Journal of Animal and Feed Sciences, 9, 2000, 213 – 246

The structure and activity of cyclic AMP-dependent protein kinase A

A. Ostrowska

Institute of Ecology, Polish Academy of Sciences
Konopnickiej, 1, Dziekanów Leśny near Warsaw
05-092 Łomianki, Poland

(Received 11 April 1999; accepted 8 May 2000)

ABSTRACT

Recent data concerning the activity of protein kinase A (cyclic adenosine 3',5' monophosphate-dependent protein kinase) in processes associated with gene expression and regulation, as well as general information concerning protein kinase A structure, synthesis and activity are presented in this article. Protein kinase A is a tetrameric protein comprising two regulatory and two catalytic subunits. The enzyme is activated by binding cAMP to the regulatory subunit, dissociation of the holoenzyme, and liberation of free catalytic subunits. Cyclic AMP is synthesized after adenylyl cyclase is activated by extracellular stimuli. Two types of protein kinase A are known, protein kinase A I and protein kinase A II. They differ in their regulatory subunits, R I and R II. Each regulatory subunit may occur in two isoforms, R I α, R I β, R II α and R II β. Three variants of catalytic subunits exist, C α, C β and C γ; the C β subunit may occur in three subisoforms, C β1, C β2 and C β3. The R I α, R II α, and C α subunits are expressed ubiquitously, while the R I β, R II β, C β and C γ subunits are expressed in endocrine, neuroendocrine, neural and leukemic tissues. The activity of protein kinase A depends on cellular localization, which determines the access of the enzyme to cAMP and substrate, and on the proportions of the regulatory and catalytic subunits. Protein kinase A II is associated with cellular structures through the regulatory subunit R II, which is bound to protein kinase A anchor proteins (AKAP) or to microtubule-associated protein (MAP-2). Protein kinase A I is soluble in cellular cytosol. The protein kinase A subunits, R I α, R I β, R II α, R II β C α, C β, and C γ are encoded by separate genes whose promoters are activated by cAMP and bind Sp 1; they lack TATA and CAAT sequences and have several transcription start sites. Protein kinase A is involved in the expression of various proteins through the regulation of the activity and synthesis of transcription factors, cycline A, cycline-dependent protein kinase inhibitors, and phosphorylation of microtubule-associated proteins. Protein kinase A is involved in hormone synthesis and secretion. Basal cellular metabolism is affected by protein kinase A through phosphorylation and regulation of the activity of protein kinases, phosphorylases, phosphatases, protease inhibitors, and through

influencing their synthesis. The cellular compartmentalization of diverse isoforms of protein kinase A, differing in activity and substrate specificity, brings about a variety of cAMP-mediated cellular responses to different hormonal stimuli.

KEY WORDS: cyclic AMP, regulatory subunit, catalytic subunit, structure, biosynthesis, transcriptional activation

ABBREVIATIONS

AKAP, protein kinase A anchor protein
AP-1, activator protein 1
AP-2, activator protein 2
ATP, adenosine 5'-triphosphate
C, catalytic subunit of protein kinase A
CAP, catabolite gene activator protein
cAMP, cyclic adenosine 3',5'-monophosphate
cDNA, complementary DNA
CBP, CREB binding protein
cdk, cycline dependent protein kinase
CRE, cAMP response element
CREB, CRE binding protein
Ht 31, a 31 kDa human thyroid anchor protein
MAP, mitogen activated protein kinase
MAP-2 microtubule associated protein 2
mRNA, messenger RNA
p75, bovine; p150, rat brain proteins binding protein kinase A, with respective molecular weights of 75 kDa and 150 kDa
Protein kinase A, adenosine 3',5'-monophosphate-dependent protein kinase (ATP-protein phosphotransferase, EC 2.7.1.37)
R, regulatory subunit of protein kinase A
TF, transcription factor;
TBP, TATA-box binding protein

INTRODUCTION

Adenosine 3',5'-monophosphate-dependent protein kinase (protein kinase A, ATP-protein phosphotransferase; EC 2.7.1.37) is known to play an important regulatory role in many biochemical processes. The enzyme is activated by cAMP binding to its regulatory (R) subunit, which results in the dissociation of protein kinase A and liberation of active, catalytic (C) subunit. Cyclic AMP is accumu-

lated following stimulation of membrane receptor adenylyl cyclase coupled to the stimulatory G protein α subunit or βγ subunits of membrane receptors (Taussing and Gilman, 1995; Daaka et al., 1997; Dessauer and Gilman, 1997; Dessauer et al., 1997; Bayewitch et al., 1998). Protein kinase A mediates the effects of cAMP through phosphorylation of specific protein substrates. A general review of protein phosphorylation was presented earlier (Ostrowska, 1987), and new data concerning cAMP-mediated phosphorylation and regulation of transcriptional processes have recently been published in Polish literature (Ostrowska, 1999).

PROTEIN KINASE A STRUCTURE AND ISOENZYMES

Protein kinase A is a tetrameric holoenzyme comprising two regulatory and two catalytic subunits (Gill and Garren, 1970; Hofmann et al., 1975; McKnight et al., 1988; Taylor et al., 1990). A diagrammatic representation of protein kinase A structure is presented in Figure 1. Each of the four known R subunit retains the general features of the molecule. The amino-terminal (N-terminal) region of the R subunit contains a dimer interaction site encompassing approximately eighty amino acid residues, rich in hydrophilic and charged amino acids. These are followed by two antigenic sites separated by a „hinge", a pseudosubstrate region. The hinge region is an auto-inhibitor substrate-like site in which the R subunit binds to the C subunit. The carboxyl-terminal (C-terminal) part of the R subunit contains two tandem cAMP-binding sites, A and B. Site A is located inside of the molecule, site B is situated at the C-terminal. The spatial structure of the cAMP-binding sites is similar to catabolite gene activator protein (CAP) from *Escherichia coli* (CAP binds to the lac promoter in the presence of cAMP, thus promoting the transcription of the lac gene). The model of cAMP binding sites in the R subunit was built by fitting the R subunit amino acid sequence into the crystallographic coordinates of CAP. The cAMP-binding site is a β-barrel composed of eight β-strands and environed by three α-helices. Cyclic AMP is bound inside of the β-barrel due to interactions between glycine, glutamic acid and arginine (see section cAMP-binding sites), located on the strands of β-barrel and exocyclic oxygen of the phosphate moiety and 2-OH' of the ribose of cAMP (Taylor et al., 1990; Ostrowska, 1999). Recently, a crystallographic study of the fragment of the R subunit of protein kinase A revealed a similar structure for the A cAMP binding site. Site B, located at the C-terminal, is less shielded by β-strands and more accessible to cAMP (Gibson and Taylor, 1997).

The catalytic subunit is a slightly elongated molecule comprising two lobes, smaller and larger, separated by a deep cleft. The smaller lobe is associated primarily with the binding of MgATP. It corresponds to the N-terminal segment

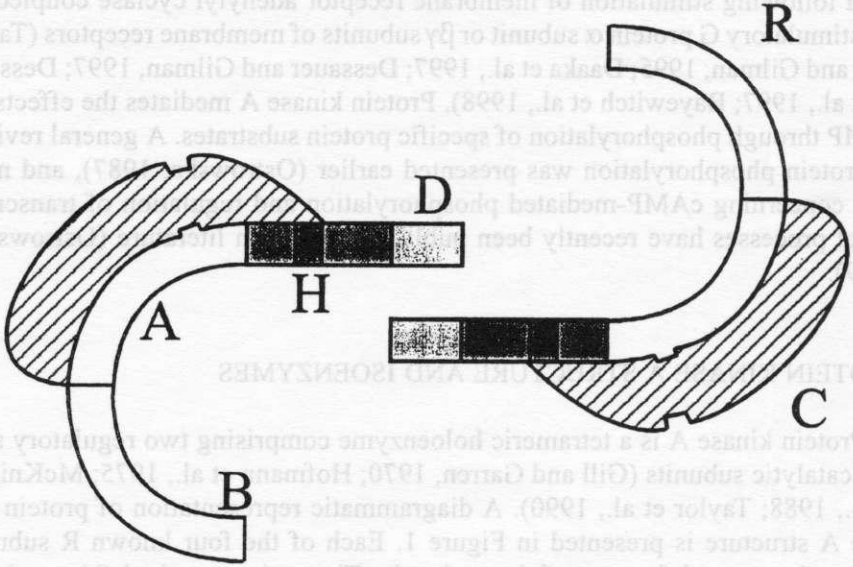


Figure 1. The scheme of protein kinase A structure. R, regulatory subunit, C, catalytic subunit, D, dimerization domain, H, hinge region, A and B, the sites binding cAMP (according to data presented by McKnight et al., 1988; Scott et al., 1990; Taylor et al., 1990; Gibson and Taylor, 1997 and as presented in Ostrowska, 1999)

(residues 15-127). This domain is dominated by a β-sheet consisting of five anti-parallel β-strands. The only helical element is inserted between β-strands 3 and 4. The N-terminal of the C subunit begins with an amphipathic α helix that lies primarily along the surface of the larger lobe and is not visible in the crystal structure. In mammalian species, the N-terminal glycine is myristoylated. The myristoyl group stabilizes the C subunit (Taylor et al., 1990).

