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

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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 be contributed to features of the PKA signaling pathway.

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 motility [13], as well as ion channel conductivity [14]. Therefore, a major challenge has been to understand

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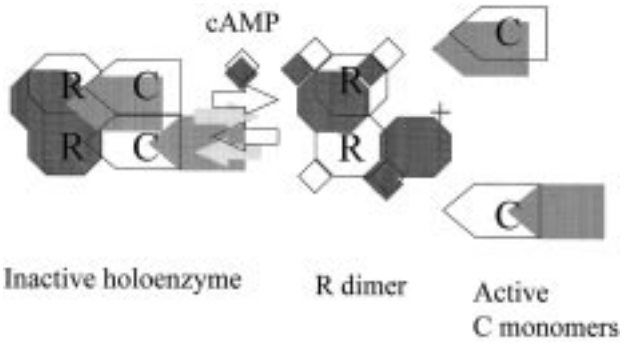

Fig. 1. Cyclic AMP-dependent protein kinase (PKA) is a holoenzyme consisting of a regulatory (R) subunit dimer and two catalytic (C) subunits. Activation of PKA occurs when four molecules of cAMP bind to the R subunit dimer, two to each subunit, in a positive cooperative fashion. When both cAMP binding sites (A and B) are occupied the R subunit adopt a confirmation with low affinity for the C subunit and the holoenzyme dissociate. 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.

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.

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 pachetyne 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 of the RII subunit does not involve cysteines, but the domain responsible for dimerization resides in the amino terminal part of the protein (amino acids 1–30) [39]. Despite that residues in the amino terminus (amino acids 1–5) of the RII dimer interact with anchoring proteins, it is assumed that additional contact points within the region 1–82 may exist [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 rela-

tive 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, the Cγ subunit is mutated 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 that 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 [16].

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 through primarily gene transcription [55,56] but also influences mRNA stability [57]. At the protein level stability's 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 in. 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 (cyclohexi-

mide). 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].

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 RIα_2Cβ_2 and RIIα_2Cβ_2, but lack RIIβ_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) [94–98]. 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, hAKAP150) 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) function as signaling scaffold proteins by binding and assembly of different signaling proteins such as phosphates 2B (Calsineurin) and PKC in addition to PKAII [110]. 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. However, a major breakthrough in understanding the role of various isozymes of PKA in vivo, was first 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] are 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 prolifer-

ation 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 strongly 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 that cAMP-induces 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], whereas RIIβ has recently been shown to serve as a substrate for cdc2 kinase in vitro [120]. 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 AKAP79 by overexpression of a soluble AKAP79, competed the effect of cAMP on the reported gene.

6.3. Specific effects of C subunits

A recent report demonstrates defects in synaptic plasticity in neurons of mice that are null mutant for

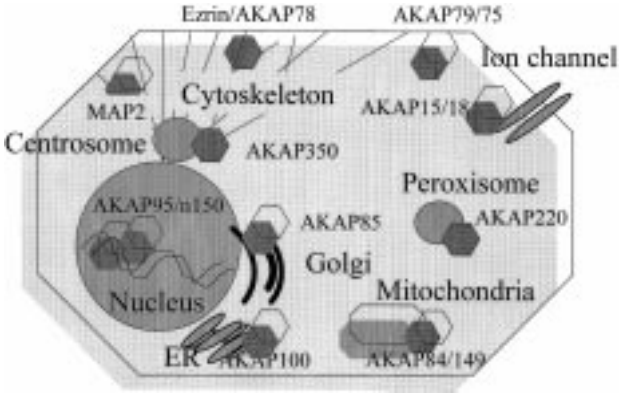

Fig. 2. Cyclic AMP-dependent protein kinase II (PKAII) is targeted to different subcellular compartments through binding to A kinase anchoring proteins (AKAPs). At present more than 20 AKAPs have been cloned and it has been suggested that some cells may express as many as 10–15 different AKAPs located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

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.

References

[1] E.H. Fischer, E.G. Krebs, Conversion of phosphorylase β to phosphorylase a in muscle extracts, J. Biol. Chem 216 (1955) 121–132.

[2] E.W. Sutherland, W.D. Wosilait, Inactivation and activation of liver phosphorylase, Nature 175 (1955) 169–170.

[3] E.W. Sutherland, T.W. Rall, Fractionation and characterization of a cyclic adenonsine ribonucleotide formed by tissue particles, J. Biol. Chem 232 (1958) 1077–1091.

