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Protein kinase A catalytic subunit isoform *PRKACA*; history, function and physiology

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Abstract

Our appreciation of the scope and influence of second messenger signaling has its origins in pioneering work on the cAMP-dependent protein kinase. Also called protein kinase A (PKA), this holoenzyme exists as a tetramer comprised of a regulatory (R) subunit dimer and two catalytic (C) subunits. Upon binding of two molecules of the second messenger cAMP to each R subunit, a conformational change in the PKA holoenzyme occurs to release the C subunits. These active kinases phosphorylate downstream targets to propagate cAMP responsive cell signaling events. This article focuses on the discovery, structure, cellular location and physiological effects of the catalytic subunit alpha of protein kinase A (encoded by the gene *PRKACA*). We also explore the potential role of this essential gene as a molecular mediator of certain disease states.

Keywords

Protein kinase A signaling; Phosphorylation; Regulatory subunits; Catalytic subunits; A-kinase anchoring proteins; Pathological cAMP signaling

1. Introduction

Since the discovery of protein phosphorylation as a universal means for cell communication, it has become apparent that protein kinases play crucial roles in all aspects of cellular life (1). These enzymes are responsible for the transfer of the γ -phosphate group of adenosine 5'-triphosphate (ATP) to a protein substrate, usually a serine, threonine or tyrosine but can also transfer to a histidine or lysine (2, 3). Bioinformatic analyses have identified on the order of 540 different protein kinase genes that comprise the human kinome (4). This represents about 2% of the genome (4). Although the number of kinases varies across species, it is worthy to note that gene duplication events have enlarged several plant, Dinoflagellata and Arabidopsis kinomes (5). Irrespective of their size, kinomes can be divided into 9 sub-families, one of which is called the AGC group. This subgroup of the kinome includes the protein kinase A, G and C families of serine/threonine kinases. The common feature of these

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enzymes is that they are responsive to changes in local concentrations of cytoplasmic second messengers such as cyclic AMP or lipids. In humans, this group consists of 63 protein kinases corresponding to the protein kinase G and protein kinase C family of kinases, Akt/protein kinase B, Aurora kinases, ribosomal protein S6 kinases, as well as the phosphoinositide-dependent kinases (4). AGC kinases regulate a multitude of cellular processes including glucose metabolism, cell division and development, the stress responses and molecular aspects of synaptic transmission and contextual memory (6–10). A prominent member of the AGC kinases is the cAMP-dependent protein kinase, also called protein kinase A (PKA) (11). This review article focuses exclusively on the structure, function, cellular location and pathophysiological effects of this key enzyme.

2. History

Our appreciation of mammalian PKA signaling has its origins in the pioneering work of biochemists Edmond H. Fischer and Edwin G. Krebs on the role of protein phosphorylation and the control of glycogen metabolism. Fischer and Krebs were working together on glycogen phosphorylase, an enzyme that is activated by a protein kinase called phosphorylase kinase (12). One of their key breakthroughs was realizing that activation of phosphorylase kinase was catalyzed by another enzyme that was dependent on the recently discovered second messenger, cAMP, reported by Earl Sutherland a few years earlier (13–15). Accordingly Fischer and Krebs named their new enzyme cAMP-dependent protein kinase and purified the kinase in 1968 (16). Fischer and Krebs won the Nobel Prize in Physiology or Medicine in 1992 for this discovery and subsequent work on phosphorylation and dephosphorylation, particularly how it relates to cAMP dependent protein kinase activity (12). Today, the cAMP-dependent protein kinase is more frequently denoted as protein kinase A, or simply PKA. PKA is a serine/threonine kinase that is responsible for phosphorylating a broad array of downstream substrates, the extent of which depends on cellular localization (17, 18). Not surprisingly PKA is considered as an essential regulator of many cell signaling events (19, 20).

Shortly after the discovery of PKA, there was a concerted effort by several investigators including Edwin Krebs, Jackie Corbin, and Susan Taylor, to discern the molecular components and stoichiometry of the bovine and rabbit enzyme complexes. In retrospect perhaps the most surprising finding was that the catalytic activity of PKA resided in a dedicated catalytic (C) subunit that was constrained in an inactive state by a range of regulatory (R) subunits (21–25). Elegant biochemical studies demonstrated the R subunits of PKA exist in two forms, designated as RI and RII on the basis of their elution from ion exchange columns (21–25). Subsequently, it was discovered that there are two RI subunits (termed RI α and RI β) and two RII subunits (termed RII α and RII β). The intact PKA holoenzyme exists as a tetramer of two regulatory subunits in contact with two catalytic subunits (26). This tetrameric protein complex constitutes the type I and type II PKA holoenzymes (11). The uniqueness of this holoenzyme configuration was not fully appreciated until the beginning of kinome analyses when it was realized that of the over 540 human protein kinases, only one other AGC kinase, called CK2, exists in a tetramer complex. In contrast, the vast majority of protein kinases contain intramolecular regulatory

sequences that reside within the same polypeptide chain as the catalytic core of the enzyme (4).