The larger lobe is associated with peptide substrate binding and catalysis. It is predominantly helical and has seven α-helices. The only β structure region is located on the surface of the cleft at the interface between the two lobes where four anti-parallel strands form a sheet. The regions important for recognition of the peptide and catalysis are located within this large lobe (from Glu 127 to Glu 331). The seventy C-terminal amino acids (281- 350) extend over a large portion of the surface of the enzyme from the bottom of the larger lobe to the top of the smaller one. A part of this region appears to participate in the recognition of both the protein substrate and ATP. The catalytic subunit contains several phosphorylation sites Ser 14, Ser 139, Thr 197, Ser 338. The interaction between R and C subunits occurs through amino acids located in the pseudo-substrate region and cAMP-binding site A of the regulatory subunit and His 87, Trp 196, Lys 213 of the cata-

lytic subunit (Knighton et al., 1991; Taylor et al., 1992; Gibson et al., 1997; Gibson and Taylor, 1997).

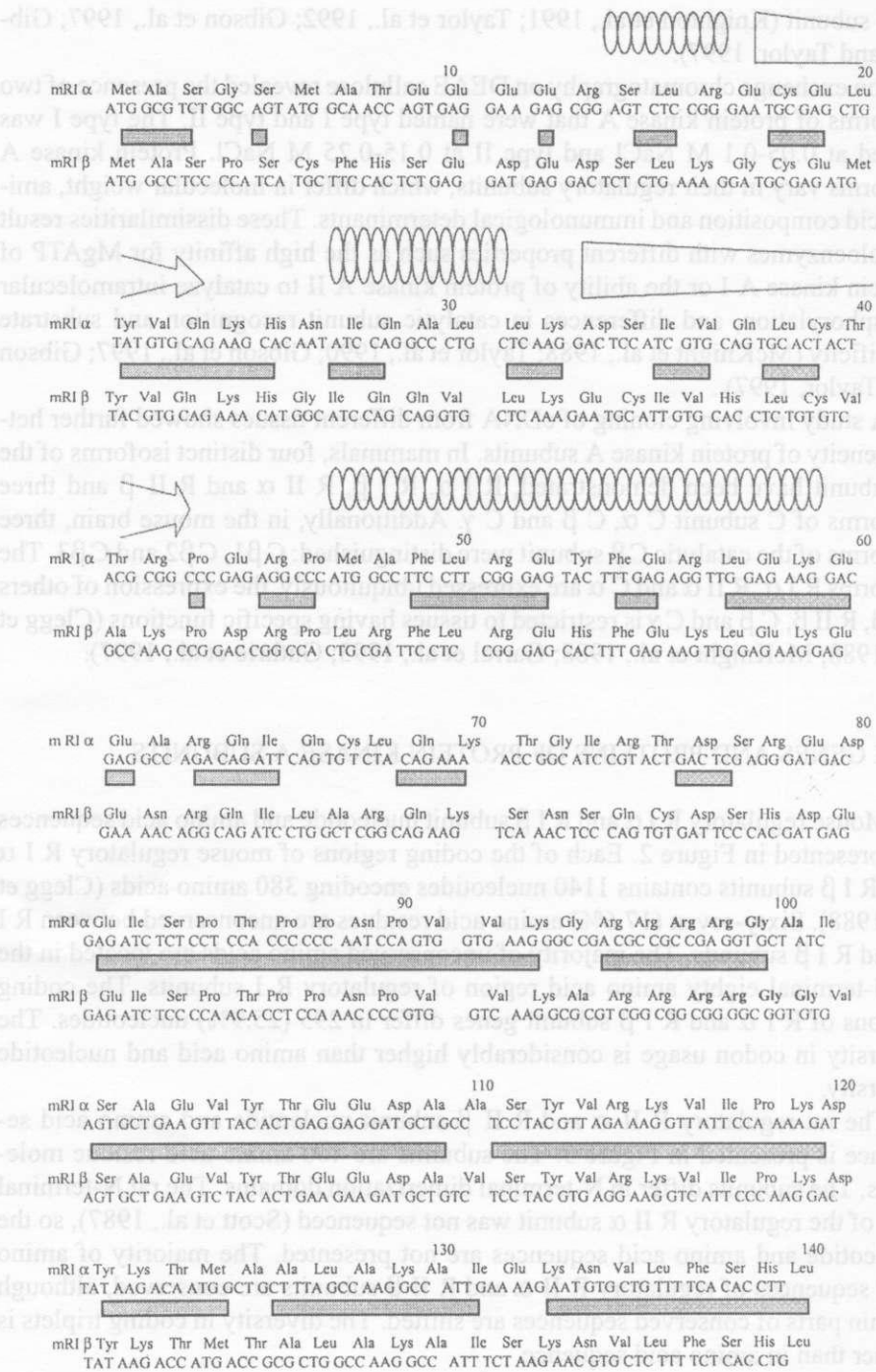
Ion exchange chromatography on DEAE cellulose revealed the presence of two isoforms of protein kinase A that were named type I and type II. The type I was eluted at 0.05-0.1 M NaCl and type II at 0.15-0.25 M NaCl. Protein kinase A isoforms vary in their regulatory subunits, which differ in molecular weight, amino acid composition and immunological determinants. These dissimilarities result in holoenzymes with different properties such as the high affinity for MgATP of protein kinase A I or the ability of protein kinase A II to catalyze intramolecular phosphorylation, and differences in catalytic subunit recognition and substrate specificity (McKnight et al., 1988; Taylor et al., 1990; Gibson et al., 1997; Gibson and Taylor, 1997) .

