

PKA & PKG

Key References

Amieue, P.S. and McKnight, G.S. The essential role of RI alpha in the maintenance of regulated PKA activity., *Ann. N.Y. Acad. Sci.*, **968**, 75-95 (2002).

Bos, J.L., Epac: a new cAMP target and new avenues in cAMP research., *Nat. Rev. Mol. Cell Biol.*, **4**, 733-738 (2003).

Francis, S.H., et al., Mechanisms of autoinhibition in cyclic nucleotide-dependent protein kinases., *Front. Biosci.*, **7**, D580-D592 (2002).

Lohmann, S.M., et al., Distinct and specific functions of cGMP-dependent protein kinases., *Trends Biochem. Sci.*, **22**, 307-312 (1997).

Michel, J.J. and Scott, J.D., AKAP mediated signal transduction., *Annu. Rev. Pharmacol. Toxicol.*, **42**, 235-257, (2002).

Münzel, T., et al., Physiology and pathophysiology of vascular signaling controlled by guanosine 3',5'-cyclic monophosphate-dependent protein kinase., *Circulation*, **108**, 2172-2183 (2003).

Skalhegg, B.S. and Tasken, K., Specificity in the cAMP/PKA signaling pathway. Differential expression, regulation, and subcellular localization of subunits of PKA., *Front. Biosci.*, **5**, D678-D693 (2000).

Taylor, S.S., et al., PKA: a portrait of protein kinase dynamics., *Biochim. Biophys. Acta.*, **1697**, 259-269 (2004).

Tegeer, I., et al., Reduced inflammatory hyperalgesia with preservation of acute thermal nociception in mice lacking cGMP-dependent protein kinase I., *Proc. Natl. Acad. Sci. USA*, **101**, 3253-3257 (2004).

Tischkau, S.A., et al., Protein kinase G type II is required for night-to-day progression of the mammalian circadian clock., *Neuron*, **43**, 539-549 (2004).

Vaandrager, A.B., Differential role of cyclic GMP-dependent protein kinase II in ion transport in murine small intestine and colon., *Gastroenterology*, **118**, 108-114 (2000).

Veugeliers, M., et al., Comparative PRKAR1A genotype-phenotype analyses in humans with Carney complex and *prkar1a* haploinsufficient mice., *Proc. Natl. Acad. Sci. USA*, **101**, 14222-14227 (2004).

Overview

Cyclic AMP-dependent protein kinase (PKA or cAK) and cyclic GMP-dependent protein kinase (PKG or cGK) transfer the γ -phosphate of ATP to serine and threonine residues of many cellular proteins. PKAs are present in most cells and function as effectors of many cAMP-elevating first messengers such as hormones and neurotransmitters. PKG is highly expressed in special cell types such as smooth muscle cells, platelets and cerebellar Purkinje cells (PKG I), as well as intestinal, kidney and brain cells (PKG II). cGMP-elevating agents include NO, natriuretic peptides and guanylin. In most tissues, PKGs are much less abundantly expressed than PKAs.

In the absence of its activating ligand cAMP, PKA exists as an inactive holoenzyme of two regulatory (R) and two catalytic (C) subunits. Following an increase in intracellular cAMP, the R-subunits bind cAMP resulting in the dissociation of the holoenzyme and the release of two free active C-subunits. Active C-subunit phosphorylates peptide substrates containing the -R-R/K-X-S/T- substrate consensus amino acid sequence (although exceptions to this consensus sequence have been observed). The PKA type I holoenzymes (RI α C₂, RI β C₂) are predominantly cytoplasmic, whereas the majority of type II PKA (RII α C₂, RII β C₂) associates with cytoskeletal structures, organelles and membranes. The holoenzymes can be anchored to specific compartments via interaction of their regulatory subunits with specific PKA anchoring proteins (AKAPs - most of which are identified for PKA II so far).

In contrast to PKA, the regulatory and catalytic regions of the PKG enzyme are present in one polypeptide. Binding of cGMP to the two cGMP-binding sites

is thought to release the autoinhibitory N-terminal domain from binding to the C-terminal catalytic domain, thus enabling substrate binding and heterophosphorylation. The substrate consensus amino acid sequence for PKGs appears to require more multiple basic residues than does PKA (consensus -R/K₂₋₃-X-S/T-). However, *in vitro*, many substrate proteins can be phosphorylated by both kinases. In addition to phosphorylating other proteins (heterophosphorylation), each of the PKGs and type II PKA phosphorylate themselves (autophosphorylation). Within the cell, the specific localization of the kinases and their substrates has been shown to restrict some of the possible interactions suggested by *in vitro* data. PKG I is localized mainly in the cytoplasm and a number of PKG anchoring proteins (GKAPs, especially for the type α enzyme) have been identified. The PKG II enzyme is anchored to membranes via its myristoylated N-terminus.