During the characterization of the PKA holoenzymes, the Krebs lab discovered an endogenous protein inhibitor of the enzyme that acted independent of cAMP (27). This 75 amino acid heat stable protein kinase inhibitor termed PKI was proposed to modulate the free catalytic subunit, but only after cAMP-dependent dissociation from the regulatory subunit (28, 29). The mechanistic core of PKI resides in a pseudosubstrate sequence, RRNAI, that binds with high affinity to the active site of the C subunit (30). In fact, a peptide that encompasses this region, PKI-(5–24) potently inhibits the C subunit with an inhibition constant of 2–4 nM (30).

With the advent of recombinant DNA technology, the extent and diversity of the mammalian PKA subunits was soon realized. Cloning studies by Stan McKnight and others identified four possible C subunit genes (C α , C β , C γ and the related gene C χ) and confirmed the presence of the four R subunit genes (31–37). Moreover, the overexpression and purification of these recombinant proteins from bacteria made them ideal targets for structural analysis. A major milestone was the crystallization of the PKA C α subunit by Susan Taylor and colleagues in 1991 (38, 39). This revealed for the first time the bi-lobed structure of the kinase core, a protein fold that has now been observed in well over 100 kinase structures (11).

3. PRKACA

The human *PRKACA* gene is located on chromosome 19 at p13.1 on the reverse strand (40). This gene, which is approximately 26,000 nucleotides long, encodes the PKA catalytic subunit alpha (C α) isoform. *PRKACA* has 10 exons which transcribe into a 351 amino acid protein (40kDa)(41, 42).

Three alternately spliced transcripts of human *PRKACA* have been identified, denoted C α 1, C α 2 (also known as C α s) and C α 3. The canonical C α 1 form that is ubiquitously expressed throughout human tissue encodes the 351 amino acid protein, while C α 2 is primarily expressed in sperm cells and contains a different 5' exon that initiates transcription at an alternate start codon (Figure 1A) (43–47). This results in C α 2 differing in the first 15 amino acids as compared to C α 1 (Figure 1B). A third variant of C α called isoform 3 has also been reported, but has yet to be characterized (48).

As previously mentioned there are three isoforms of the catalytic subunit of PKA; C α , which is thought to be the predominant isoform and expressed in most tissues; C β , which is also expressed in various tissues, coded by the gene *PRKACB* (33). In contrast C γ , encoded by *PRKACG* gene is most likely expressed only in the testis, (49). Yet there is little if any evidence that the C γ protein is transcribed or has any functional significance in testis physiology. It is also worth noting that a fourth C subunit related gene *PRKX* has also been identified. Although the physiological significance of this putative C subunit isozyme is unclear, a number of decisive biochemical studies have conclusively demonstrated that C χ can interact with the R subunits to form a modified PKA holoenzyme (50).

Over 90 organisms are known to have *PRKACA* orthologs (42). The primary structure of the protein is conserved from humans to mice, zebrafish, and yeast. In mice, targeted deletion of this gene results in growth retardation in the small number of animals that survive (51), while a Ca deficiency has also been linked to spinal neural tube defects (52). On the other hand, a double knockout of Ca and $\text{C}\beta$ or haploinsufficiency of $\text{C}\beta$ in the context of a Ca total knockout is embryonic lethal. In contrast, deletion of $\text{C}\beta$ results in phenotypically normal mice (51, 53). These seemingly contradictory results argue for a high degree of redundancy in the C subunit genes and a complex utilization of C subunit isozymes in different tissues (54).

4. PKA signaling

4.1 Tetrameric holoenzymes

Inactive PKA exists as a holoenzyme, comprised of two regulatory (R) subunits and two catalytic subunits (55). In the presence of cAMP, the holoenzyme becomes active by binding two cAMP molecules cooperatively to each R subunit, resulting in a conformational change in the R subunits, thus releasing the two C subunits to phosphorylate downstream targets (Figure 1C) (56). There are two distinct types of R subunits, RI and RII, of which there are two isoforms of each type, $\text{RI}\alpha$, $\text{RI}\beta$, $\text{RII}\alpha$, and $\text{RII}\beta$. PKA holoenzymes that contain either RI or RII are termed PKA type I or type II, respectively. The RI and RII isoforms differ in their localization, expression level and are believed by some to have differential sensitivities for cAMP (21, 22, 57). More recently it has been realized that the differential localization of the type I and type II PKA holoenzymes is an important determining factor that dictates their physiological roles (17, 18, 58).