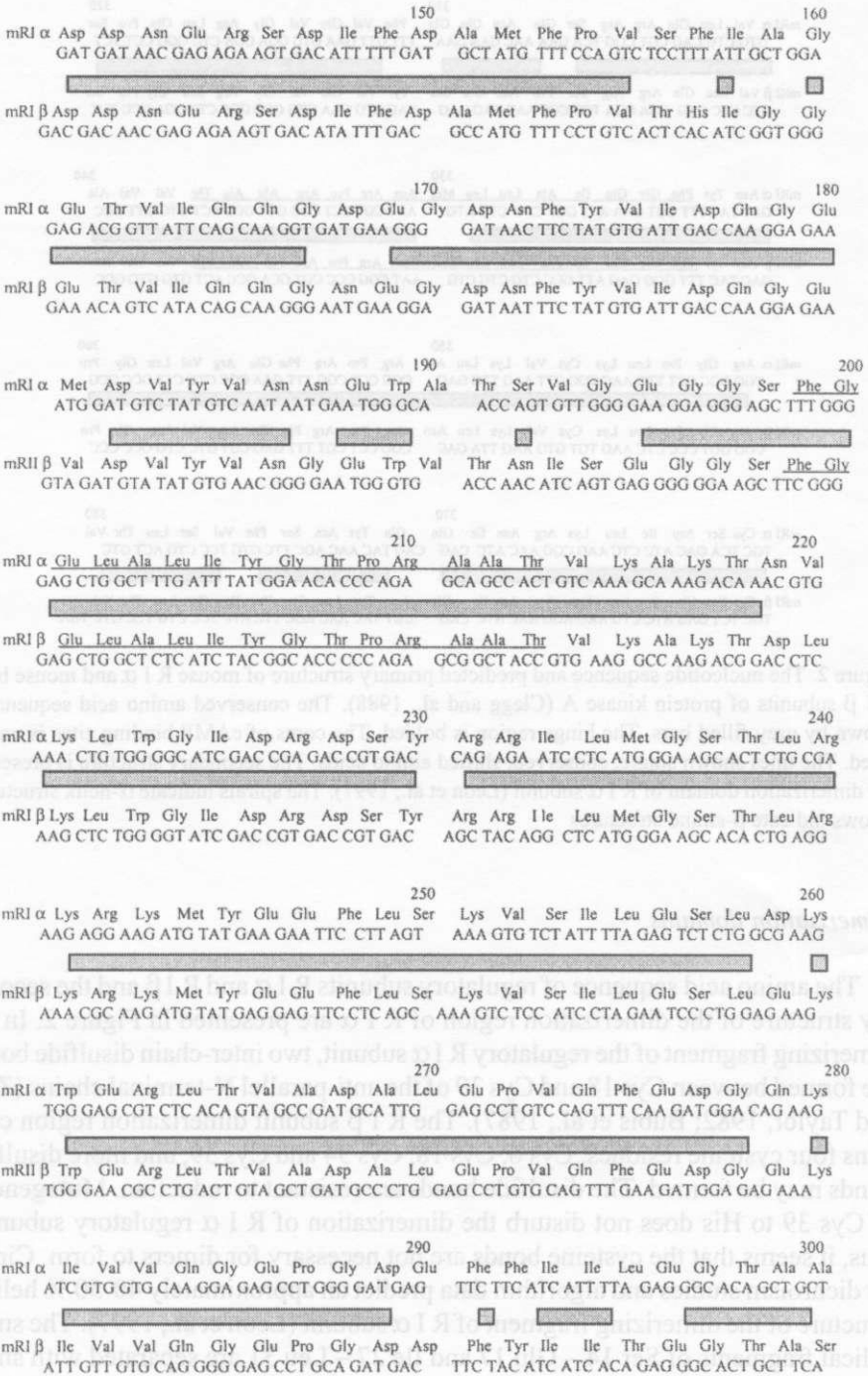
A study involving cloning of cDNA from different tissues showed further heterogeneity of protein kinase A subunits. In mammals, four distinct isoforms of the R subunit have been demonstrated, R I α, R I β, R II α and R II β and three isoforms of C subunit C α, C β and C γ. Additionally, in the mouse brain, three isoforms of the catalytic C β subunit were distinguished: C β1, C β2 and C β3. The isoforms R I α, R II α and C α are expressed ubiquitously, the expression of others R I β, R II β, C β and C γ is restricted to tissues having specific functions (Clegg et al., 1988; McKnight et al., 1988; Garrel et al., 1993; Guthrie et al., 1997).

THE GENES AND PROTEINS OF PROTEIN KINASE A SUBUNITS

Mouse regulatory R I α and R I β subunit nucleotide and amino acid sequences are presented in Figure 2. Each of the coding regions of mouse regulatory R I α and R I β subunits contains 1140 nucleotides encoding 380 amino acids (Clegg et al., 1988). Sixty-seven (17.6%) amino acid residues are unconserved between R I α and R I β subunits. The majority of unconserved amino acids are located in the in N-terminal eighty amino acid region of regulatory R I subunits. The coding regions of R I α and R I β subunit genes differ in 295 (25.9%) nucleotides. The diversity in codon usage is considerably higher than amino acid and nucleotide diversity.

The rat regulatory R II α and R II β subunit nucleotide and amino acid sequence is presented in Figure 3. The subunits are 400 amino acid residue molecules. The subunits differ in N-terminal dimerization domains. The rat N-terminal part of the regulatory R II α subunit was not sequenced (Scott et al., 1987), so the nucleotide and amino acid sequences are not presented. The majority of amino acid sequences of regulatory R II α and R II β subunits are conserved, although certain parts of conserved sequences are shifted. The diversity in coding triplets is higher than in amino acid sequence.


10
20
mRI α Met Ala Ser Gly Ser Met Ala Thr Glu Glu Glu Glu Arg Ser Leu Arg Glu Cys Glu Leu
ATG GCG TCT GGC AGT ATG GCA ACC AGT GAG GA A GAG CGG AGT CTC CGG GAA TGC GAG CTG
mRI β Met Ala Ser Pro Ser Cys Phe His Ser Glu Asp Glu Asp Ser Leu Lys Gly Cys Glu Met
ATG GCC TCC CCA TCA TGC TTC CAC TCT GAG GAT GAG GAC TCT CTG AAA GGA TGC GAG ATG
30
40
mRI α Tyr Val Gln Lys His Asn Ile Gln Ala Leu Leu Lys A sp Ser Ile Val Gln Leu Cys Thr
TAT GTG CAG AAG CAC AAT ATC CAG GCC CTG CTC AAG GAC TCC ATC GTG CAG TGC ACT ACT
mRI β Tyr Val Gln Lys His Gly Ile Gln Gln Val Leu Lys Glu Cys Ile Val His Leu Cys Val
TAC GTG CAG AAA CAT GGC ATC CAG CAG GTG CTC AAA GAA TGC ATT GTG CAC CTC TGT GTC
50
60
mR I α Thr Arg Pro Glu Arg Pro Met Ala Phe Leu Arg Glu Tyr Phe Glu Arg Leu Glu Lys Glu
ACG CGG CCC GAG AGG CCC ATG GCC TTC CTT CGG GAG TAC TTT GAG AGG TTG GAG AAG GAC
mRI β Ala Lys Pro Asp Arg Pro Leu Arg Phe Leu Arg Glu His Phe Glu Lys Leu Glu Lys Glu
GCC AAG CCG GAC CGG CCA CTG CGA TTC CTC CGG GAG CAC TTT GAG AAG TTG GAG AAG GAG
70
80
m RI α Glu Ala Arg Gln Ile Gln Cys Leu Gln Lys Thr Gly Ile Arg Thr Asp Ser Arg Glu Asp
GAG GCC AGA CAG ATT CAG TG T CTA CAG AAA ACC GGC ATC CGT ACT GAC TCG AGG GAT GAC
mRI β Glu Asn Arg Gln Ile Leu Ala Arg Gln Lys Ser Asn Ser Gln Cys Asp Ser His Asp Glu
GAA AAC AGG CAG ATC CTG GCT CGG CAG AAG TCA AAC TCC CAG TGT GAT TCC CAC GAT GAG
90
100
mRI α Glu Ile Ser Pro Thr Pro Pro Asn Pro Val Val Lys Gly Arg Arg Arg Arg Gly Ala Ile
GAG ATC TCT CCT CCA CCC CCC AAT CCA GTG GTG AAG GGC CGA CGC CGC CGA GGT GCT ATC
mRI β Glu Ile Ser Pro Thr Pro Pro Asn Pro Val Val Lys Ala Arg Arg Arg Arg Gly Gly Val
GAG ATC TCC CCA ACA CCT CCA AAC CCC GTG GTC AAG GCG CGT CGG CGG CGG GGC GGT GTG
110
120
mRI α Ser Ala Glu Val Tyr Thr Glu Glu Asp Ala Ala Ser Tyr Val Arg Lys Val Ile Pro Lys Asp
AGT GCT GAA GTT TAC ACT GAG GAG GAT GCT GCC TCC TAC GTT AGA AAG GTT ATT CCA AAA GAT
mRI β Ser Ala Glu Val Tyr Thr Glu Glu Asp Ala Val Ser Tyr Val Arg Lys Val Ile Pro Lys Asp
AGT GCT GAA GTC TAC ACT GAA GAA GAT GCT GTC TCC TAC GTG AGG AAG GTC ATT CCC AAG GAC
130
140
mRI α Tyr Lys Thr Met Ala Ala Leu Ala Lys Ala Ile Glu Lys Asn Val Leu Phe Ser His Leu
TAT AAG ACA ATG GCT GCT TTA GCC AAG GCC ATT GAA AAG AAT GTG CTG TTT TCA CAC CTT
mRI β Tyr Lys Thr Met Thr Ala Leu Ala Lys Ala Ile Ser Lys Asn Val Leu Phe Ser His Leu
TAT AAG ACC ATG ACC GCG CTG GCC AAG GCC ATT TCT AAG AAC GTG CTC TTT TCT CAC CTG


