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Cyclic-AMP-dependent protein kinase (PKA) in testicular cells. Cell specific expression, differential regulation and targeting of subunits of PKA ☆

Vidar Hansson, Bjørn S. Skålhegg*, Kjetil Taskén

Institute of Medical Biochemistry, University of Oslo, P.O. Box 1112, N-0317, Oslo, Norway

Abstract

LH and FSH regulate via cyclic adenosine 3′5′ cyclic monophosphate (cAMP) and cAMP-dependent protein kinase (PKA), steroid biosynthesis is Leydig and Sertoli cells, respectively. Cyclic AMP also regulates a number of different cellular processes such as cell growth and differentiation, ion channel conductivity, synaptic release of neurotransmitters, and gene transcription. The principle intracellular target for cAMP in mammalian cells is the PKA. The fact that this broad specificity protein kinase mediates a number of discrete physiological responses following cAMP engagement, has raised the question of how specificity is maintained in the cAMP/PKA system. Here we describe features of this signaling pathway that may contribute to explain how differential effects of cAMP may occur.

1. Cyclic AMP and the cAMP-dependent protein kinase (PKA) signaling system

Reversible protein phosphorylation is a key regulatory mechanism in eukaryotic cells. Protein phosphorylation was first demonstrated to regulate the activity of glycogen phosphorylase in response to glucagon [1,2]. A heat-stable factor mediating the effect of glucagon on the phosphorylation status of glycogen phosphorylase was next identified as 3′,5′-cyclic adenosine monophosphate (cAMP) [3], and the concept of cAMP as an intracellular second messenger to a wide range of hormones (including gonadotropins), neurotransmitters, and other signaling substances was developed [4]. The target for cAMP was purified and identified as a cAMP regulated protein kinase [5], termed cAMP-dependent protein kinase (PKA; EC 2.7.1.37). In the absence of cAMP, PKA is an enzymatically inactive tetrameric holoenzyme consisting of two catalytic subunits (C) bound to a regulatory subunit (R) dimer (Fig. 1). Cyclic AMP binds cooperatively to two sites on each R promoter [for review, see 6,7]. Upon binding of four molecules of cAMP, the enzyme dissociates into an R subunit dimer with four molecules of cAMP bound and two free, active C subunits that phosphorylate serine and threonine residues on specific substrate proteins.

At present, the cAMP/PKA signaling pathway is known to be activated by a number of different receptors that upon binding of their respective ligands, transduce their signals over the cell membrane by coupling to G-proteins. These G-proteins interact with adenylyl cyclase on the inner membrane surface either to activate or to inhibit the production of cAMP. Receptors that activates PKA through generation of cAMP, regulate a vast number of cellular processes. In addition to steroidogenesis, cAMP/PKA regulate metabolism [8], gene activity [9], cell growth and division [10], cell differentiation [11,12], and sperm moti-

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* Corresponding author. Tel.: +47 22851454; Fax: +47 22851497.
E-mail address: bjorn.skalhegg@basalmed.uio.no (B.S. Skålhegg).

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lity [13], as well as ion channel conductivity [14]. Therefore, a major challenge has been to understand how specificity can be maintained in this second messenger system.

2. Isozymes of PKA

Initially, two different isozymes of PKA, termed I and II (PKAI and PKAII, respectively), were identified based on their pattern of elution from DEAE-cellulose columns [15,16]. The PKAI and PKAII, eluting at salt concentrations between 25 and 50 mM and 150 and 200 mM NaCl, respectively, were shown to contain C subunits associated with two different R subunits, termed RI and RII [6]. However, over the last 10 years molecular cloning techniques have revealed a great heterogeneity in both R and C subunits which reveal the potential of multiple isozymes of PKA.

2.1. Multiple isoforms of regulatory and catalytic subunits of PKA

Cloning of cDNAs for regulatory subunits has identified two RI subunits termed RIα [17,18] and RIβ [19,20] and two RII subunits termed RIIα [21,22] and RIIβ [23,24] as separate gene products. The RIα and RIβ subunits are dissimilar, but reveal high homology (81% identify at the amino acid level) as do the RIIα and RIIβ subunits (68% identify at the amino acid level). Recently, alternative splice variants of the RIα subunit has been demonstrated. RIα cDNAs with different leader exons and differentially regulated initiation from two promoters of the RIα gene were shown [25]. Furthermore, RIα and RIIβ are stimulated by cAMP both in Leydig cells and Sertoli cells, and thus tune cAMP/PKA responsiveness within its own signaling system.