Structural elucidation of the R subunits has always lagged behind our understanding of the catalytic subunits, in no small part because these modular proteins are flexible in nature (59–65). Nonetheless, a concerted effort over the past 20 years has resulted in high-resolution X ray structures for the cAMP binding sites and fragments of the RI and RII subunits in complex with Ca . Although an X ray structure of an intact PKA holoenzyme has eluded investigators, the amino-terminal docking and dimerization domains have been resolved to reveal a four helix bundle that not only forms the interface for R subunit dimerization but also created a pocket for the association of mammalian RI or RII with a family of A-kinase anchoring proteins (AKAPs) (63, 64, 66, 67). As will be discussed later, this latter group of proteins is critical for the subcellular tethering of PKA in proximity to preferred substrates. More recently, negative stain single particle electron microscopy has been used to resolve the structure of an intact PKA holoenzyme in complex with an AKAP. This structure revealed that there are regions of intrinsic disorder within the R subunits of PKA that impart a high degree of flexibility and rotation to the C subunit when it is part of the holoenzyme complex (68). In fact, the anchored C subunit of PKA can move with relative freedom within a zone of 160 angstroms when bound to the R subunit dimer (58). Ongoing studies are trying to establish the functional significance of this new model for anchored PKA in a cellular context.

4. 2 AKAPs

Specificity of PKA signaling is attained through the binding of the R subunits to scaffolding proteins called A-kinase anchoring proteins, or AKAPs (69). AKAPs are a diverse family of about 50 proteins that vary in localization and structure that all share the ability to bind the R subunits of PKA (19, 70). The trademark feature in all AKAPs is the amphipathic helix that binds with high affinity to the R subunits (71). AKAPs target the PKA holoenzyme to different subcellular locations in proximity to other proteins to optimize signal transduction. This process allows for local cAMP-responsive events to occur within specific compartments of the cell (72). Recently, AKAP-PKA complexes were evaluated with electron microscopy and a three-dimensional reconstruction of the holoenzyme revealed that the AKAP-PKA complex has some flexibility, which might further allow PKA to adopt the optimal conformation for substrate phosphorylation (68). Although the majority of AKAPs preferentially associate with the RII subunit, there is reason to believe that RI subunits can also be compartmentalized through interaction with anchoring proteins (73, 74). In addition there are a few RI selective anchoring proteins that only compartmentalize the type I PKA (75, 76).

Perhaps the most biologically relevant feature of AKAPs is their ability to cluster PKA with other classes of signaling enzyme (18, 77–80). By constraining broad specificity enzymes such as PKA, PKC, and the protein phosphatases PP1 and PP2B in customized macromolecular units, AKAP complexes allow cells to efficiently and accurately respond to the transient production of diffusible second messenger signals. This concept was first proposed in 1995 when it was shown that the anchoring protein AKAP79/150 not only compartmentalized PKA, but also sequestered the protein phosphatase PP2B (81). As a result, the opposing actions of a protein kinase and a protein phosphatase were constrained within the confines of the same macromolecular complex (81). It was subsequently shown that the proximity of both enzymes to phosphoprotein substrates such as ion channels and cytoskeletal components enhanced the bi-directional control of neuronal events that underlie synaptic transmission, glucose homeostasis, cardiac transcriptional regulation and higher-order cognitive function in genetically modified mice (82–88).

5. *PRKACA* and Disease

5.1 Cardiovascular diseases

Given its prominent role in normal physiology it is not surprising that there has been much interest in how PKA phosphorylation goes wrong in disease (89). Probably the most common pathological effects of defective cAMP signaling underlie the onset of coronary heart disease and cardiomyopathies (90–93). It has been known since the early 1960's that blocking β adrenergic stimulation of cAMP synthesis is protective against heart disease (94). Moreover, prolonged elevation of cAMP is observed in heart failure and the onset of several cardiomyopathies (95, 96). Yet despite the prominence of heart disease as a global health problem in the first world, less is known about the specific role of PKA in this process. That being said there are two aspects of cardiac pathophysiology where PKA phosphorylation events are known to be mis-regulated. First, excitation-contraction coupling of cardiomyocytes requires the rhythmic and coordinated action of the calcium and cAMP