150 160
mRI α Asp Asp Asn Glu Arg Ser Asp Ile Phe Asp Ala Met Phe Pro Val Ser Phe Ile Ala Gly
GAT GAT AAC GAG AGA AGT GAC ATT TTT GAT GCT ATG TTT CCA GTC TCCTTT ATT GCT GGA
mRI β Asp Asp Asn Glu Arg Ser Asp Ile Phe Asp Ala Met Phe Pro Val Thr His Ile Gly Gly
GAC GAC AAC GAG AGA AGT GAC ATA TTT GAC GCC ATG TTT CCT GTC ACT CAC ATC GGT GGG
170 180
mRI α Glu Thr Val Ile Gln Gln Gly Asp Glu Gly Asp Asn Phe Tyr Val Ile Asp Gln Gly Glu
GAG ACG GTT ATT CAG CAA GGT GAT GAA GGG GAT AAC TTC TAT GTG ATT GAC CAA GGA GAA
mRI β Glu Thr Val Ile Gln Gln Gly Asn Glu Gly Asp Asn Phe Tyr Val Ile Asp Gln Gly Glu
GAA ACA GTC ATA CAG CAA GGG AAT GAA GGA GAT AAT TTC TAT GTG ATT GAC CAA GGA GAA
190 200
mRI α Met Asp Val Tyr Val Asn Asn Glu Trp Ala Thr Ser Val Gly Glu Gly Gly Ser Phe Gly
ATG GAT GTC TAT GTC AAT AAT GAA TGG GCA ACC AGT GTT GGG GAA GGA GGG AGC TTT GGG
mRII β Val Asp Val Tyr Val Asn Gly Glu Trp Val Thr Asn Ile Ser Glu Gly Gly Ser Phe Gly
GTA GAT GTA TAT GTG AAC GGG GAA TGG GTG ACC AAC ATC AGT GAG GGG GGA AGC TTC GGG
210 220
mRI α Glu Leu Ala Leu Ile Tyr Gly Thr Pro Arg Ala Ala Thr Val Lys Ala Lys Thr Asn Val
GAG CTG GCT TTG ATT TAT GGA ACA CCC AGA GCA GCC ACT GTC AAA GCA AAG ACA AAC GTG
mRI β Glu Leu Ala Leu Ile Tyr Gly Thr Pro Arg Ala Ala Thr Val Lys Ala Lys Thr Asp Leu
GAG CTG GCT CTC ATC TAC GGC ACC CCC AGA GCG GCT ACC GTG AAG GCC AAG ACG GAC CTC
230 240
mRI α Lys Leu Trp Gly Ile Asp Arg Asp Ser Tyr Arg Arg Ile Leu Met Gly Ser Thr Leu Arg
AAA CTG TGG GGC ATC GAC CGA GAC CGT GAC CAG AGA ATC CTC ATG GGA AGC ACT CTG CGA
mRI β Lys Leu Trp Gly Ile Asp Arg Asp Ser Tyr Arg Arg Ile Leu Met Gly Ser Thr Leu Arg
AAG CTC TGG GGT ATC GAC CGT GAC CGT GAC AGC TAC AGG CTC ATG GGA AGC ACA CTG AGG
250 260
mRI α Lys Arg Lys Met Tyr Glu Glu Phe Leu Ser Lys Val Ser Ile Leu Glu Ser Leu Asp Lys
AAG AGG AAG ATG TAT GAA GAA TTC CTT AGT AAA GTG TCT ATT TTA GAG TCT CTG GCG AAG
mRI β Lys Arg Lys Met Tyr Glu Glu Phe Leu Ser Lys Val Ser Ile Leu Glu Ser Leu Glu Lys
AAA CGC AAG ATG TAT GAG GAG TTC CTC AGC AAA GTC TCC ATC CTA GAA TCC CTG GAG AAG
270 280
mRI α Trp Glu Arg Leu Thr Val Ala Asp Ala Leu Glu Pro Val Gln Phe Glu Asp Gly Gln Lys
TGG GAG CGT CTC ACA GTA GCC GAT GCA TTG GAG CCT GTC CAG TTT CAA GAT GGA CAG AAG
mRII β Trp Glu Arg Leu Thr Val Ala Asp Ala Leu Glu Pro Val Gln Phe Glu Asp Gly Glu Lys
TGG GAA CGC CTG ACT GTA GCT GAT GCC CTG GAG CCT GTG CAG TTT GAA GAT GGA GAG AAA
290 300
mRI α Ile Val Val Gln Gly Glu Pro Gly Asp Glu Phe Phe Ile Ile Leu Glu Gly Thr Ala Ala
ATC GTG GTG CAA GGA GAG CCT GGG GAT GAG TTC TTC ATC ATT TTA GAG GGC ACA GCT GCT
mRI β Ile Val Val Gln Gly Glu Pro Gly Asp Asp Phe Tyr Ile Ile Thr Glu Gly Thr Ala Ser
ATT GTT GTG CAG GGG GAG CCT GCA GAT GAC TTC TAC ATC ATC ACA GAG GGC ACT GCT TCA