[4] G.A. Robinson, R.W. Butcher, E.W. Sutherland, Cyclic AMP, Academic Press, New York, 1971.
[5] D.A. Walsh, J.P. Perkins, E.G. Krebs, An adenosine 3′,5′-monophosphate-dependent protein kinase from rabbit skeletal muscle, J. Biol. Chem 243 (1968) 2867–2873.
[6] S.J. Beebe, J.D. Corbin, Cyclic nucleotide-dependent protein kinases, The Enzymes 17 (1986) 11–43.
[7] S.O. Doøskeland, E. Maronde, B.T. Gjertsen, The genetic subtypes of cAMP-dependent protein kinase—functionally different or redundant?, Biochim. Biophys. Acta 1178 (1993) 249–258.
[8] E.G. Krebs, J.A. Beavo, Phosphorylation-dephosphorylation of enzymes, Annu Rev. Biochem 48 (1979) 923–959.
[9] W.J. Roesler, G.R. Vanderbark, R.W. Hanson, Cyclic AMP and the induction of eukaryotic gene transcription, J. Biol. Chem 263 (1988) 9063–9066.
[10] A.L. Boynton, J.F. Whitfield, The role of cAMP in cell proliferation: A critical assessment of the evidence, Adv. Cyclic Nucleotide Res 15 (1983) 193–294.
[11] A.Y-C. Liu, Differentiation-specific increase of cAMP-dependent protein kinase in 3T3-L1 cells, J. Biol. Chem 257 (1982) 298–306.
[12] D.A. Schwartz, C.S. Rubin, Regulation of cAMP-dependent protein kinase subunit levels in Friend erythroleukemic cells, J. Biol. Chem 258 (1983) 777–784.
[13] J.S. Tash, S.S. Kakar, A.R. Means, Flagellar motility requires the cAMP-dependent phosphorylation of a heat-stable NP-40 soluble 56 kDa protein, axokinin, Cell 38 (1984) 551–559.
[14] M. Li, J.W. West, R. Numann, B.J. Murphy, T. Scheuer, W.A. Catterall, Convergent regulation of sodium channels by protein kinase C and cAMP-dependent protein kinase, Science 261 (1993) 1439–1442.
[15] J.D. Corbin, S.L. Keely, C.R. Park, The distribution and dissociation of cyclic adenosine 3′:5′-monophosphate-dependent protein kinases in adipose, cardiac, and other tissues, J. Biol. Chem 250 (1975) 218–225.
[16] E.M. Reimann, D.A. Walsh, E.G. Krebs, Purification and properties of rabbit skeletal muscle adenosine 3′:5′-monophosphate-dependent protein kinases, J. Biol. Chem 246 (1971) 1986–1995.
[17] D.C. Lee, D.F. Carmichael, E.G. Krebs, G.S. McKnight, Isolation of a cDNA clone for the type I regulatory subunit of bovine cAMP-dependent protein kinase, Proc. Natl Acad. Sci., USA 80 (1983) 3608–3612.
[18] M. Sandberg, K. Taskén, O. Øyen, V. Hannson, T. Jahnsen, Molecular cloning, cDNA structure and deduced amino acid sequence for a type I regulatory subunit of cAMP-dependent protein kinase from human testis, Biochem. Biophys. Res. Commun 149 (1987) 939–945.
[19] C.H. Clegg, G.H. Cadd, G.S. McKnight, Genetic characterization of a brain-specific form of the type I regulatory subunit of cAMP-dependent protein kinase, Proc. Natl Acad. Sci. USA 85 (1988) 3703–3707.
[20] R. Solberg, K. Taskén, A. Keiserud, T. Jahnsen, Molecular cloning, cDNA structure and tissue-specific expression of the human regulatory subunit RIβ of cAMP-dependent protein kinases, Biochem. Biophys. Res. Commun 176 (1991) 166–172.
[21] O. Øyen, F. Myklebust, J.D. Scott, V. Hansson, T. Jahnsen, Human testis cDNA for the regulatory subunit RII_a of cAMP-dependent protein kinase encodes an alternate amino-terminal region, FEBS Lett 246 (1989) 57–64.
[22] J.D. Scott, M.B. Glaccum, M.J. Zoller, M.D. Uhler, D.M. Helfman, G.S. McKnight, E.G. Krebs, The molecular cloning of a type II regulatory subunit of the cAMP-dependent protein kinase from rat skeletal muscle and mouse brain, Proc. Natl Acad. Sci., USA 84 (1987) 5192–5196.
[23] T. Jahnsen, L. Hedin, V.J. Kidd, W.G. Beattie, S.M. Lohmann, U. Walter, J. Durica, T.Z. Schulz, E. Schiltz, M. Browner, C.B. Lawrence, D. Goldman, S.L. Ratoosh, J.S. Richards, Molecular cloning, cDNA structure and regulation of the regulatory subunit of type II cAMP-dependent protein kinase from rat ovarian granulosa cells, J. Biol. Chem 261 (1986) 12,352–12,361.
[24] F.O. Levy, O. Øyen, M. Sandberg, K. Taskén, W. Eskild, V. Hannson, T. Jahnsen, Cloning complementary deoxyribonucleic acid structure and predicted full-length amino acid sequence of the hormone-inducible regulatory subunit of 3′,5′-cyclic adenosine monophosphate-dependent protein kinase from human testis, Molec. Endocr 2 (1988) 1364–1373.
[25] R. Solberg, M. Sandberg, V. Natarajan, P.A. Torjesen, V. Hansson, T. Jahnsen, K. Taskén, The human gene for the regulatory subunit RIα of cAMP-dependent protein kinase—Two distinct promoters provide differential regulation of alternately spliced mRNAs, Endocrinology 138 (1997) 169–181.
[26] M.D. Uhler, D.F. Carmichael, D.C. Lee, J.C. Chrivia, E.G. Krebs, G.S. McKnight, Isolation of cDNA clones coding for the catalytic subunit of mouse cAMP-dependent protein kinase, Proc. Natl Acad. Sci., USA 83 (1986) 1300–1304.
[27] M.O. Showers, R.A. Maurer, A cloned bovine cDNA encodes an alternate form of the catalytic subunit of cAMP-dependent protein kinase, J. Biol. Chem 261 (1986) 16,288–16,291.
[28] M.D. Uhler, J.C. Chrivia, G.S. McKnight, Evidence for a second isoform of the catalytic subunit of cAMP-dependent protein kinase, J. Biol. Chem 261 (1986) 15,360–15,363.
[29] S.J. Beebe, O. Øyen, M. Sandberg, A. Frøysa, V. Hansson, T. Jahnsen, Molecular cloning of a tissue-specific protein kinase (C gamma) from human tests—representiing a third isoform for the catalytic subunit of cAMP-dependent protein kinase, Molec. Endocr 4 (1990) 465–475.
[30] N. Reinton, T.B. Haugen, S. Ørstavik, B.S. Skålhegg, V. Hansson, T. Jahnsen, K. Taskén, The gene encoding the C gamma catalytic subunit of cAMP-dependent protein kinase is a transcribed retroposon, Genomics 15 (1998) 287–290.
[31] D.C. Thomis, G. Floyd-Smith, C.E. Samuel, Mechanism of interferon action. cDNA structure and regulation of a novel splice-site variant of the catalytic subunit of human protein kinase A from interferon-treated human cells, J. Biol. Chem 267 (1992) 10,723–10,728.
[32] S. Wiemann, V. Kinzel, W. Pyerin, Isoform Cβ2, an unusual form of the bovine catalytic subunit of cAMP-dependent protein kinase, J. Biol. Chem 266 (1991) 5140–5146.
[33] G.R. Guthrie, B.S. Skålhegg, G.S. McKnight, Two novel brain-specific splice variants of the murine C beta gene of cAMP-dependent protein kinase, J. Biol. Chem 272 (1998) 29,560–29.
[34] G.S. McKnight, R.L. Idzerda, E.R. Kandel, E.P. Brandon, M. Zhuo, M. Qi, R. Bourtchouladze, Y.Y. Huang, K.A. Burton, B.S. Skålhegg, D.E. Cummings, L. Vashavsky, J.V. Planas, K. Motamed, K.A. Gerhold, P.S. Amieux, C.R. Guthrie, K.M. Millet, M. Belyamani, T. Su, Targeted disruption of the protein kinase A system in mice, in: V. Hansson, F.O. Levy, K. Tasken (Eds.), Signal transduction in testicular cells Basic and Clinical Aspects, vol. vol. 1, Springer-Verlag, Berlin, 1996, pp. 95–122.
[35] J.D. Corbin, P.H. Sugden, L. West, D.A. Flockhardt, T.M. Lincoln, D. McCarthy, Studies on the properties and mode of action of purified regulatory subunit of bovine heart adenosine 3′:5′-monophosphate-dependent protein kinase, J. Biol. Chem 253 (1978) 3997–4003.
[36] S.O. Døskeland, Evidence that rabbit muscle protein kinase has two kineatically distinct binding sites for adenosine 3′:5′-cyclic monophosphate, Biochem. Biophys. Res. Commun 83 (1978) 542–549.