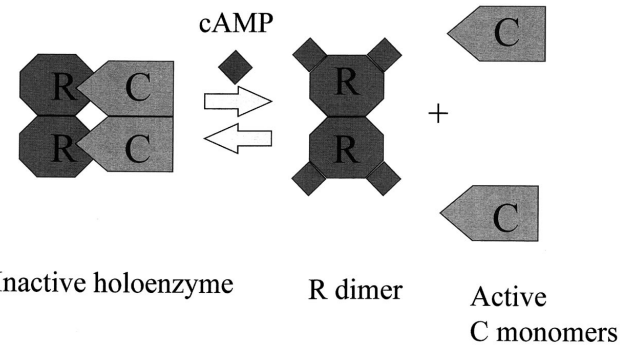

Fig. 1. Cyclic AMP-dependent protein kinase (PKA) is a holoenzyme consisting of a regulatory (R) subunit dimer and two catalytic (C) subunits. Activation of PKA occurs when four molecules of cAMP bind to the R subunit dimer, two to each subunit, in a positive cooperative fashion. When both cAMP binding sites (A and B) are occupied the R subunit adopt a confirmation with low affinity for the C subunit and the holoenzyme dissociates. The relation between free C subunits, the R subunit dimer and the intact holoenzyme is an equilibrium which is determined by several factors, that include the relative concentration of PKA subunits, cAMP in addition to salt concentration, pH and temperature.

Two distinct C subunits were initially identified by molecular cloning, and were designated Cα [26] and Cβ [27,28]. The cloning of the Cα and Cβ subunits from human testis by low homology screening also revealed an additional C subunit, designated Cγ, expressed only in late pachytene spermatocytes and early haploid germ cells in primates [29,30]. Recent work has also revealed the existence of splice variants of the human form of Cα(Cα2), which is catalytically inactive due to truncation of the C-terminal region [31]. Furthermore, a splice variant of the bovine form of Cβ (Cβ2) where the mRNA encodes a protein with an additional amino terminal 47 amino acids has been identified [32]. Recently, two novel brain specific splice variants of the mouse Cβ form (Cβ2 and Cβ3) have been cloned [33,34]. The previously described Cβ has now been designated Cβ1 [28]. Cβ2 and Cβ3 represent N-terminal truncated splice variants and are catalytically fully active.

2.2. Features of the regulatory and the catalytic subunits of PKA

2.2.1. Structure of the regulatory subunits

The RI and RII subunits contain an amino terminal dimerization domain, a region responsible for interaction with the C subunit, and in the carboxy terminus, two tandem cAMP binding sites, termed sites A and B [35,36]. Dimerization was initially discovered by the fact that proteolytic cleavage in the hinge region of the molecule would produce a monomeric R subunit with a cAMP binding activity [37]. For the RI subunits, dimerization involves two disulfide bridges (Cys16 and Cys37) [38]. Dimerization and anchoning of the RII subunit resides in an x-type four helix bundle domain (amino acid 1–44) [39]. The hinge region of the molecule, that has a site sensitive to proteolysis, is involved in binding to the substrate binding site of the C subunit. The RII subunits serve as true substrates and are phosphorylated by the C subunits of PKA. In contrast, the RI subunits are not phosphorylated and bind C's as pseudosubstrates. Of the two tandem cAMP binding sites, only site B is exposed in the inactive tetrameric PKA complex [reviewed in 7]. Binding of cAMP to this site enhances binding of cAMP to the A site in a postively cooperative fashion, as a result of the conformational change in the molecule. The characteristics of the two cAMP binding sites have been described in detail elsewhere [reviewed in 6,7,40], as have the relative affinities and site selectivity's of a wide array of chemically modified cAMP analogs [41].