PKA has been shown to mediate many cellular responses to the intracellular second messenger cAMP in eukaryotes. Other important effectors of cAMP are the guanine nucleotide exchange factors Epac 1 and 2, which activate Rap1, a member of the Ras family of small GTPases. cAMP is also known to directly regulate ion channels. PKA I has been shown to mediate cAMP effects on inhibition of lymphocyte cell proliferation and immune response as well as long term depression in the hippocampus and sensory nerve transmission. PKA II is involved in the regulation of neuronal gene expression and motor learning as well as lipolysis and sperm motility. However, differences between PKA I and II functions are probably mainly due to differences in levels of expression in specific cells and the relative ability of each kinase

subtype to localize near substrates by binding to scaffolding proteins (AKAPs). For example PKA II can be localized via AKAPs to the Golgi-centrosomal area, to receptors and ion channels, to the cytoskeleton and the nucleus.

The second messenger cGMP has three major effector systems within the cell: cGMP-regulated ion channels, cGMP-regulated phosphodiesterases and PKGs. PKG I mediates cGMP-induced smooth muscle cell relaxation and inhibition of platelet aggregation. These effects correlate at least partly with an inhibition of calcium release from intracellular stores. In addition PKG I can inhibit cardiac myocyte contractility and has also been shown to regulate proliferation and gene expression in various cell types. PKG II stimulates intestinal chloride secretion, inhibits renin release from juxtaglomerular cells, stimulates renal calcium reabsorption and regulates endochondral ossification.

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FAMILY MEMBERS	PKA I (R ₁ C ₂) and PKA II (R ₁ C ₂) (P5511 (b))	PKA I (R ₁ C ₂) and PKA II (R ₁ C ₂) (P5511 (b))	PKA I (R ₁ C ₂) and PKA II (R ₁ C ₂) (P5511 (b))
OTHER NAMES	cAK I and cAK II	cAK I and cAK II	cAK I and cAK II
MOLECULAR WEIGHT/ STRUCTURAL DATA	43-47 kDa (R I) Tetramer of 2 regulatory (R) and two catalytic (C) subunits R I	49-55 kDa (R II) Tetramer of 2 regulatory (R) and two catalytic (C) subunits R II	40 kDa (C) Tetramer of 2 regulatory (R) and two catalytic (C) subunits C (P2645 (b), P6998 (h), C8482 (h))
ISOFORMS	R1α: 381 aa, R1β: 381 aa	R11α: 404 aa, R11β: 418 aa	Cα: 351 aa, Cβ: 351 aa, Cγ: 351 aa
SPECIES	Human	Human	Human
DOMAIN ORGANIZATION	N-terminal dimerization/AKAP-binding and autoinhibitory region, followed by two cAMP binding domains (similar organization in RI and RII)	N-terminal dimerization/AKAP-binding and autoinhibitory region, followed by two cAMP binding domains (similar organization in RI and RII)	Kinase domain
PHOSPHORYLATION SITES	Not known	Ser ⁹⁹ (R11α), autophosphorylation Thr ⁵⁴ (R11α), phosphorylated by CDK1 Ser ¹¹⁴ (R11β), autophosphorylation	Thr ¹⁹⁷ , Ser ³³⁸ (associated with activation)
TISSUE DISTRIBUTION	R1α: expressed in all tissues R1β: preferentially expressed in neurons	R11α: expressed in all tissues R11β: preferentially expressed in fat and neurons	Ubiquitous (Cα1, Cβ1), brain (Cβ2,3), testis (Cα2, Cγ), lymphocytes (Cα1, Cβ2)
SUBCELLULAR LOCALIZATION	Cytoplasm, organelles	Cytoskeletal structures, organelles, membranes	Same as R-subunits
BINDING PARTNERS/ ASSOCIATED PROTEINS	AKAPs (D-AKAP1, AKAP220)	AKAPs (MAP2, AKAP-79, AKAP95, S-AKAP84/D-AKAP1, AKAP220, AKAP450, AKAP18, WASP, mA-KAP and many others)	N-terminal myristoylation
UPSTREAM ACTIVATORS	cAMP	cAMP	Not known
DOWNSTREAM ACTIVATION	Not known	Not known	Multiple substrates
ACTIVATORS	cAMP (A6885), ^a 8-Br-cAMP (B7880), ^a 6-Bnz-cAMP (B4560), ^b 6-MB-cAMP	cAMP (A6885), ^a Sp-5,6-DCl-cBIMPS (S6069), ^a 6-Bnz-cAMP ^b	See R-subunits
INHIBITORS	Rp-8-Br-cAMPS (B2432), Rp-8-Cl-cAMPS (C0610), Rp-cAMPS (A165)	Rp-8-pCPT-cAMPS (C0735), Rp-cAMPS (A165)	PKI (P0300), H89 (B1427), H8 (M9656), KT5720 (K3761)