[37] R.L. Potter, P.H. Stafford, S.S. Taylor, Regulatory subunit of cAMP-dependent protein kinase I from porcine skeletal muscle: purification and proteolysis, Arch. Biochem. Biophys 190 (1978) 174–180.

[38] J. Bubis, T.S. Vedvick, S.S. Taylor, Antiparallel alignment of the two promoters of the regulatory subunit dimer of cAMP-dependent protein kinase I, J. Biol. Chem 262 (1987) 14,961–14,966.

[39] J.D. Scott, R.E. Stofko, J.R. McDonald, J.D. Comer, E.A. Vitalis, J.A. Mangili, Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase, J. Biol. Chem 265 (1990) 21,561–21,566.

[40] J.D. Scott, Cyclic nucleotide-dependent protein kinases, Pharmacol. Ther 50 (1991) 123–145.

[41] D. Øgreid, R. Ekanger, R.H. Suva, J.P. Miller, S.O. Døskeland, Comparison of the two classes of binding sites (A and B) of type I and type II cyclic-AMP-dependent protein kinases by using cyclic nucleotide analogs, Eur. J. Biochem 181 (1989) 19–31.

[42] Y. Su, S.S. Taylor, W.R.G. Dostmann, N.H. Xuong, K.I. Varughese, Crystallization of a deletion mutant of the R-subunit of cAMP dependent protein kinase, J. Molec. Biol 230 (1993) 1091–1093.