The crystal structure of a monomeric RI deletion mutant (Δ1–91) has been reported [42], and this provides a model for cAMP-binding.

2.2.2. Structure of the catalytic subunits

All the C subunits (Cα, Cβ, Cγ) have catalytic core motifs that are common to all protein kinases [43,44], and involve a MgATP binding site as well as a peptide binding site. The crystal structure of the murine Cα subunit was the first protein kinase crystal structure available [45]. This has served as a template for modeling all other kinases. The catalytic subunit is a nearly globular protein with two lobes. The small, amino terminal lobe is involved in MgATP-binding, whereas the larger carboxy terminal lobe is involved in peptide binding and catalysis. Both MgATP and the peptide come together for catalysis in the cleft between the two lobes. The C subunit (except the inactive Cα2) contain a domain that involves additional sites apart from the peptide binding site [46]. This site is capable of binding the heat stable protein kinase inhibitor (PKI). PKI, which contains a NES (nuclear export signal), has the ability of transporting the C subunit from the nucleus to the cytosol and serves as a major regulator of C subunit activity [47]. Interestingly, when compared to Cα the Cγ subunit has a substitution at amino acid 133 and does not bind PKI, and may thus not be exported from the nucleus [47,48]. Furthermore, all the C subunits except bovine Cβ2 and mouse Cβ2, have the potential of being myristoylated at the N-terminus. This modification may serve to stabilize the C subunit secondary structure [46,49]. Despite that the bovine Cβ2 lacks a myristoylation site, the N-terminal extension which is hydrophobic, may serve the same function [32,49].

3. Regulation of levels and expression of the regulatory and catalytic subunits

In a number of different cells and tissues extensive studies have been performed in order to demonstrate differential expression of R and C subunits. Levels of expression of the different PKA subunits are subject to regulation by hormones acting through G-protein coupled receptors [50–52], mitogen signals through receptors associated with protein tyrosine kinases (PTK) [53], as well as by steroid hormones [54]. Regulation of PKA by hormones acting through cAMP may serve as an autologous sensitization–desensitization mechanism of the cAMP effector system.

Cyclic AMP mediated regulation of levels of PKA subunits acts primarily through gene transcription [55,56] but also influences mRNA stability [57]. At the protein level, stability of R and C subunits are heavily dependent on the degree of dissociation of the holoenzyme, which is regulated by cAMP [55,58].

3.1. Developmental expression of regulatory and catalytic subunits of PKA

Gonadal tissues have a high level of α subunits as well as β subunits of PKA. Age studies of whole rat testes revealed distinct developmental changes in the expression of PKA subunits [57,59,60]. At a prepubertal stage high levels of RIα (2.8 and 3.2 kb), RIIα (6.0 kb), RIIβ (3.2 kb), and Cα (2.4 kb) mRNAs was detected. These are the mRNA species primarily seen in somatic cells. During puberty germ cells increase exponentially and haploid cells appear. At later stage the large number of germ cells dominate the testis and dilute signals from somatic cells. During this time period small germ cell mRNA's encoding RIα, RIIα, and RIIβ and mRNAs appear. These shorter messages result from germ cell specific use of alternative polyadenylation site signals and may be important for long term storage of mRNA [60]. Whereas the RIα, RIβ, and Cα subunits in germ cells are induced at premeiotic and meiotic stages, the RII subunits are induced only during spermatid elongation. The Cβ mRNA was detected in peritubular cells and Leydig tumor cells but not in Sertoli cells or germ cells [60].

3.2. Regulation of PKA subunits in rat Sertoli cells and Leydig cells

Rat Sertoli cells serves as a good model system for studies of hormone responsiveness in general and of PKA regulation in particular. FSH and cAMP induce aromatization of testosterone to estradiol-17β and stimulate both regulatory and catalytic subunits of PKA. The up-regulation of RIα, RIIβ, and Cα mRNAs after treatment by cAMP is, at least partly, due to an increased transcriptional activity [56], and in the case of RIIβ also involves increased stability of the mRNA [57]. Very similar effects are observed after stimulation of tumor Leydig cells (MA-10) with hCG or cAMP [61]. In Sertoli cells, similar regulatory changes are observed in RIα, RIIα and RIIβ protein [62].