FOOTNOTES

^a cAMP and most cAMP analogs also activate Epac. To distinguish PKA and Epac effects in intact cells the Epac-specific activators 8-pCPT-2'-O-Me-cAMP or 8-Br-2'-O-Me-cAMP can be used.
^b 6-modified cAMP analogs have a higher affinity for PKA over Epac.

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ISOZYME SELECTIVE ACTIVATORS	6-Bnz-cAMP or 8-PIP-cAMP cAMP (P0872) in combination with 8-AHA-cAMP (P2104) or 8-MA-cAMP (P0747)	6-Bnz-cAMP or 6-Phe-cAMP In combination with Sp-5,6-DCl-cBIMPS or Sp-8-pCPT-cAMPs (C8990)	Not known
PHYSIOLOGICAL FUNCTION	Inhibition of T-cell activation (R1 α), nociceptive processing in the spinal cord (R1 β), long-term depression in the hippocampus (R1 β), mesoderm development (R1 α)	Lipolysis (R11 β), increased myocardial contractility, inhibition of T-cell activation, long-term depression in the visual cortex (R11 β), motor learning (R11 β), neuronal gene expression (R11 β)	Gene expression, growth and differentiation, cytoskeleton dynamics, ion channel conductivity, sperm motility (C α 2)
DISEASE RELEVANCE	Carney complex (R1 α)	Diabetes (R11 β)	See R-subunits

PKA & PKG (continued)

FAMILY MEMBERS	PKG I	PKG II
OTHER NAMES	cGK I	cGK II
MOLECULAR WEIGHT/ STRUCTURAL DATA	74-80 kDa Dimer	86 kDa Dimer
ISOFORMS	PKG I α : 671 aa (P3488 (b)) PKG I β : 686 aa (P3738 (h))	PKG II: 762 aa (P2363 (rat))
SPECIES	Human	Human
DOMAIN ORGANIZATION	N-terminal regulatory domain containing dimerization/GKAP-binding and auto-inhibitory sites, two central cGMP binding domains, C-terminal kinase domain	N-terminal regulatory domain containing dimerization/GKAP-binding and auto-inhibitory sites, two central cGMP binding domains, C-terminal kinase domain
PHOSPHORYLATION SITES	Ser ⁵¹ , Thr ⁵⁹ , Ser ⁶⁵ , Ser ⁷³ (PKG I α), autophosphorylation Ser ⁶⁴ , Ser ⁸⁰ (PKG I β), autophosphorylation	Ser ¹¹⁰ , Ser ¹¹⁴ , Ser ¹²⁶ , Ser ⁴⁴⁵ , autophosphorylation
TISSUE DISTRIBUTION	All types of smooth muscle, platelets (I β), cerebellar Purkinje cells, lung (I α), lymphocytes (I β), cardiac myocytes (I α), endothelial cells (not all)	Intestinal mucosa, kidney, adrenal cortex, brain, bone (chondrocytes), lung (Clara cells)
SUBCELLULAR LOCALIZATION	Cytoplasm, cytoskeletal structures	Membranes

FOOTNOTES

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BINDING PARTNERS/ ASSOCIATED PROTEINS	GKAPs, (MBS (I α), troponin T (T0175), GKAP-42 (I α), IRAG (I β), RGS-2 (I α))	N-terminal myristoylation, NHERF2
UPSTREAM ACTIVATORS	cGMP	cGMP
DOWNSTREAM ACTIVATION	Many substrates, e.g. VASP, IRAG, BK _{Ca} channel, MBS, PDE5, G-substrate	Substrates, e.g. CFTR
ACTIVATORS	cGMP (G6129), PET-cGMP, 8-Br-cGMP (B1381), 8-pCPT-cGMP (C5438)	cGMP (G6129), 8-pCPT-cGMP (C5438), 8-Br-cGMP (B1381), PET-cGMP (P0622)
INHIBITORS	Rp-8-Br-PET-cGMPS (B6684), Rp-8-pCPT-cGMPS (C240), KT5823 (K1388) (<i>in vitro</i> only) DT-2, DT-3 (peptide-based)	Rp-8-pCPT-cGMPS (C240), Rp-8-Br-PET-cGMPS (B6684)
SELECTIVE ACTIVATORS	Not known	Not known
PHYSIOLOGICAL FUNCTION	Smooth muscle relaxation (e.g. vasodilation, penile erection), inhibition of platelet aggregation, facilitation of nociceptive transmission in the spinal cord, inhibition of T-cell proliferation, negative inotropic and antihypertrophic effect in heart, regulation of gene expression	Cl ⁻ secretion and inhibition of Na ⁺ reabsorption (small intestine), inhibition of renin secretion, stimulation of renal Ca ²⁺ reabsorption, stimulation of aldosterone production, progression of circadian clock, endochondral ossification
DISEASE RELEVANCE	Atherosclerosis	STa-induced diarrhea