[43] S.K. Hanks, A.M. Quinn, T. Hunter, The protein kinase family: Conserved features and deduced phylogeny of the catalytic domains, Science 241 (1988) 42–52.

[44] S.S. Taylor, D.R. Knighton, J. Zheng, L.F. Ten Eyck, J.M. Sowadski, Structural framework for the protein kinase family, Annu Rev. Cell. Biol 8 (1992) 429–462.

[45] D.R. Knighton, J.H. Zheng, L.F. Ten Eyck, V.A. Ashford, N.H. Xuong, S.S. Taylor, J.M. Sowadski, Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase, Science 253 (1991) 407–414.

[46] D.R. Knighton, N.H. Xuong, S.S. Taylor, J.M. Sowadski, Crystallization studies of cAMP-dependent protein kinase. Cocrystals of the catalytic subunit with a 20 amino acid residue peptide inhibitor and MgATP diffract to 3.0 A resolution, J. Mol. Biol 220 (1991) 217–220.

[47] W. Wen, J.L. Meinkoth, R.Y. Tsien, S.S. Taylor, Identification of a signal for rapid export of proteins from the nucleus, Cell 82 (1995) 463–473.

[48] S.J. Beebe, P. Salomonsky, T. Jahnsen, Y. Li, The C gamma subunit is a unique isozyme of the cAMP-dependent protein kinase, J. Biol. Chem 267 (1992) 25,505–25,512.

[49] C.H. Clegg, W. Ran, M.D. Uhler, G.S. McKnight, A mutation in the catalytic subunit of protein kinase A prevents myristylation but does not inhibit biological activity, J. Biol. Chem 264 (1989) 20,140–20,146.

[50] T. Jahnsen, S.M. Lohmann, U. Walter, L. Hedin, J.S. Richards, Purification and characterization of hormone-regulated isoforms of the regulatory subunit of type II cAMP-dependent protein kinase from rat ovaries, J. Biol. Chem 260 (1985) 15,980–15,987.

[51] B.F. Landmark, O. Øyen, B.S. Skålhegg, B. Fauske, T. Jahnsen, V. Hansson, Cellular localization and age-dependent changes of the regulatory subunits of cAMP-dependent protein kinase in rat testis, J. Reprod. Fertil 99 (1993) 323–334.

[52] O. Øyen, M. Sandberg, W. Eskild, F.O. Levy, G. Knutsen, S. Beebe, V. Hansson, T. Jahnsen, Differential regulation of messenger ribonucleic acids for specific subunits of cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent protein kinase by cAMP in rat Sertoli cells, Endocrinology 122 (1988) 2658–2666.

[53] B.S. Skålhegg, K. Taskén, A.M. Rasmussen, V. Hansson, T. Jahnsen, T. Lea T-cell activation through the TCR/CD3 complex is associated with regulatory effects on subunits of cAMP-dependent protein kinase (PKA). Involvement of tyrosine kinases, protein kinase C and cAK. Manuscript 1998.

[54] F.O. Levy, A.H. Ree, L. Eikvar, M.V. Govindan, T. Jahnsen, V. Hansson, Glucocorticoid receptors and glucocorticoid effects in rat sertoli cells, Endocrinology 124 (1989) 430–436.

[55] K. Taskén, K.B. Andersson, B. Skålhegg, K.A. Taskén, V. Hansson, T. Jahnsen, H.K. Blomhoff, Reciprocal regulation of mRNA and protein for subunits of cAMP-dependent protein kinase (RIα and Cα) by cAMP in a neoplastic B cell line (Reh), J. Biol. Chem 268 (1993) 23,483–23,489.

[56] K.A. Taskén, H.K. Knutsen, H. Attramadal, K. Taskén, T. Jahnsen, V. Hansson, W. Eskild, Different mechanisms are involved in cAMP-mediated induction of mRNAs for subunits of cAMP-dependent protein kinases, Molec. Endocr 5 (1991) 21–28.

[57] H.K. Knutsen, K.A. Taskén, W. Eskild, T. Jahnsen, V. Hansson, Adenosine 3′,5′-monophosphate-dependent stabilization of messenger ribonucleic acids (mRNAs) for protein kinase-A (PKA) subunits in rat Sertoli cells: rapid degradation of mRNAs for PKA subunits is dependent on ongoing RNA and protein synthesis, Endocrinology 129 (1991) 2496–2502.

[58] G. Houge, O.K. Vintermyr, S.O. Døskeland, The expression of cAMP-dependent protein kinase subunits in primary rat hepatocyte cultures. Cyclic AMP down-regulates its own effector system by decreasing the amount of catalytic subunit and increasing the mRNAs for the inhibitory (R) subunits of cAMP-dependent protein kinase, Molec. Endocr 4 (1990) 481–488.

[59] O. Øyen, A. Frøysa, M. Sandberg, W. Eskild, D. Joseph, V. Hansson, T. Jahnsen, Cellular localization and age-dependent changes in mRNA for cyclic adenosine 3′,5′-monophosphate-dependent protein kinases in rat testis, Biol. Reprod 37 (1987) 947–956.

