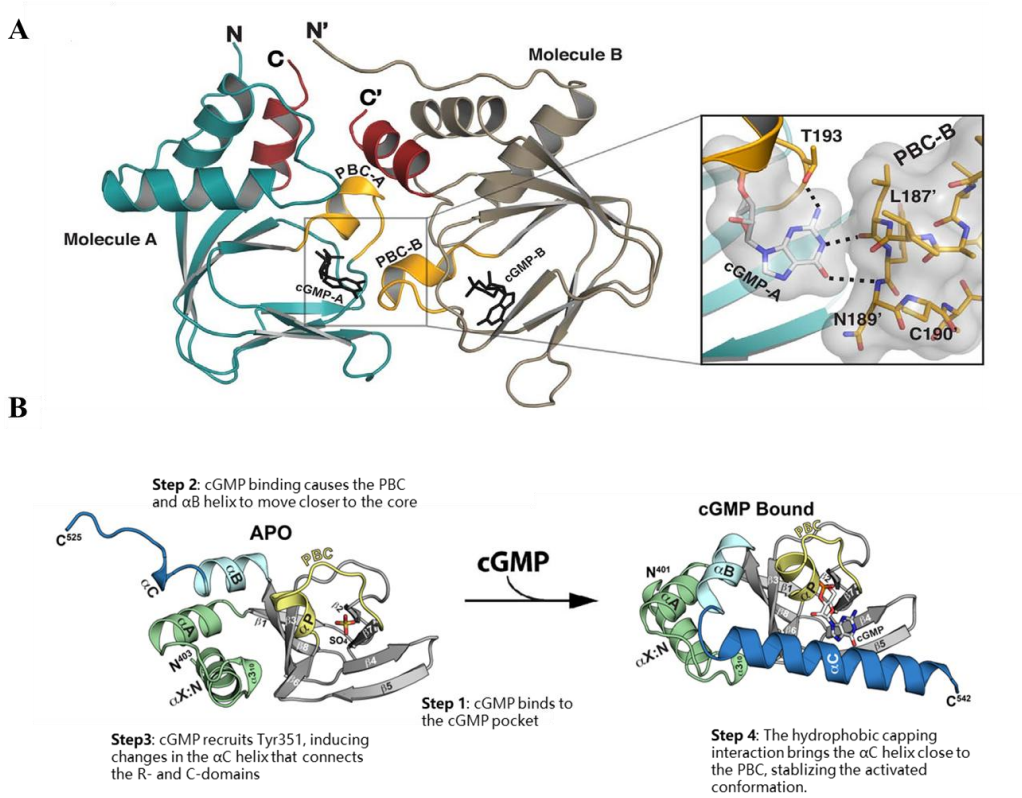
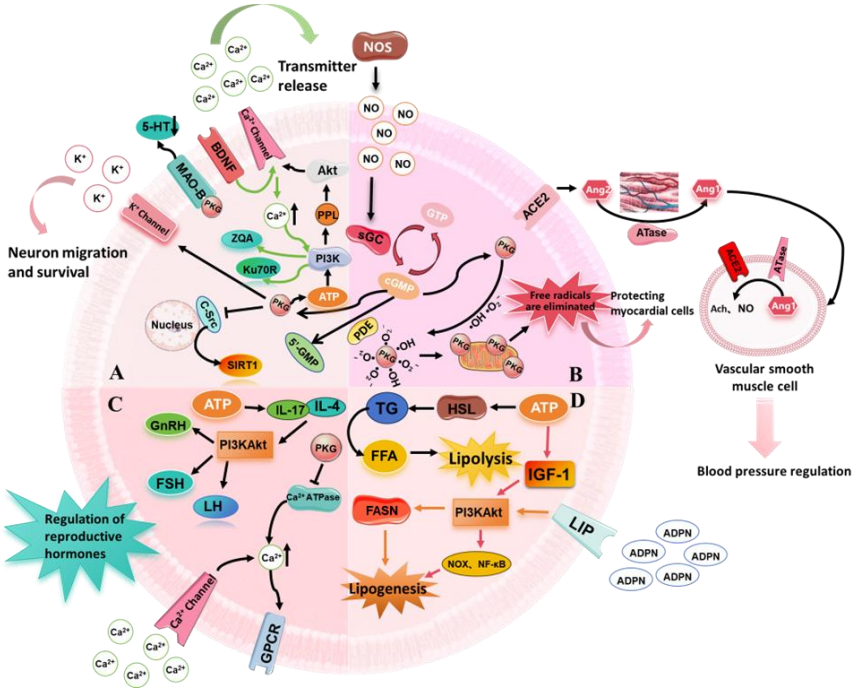