Different mechanisms are involved in the regulation of the RIIβ and RIα genes. Whereas transcriptional activity of the RIα gene (maximal at 30 min) is induced with similar kinetics as that of the *c-fos* gene, the induction of the RIIβ gene is increasing throughout the observation period (120 min). Furthermore, the RIα mRNA is superinduced by combined treatment with cAMP and a protein synthesis inhibitor (cycloheximide). In contrast, inhibition of protein synthesis almost completely blocks the cAMP-mediated induction of the

RIIβ gene [56]. Regulation of the RIIα gene appears to be qualitatively similar to that of RIIβ, but is quantitatively less pronounced.

The RIα and RIIβ genes are also subject to regulation by PKC [63]. Again the mechanisms of regulation appear to be different. PKC-dependent activation of RIα is unaffected by cycloheximide whereas induction of RIIβ is dependent of on-going protein synthesis [63]. Cyclic AMP and TPA have additive effects on the regulation of the RIα message, whereas TPA inhibits the cAMP-mediated induction of the RIIβ gene.

Thus, there is extensive evidence showing differential mechanisms of regulation of the R subunit genes. The RIα gene seems to be regulated by cAMP with similar characteristics as the cAMP response element (CRE) regulated *c-fos* gene. The 5′-flanking sequence of the RIα gene contains a consensus CRE that is conserved between pig [64] and man [25]. Furthermore, cloning of an alternatively spliced mRNA with a different leader exon leads to the identification of two alternatively initiated promoters in the RIα gene that are differentially regulated [25]. In contrast, the RIIβ gene has a regulation by cAMP distinct from that of RIα and *c-fos*, and belongs to a group of genes which respond to cAMP with slower kinetics and have a cAMP-responsive regions distinct from the classical CRE, TRE, and AP-2 elements [65–67], that involve up regulation of the CAAT enhancer binding protein C/EBP [127].

3.3. Regulation of PKA subunits by mitogens

While activation of PKA leads to a mitogenic response in Leydig cells and Sertoli cells, the opposite is seen in many other cell types e.g., lymphocytes. Such cells are different from steroidogenic cells and express both $RI\alpha_2C\beta_2$ and $RII\alpha_2C\beta_2$, but lack $RII\beta_2C_2$ [68].

Upon T cell receptor triggering, an initial peak of cAMP and PKA activity [53,69] is observed that may serve as an acute negative modulator and a negative feedback of signaling through TCR. This is followed by regulatory changes of R and C subunit levels within hours of stimulation. The biological implication of this regulation may be that the R/C ratio is transiently increased, leading to a down-regulation of PKAI activity, which may be important for the G/S transition of the cell cycle, following TCR-induced mitogen stimulation [68]. Similar reciprocal regulation of level of RIα and mRNA and protein was observed in a panel of lymphoid cell lines investigated for PKA regulation, levels of cAMP and cell growth [70]. In contrast, activation of PKA by cAMP in both Leydig cells and Sertoli cells leads to a mitogenic effect with subsequent increase in RIIβ mRNA and protein [51].

3.4. Transcriptional regulation of the genes for PKA subunits

Upstream regulatory sequences have been reported for the genes encoding RIα [25,64], RIβ [71], RIIα [72], RIIβ [73,74], Cα [75], and Cβ [75]. All these genes have GC-rich and TATA-less promoters. Furthermore, the human gene for RIα has two promoters directing expression of alternatively initiated RIα mRNAs with different 5′ non-translated regions. The two different promoters provide a more complex regulation of the RIα mRNA and proteins [25,76].

Regulation of the RIIβ gene have been subject to extensive studies. RIIβ was first isolated and cloned from rat granulosa cells [23] where a 6–10-fold induction of its mRNA by cAMP is seen [77]. Studies of the 5′-flanking region of the rat RIIβ gene in ovarian granulosa cells revealed that the cAMP-responsiveness resided within a distinct region (-395 to -293) upstream of the translation initiation codon [73].