[60] O. Øyen, F. Myklebust, J.D. Scott, G.G. Cadd, G.S. McKnight, V. Hansson, T. Jahnsen, Subunits of cyclic adenosine 3′,5′-monophosphate-dependent protein kinase show differential and distinct expression patterns during germ cell differentiation: alternative polyadenylation in germ cells gives rise to unique smaller-sized mRNA species, Biol. Reprod 43 (1990) 46–54.

[61] A.H. Ree, A. Froysa, W. Eskild, T. Jahnsen, V. Hansson, Biphasic regulation of the messenger ribonuleic acid coding for the estrogen receptor by cyclic adenosine 3′:5′-monophosphate in tumor Leydig cells, Cancer Res 50 (1990) 1528–1531.

[62] B.F. Landkmark, B. Fauske, W. Eskild, B. Skälhegg, S.M. Lohmann, V. Hansson, T. Jahnsen, S.J. Beebe, Identification, characterization, and hormonal regulation of 3′,5′-cyclic adenosine monophosphate-dependent protein kinases in rat Sertoli cells, Endocrinology 129 (1991) 2345–2354.

[63] K.A. Taskén, H.K. Knutsen, L. Eikvar, K. Taskén, W. Eskild, T. Jahnsen, V. Hansson, Protein kinase C activation by 12-O-tetradecanoylphorbol 13-acetate modulates messenger ribonucleic acid levels for two of the regulatory subunits of 3′,5′-cyclic adenosine monophosphate-dependent protein kinases (RIIβ and RIα) via multiple and distinct mechanisms, Endocrinology 130 (1992) 1271–1280.

[64] I. Nowak, K. Seipel, M. Schwarz, D.A. Jans, B.A. Hemmings, Isolation of a cDNA and characterization of the 5′ flanking region of the gene encoding the type I regulatory subunit of the cAMP-dependent protein kinase, Eur. J. Biochem 167 (1987) 27–33.

[65] N. Kagawa, M.R. Waterman, cAMP-dependent transcription of the human CYP21B (P-450C21) gene requires a cis-regulatory element distinct from the consensus cAMP-regulatory element, J. Biol. Chem 265 (1990) 11,299–11,305.

[66] J. Lund, R. Ahlgren, D.H. Wu, M. Kagimoto, E.R. Simpson,

M.R. Waterman, Transcriptional regulation of the bovine CYP17 (P-450(17)alpha) gene. Identification of two cAMP regulatory regions lacking the consensus cAMP-responsive element (CRE), J. Biol. Chem 265 (1990) 3304–3312.

[67] J.M. Richardson, P. Howard, J.S. Massa, R.A. Maurer, Post-transcriptional regulation of cAMP-dependent protein kinase activity by cAMP in GH3 pituitary tumor cells. Evidence for increased degradation of catalytic subunit in the presence of cAMP, J. Biol. Chem 265 (1990) 13,635–13,640.

[68] B.S. Skålhegg, B.F. Landmark, S.O. Døskeland, V. Hansson, T. Lea, T. Jahnsen, AMP-dependent protein kinase type I mediates the inhibitory effects of 3′,5′-cyclic adenosine monophosphate on cell replication in human T lymphocytes, J. Biol. Chem 267 (1992) 15,707–15,714.

[69] D. Laxminarayana, G.M. Kammer, Activation of type I protein kinase A during receptor-mediated human T lymphocyte activation, J. Immunol 156 (1996) 497–506.

[70] B.S. Skålhegg, A.K. Johansen, F.O. Levy, K.B. Andersson, E.M. Aandahl, H.K. Blomhoff, V. Hansson, K. Taskén, Isozymes of cyclic AMP-dependent protein kinases (PKA) in human lymphoid cell lines. Levels of endogenous cAMP influence levels of PKA subunits and growth in lymphoid cell lines, J. Cell. Physiol (1998) (in press).

[71] K.V. Rogers, L.F. Boring, G.S. McKnight, C.H. Clegg, Promoter for the regulatory type Ib subunit of the 3′,5′-cyclic adenosine monophosphate-dependent protein kinase directs transgene expression in the central nervous system, Molec. Endocr 6 (1992) 1756–1765.

[72] K.B. Foss, R. Solberg, J. Simard, F. Myklebust, V. Hansson, T. Jahnsen, K. Taskén, Molecular cloning, upstream sequence and promoter studies of the human gene for the regulatory subunit RIIα of cAMP-dependent protein kinase, Biochim. Biophys. Acta 1350 (1997) 98–108.

[73] R.C. Kurten, L.O. Levy, J. Shey, J.M. Durica, J.S. Richards, Identification and characterization of the GC-rich and cyclic adenosine 3′,5′-monophosphate (cAMP)-inducible promoter of the type IIb cAMP-dependent protein kinase regulatory subunit gene, Molec. Endoc 6 (1992) 536–550.

[74] I.S. Singh, Z.J. Luo, A. Eng, J. Erlichman, Molecular cloning and characterization of the promoter region of the mouse regulatory subunit RIIβ of type II cAMP-dependent protein kinase, Biochem. Biophys. Res. Commun 178 (1991) 221–226.