Figure 2 Schematic diagram of the Activation of PKG. (A) Schematic diagram of the structure of cGMP binding to PKG Iβ. (B) Schematic diagram of PKG Iβ activation process. PKG, Protein Kinase G; PBC, phosphate-binding box; cGMP, cyclic guanosine monophosphate. Reproduced with permission. Kim JJ, Casteel DE, Huang G, Kwon TH, Ren RK, et al. Co-Crystal Structures of PKG Ib (92–227) with cGMP and cAMP Reveal the Molecular Details of Cyclic-Nucleotide Binding. *PLoS ONE* 2011;6(4): e18413. Reproduced with permission Kim JJ, Flueck C, Franz E, Sanabria-Figueroa E, et al. Crystal Structures of the Carboxyl cGMP Binding Domain of the Plasmodium falciparum cGMP-dependent Protein Kinase Reveal a Novel Capping Triad Crucial for Merozoite Egress. *PLoS Pathog* 2015;11(2): e1004639. Copyright 2015, Public Library of Science.



5-HT：5-hydroxytryptamine
MAO-B：Monoamine Oxidase B
BDNF：Brain-Derived Neurotrophic Factor Receptor
ZQA：Zinc-Dependent Quinone Acceptor
Ku70R：Ku70 heterodimer Receptor
PPL：Phospholipase P
PI3k：Phosphoinositide 3-Kinase
c-Src：cellular Src tyrosine kinase
SIRT1：Sirtuin 1
NOS：Nitric Oxide Synthase
sGC：soluble Guanylate Cyclase
PDE：Phosphodiesterase
ACE2：Angiotensin-Converting Enzyme 2
Ang1：Angiotensin 1
Ang2：Angiotensin 2
ATase：Adenosine Triphosphatase
HSL：Hormone-Sensitive Lipase
TG：Triglyceride
FFA：Free Fatty Acids
IGF-1：Insulin-like Growth Factor 1
NOX：NADPH Oxidase
NF-κB：Nuclear Factor kappa-light-chain-enhancer of activated B cells
FASN：Fatty Acid Synthase
LIP：Lipase
ADPN：Adenosine Diphosphate Ribose
IL-4：Interleukin-4
IL-17：Interleukin-17
GnRH：Gonadotropin-Releasing Hormone
FSH：Follicle-Stimulating Hormone
LH：Luteinizing Hormone
GPCR：G-Protein Coupled Receptor

Figure 3 Schematic diagram of the metabolic pathways and physiological functions of PKG in (A) nervous, (B) cardiovascular, and (C, D) endocrine systems.

PKG in the nervous system

In the nervous system, PKG plays a regulatory role in the behavior and functions of various functional cells. Firstly, PKG participates in the regulation of the growth, differentiation, and synaptic plasticity of neurons, influencing the strengthening or weakening of synapses. This is of great significance in the learning and memory processes [5, 34]. Secondly, within the central nervous system, PKG has been observed to regulate the synthesis and release of specific neurotransmitters by phosphorylating associated proteins. This regulatory process is believed to play a crucial role in emotional disorders, such as depression and anxiety [35, 36]. Collectively, these findings suggest that PKG may have a significant impact on emotion-related signaling pathways. Additionally, PKG has been shown to possess a neuroprotective effect [37]. In states of nerve injury or disease, PKG can protect neurons by regulating the intracellular oxidative stress response to reduce cell damage.