Abbreviations

AHA: Aminohexyl amino

8-AHA-cAMP: 8-(6-Aminohexyl)aminoadenosine-3',5'-cyclic monophosphate

AKAP: PKA-anchoring protein

BKCa: Ca²⁺-regulated potassium channel

Bnz: Benzoyl

6-Bnz-cAMP: N⁶-Benzoyladeniosine-3',5'-cyclic monophosphate

Br: Bromo

cAMP: Adenosine-3',5'-cyclic monophosphate

cAMPS: Adenosine-3',5'-cyclic monophosphorothioate

CFTR: Cystic fibrosis transmembrane conductance regulator

cGMP: Guanosine-3',5'-cyclic monophosphate

cGMPS: Guanosine-3',5'-cyclic monophosphorothioate

CPT: Chlorophenylthio

Epac: Exchange protein directly activated by cAMP

GKAP: PKG-anchoring protein

H8: N-(2-[Methylamino]ethyl)-5-isoquinoline-sulfonamide

H89: N-(2-[p-Bromocinnamylamino]ethyl)-5-isoquinolinesulfonamide

IRAG: IP₃-receptor-associated cGMP kinase substrate

KT5720: (9S,10R,12R)-2,3,9,10,11,12-Hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-ij][1,6]benzodiazocine-10-carboxylic acid hexyl ester

KT5823: (9S,10R,12R)-2,3,9,10,11,12-Hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-ij][1,6]benzodiazocine-10-carboxylic acid methyl ester

MA: Methylamino

8-MA-cAMP: 8-Methylaminoadenosine-3',5'-cyclic monophosphate

MB: Monobutyladenosine

MAP: Microtubule-associated protein

MBS: Myosin-binding subunit of myosin light chain phosphatase

NHERF2: Na⁺/H⁺ exchanger regulatory factor 2

PET: β -Phenyl-1,N²-ethenol

PET-cGMP: β -Phenyl-1,N²-ethenoguanosine 3',5'-monophosphate

Phe: Phenyladenosine

PIP: Piperidino

8-PIP-cAMP: 8-Piperinoadenosine 3',5'-cyclic monophosphate

PKA: cAMP-dependent protein kinase

PKG: cGMP-dependent protein kinase

PKI: cAMP-dependent protein kinase inhibitor peptide

RGS-2: Regulator of G-protein signaling-2

Rp-8-Br-cAMPS: 8-Bromoadenosine-3',5'-cyclic monophosphorothioate, Rp isomer

Rp-8-Cl-cAMPS: 8-Chloroadenosine-3',5'-cyclic monophosphorothioate, Rp isomer

Rp-cAMPS: Adenosine 3',5'-cyclic monophosphorothioate, Rp-isomer

Rp-8-pCPT-cAMPS: 8-(4-Chlorophenylthio)adenosine-3',5'-cyclic monophosphorothioate, Rp isomer

Rp-8-Br-PET-cGMPS: b-Phenyl-1,N²-etheno-8-bromoguanosine-3',5'-cyclic monophosphorothioate, Rp isomer

Rp-8-pCPT-cGMPS: 8-(4-Chlorophenylthio)guanosine-3',5'-cyclic monophosphorothioate, Rp isomer

Sp-5,6-DCI-cBIMPS: 5,6-Dichloro-1-b-D-ribofuranosylbenzimidazole-3',5'-cyclic monophosphorothioate, Sp-isomer

Sp-8-pCPT-cAMPS: 8-(4-Chlorophenylthio)adenosine-3',5'-cyclic monophosphorothioate, Sp-isomer

STa: heat-stable enterotoxin secreted by enteropathogenic bacteria

VASP: vasodilator-stimulated phosphoprotein

WASP: Wiskott-Aldrich syndrome protein

FOOTNOTES