[75] J.C. Chrivia, M.D. Uhler, G.S. McKnight, Characterization of genomic clones coding for the Ca and Cb subunits of mouse cAMP-dependent protein kinase, J. Biol. Chem 263 (1988) 5739–5744.

[76] R. Solberg, M. Sandberg, A. Spurkland, T. Jahnsen, Isolation and characterization of a human pseudogene for the regulatory subunit RIα of cAMP-dependent protein kinases and its sublocalization on chromosome 1, Genomics 15 (1993) 591–597.

[77] S.L. Ratoosh, J. Lifka, L. Hedin, T. Jahnsen, J.S. Richards, Hormonal regulation of the synthesis and mRNA content of the regulatory subunit of cyclic AMP-dependent protein kinase type II in cultured rat ovarian granulosa cells, J. Biol. Chem 262 (1987) 7306–7313.

[78] H.K. Knutsen, K. Taskén, W. Eskild, J.S. Richards, R.C. Kurten, P.A. Torjesen, T. Jahnsen, V. Hansson, S.L. Guérin, K.A. Taskén, Characterization of the 5′-flanking region of the gene for the cAMP-inducible protein kinase A subunit, RIIb, in Sertoli cells, Molecular and cellular endocrinology 129 (1997) 101–114.

[79] W.R. Dostmann, S.S. Taylor, H.G. Genieser, B. Jastorff, S.O. Døskeland, D. Øgreid, Probing the cyclic nucleotide binding sites of cAMP-dependent protein kinases I and II with analogs of adenosine 3′,5′-cyclic phosphorothioates, J. Biol. Chem 265 (1990) 10,484–10,491.

[80] A.D. Otten, G.S. McKnight, Overexpression of the type II regulatory subunit of the cAMP-dependent protein kinase eliminates the type I holoenzyme in mouse cells, J. Biol. Chem 264 (1989) 20,255–20,260.

[81] P.S. Amieux, D.E. Cummings, K. Motamed, E.P. Brandon, L.A. Wailes, K. Le, R.L. Idzerda, G.S. McKnight, Compensatory regulation of RIα protein levels in protein kinase A mutant mice, J. Biol. Chem 272 (1997) 3993–3998.

[82] G. Houge, R.A. Steinberg, D. Øgreid, S.O. Døskeland, The rate of recombination of the subunits (RI and C) of cAMP-dependent protein kinase depends on whether one or two cAMP molecules are bound per RI monomer, J. Biol. Chem 265 (1990) 19,507–19,516.

[83] R. Solberg, K. Taskén, W. Wen, V.M. Coghlan, J.L. Meinkoth, J.D. Scott, T. Jahnsen, S.S. Taylor, Human regulatory subunit RIb of cAMP-dependent protein kinases: Expression, holoenzyme formation, and microinjection into living cells, Exp. Cell Res 214 (1994) 595–605.

[84] K. Taskén, R. Kopperud, A.E. Christensen, L.K. Dybdahl, B. Fauske, B.T. Gjertsen, R. Solberg, V. Hansson, T. Jahnsen, S.O. Døskeland Kinetic properties of human regulatory subunits (RIα, RIβ, RIIα, and RIIβ) of cAMP-dependent protein kinase. Affinity for C distinguishes RIβ from RIα. Manuscript 1998.

[85] A.D. Otten, L.A. Parenteau, S.O. Døskeland, G.S. McKnight, Hormonal activity of gene transcription in ras-transformed NIH3T3 cells overexpressing RIIα and RIIβ subunits of the cAMP-dependent protein kinase, Biol. Chem 266 (1991) 23,074–23,082.

[86] K. Taskén, B.S. Skålhegg, R. Solberg, K.B. Andersson, S.S. Taylor, T. Lea, H.K. Blomhoff, T. Jahnsen, V. Hansson, Novel isozymes of cAMP-dependent protein kinase exist in human cells due to formation RIα-RIβ heterodimeric complexes, J. Biol. Chem 268 (1993) 21,276–21,283.

[87] J.D. Scott, S. McCartney, Localization of A-kinase through anchoring proteins, Molec. Endocr 8 (1994) 5–11.

[88] F.O. Levy, A.M. Rasmussen, K. Taskén, B.S. Skålhegg, H.S. Huitfeldt, S. Funderud, E.B. Smeland, V. Hansson, Cyclic AMP-dependent protein kinase (cAK) in human B cells: co-localized of type I cAK ($RI\alpha_2C_2$) with the antigen receptor during anti-immunoglobulin-induced B cell activation, Eur. J. Immunol 26 (1996) 1290–1296.

[89] B.S. Skålhegg, K. Taskén, V. Hansson, H.S. Huitfeldt, T. Jahnsen, T. Lea, Location of cAMP-dependent protein kinase type I with the TCR/CD3 complex, Science 263 (1994) 84–87.