For transfection in Sertoli cells, 5′-deletions of the RIIβ flanking region were inserted in front of a CAT reporter gene. Basal CAT activity directed from the different constructs was reduced to approximately 50% when the region -723 to -395 was included. The same region conferred cAMP responsiveness to the CAT reporter gene. In contrast, transfections of the same constructs into rat testis peritubular cells revealed that the cAMP-responsiveness as well as the inhibition of basal activity that resided within the region -723 to -395 was specific to Sertoli cells. Mapping of the cAMP-responsive region by gel retardation and DNAse I footprinting experiments identified several protected regions that are candidates for novel cAMP responsive elements [78].

4. PKA isozyme composition and characteristics

It is generally assumed that the catalytic subunits associate freely with homodimers of all the R subunits. However, PKAI holoenzymes are more readily dissociated by cAMP in vitro than PKAII holoenzymes [6,79]. Furthermore, when RII is overexpressed in 3T3 cells, the C subunit will preferably be bound to RII, whereas RI will be present as free dimer [80]. This indicates that PKAII holoenzyme forms preferentially compared to PKAI under physiological circumstances either due to lower sensitivity to cAMP or due to kinetics of association/dissociation influenced by salt and MgATP [reviewed in 7]. This observation is confirmed in mice that are genetically null mutant for the RIIβ subunit where RIα is induced and PKAI is formed, not as a result of increased transcription of the RIα gene, but rather due to an increased half life (up to 5-fold) of the RIα protein when associated with C [81].

Furthermore, the PKAI ($RI\alpha_2C_2$ and $RI\beta_2C_2$) and PKAII ($RII\alpha_2$ and $RII\beta_2C_2$) holoenzymes have distinct biochemical properties. RIβ holoenzymes are 2- to 7-fold more sensitive to cyclic nucleotides than RIα holoenzymes [82–84]. RIIα and RIIβ holoenzymes elute from DEAE-cellulose columns at different positions in the PKAII area, and RIIα expressed at high levels will compete with RIIβ in binding the C subunit, indicating either a higher affinity for the C subunit or a higher threshold for cAMP induced dissociation [85].

Characterization of a cell line almost completely devoid of PKAII, revealed the presence of an isozyme consisting of an RIα–RIβ heterodimer with associated phosphotransferase activity. This isozyme elutes in the position of PKAII by DEAE-cellulose chromatography [86]. Formation of RIα–RIβ heterodimeric complexes was also demonstrated in vitro by coimmunoprecipitation, using recombinant proteins [86]. How PKA isozyme composition relates to steroigenic responses remains to be elucidated.

5. Subcellular localization of PKA

Compartmentalization of PKA is mediated through binding of the R subunit to subcellular components [87]. In general, PKAI ($RI\alpha_2C_2$, $RI\beta_2C_2$) is soluble and is preferentially located to the cytosol. However, there are an increasing number of reports of RIα association with subcellular components of the cell. In lymphocytes, RIα associates with the antigen receptor during activation and capping [88,89], and in muscle RIα has been implicated at the neuromuscular junction [90]. Moreover, it was recently demonstrated that RIα binds to the adapter protein Grb2, an association which allows PKAI to interact with the epidermal growth factor receptor in epithelial MCF-10A cells [91]. Furthermore, a recent report demonstrated a dual-specificity. A kinase anchoring protein (AKAP) for both RIα and RIIα. This AKAP is designated D-AKAP1 [92]. In contrast to PKAI, PKAII isozymes ($RII\alpha_2C_2$, $RII\beta_2C_2$) are generally associated with the particulate fraction of the cell through the hydrophobic interaction of AKAPs with the dimerization domain of RII [93]. A number of different anchoring proteins have been identified and serve to sequester PKAII with the cytoskeletal elements such as microtubules (MAP2), postsynaptic densities and cortical actin (AKAP79/75), filopodia (Gravin/AKAP250), actin-binding proteins (ezrin/AKAP78) and centrosomes (AKAP350 AKAP450) [94–98,128]. Also membrane anchored and organelle associated AKAPs have been identified, such as AKAP100 of the smooth sarcoplasmatic reticulum, AKAP220 on peroxisomes, AKAP85 bound to the Golgi, AKAP84/149 in mitochondria and AKAP15/18 membrane anchored and associated with the L-type Ca^{2+} channel [99–106]. Furthermore, despite the absence of PKA R subunits from the nucleus, nuclear AKAPs (AKAP95, nAKAP150) have been identified, the biological significance of these AKAP are still elusive as PKAII holoenzyme complex is excluded from the nuclei in interphase [107]. However, AKAP95 detaches from nuclear matrix at mitosis and colocalizes with RIIα outside the metaphase plate [108]. As a further refinement of specificity in binding of PKAII to AKAPs, it has been demonstrated preferential association of AKAP95 with RIIα and not RIIβ [107], and that RIIα but not RIIβ associate with the Golgi apparatus where as RIIβ preferentially associate with centrosomes [109]. Interestingly, it has recently been reported that some AKAPs (AKAP79, Gravin AKAP220) function as signaling scaffold proteins by binding and assembly of different signaling proteins such as phosphates 2B (Calsineurin), PKC and PP1 (protein phosphatase 1) in addition to PKAII [110,129]. Whether such "signaling units" play a role in steroidigenic responses is not known.