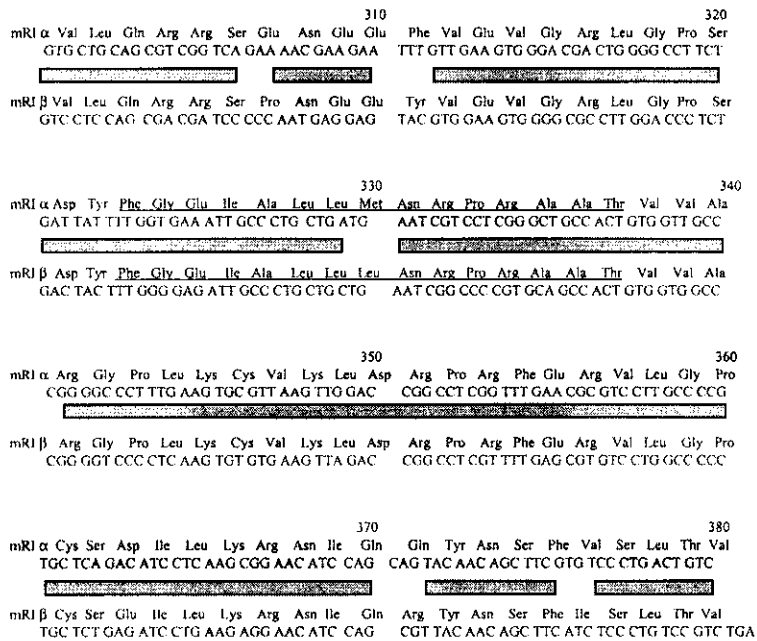
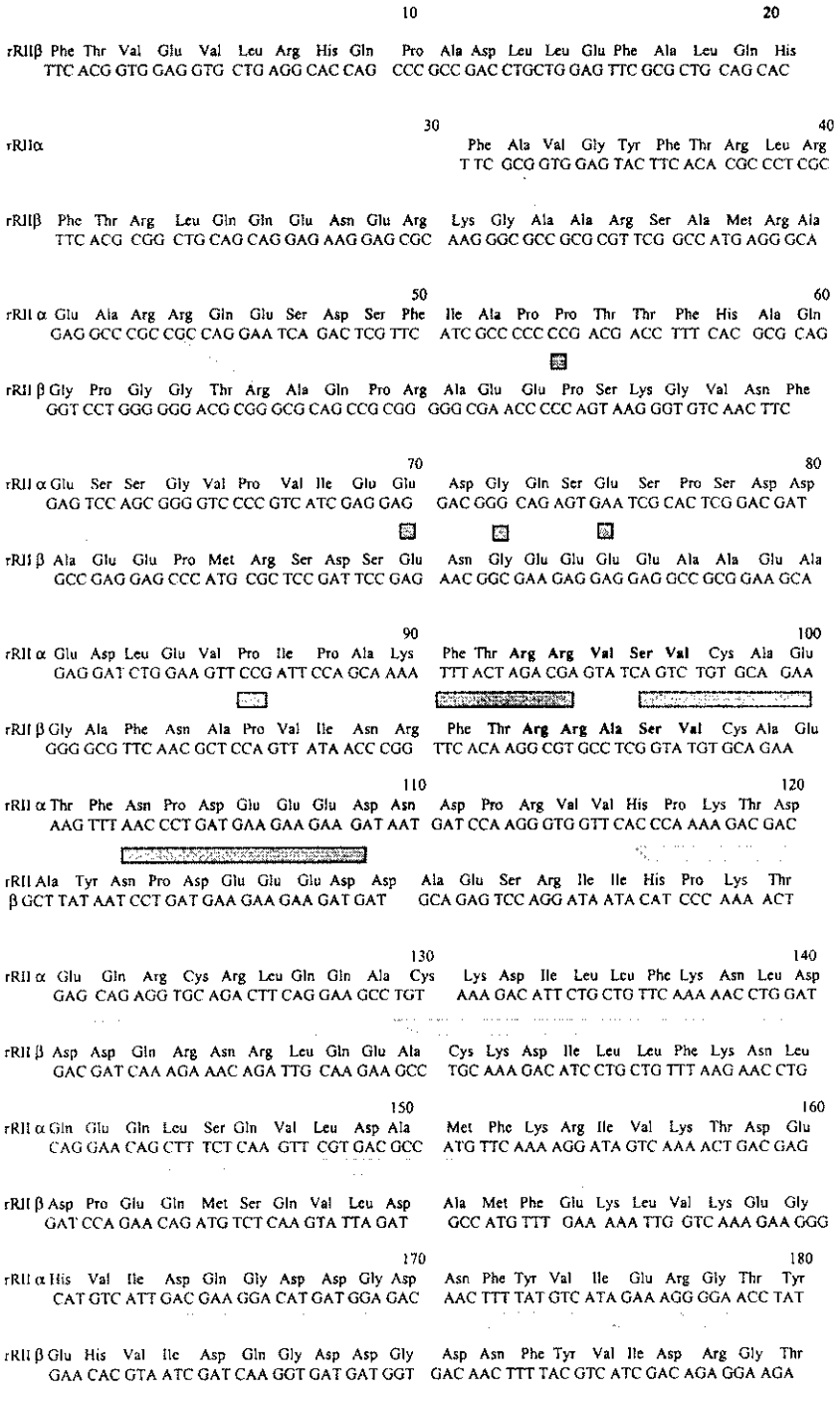
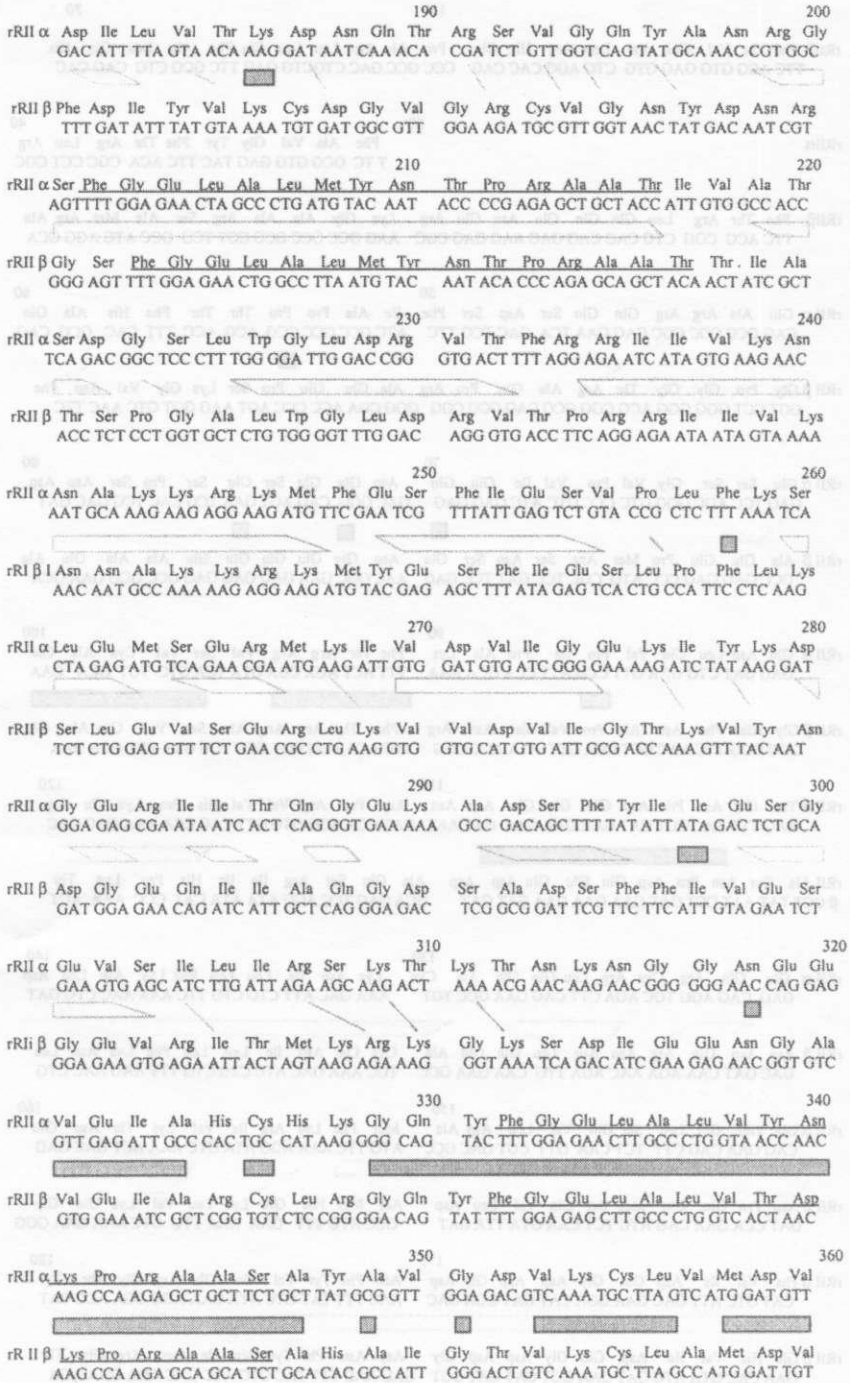


Figure 2. The nucleotide sequence and predicted primary structure of mouse R I α and mouse brain R I β subunits of protein kinase A (Clegg and al., 1988). The conserved amino acid sequence is shown by grey filled bars. The hinge region is bolded. The cores of cAMP binding sites is underlined. The lines shown single, conserved, shifted amino acids. The secondary structure is presented for dimerization domain of R I α subunit (Leon et al., 1997). The spirals indicate α-helix structures, arrows indicate β-strand structures

Dimerization domains

The amino acid sequence of regulatory subunits R I α and R I β and the secondary structure of the dimerization region of R I α are presented in Figure 2. In the dimerizing fragment of the regulatory R I α subunit, two inter-chain disulfide bonds are formed between Cys 18 and Cys 39 of the anti-parallel N-terminal chains (Zick and Taylor, 1982; Bubis et al., 1987). The R I β subunit dimerization region contains four cysteine residues, Cys 6, Cys 18, Cys 34 and Cys 39, and more disulfide bonds may be formed. The disulfide bonds are resistant to reduction. Mutagenesis of Cys 39 to His does not disturb the dimerization of R I α regulatory subunits, thus, it seems that the cysteine bonds are not necessary for dimers to form. Circular dichroism studies and algorithm data predict an approximately 40-50 % helical structure of the dimerizing fragment of R I α subunit (Leon et al., 1997). The small helical fragments of Ser 14 – Glu 17 and Ile 27 – Leu 31 are separated with small