[90] T. Imaizumi-Scherrer, D.M. Faust, J.C. Benichou, R. Hellio, M.C. Weiss, Accumulation in fetal muscle and localization to the neuromuscular junction of cAMP-dependent protein kinase A regulatory and catalytic subunits RI alpha and C alpha, J. Cell Biol 134 (1996) 1241–1254.

[91] G. Tortora, V. Damiano, C. Bianco, G. Baldassarre, A.R. Bianco, L. Lanfrancone, P.G. Pelicci, F. Ciardiello, The RIα subunit of protein kinase A (PKA) binds to Grb2 and allows PKA interaction with the activated EGF-receptor, Oncogene 14 (1997) 923–928.

[92] L.J. Huang, K. Durick, J.A. Weiner, J. Chun, S.S. Taylor, Identification of a novel protein kinase A anchoring protein that binds both type I and II regulatory subunits, J. Biol. Chem 272 (1997) 8057–8064.

[93] Z.E. Hausken, J.D. Scott, Properties of A-kinase anchoring proteins, Biochem. Soc. Trans 24 (1996) 986–991.

[94] D.W. Carr, Z.E. Hausken, I.D. Fraser, R.E. Stofko-Hahn, J.D. Scott, Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein. Cloning and characterization of the RII-binding domain, J. Biol. Chem 267 (1992) 13,376–13,382.

[95] G. Keryer, R.M. Rios, B.F. Landmark, B.S. Skålhegg, S.M.

Lohmann, M. Bornens, A high-affinity binding protein for the regulatory subunit of cAMP-dependent protein kinase II in the centrosome of human cells, Exp. Cell Res 204 (1993) 230–240.

[96] Y. Li, C. Ndubuka, C.S. Rubin, A kinase anchor protein 75 targets regulatory (RII) subunits of cAMP-dependent protein kinase to the cortical actin cytoskeleton in non-neuronal cells, J. Biol. Chem 271 (1996) 16,862–16,869.

[97] S.M. Lohmann, P. DeCamilli, I. Einig, U. Walter, High-affinity binding of the regulatory subunit (RII) of cAMP-dependent protein kinase to microtubule-associated and other cellular proteins, Proc. Natl Acad. Sci., USA 81 (1984) 6723–6727.

[98] J.B. Nauert, T.M. Klauck, L.K. Langeberg, J.D. Scott, Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein, Curr. Biol 7 (1997) 52–62.

[99] I.D.C. Fraser, S.J. Tavalin, L.B. Lester, L.K. Langeberg, A.M. Westphal, R.A. Dean, N.V. Marrison, J.D. Scott, A novel lipid-anchored A-kinase anchoring protein facilitates cAMP-responsive membrane events, EMBO J 15 (1998) 2261–2272.

[100] P.C. Gray, B.D. Johnson, R.E. Westenbroek, L.G. Hays, J.R3. Yates, T. Sceuer, W.A. Catterall, B.J. Murphy, Primary structure and function of an A kinase anchoring protein associated with calcium channels, Neuron 20 (1998) 1017–1026.

[101] L.B. Lester, V.M. Coghlan, B. Nauert, J.D. Scott, Cloning and characterization of a novel A-kinase anchoring protein. AKAP 220, association with testicular peroxisomes, J. Biol. Chem 271 (1996) 9460–9465.

[102] R-Y. Lin, S.B. Moss, C.S. Rubin, Characterization of S-AKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells, J. Biol. Chem 270 (1995) 27,804–27,811.

[103] S. McCartney, B.M. Little, L.K. Langeberg, J.D. Scott, Cloning and characterization of A-kinase anchor protein 100 (AKAP100). A protein that targets A-kinase to the sarcoplasmic reticulum, J. Biol. Chem 270 (1995) 9327–9333.

[104] S. McCartney, B.M. Little, J.D. Scott, Analysis of a novel A-kinase anchoring protein 100, (AKAP100), Biochem. Soc. Trans 23 (1995) 268S.

[105] R.M. Rios, C. Celati, S.M. Lohmann, M. Bornens, G. Keryer, Identification of a high affinity binding protein for the regulatory subunit RIIβ of cAMP-dependent protein kinase in Golgi enriched membranes of human lymphoblasts, EMBO J 11 (1992) 1723–1731.

[106] G. Trendelenburg, M. Hummel, E-O. Riecken, C. Hanski, Molecular characterization of AKAP149, a novel A kinase anchor protein with a KH domain, Biochem. Biophys. Res. Commun 225 (1996) 313–319.

[107] V.M. Coghlan, L.K. Langeberg, A. Fernandez, N.J. Lamb, J.D. Scott, Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase, J. Biol. Chem 269 (1994) 7658–7665.

[108] T. Eide, V.M. Coghlan, S. Orstavik, C. Holsve, R. Solberg, B.S. Skålhegg, N.J. Lamb, L.K. Langeberg, A. Fernandez, J.D. Scott, T. Jahnsen, K. Taskén, Molecular cloning, chromosal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95, Exp. Cell Res 1 (1998) 305–316.