6. Effects of cAMP mediated by specific isozymes of PKA

Since the demonstration of a multitude of PKA isozymes, a key question has been to what extent different effects of cAMP may be mediated by specific isozymes. Approaches such as selective activation of one PKA isozyme by the use of combinations of cAMP analogs to complement each other in the preferential activation of PKAI or PKAII has demonstrated isozyme-specific effects of cAMP in cells. Furthermore, a major breakthrough in understanding the role of various isozymes of PKA in vivo, was made by creating mice that are null mutant for specific PKA subunits.

6.1. Cyclic AMP effects mediated by PKAI

It is generally assumed that specific isozymes of PKA localized to subcellular structures, mediates distinct effects of cAMP. The PKAI isozymes ($RI\alpha_2C_2$, $RI\beta_2C_2$) appears generally soluble and freely distributed in the cytoplasm [111]. Thus, it may appear that PKAI is promiscuous in its phosphorylation of proteins and regulates all activities that are triggered by cAMP. However, lymphoid cells have proved to be good model systems to demonstrate the specificity in cAMP signaling. Cell growth of Reh cells which are practically devoid of PKAII [86] is inhibited by cAMP. In Reh cells stable transfection with Cα, proliferation was specifically inhibited, an effect that could be counteracted by cotransfection of a dominant negative mutant of RIα, that does not bind cAMP [112]. These results testify to the role of the C subunit in mediating

cAMP-dependent inhibition of cell proliferation in lymphoid cells, but do not define the PKA holoenzyme responsible for mediating the cAMP effect. However, since Reh cells contains exclusively PKAI, this result indicate that the inhibitory effect of cAMP on lymphoid cell proliferation can be mediated via this isozyme. The inhibitory effect of cAMP through PKAI on cell proliferation have further been verified in T and B cells. Both these cells contain PKAI ($RI\alpha_2C\beta_2$) and PKAII ($RII\alpha_2C\beta_2$) in a proportion of 3:1 [68,88]. In resting cells the PKAI is 75% soluble whereas 75–90% of the PKAII is particulate. Quiescent cells can be activated to proliferate by cross-linking antigen receptor complexes (TCR/CD3 and BCR/Ig-complex, respectively). To test whether PKAI or PKAII mediates the inhibitory effect on proliferation of lymphoid cells, chemically modified cAMP analogs selective for either site A or site B of PKAI and PKAII [41,113] were used. The combination of 8-piperidino-cAMP (8-pip) and 8-aminohexylamino-cAMP (8-AHA) synergized in inhibiting incorporation of [^{3}H]thymidine in proliferating cells when compared to the effect of 8-AHA alone. No such synergism was observed when inhibition by 8-(4-chlorophenylthio) cAMP (8-CPT) was examined in the absence and presence of a small priming dose of N^6-benzoyl-cAMP (N^6-Bnz) that by itself had no effect on T and B cell proliferation. The combination 8-pip/8-AHA synergies strongly in the activation of PKAI. This is contrary to activation of PKAII where both 8-pip and 8-AHA compete for binding to the B site. In contrast, the combination of N^6-Bnz and 8-CPT tends primarily to activate PKAII. This is because 8-CPT binds to the B site of RII with much higher affinity than to the PKAI B site and N^6-Bnz binds to the A site of both RI and RII. Thus, inhibition of cell proliferation by cAMP appears to be a PKAI-mediated effect. Furthermore, using the same approach on metabolic responses, we demonstrated that cAMP-dependent inhibition of NK cell cytotoxicity is mediated by PKAI [114]. In addition, isozyme-specific effects of PKAI has been demonstrated in cAMP-induced apoptosis of a myeloid leukemia cell line (IPC-81) [115].