Through comprehensive proteomic analysis, Chen et al. elucidated that PKG modulates several signaling pathways within the nervous system, including PI3K/Akt, PI3K/GSK3β, PI3K/ERK, and MAPK, by scavenging reactive oxygen species (ROS) [38]. Elevated levels of free radicals are capable of engaging with the substrate-binding domain of PI3K, thereby obstructing the execution of its catalytic processes and undermining the enzyme's physiological efficacy. Elevated ROS impair oxidative phosphorylation processes, leading to a decline in ATP synthesis. PI3K signaling activation requires substantial bioenergetic support. ATP deficits consequently establish indirect regulation, attenuating PI3K enzymatic activity through energy deprivation-mediated suppression (Figure 3A). Due to its structural characteristics, PKG can bind to ROS, thereby reducing ROS levels. This process alleviates the inhibition of PI3K by free radicals, promotes neuronal migration, and enhances neuronal excitability. Additionally, PKG also promotes signaling pathways such as MAO, BDNF, and MAPK [39]. Zhou X. and colleagues found that PKG is involved in regulating the secretion of neurotransmitters 5-HT (serotonin), GABA, and NE (acetylcholine). PKG can bind to the MAO-B receptors on the postsynaptic membrane, inhibiting the breakdown of 5-HT into 5-OH-Q by MAO-B, and promoting the release of neurotransmitters such as 5-HT, NE, and GABA. The release of neurotransmitters contributes to the activation of the BDNF signaling pathway, increasing intracellular calcium ion concentration, and subsequently affecting the activation of downstream proteins like PI3K, ZQA, Ku70R, etc. This process is of great significance for synaptic transmitter release, postsynaptic membrane excitability, and neuronal survival [35, 36].

PKG in the cardiovascular system

PKG plays a critical role in the cardiovascular system. The activation of PKG can promote the relaxation of smooth muscle cells and reduce blood pressure. Additionally, by inhibiting the production of certain pro-inflammatory cytokines, it alleviates inflammatory responses in cardiovascular diseases [40–42].

PKG regulates blood pressure through the ACE2 signaling pathway (Figure 3B) [43]. PKG binds to the ACE2 receptor on the surface of cardiomyocytes, promoting the release of Ang2 molecules. Ang2 is transported through the blood to the perivascular tissue and binds to angiotensin-converting enzyme (ATase), generating Ang1 molecules. Ang1 is transported via the blood to vascular smooth muscle cells and enters the cells with the help of plasma proteins. Ang1 binds to specific target receptors (such as ATase and ACE2) inside the smooth muscle cells, releasing molecules such as nitric oxide (NO), acetylcholine, etc. that can cause vasodilation, thereby reducing blood pressure and enhancing myocardial contractility. Lowering blood pressure is a key measure to reduce the risk of cardiovascular diseases (such as myocardial infarction, stroke, etc.), so the research on PKG in lowering blood pressure has important theoretical significance and potential clinical application value [44, 45].

PKG is involved in the scavenging process of ROS in the cardiovascular system [46]. Studies have shown that the accumulation of free radicals in the cardiovascular system is closely related to various diseases such as thrombosis and myocardial inflammation. Moraes et al. found that PKG achieves ROS scavenging through its unique structure [46]. PKG contains active sites of phosphate choline receptors, catalase carriers, and superoxide dismutase, enabling it to capture and remove free radicals from plasma (Figure 3B). The phosphorylcholine receptor binds to ROS in plasma to form complexes, which are subsequently converted by catalase or superoxide dismutase carried by PKG into water ($\bullet OH \rightarrow H_2O$) or superoxide anions ($\bullet O_2^-$). Subsequently, PKG binds to and embeds itself within the mitochondrial membrane. The mitochondria, rich in antioxidant enzymes, further decompose free radicals while generating energy. Through this radical-scavenging mechanism, PKG effectively reduces plasma concentrations of $\bullet OH$ and $\bullet O_2^-$, and diminishes intracellular and intramitochondrial free radical accumulation, thereby mitigating oxidative stress-induced damage to cardiomyocytes. PKG protects cardiomyocytes from ROS oxidative damage to critical structures such as cell membranes and mitochondrial membranes, preventing osmotic imbalance and functional impairment. Furthermore, PKG suppresses the bioactivity of inflammatory cytokines such as interleukin-1β (IL-1β), effectively blocking free radical-triggered inflammatory cascades and consequently ameliorating cardiac inflammatory pathogenesis [47, 48]. PKG protects heart function comprehensively by scavenging free radicals, safeguarding mitochondrial function, and regulating inflammatory responses.