signaling pathways (95, 97). Although some consider it controversial, it has been postulated that changes in the phosphorylation status of the L-type calcium channel (Ca_v1.x), the ryanodine receptor, and the SERCA modulating protein phospholamban underlie the onset of various heart ailments including dilated cardiomyopathies (98–100). Whether these defects occur at the level of the channels, cAMP metabolism, or mis-localization of anchored PKA remains a matter of contentious debate. Nonetheless, it is evident that modulation of PKA activation is a therapeutic strategy that manages some stages of progressive and congestive heart failure (101–104). Second, membrane depolarization by cardiac potassium channels is key to the transduction of electrical impulses from the sino atrial node of the heart. An elegant series of studies suggest that mutations in a gene encoding the A-kinase anchoring protein Yotiao/AKAP6 underlie the onset of arrhythmias (105, 106). Interestingly, this anchoring protein associates with the KCNQ1 subunit of potassium channel (107). Further study is necessary to more precisely pinpoint the functional consequences of the genetic lesion and whether or not anchored PKA is a driver or a passive player in this process. Nonetheless, it is possible that the mis-localization or mis-regulation of PKA in the sinoatrial node may be a contributing factor to the onset of certain arrhythmias (108).

5.2 Tumors of the Adrenal Cortex

Somatic mutations have been found in *PRKACA* that result in Cushing's syndrome. Cushing's syndrome is a disease caused by excess glucocorticoid production that can be a result of adrenocortical (ACT) and ACTH-producing pituitary tumors (109). These tumors include adrenocortical adenomas (ACA), adrenocortical carcinomas (ACC) and bilateral adrenal hyperplasia (BAH). Overproduction of glucocorticoids caused by Cushing's syndrome can result in other disorders, like hypertension, osteoporosis, diabetes as well as interfering with hormone production from the pituitary. Recently several groups have independently reported that whole-exome sequencing of cortisol-producing ACTs resulted in the identification of c.T617G heterozygous mutation that results in an amino acid substitution, Leu206Arg (110–113). This mutation was only found in ACAs, with a varying frequency of approximately 20% to 70% depending on the study. This mutation L206R is found in the p+1 loop of PKA, and interferes with binding between the catalytic subunit and the R subunits of PKA in the presence of cAMP. Further studies have confirmed this mutation in *PRKACA*, and concluded that the Leu206Arg mutation of PKA results in higher basal PKA activity (113, 114).

Three insertions have been found in patients with adrenocortical adenomas; c. 595_596insCAC that results in an insertion of a tryptophan (Leu199_Cys200insTrp), c. 600_601insGTG that results in a insertion of valine (p.Cys200_Gly201insVal), and a missense mutation, c.639C>G that changes Ser213 to Arg while inserting four amino acids (114, 115). This mutation is found in a highly conserved region of the catalytic subunit of PKA, and is thought to elevate basal activity of PKA. In contrast, the Leu206Arg, Leu199_Cys200insTrp, and the Cys200_Gly201insVal mutations are all found near the autoinhibitory sequence of the regulatory subunit (114). This region is involved in keeping the catalytic subunit inactive by blocking the active site in the absence of cAMP (114). Collectively these findings provide further evidence that disruption of this conserved

structure of the catalytic subunit of PKA can lead to aberrant and unregulated kinase activity (116). Germline gene amplification of *PRKACA* has also been recently reported in Cushing's syndrome patients that resulted in bilateral adrenal hyperplasia. Interestingly, exome sequencing revealed that none of these harbored mutations in the *PRKACA* gene, but rather exhibited increased copy numbers of *PRKACA* transcript (117, 118).

Carney complex (CNC) is another disease of the adrenal gland, which presents as somatotroph-pituitary adenomas, testicular Sertoli cell calcified tumors, benign thyroid nodules, differentiated thyroid cancer, and most commonly, primary pigmented nodular adrenocortical disease (119). Inactivating mutations in the gene that encodes R1 α (*PRKARIA*) have been identified in over 60% of patients with CNC (115, 120, 121). While the exact mutations differ from family to family, these mutations usually lead to a premature stop codon and the RNA gets promptly degraded. One molecular explanation is that a decrease in the cellular R1 α pool allows the catalytic subunit to roam unregulated and increases the kinase activity in tissues.

When taken together all of these above findings infer that increased transcripts of PKA, disruption of the C subunit binding to the R subunits or pathological changes in the cellular stoichiometry of PKA subunits can underlie a variety of diseases. Thus it would appear that each of these endocrine disorders are perpetuated by defective cAMP signaling and misregulated PKA activity.