10 20
rRIIβ Phe Thr Val Glu Val Leu Arg His Gln Pro Ala Asp Leu Leu Glu Phe Ala Leu Gln His
TTC ACG GTG GAG GTG CTG AGG CAC CAG CCC GCC GAC CTGCTG GAG TTC GCG CTG CAG CAC
30 40
rRIIα Phe Ala Val Gly Tyr Phe Thr Arg Leu Arg
T TC GCG GTG GAG TAC TTC ACA CGC CCT CGC
rRIIβ Phe Thr Arg Leu Gln Gln Glu Asn Glu Arg Lys Gly Ala Ala Arg Ser Ala Met Arg Ala
TTC ACG CGG CTG CAG CAG GAG AAG GAG CGC AAG GGC GCC GCG CGT TCG GCC ATG AGG GCA
50 60
rRII α Glu Ala Arg Arg Gln Glu Ser Asp Ser Phe Ile Ala Pro Pro Thr Thr Phe His Ala Gln
GAG GCC CGC CGC CAG GAA TCA GAC TCG TTC ATC GCC CCC CCG ACG ACC TTT CAC GCG CAG
rRII β Gly Pro Gly Gly Thr Arg Ala Gln Pro Arg Ala Glu Glu Pro Ser Lys Gly Val Asn Phe
GGT CCT GGG GGG ACG CGG GCG CAG CCG CGG GGG CGA ACC CCC AGT AAG GGT GTC AAC TTC
70 80
rRII α Glu Ser Ser Gly Val Pro Val Ile Glu Glu Asp Gly Gln Ser Glu Ser Pro Ser Asp Asp
GAG TCC AGC GGG GTC CCC GTC ATC GAG GAG GAC GGG CAG AGT GAA TCG CAC TCG GAC GAT
rRII β Ala Glu Glu Pro Met Arg Ser Asp Ser Glu Asn Gly Glu Glu Glu Glu Ala Ala Glu Ala
GCC GAG GAG CCC ATG CGC TCC GAT TCC GAG AAC GGC GAA GAG GAG GAG GCC GCG GAA GCA
90 100
rRII α Glu Asp Leu Glu Val Pro Ile Pro Ala Lys Phe Thr Arg Arg Val Ser Val Cys Ala Glu
GAG GAT CTG GAA GTT CCG ATT CCA GCA AAA TTT ACT AGA CGA GTA TCA GTC TGT GCA GAA
rRII β Gly Ala Phe Asn Ala Pro Val Ile Asn Arg Phe Thr Arg Arg Ala Ser Val Cys Ala Glu
GGG GCG TTC AAC GCT CCA GTT ATA ACC CGG TTC ACA AGG CGT GCC TCG GTA TGT GCA GAA
110 120
rRII α Thr Phe Asn Pro Asp Glu Glu Glu Asp Asn Asp Pro Arg Val Val His Pro Lys Thr Asp
AAG TTT AAC CCT GAT GAA GAA GAA GAT AAT GAT CCA AGG GTG GTT CAC CCA AAA GAC GAC
rRII Ala Tyr Asn Pro Asp Glu Glu Glu Asp Asp Ala Glu Ser Arg Ile Ile His Pro Lys Thr
β GCT TAT AAT CCT GAT GAA GAA GAA GAT GAT GCA GAG TCC AGG ATA ATA CAT CCC AAA ACT
130 140
rRII α Glu Gln Arg Cys Arg Leu Gln Gln Ala Cys Lys Asp Ile Leu Leu Phe Lys Asn Leu Asp
GAG CAG AGG TGC AGA CTT CAG GAA GCC TGT AAA GAC ATT CTG CTG TTC AAA AAC CTG GAT
rRII β Asp Asp Gln Arg Asn Arg Leu Gln Glu Ala Cys Lys Asp Ile Leu Leu Phe Lys Asn Leu
GAC GAT CAA AGA AAC AGA TTG CAA GAA GCC TGC AAA GAC ATC CTG CTG TTT AAG AAC CTG
150 160
rRII α Gln Glu Gln Leu Ser Gln Val Leu Asp Ala Met Phe Lys Arg Ile Val Lys Thr Asp Glu
CAG GAA CAG CTT TCT CAA GTT CGT GAC GCC ATG TTC AAA AGG ATA GTC AAA ACT GAC GAG
rRII β Asp Pro Glu Gln Met Ser Gln Val Leu Asp Ala Met Phe Glu Lys Leu Val Lys Glu Gly
GAT CCA GAA CAG ATG TCT CAA GTA TTA GAT GCC ATG TTT GAA AAA TTG GTC AAA GAA GGG
170 180
rRII α His Val Ile Asp Gln Gly Asp Asp Gly Asp Asn Phe Tyr Val Ile Glu Arg Gly Thr Tyr
CAT GTC ATT GAC GAA GGA CAT GAT GGA GAC AAC TTT TAT GTC ATA GAA AGG GGA ACC TAT
rRII β Glu His Val Ile Asp Gln Gly Asp Asp Gly Asp Asn Phe Tyr Val Ile Asp Arg Gly Thr
GAA CAC GTA ATC GAT CAA GGT GAT GAT GGT GAC AAC TTT TAC GTC ATC GAC AGA GGA AGA


190 200
rRII α Asp Ile Leu Val Thr Lys Asp Asn Gln Thr Arg Ser Val Gly Gln Tyr Ala Asn Arg Gly
GAC ATT TTA GTA ACA AAG GAT AAT CAA ACA CGA TCT GTT GGT CAG TAT GCA AAC CGT GGC
rRII β Phe Asp Ile Tyr Val Lys Cys Asp Gly Val Gly Arg Cys Val Gly Asn Tyr Asp Asn Arg
TTT GAT ATT TAT GTA AAA TGT GAT GGC GTT GGA AGA TGC GTT GGT AAC TAT GAC AAT CGT
210 220
rRII α Ser Phe Gly Glu Leu Ala Leu Met Tyr Asn Thr Pro Arg Ala Ala Thr Ile Val Ala Thr
AGTTTT GGA GAA CTA GCC CTG ATG TAC AAT ACC CCG AGA GCT GCT ACC ATT GTG GCC ACC
rRII β Gly Ser Phe Gly Glu Leu Ala Leu Met Tyr Asn Thr Pro Arg Ala Ala Thr Thr . Ile Ala
GGG AGT TTT GGA GAA CTG GCC TTA ATG TAC AAT ACA CCC AGA GCA GCT ACA ACT ATC GCT
230 240
rRII α Ser Asp Gly Ser Leu Trp Gly Leu Asp Arg Val Thr Phe Arg Arg Ile Ile Val Lys Asn
TCA GAC GGC TCC CTT TGG GGA TTG GAC CGG GTG ACT TTT AGG AGA ATC ATA GTG AAG AAC
rRII β Thr Ser Pro Gly Ala Leu Trp Gly Leu Asp Arg Val Thr Pro Arg Arg Ile Ile Val Lys
ACC TCT CCT GGT GCT CTG TGG GGT TTG GAC AGG GTG ACC TTC AGG AGA ATA ATA GTA AAA
250 260
rRII α Asn Ala Lys Lys Arg Lys Met Phe Glu Ser Phe Ile Glu Ser Val Pro Leu Phe Lys Ser
AAT GCA AAG AAG AGG AAG ATG TTC GAA TCG TTTATT GAG TCT GTA CCG CTC TTT AAA TCA
rRI β I Asn Asn Ala Lys Lys Arg Lys Met Tyr Glu Ser Phe Ile Glu Ser Leu Pro Phe Leu Lys
AAC AAT GCC AAA AAG AGG AAG ATG TAC GAG AGC TTT ATA GAG TCA CTG CCA TTC CTC AAG
270 280
rRII α Leu Glu Met Ser Glu Arg Met Lys Ile Val Asp Val Ile Gly Glu Lys Ile Tyr Lys Asp
CTA GAG ATG TCA GAA CGA ATG AAG ATT GTG GAT GTG ATC GGG GAA AAG ATC TAT AAG GAT
rRII β Ser Leu Glu Val Ser Glu Arg Leu Lys Val Val Asp Val Ile Gly Thr Lys Val Tyr Asn
TCT CTG GAG GTT TCT GAA CGC CTG AAG GTG GTG CAT GTG ATT GCG ACC AAA GTT TAC AAT
290 300
rRII α Gly Glu Arg Ile Ile Thr Gln Gly Glu Lys Ala Asp Ser Phe Tyr Ile Ile Glu Ser Gly
GGA GAG CGA ATA ATC ACT CAG GGT GAA AAA GCC GACAGC TTT TAT ATT ATA GAC TCT GCA
rRII β Asp Gly Glu Gln Ile Ile Ala Gln Gly Asp Ser Ala Asp Ser Phe Phe Ile Val Glu Ser
GAT GGA GAA CAG ATC ATT GCT CAG GGA GAC TCG GCG GAT TCG TTC TTC ATT GTA GAA TCT
310 320
rRII α Glu Val Ser Ile Leu Ile Arg Ser Lys Thr Lys Thr Asn Lys Asn Gly Gly Asn Glu Glu
GAA GTG AGC ATC TTG ATT AGA AGC AAG ACT AAA ACG AAC AAG AAC GGG GGG AAC CAG GAG
rRIi β Gly Glu Val Arg Ile Thr Met Lys Arg Lys Gly Lys Ser Asp Ile Glu Glu Asn Gly Ala
GGA GAA GTG AGA ATT ACT AGT AAG AGA AAG GGT AAA TCA GAC ATC GAA GAG AAC GGT GTC
330 340
rRII α Val Glu Ile Ala His Cys His Lys Gly Gln Tyr Phe Gly Glu Leu Ala Leu Val Tyr Asn
GTT GAG ATT GCC CAC TGC CAT AAG GGG CAG TAC TTT GGA GAA CTT GCC CTG GTA ACC AAC
rRII β Val Glu Ile Ala Arg Cys Leu Arg Gly Gln Tyr Phe Gly Glu Leu Ala Leu Val Thr Asp
GTG GAA ATC GCT CGG TGT CTC CGG GGA CAG TAT TTT GGA GAG CTT GCC CTG GTC ACT AAC
350 360
rRII α Lys Pro Arg Ala Ala Ser Ala Tyr Ala Val Gly Asp Val Lys Cys Leu Val Met Asp Val
AAG CCA AGA GCT GCT TCT GCT TAT GCG GTT GGA GAC GTC AAA TGC TTA GTC ATG GAT GTT
rR II β Lys Pro Arg Ala Ala Ser Ala His Ala Ile Gly Thr Val Lys Cys Leu Ala Met Asp Val
AAG CCA AGA GCA GCA TCT GCA CAC GCC ATT GGG ACT GTC AAA TGC TTA GCC ATG GAT TGT