Further evidence for specific roles of PKA in vivo was first obtained when mice null mutant for the RIβ subunit were generated. These animals appeared healthy and fertile, but examination of brain slices revealed that they had lost the ability to undergo long term depression (LTD) in the Schaffer Collateral pathway. The RIα, RIIα and RIIβ are expressed in the hippocampus [116] but appears unable to compensate functionally for the loss of RIβ [117].

6.2. Cyclic AMP effects mediated by PKAII

Both RIIα and RIIβ have been reported to localize to the Golgi-centrosomal area of different cell types [118] and the RIIβ subunit is subject to extensive regulation by cAMP in steroidogenic cells. Centrosomal localization of the RII subunits is in agreement with the observations in T cells, and may suggest involvement of PKAII in cell cycle control and formation of the spindle apparatus. Colocalization and coimmunoprecipiation of RIIα of PKAII with $p34^{cdc2}$ kinase has also been reported [119], and both RIIα and RIIβ have recently been shown to serve as a substrate for cdc2 kinase in vitro [120,130]. However, a specific function of PKAII from these studies that can be ascribed to this localization remains to be shown. Furthermore, a previous study [121] showed that PKAII activity was associated with regulation of AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid)/kainate Ca^{2+} channels. Disruption of PKAII (RII) binding to the AKAP associated with the AMPA receptor impairs the PKAII-dependent regulatory effect on the Ca^{2+} flux in cultured hippocampal neurons. Similarly, specific anchoring of PKAII was necessary for cAMP-mediated modulation of the L-type calcium channel in heart skeletal muscle [37]. Also, PKAII ($RII\beta_2C_2$) has been shown to mediate cAMP-dependent activation of lipolysis and glycerol release from adipocytes in vitro [122]. Interestingly, similar effects have been shown in vivo in adipocytes of mice lacking the RIIβ subunit [123]. Disruption of the mouse RIIβ gene leads to a profound change in PKA composition in both white and brown adipose tissue (WAT, BAT), where RIIβ normally is the principal R subunit. WAT was significantly diminished in these animals despite normal food uptake and the animals were protected against diet-induced obesity and fatty liver. In the RIIβ null mutated mice, levels of RIα in brown adipose tissue were induced, generating an isozyme switch from PKAII to PKAI. Moreover, these studies also showed that the RIα containing holoenzyme is more readily activated by cAMP and causes an induction of uncoupling protein (UCP), increased metabolic rate and elevated body temperature, which together contribute to a chronically lean phenotype of RIIβ null mutant mice. These results are the first to demonstrate a specific effect of PKAII ($RII\beta_2C_2$) in vivo which was not compensated for by upregulation of PKAII holoenzymes. Finally, recent studies have revealed that cAMP-mediated signaling to the nucleus may partly depend on specific anchoring of PKAII isozymes as overexpression of AKAP increased cAMP-regulated reported gene activity. In contrast, an over all disruption of AKAP75 by overexpression of a soluble AKAP75, competed the effect of cAMP on the reported gene.