[109] G. Keryer, B.S. Skålhegg, B.F. Landmark, V. Hansson, T. Jahnsen, K. Taskén Differential localization of the type II regulatory subunits, RIIα and RIIβ, of cAMP-dependent protein kinase in Golgi-centrosomal area—Characterization of monospecific antibodies to RIIα and RIIβ. Exp. Cell. Res. in press (1999).

[110] T.M. Klauck, M.C. Faux, K. Labudda, L.K. Langeberg, S. Jaken, J.D. Scott, Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein, Science 271 (1996) 1589–1592.

[111] J.L. Meinkoth, Y. Ji, S.S. Taylor, J.R. Feramisco, Dynamics of the distribution of cyclic AMP-dependent protein kinase in living cells, Proc. Natl Acad. Sci., USA 87 (1990) 9595–9599.

[112] K. Taskén, K.B. Andersson, B.K. Erikstein, V. Hansson, T. Jahnsen, H.K. Blomhoff, Regulation of growth in a neoplastic B cell line (Reh) by transfected subunits of cAMP-dependent protein kinase, Endocrinology 135 (1994) 2109–2119.

[113] S.O. Døskeland, R. Bøe, T. Bruland, O.K. Vintermyr, B. Jastorff, M. Lanotte, Criteria used to judge that a cellular response is mediated by cAMP, in: E. Reid, G.M.W. Cook, J.P. Luzio (Eds.), Cell Signalling: Experimental Strategies, Royal Society of Chemistry, Cambridge, UK, 1991, pp. 103–114.

[114] K.M. Torgersen, J.T. Vaage, F.O. Levy, V. Hansson, B. Rolstad, K. Taskén, Selective activation of cAMP-dependent protein kinase type I inhibits rat Natural Killer cell cytotoxicity, J. Biol. Chem 272 (1997) 5495–5500.

[115] M. Lanotte, J.B. Riviere, S. Hermouet, G. Houge, O.K. Vintermyr, B.T. Gjertsen, S.O. Døskeland, Programmed cell death (apoptosis) is induced rapidly and with positive cooperativity by activation of cyclic adenosine monophosphate-kinase I in a myeloid leukemia cell line, J. Cell Physiol 146 (1991) 73–80.

[116] G. Cadd, G.S. McKnight, Distinct patterns of cAMP-dependent protein kinase gene expression in mouse brain, Neuron 3 (1989) 71–79.

[117] E.P. Brandon, M. Zhuo, Y.Y. Huang, M. Qi, K.A. Gerhold, K.A. Burton, E.R. Kandel, G.S. McKnight, R.L. Idzerda, Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RI beta subunit of cAMP-dependent protein kinase, Proc. Natl Acad. Sci. USA 92 (1995) 8851–8855.

[118] M. Boshart, Weih, M. Nichols, G. Schütz, The tissue-specific extinguisher locus TSE1 encodes a regulatory subunit of cAMP-dependent protein kinase, Cell 66 (1991) 849–859.

[119] J.H. Zheng, D.R. Knighton, J. Parello, S.S. Taylor, J.M. Sowadski, Crystallization of catalytic subunit of adenosine cyclic monophosphate-dependent protein kinase, Methods Enzymol 200 (1991) 508–521.

[120] G. Keryer, Z. Luo, J.C. Cavadore, J. Erlichman, M. Bornens, Phosphorylation of the regulatory subunit of type IIβ cAMP-dependent protein kinase by cyclin B/p34^{cdc2} kinase impairs its binding to microtubule-associated protein 2, Proc. Natl Acad. Sci., USA 90 (1993) 5418–5422.

[121] C. Rosenmund, D.W. Carr, S.E. Bergeson, G. Nilaver, J.D. Scott, G.L. Westbrook, Anchoring of protein kinase A is required for modulation of AMPA/kainate receptors on hippocampal neurons, Nature 368 (1994) 853–856.

[122] S.J. Beebe, R. Holloway, S.R. Rannels, J.D. Corbin, Two classes of cAMP analogs which are selective for the two different cAMP-binding sites of type II protein kinase demonstrate synergism when added together to intact adipocytes, J. Biol. Chem 259 (1984) 3539–3547.

[123] D.E. Cummings, E.P. Brandon, J.V. Planas, K. Motamed, R.L. Idzerda, G.S. McKnight, Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A, Nature 382 (1996) 622–626.

[124] M. Qi, M. Zhuo, B.S. Skålhegg, E.P. Brandon, E.R. Kandel, G.S. McKnight, R.L. Idzerda, Impaired hippocampal plasticity in mice lacking the Cbetal subunit of cAMP-dependent protein kinase, Proc. Natl Acad. Sci., USA 93 (1996) 1571–1576.

[125] W. Wen, A.T. Harootunian, S.R. Adams, J. Feramisco, R.Y. Tsien, J.L. Meinkoth, S.S. Taylor, Heat-stable inhibitors of

cAMP-dependent protein kinase carry a nuclear export signal, J. Biol. Chem 269 (1994) 32,214–32,220.
[126] H. Zhong, H. SuYang, H. Erdjument-Bromage, P. Tempst, S. Ghosh, The transcriptional activity of NFkappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-dependent mechanism, Cell 89 (1997) 413–424.