5.3 *PRKACA* and cancer

Although PKA is not traditionally considered to be an oncogene, there is a growing body of literature that suggests this kinase may contribute in some ways to the progression of cancer. For example, extracellular PKA catalytic subunit has been detected in the serum of a variety of cancer patients, including colon, renal, rectal, prostate, lung, adrenal carcinomas and lymphomas (122–124). Increased *PRKACA* transcripts have also been observed in breast cancer, specifically in patients that are resistant to trastuzumab, which is a common treatment for HER2 positive breast cancer (125). In these instances, *PRKACA* can be used as a biomarker for cancer as well as a prognostic tool (125).

More recently, there has been a lot of interest in fibrolamellar hepatocellular carcinoma (FL-HCC) and *PRKACA*. This began when Honeyman *et. al.* described a 400kb deletion on chromosome 19 in 100% of FL-HCC patients (126). This genetic lesion resulted in an in-frame fusion of two genes- *DNAJB1*, which is the gene that codes for the heat shock protein 40, and *PRKACA*. Other groups have also validated this deletion, and confirmed the fusion of the first exon of *DNAJB1* to exons two through ten of *PRKACA*, shown in Figure 2 (127–129). Further analyses of FL-HCC tissues show an increase in protein levels in tumor tissues. This increase in transcript is believed due to the fusion protein being under the control of the *DNAJB1* promoter (126). Again, this is consistent with the above hypothesis that increased PKA levels in tissues can lead to disease.

6. Conclusion

As should be evident from this synopsis of published reports and personal perspectives, the field of PKA signaling has grown from a purely biochemical curiosity into a multifaceted discipline with clear implications for disease. In the next few years it will be imperative that we make an effort to translate the biochemical breakthroughs in our understanding of the structure and function of this ubiquitous enzyme into a more detailed appreciation of how PKA signaling falls apart in disease states.

Although there are many promising leads to follow up, conceivably the most intriguing challenge comes in the development of therapeutics to combat fibrolamellar hepatocellular carcinoma (FL-HCC). The recent discovery of the DNAJ-PKAc fusion as an oncogenic element beautifully underscores the advantages of combining state of the art genomics with detailed structural information (126, 129). Yet, it remains to be seen if DNAJ-PKAc is the genetic driver in the etiology of FL-HCC. It will be interesting to see if the detailed structural information on the DNAJ-PKAc reveals a mechanism that impacts the substrate specificity or how subcellular location of this pathological form of the enzyme is controlled.

It is worthy to note that FL-HCC is not the sole target for PKA directed therapies. For example, developing strategies that target some of the PKA C subunit mutant forms that underlie Cushing's syndrome may hold some therapeutic promise, as will small molecules to help reform the type I PKA holoenzymes in patients with Carney complex (113, 115). That being said, only time will tell if PKA represents a disease locus or a viable target for drug discovery.

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Abbreviations

PKA	cyclic-AMP dependent protein kinase/protein kinase A
cAMP	3',5'-cyclic adenosine monophosphate
ATP	adenosine 5'-triphosphate
C subunits	catalytic subunits
R subunits	regulatory subunits
AKAP	A-kinase anchoring protein
AGC group of kinases	protein A, protein G and protein C group of kinases

PKI

protein kinase inhibitor

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Highlights

1. Second messenger signaling events regulated by cyclic AMP.
2. An overview of the catalytic subunit alpha of protein kinase A.
3. PKA signaling in cancer, heart disease and endocrine disorders.

Figure 1. A) Alternate splicing of *PRKACA* exon 1: PKA C α 1 and PKA C α 2

This schematic depicts alternate splicing in exon 1 of *PRKACA* that gives rise to two distinct transcripts. **B) Alternate forms of PKA C α .** This schematic shows the differences in the amino terminal amino acid sequence of C α 1 and C α 2. The remainder of the sequence is identical. **C) The anchored PKA holoenzyme.** This schematic representation of the protein components of an anchored PKA holoenzyme shows the C subunits (blue) and the R subunit dimer (green) in complex with an AKAP (yellow). Following stimulation of cAMP synthesis, the second messenger (black) binds to the regulatory subunits in a manner that releases the catalytic subunits to phosphorylate target substrates.

Figure 2. A lesion in chromosome 19 underlies the formation of a chimeric DNAJ-PKAc protein in patients with fibrolamellar hepatocellular carcinoma (FL-HCC)

A 400-kilobase pair deletion in chromosome 19 is found in almost all FL-HCC tumors sequenced. This deletion event results in the in-frame fusion of the first exon of *DNAJB1* (orange) with exons two through ten of *PRKACA* (blue). This deletion event results in the production of a fusion pre-mRNA, and is eventually translated into a functional protein (DNAJ-PKAc).