PKG in the endocrine system

PKG also plays a role in the endocrine system. The physiological processes that are currently known to be regulated by PKG include the regulation of reproductive hormones [49], fat metabolism, and others [50–52]. Tian Ye et al. revealed that protein kinase G (PKG) modulates reproductive endocrine regulation by governing the secretion of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (Figure 3C) [49]. Initially, when intracellular free radicals are removed, it alleviates the inhibition of various key enzymes related to reproductive hormone release, such as IL-17 and IL-4. The activation of these key enzymes promotes signal transduction in pathways like PI3K/Akt, influencing the synthesis and release of reproductive hormones such as GnRH [53], FSH [54], and LH [55]. PKG can bind to the calcium-regulating enzyme (Ca^{2+} ATPase) and inhibit its activity, thereby increasing intracellular calcium ion concentration. The elevated calcium ion concentration can activate G protein-coupled receptors (GPCRs) [33], promoting the secretion of gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). This process affects ovarian and testicular function, ultimately regulating reproductive health and fertility.

PKG is also involved in the regulation of fat breakdown and synthesis during lipid metabolism (Figure 3D). Liu et al. discovered that lipolysis primarily relies on mitochondrial fatty acid oxidases, dehydrogenases, and other proteases [33]. As PKG scavenges ROS within adipocytes, intracellular ATP levels increase. This, on the one hand, ameliorates mitochondrial function, enhancing the activity of the lipolytic enzyme (HSL), which facilitates the release of free fatty acids (FFA) from triglycerides (TG), thereby promoting lipolysis [56]. The improvement of mitochondrial function enhances the activity of the fat decomposition enzyme (HSL), leading to the release of free fatty acids (FFA) from triglycerides (TG), thereby promoting fat breakdown. Both fat synthesis and breakdown are regulated by the PI3K/Akt and PI3K/GSK3β signaling pathways, though mediated by distinct regulatory factors [57]. When PKG elevates intracellular ATP levels, it activates the IGF-1 growth factor. This factor stimulates the activation of PI3K/Akt and PI3K/GSK3β signaling pathways, which modulate the expression of cytokines NOX and NF-κB, thereby driving fat synthesis through the PI3K/GSK3β pathway. Additionally, adiponectin (ADPN) binds to its receptor to activate the PI3K/Akt pathway, promoting the expression of lipid synthesis-related proteins in adipocytes, and ultimately facilitating fat synthesis [58].

PKG in the immune system

Emerging evidence suggests that PKG is involved in immune regulation, particularly in modulating T-cell activation, inflammation, and immune homeostasis [50, 59–61]. The Janus kinase (JAK) signaling pathway is a key mediator of PKG's function in T cells [62, 63]. The process begins with CD3ζ recognizing antigens and binding to co-stimulatory molecules on antigen-presenting cells, triggering CD3ζ phosphorylation and the release of signaling molecules such as IκBα and IL6 [64]. IL6 promotes CD73 expression by regulating the JAK/STAT3 signaling pathway, enhancing its activation [65]. The activated CD73 associates with JAK1/2 receptors, leading to the activation of JAK kinases, which in turn phosphorylate PI3K, initiating a cascade where PI3K phosphorylates Akt, thereby activating downstream signaling molecules such as Smad1/5a, Erk, and Jnk [66–68]. Through this pathway, activated Smad1/5a suppresses abnormal T cell proliferation, while Erk and Jnk promote T cell survival and functional maintenance. Overall, PKG plays a critical role in CD73 phosphorylation, facilitating JAK kinase activation and ultimately regulating the entire JAK-PI3K/Akt signaling pathway, which governs T cell activation, function, and immune responses, ensuring efficient humoral immunity [69, 70]. Despite these findings, the precise role of PKG in immune modulation remains largely undefined, with some studies indicating that it may either enhance or suppress immune responses depending on the context. Further investigation is needed to determine how PKG-targeted therapies could be utilized for treating autoimmune diseases, chronic inflammatory conditions, and even cancer immunotherapy by fine-tuning immune cell responses.