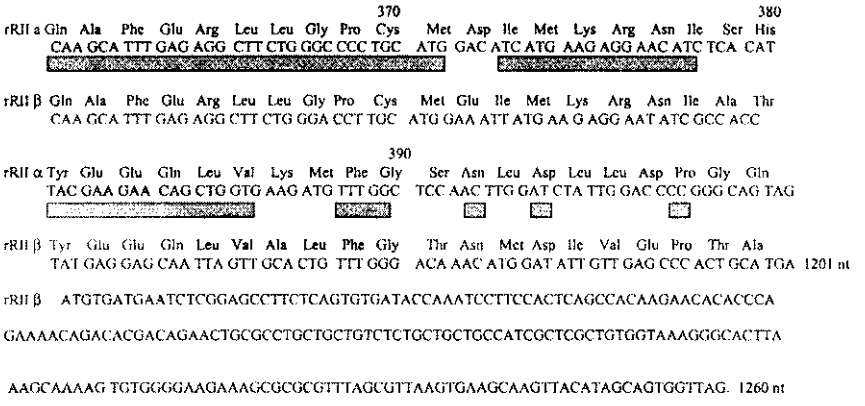


Figure 3. The nucleotide sequence and predicted primary structure of rat R II α (Scott et al., 1987) and R II β (Jahnsen et al., 1987) subunits The hinge region is bolded. The cores of cAMP binding sites A and B are underlined. The conserved amino acid sequence is shown by grey filled bars. Shifted, conserved, sequence is shown by unfilled bars. The lines shown single, conserved, shifted amino acids

β-strands Cys 18 – Gln 23 and Asp 33 – Pro 43, which are followed by a larger α-helix containing the fragment from Met 47 to Glu 60. The eighty amino acid dimerization regions of regulatory R I α and R I β subunits differ in thirty-five (45%) amino acids, however, all cysteine residues are located on β-strands, and the majority of leucine residues are conserved in α-helices.

The N-terminal fragment of the regulatory R II subunit also plays the role of a cellular protein anchor. The first fourteen amino acids are essential for regulatory R II subunit dimerization and for protein anchoring. A R II α regulatory subunit mutant lacking these amino acids is unable to dimerize and to bind MAP 2. The first thirty N-terminal amino acids seem to be sufficient for regulatory R II subunit dimerization but not for cellular protein binding. The protein fusion of thirty N-terminal amino acids and protein carrier is able to form a dimer, but it can not bind MAP 2 (Scott et al., 1990). Additionally, Phe 36 is thought to be necessary for dimer formation (Li and Rubin, 1995).

The amino acid sequence of bovine regulatory R II α and R II β subunit dimerization /anchoring domains is presented in Figure 4 A, and the secondary and tertiary structure of this region of the R II α subunit is presented in Figure 4 B. The region is constructed of β-strand (residues 1-5), β-turn I (residues 6-9), α-helix I (residues 10-23), β-turn II (residues 24-28) and α-helix II (residues 29-42). The dimerization domain is maintained by intra- and inter-subunit interactions of the aromatic ring of phenylalanine residues, and the hydrogens of residues of valines and leucines. The intra-subunit interactions are between Val 20 of α-helix I and

Phe 31 of α-helix II. The inter-subunit interactions are between Phe 36 of α-helix II of one of the R II α subunit molecule and Val 20 and Leu 13 of the α-helix I of other R II α subunit molecule (Hausken et al., 1994, 1996; Newlon et al., 1997).

The anchor protein binding domain is supposed to be located in a larger regulatory R II subunit N-terminal fragment, comprising forty-four (Lou et al., 1990; Hausken et al., 1994) or eighty-two (Scott et al., 1990) amino acid residues. Isoleucine residues 3 and 5 are required for binding anchoring proteins (Hausken et al., 1994, 1996). The amino acids of β-turns, Pro 7 and Pro 26 in regulatory R II α subunit and Ala 7 and Ala 26 in R II β subunit are thought to be responsible for the specificity of R II α and R II β subunit binding to anchor proteins. The amino acids known to be necessary for dimers to form, Leu 9, Leu 13, Val 20, Phe 31 and

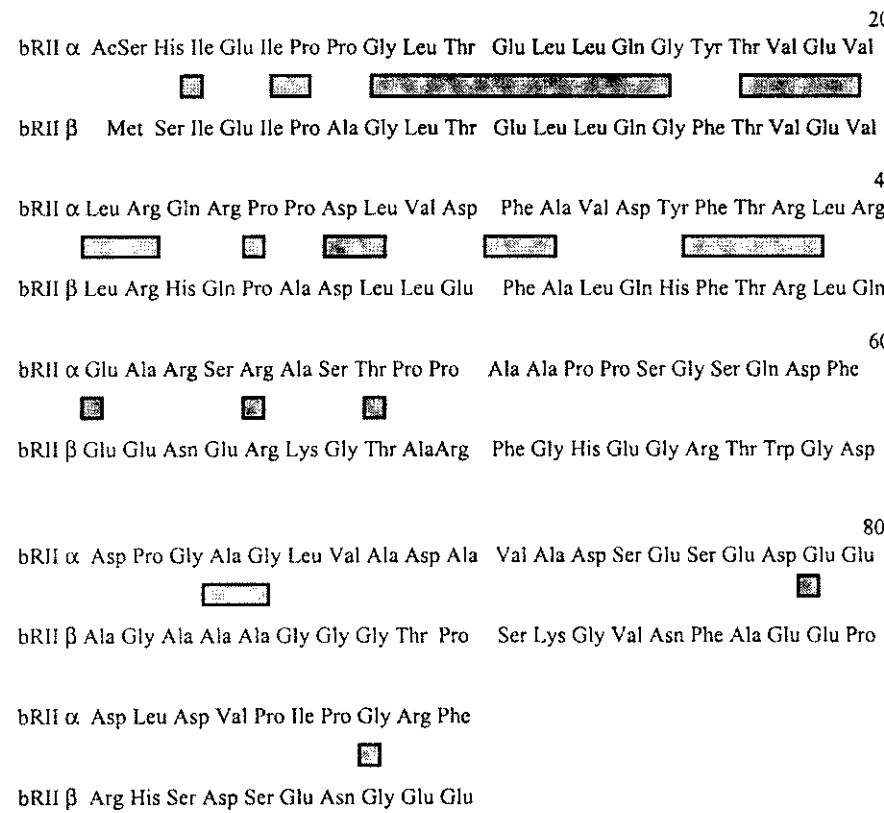


Figure 4. A. Amino acid sequence of the dimerization domains of bovine cardiac muscle regulatory R II α (Takio et al., 1982) and bovine brain R II β (Luo et al., 1990) subunits. The conserved amino acid sequence is indicated by grey filled bars