6.3. Specific effects of C subunits

A recent report demonstrates defects in synaptic

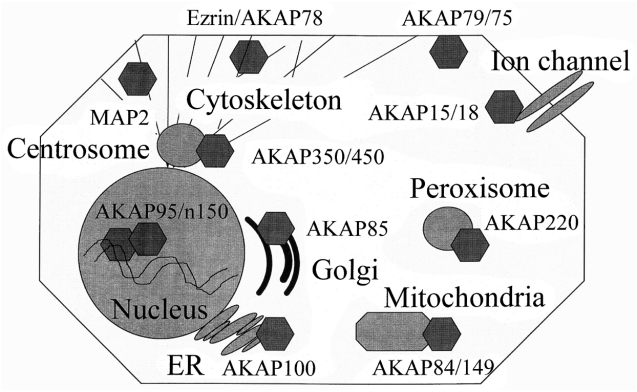

Fig. 2. Cyclic AMP-dependent protein kinase II (PKAII) is targeted to different subcellular compartments through binding to A kinase anchoring proteins (AKAPs). At present more than 20 AKAPs have been cloned and it has been suggested that some cells may express as many as 10–15 different AKAPs located to different compartments. These compartments may include the nucleus (AKAP95/n150), cytoskeleton (AKAP78, ezrin, MAP2), centrosome (AKAP350/450), ion channels (AKAP15), peroxisomes (AKAP220), the Golgi 450 (AKAP85), mitochondria (AKAP84/149), endoplasmatic reticulum (ER, AKAP100) and membranes (AKAP79/75).

plasticity in neurons of mice that are null mutant for the Cβ subunit. Interestingly, these effects could not be compensated for by the Cα subunit which quantitatively is expressed at a much higher level in the same cells [124]. Furthermore, Cγ does not bind PKI and may not be exported from the nucleus via PKI containing NES [48,125]. In addition, a very recent report demonstrates that Cα but not Cβ, Cγ nor any R subunit bind specifically to the cytosolic NFκB inhibitor IκB, which binds and sequester the transcription factor NFκB to the cytosol. In this study it was also demonstrated that Cα is activated through an cAMP-independent way through degradation of IκB [126]. Together these results demonstrate distinct effects of particular C subunits that may be either cAMP-dependent or independent.

7. Summary and perspectives

A large number of hormones, neurotransmitters, and other signaling substances that bind to G-protein coupled cell-surface receptors, converge their signals at one sole second messenger, cAMP. The question of how specificity can be maintained in a signal transduction system where many extracellular signals leading to a vast array of intracellular responses, mediate their responses through one single second messenger, cAMP, has been subject to thorough investigation and a great deal of speculation. An increasing number of PKA isozymes consisting of homo- or heterodimers of R subunits (RIα, RIβ, RIIα, RIIβ) with associated catalytic subunits (Cα, Cβ, Cγ) may contribute to the answer to this problem. Furthermore, the various PKA isozymes display distinct biochemical properties and the heterologus subunits of PKA reveal cell-specific expression and differential regulation at the level of gene transcription, mRNA stability and protein stability in response to a wide range of hormones and other signaling substances. Moreover, the existence of a number of anchoring proteins specific to either RI or RII subunits that localizes either PKAI or PKAII to distinct subcellular loci, strongly supports the idea that specific functions can be assigned to the various PKA isozymes. This is further strengthened by the demonstration that selective activation of PKAI is necessary and sufficient for cAMP-mediated inhibition of T and B cell proliferation and NK cell function which is compatible with the notion of isozyme-specific effects of PKAI. The observation that mitogenic activation is associated with redistribution and colocalization of PKAI, but not PKAII, strongly support the idea of PKA anchoring as a way of maintaining specificity of cAMP effects mediated by PKAI.

In the case of RII, a large number of AKAPs have been demonstrated that localize RII to different subcellular compartments (see Fig. 2). However, with the exception of cAMP-mediated modulation of AMPA/kainate channels in neurons and the L-type calcium channel in heart skeletal muscle no exact functions of PKAII specifically localized to distinct AKAPs have yet been demonstrated. The dramatic regulation of type IIβ PKA in stereogenic cells points to this isozyme as a candidate for mediating distinct functions in these cells. The fact the PKAI ($RI\alpha_2C_2$) can not compensate for the loss of PKAII ($RII\beta_2C_2$) in white adipose tissue in mice that are null mutant for the RIIβ subunit, indicate that a number of different cAMP effects yet to be characterized, are specifically mediated through soluble and not anchored PKA isozymes and vice versa. The fact that certain functions (synaptic plasticity, IκB binding) are mediated by specific C subunits further provides ways of achieving specificity in cAMP signaling.

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