PKG in cancer therapy

PKG has recently gained attention as a potential therapeutic target in cancer due to its role in cell cycle regulation, apoptosis, and tumor suppression. PKG can inhibit oncogenic pathways such as Akt/mTOR, thereby suppressing cancer cell proliferation and enhancing apoptosis. The Akt/mTOR signaling pathway plays a crucial role in cancer cell proliferation, where mTORC1 phosphorylates Akt, forming Akt-P Tyr531, which subsequently promotes cell growth through downstream signaling cascades. PKG interacts with Akt to prevent its phosphorylation, thereby inhibiting its activation [71]. This inhibitory process is regulated by negative feedback mechanisms, significantly reducing mTORC1 activity and suppressing cyclin-dependent kinases (CDKs) and membrane-associated protein kinases (MEKs), ultimately inhibiting cancer cell proliferation [72]. Additionally, the expression of two key genes associated with tumor growth and survival, p53 and c-Myc, is also linked to the Akt/mTOR signaling pathway and is regulated by PKG. The expression of p53 is controlled by the mTOR/MITF signaling pathway. When Akt/mTOR is inhibited, the activity of mTORC1 decreases, leading to increased p53 stability and expression. The activation of p53 promotes apoptosis by regulating key apoptotic proteins such as Apaf-1, Bcl-2, and Mcl-1, thereby enhancing cancer cell apoptosis [73]. Similarly, c-Myc, a key gene that promotes cell proliferation and migration, is positively correlated with mTORC1 activity. When Akt/mTOR is suppressed, mTORC1 activity declines, resulting in reduced c-Myc expression, thereby inhibiting cancer cell proliferation and migration [74]. However, the role of PKG in different cancer types remains complex, as some studies have reported context-dependent effects, where PKG activation may either promote or suppress tumorigenesis. Future research should focus on delineating these dual roles and developing selective PKG modulators that can be tailored for specific cancer types, either as standalone therapies or in combination with existing treatments [75].

Conclusion

PKG, a signal transduction molecule, plays a crucial regulatory role in multiple signaling pathways. As such, it is a potential therapeutic target for various diseases, including tumors [76–78], cardiovascular diseases [79], and neurodegenerative diseases [80]. Despite the extensive research conducted on the fundamental functions of PKG, numerous challenges persist. From the perspective of extraction methods, the extraction of PKG typically relies on laboratory-cultured animal models or in vitro cell culture systems. However, issues related to purity and stability persist. PKG is prone to degradation or instability in vitro due to factors such as oxidation and temperature fluctuations [17]. To address these challenges, more stable PKG purification techniques could be developed, such as chemical protection or ion exchange methods, to preserve its activity. While the structural intricacies of PKG have been the subject of extensive analysis, the intricate interplay between its catalytic and regulatory domains remains to be fully elucidated [81]. This fundamental gap in understanding hinders the development of precise models to study PKG's functions. Secondly, although the activation mechanism of PKG has been the subject of extensive research, the specific mechanism of phosphate group transfer remains to be fully elucidated. This limitation hinders the development of experimental models of PKG, consequently impeding the conduct of experimental studies such as substrate recognition and drug development through simulation. As a result, the development of drug design, disease treatment, and other related fields is constrained.

In the context of physiological function, although studies have demonstrated that PKG plays a significant role in the processes of lipolysis and gonadotropin release, its molecular mechanisms remain unclear [79–81]. PKG may interact with other signaling pathways, which can interfere with the study of its intracellular functions [82, 83]. Artificial cell models could be employed to provide a confined environment with precisely defined components and controllable experimental parameters. By extracting and reconstituting the proteins required for metabolic processes within these artificial cells, it becomes possible to reconstruct physiological processes such as lipolysis and energy metabolism [33]. This approach can help elucidate the mechanistic roles of PKG in these processes.

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