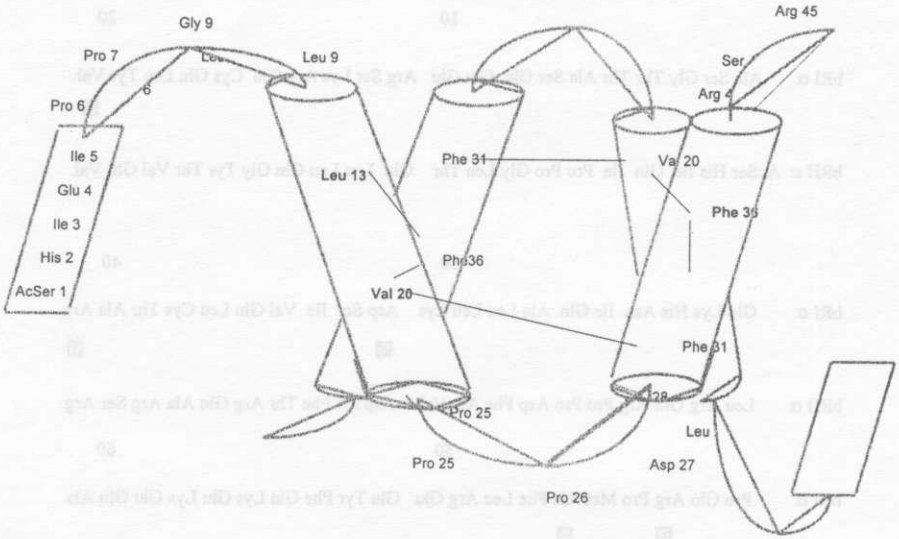


Figure 4. B. The secondary and tertiary structure of the dimerization domain of R II α subunit predicted using cirular dichroism analysis and nuclear megnetic resonance spectroscopy (according to Newlon et al., 1997). The arrows indicate β-strands, the arcs indicate β-turns and cyclinders indicate helices

Phe 36 and for cellular protein anchoring, Ile 3 and Ile 5, are conserved in both R II α and R II β regulatory subunits. The amino acids of the β-turn involved in the specific binding to cellular structures differ.

The amino acid sequences of the dimerization regions of bovine R I α and R II α and mouse R I β and R II β subunits are compared in Figures 5 A and B, respectively. The amino acid sequence is not conserved in the dimerization regions between the cytoplasmic soluble R I and cellular structure-bound R II subunits in both α and β isoenzymes. The regulatory R I α subunit does not contain phenylalanine residues in their forty amino acid N-terminal region, which maintain the dimer structure in regulatory R II subunits. The leucine residues separated by charged amino acids occur in both R I and R II subunits and may form leucine zipper-like structures one with another, or with other cellular proteins. The isoleucine residues (3 and 5), required for target protein anchoring in the regulatory R II α and R II β subunits are absent in R I α and R I β subunits. All isoforms of regulatory subunits, R I α, R I β, R II α and R II β contain of serine and threonine residues that may be potential phosphorylation sites that may influence subunit-subunit and subunit-cellular protein interactions.

Two antigenic sites separated by a „hinge", pseudo-substrate region are located next.

Figure 5. A. The amino acid sequence of the dimerization domains of bovine regulatory R I α (Titani et al., 1984) and R II α (Takio et al., 1982) subunits

Figure 5 B. The amino acid sequence of the dimerization domains of mouse regulatory R I β (Clegg et al., 1988) and R II β (Scott et al., 1987) subunits

The hinge region

The hinge region is an auto-inhibitor substrate-like site in which the regulatory subunit binds to the catalytic subunit. The hinge region is more sensitive to protease than other domains of the regulatory subunit of protein kinase A. The amino acid sequences of the hinge regions of mouse R I α and R I β; rat R I β and rat R II α and R II β subunits are presented below:

	96	97	98	99	100
mR I α	Arg	Arg	Gly	Ala	Ile
mR I β	Arg	Arg	Gly	Gly	Val
r R I β	Arg	Arg	Gly	Gly	Val

	93	94	95	96	97
rR II α	Arg	Arg	Val	Ser	Val
rR II β	Arg	Arg	Ala	Ser	Val

The hinge regions of regulatory R II α and R II β subunits may be phosphorylated by protein kinase A at Ser 96.The hinge regions of the regulatory subunits R I α and R I β do not possess a phosphorylation site.

cAMP – binding sites

The C-terminal part of the regulatory subunit contains two tandem cAMP-binding sites, A and B. Site A is located inside of the molecule, in a region of approximately 140 to 260 amino acids; site B is situated at the C-terminal. The fifteen amino acid region of the cAMP binding sites is highly conserved in all cAMP binding proteins. This region is thought to be the core of the cAMP binding site (Shabb and Corbin, 1992). The amino acid sequences of the core A and B cAMP binding sites of bovine R I α (Titani et al., 1984), mouse R I α and R I β (Clegg et al., 1988), bovine R II α (Takio et al., 1982) and bovine R II β (Luo et al., 1990) are presented below.

cAMP binding site A

198 212
bR I α Phe **Gly Glu** Leu Ala Leu Ile Tyr Gly Thr Pro **Arg** Ala Ala Thr
199 213
mR I α Phe **Gly Glu** Leu Ala Leu Ile Tyr Gly Thr Pro **Arg** Ala Ala Thr
GGA GAG AGA
mR I β Phe **Gly Glu** Leu Ala Leu Ile Tyr Gly Thr Pro **Arg** Ala Ala Thr
GGG GAG AGA

203 217
bRII α Phe **Gly Glu** Leu Ala Leu Met Tyr Asn Thr Pro **Arg** Ala Ala Thr
221 226
bR II β Phe **Gly Glu** Leu Ala Leu Met Tyr Asn Thr Pro **Arg** Ala Ala Thr
GGC GAA AGA
cAMP binding site B
322 336
bR I α Phe **Gly Glu** Ile Ala Leu Leu Met Asn Arg Pro **Arg** Ala Ala Thr
323 337
mR I α Phe **Gly Glu** Ile Ala Leu Leu Met Asn Arg Pro **Arg** Ala Ala Thr
GGT GAA CGT
mR I β Phe **Gly Glu** Ile Ala Leu Leu Met Asn Arg Pro **Arg** Ala Ala Thr
GGG GAG CGG
332 346
bRII α Phe **Gly Glu** Leu Ala Leu Val Thr Asn Lys Pro **Arg** Ala Ala Ser
350 364
bRII β Phe **Gly Glu** Leu Ala Leu Val Thr Asn Lys Pro **Arg** Ala Ala Ser
GTC GAA CGA

The amino acid residues that interact with exocyclic phosphate and ribose hydroxyl group of cAMP, Gly, Glu and Arg (bolded) are conserved in both A and B cAMP binding sites in R I α, R I β, R II α and R II β subunits. The amino acid sequence is conserved in cAMP binding site A between α and β isoforms of the same, R I or R II, regulatory subunit. The differences between site A cores of R I and R II subunits are in two amino acids (Ile/Met and Gly/Asn, underlined). The differences between site B cores of R I and R II subunits are in five amino acids (underlined). Sites A and B of all isoforms of regulatory subunits contain threonine or serine residues that may be phosphorylation sites. The site B core of R I α and R I β subunits contains one threonine residue, while other cAMP binding site cores (sites A and B of R II α and R II β, and site A of R I α and R I β) contain two amino acid residues that may be phosphorylated. The pattern of phosphorylation of cytosol soluble R I subunit may differ from that of membrane-bound R II subunit.

The conserved amino acid sequence in cAMP binding sites A and B may suggest that the sites A and B originate from a gene duplication. The amino acids known to be involved in cAMP binding are encoded by the same or different nucleotide triplets in sites A and B. The triplets coding for Gly (GGG) and Glu (GAG) are the same in sites A and B of the mR I β, and the triplets coding for Glu (GAA) are conserved in sites A and B of the bR II β subunit. However, site A Arg is encoded by AGA in mR I α , mR I β and bR II β and site B Arg is encoded by CGT

in mR I α, CGG in mR I β and CGA in bR II β. The coding triplets are partially conserved, partially changed. Gene duplication of cAMP binding sites in the regulatory subunit of protein kinase A during evolution can not be excluded.

Catalytic subunit

The nucleotide and amino acid sequences of mouse catalytic subunits C α and C β and their isoforms, C β2 and C β3 are presented in Figure 6. In the 240-nucleotide promoter region of the C α subunit gene, five transcription start sites and three DNA sequences binding Sp 1 transcription factor have been found. In the same promoter region of the C β subunit gene, four transcription start sites have been found and one Sp 1 binding sequence. Three additional Sp 1 regulated sequences are located in the region extended to – 390 nucleotides of the 5'-untransalated region.

The exon-intron composition of the mouse catalytic C α subunit is presented in Table 1, and a diagram of the gene is shown in Figure 7. The C α subunit gene comprises ten exons and nine introns. The first exon also contains 350 nucleotides of the 5'-untranslated promoter sequence (Chrivia et al., 1988).

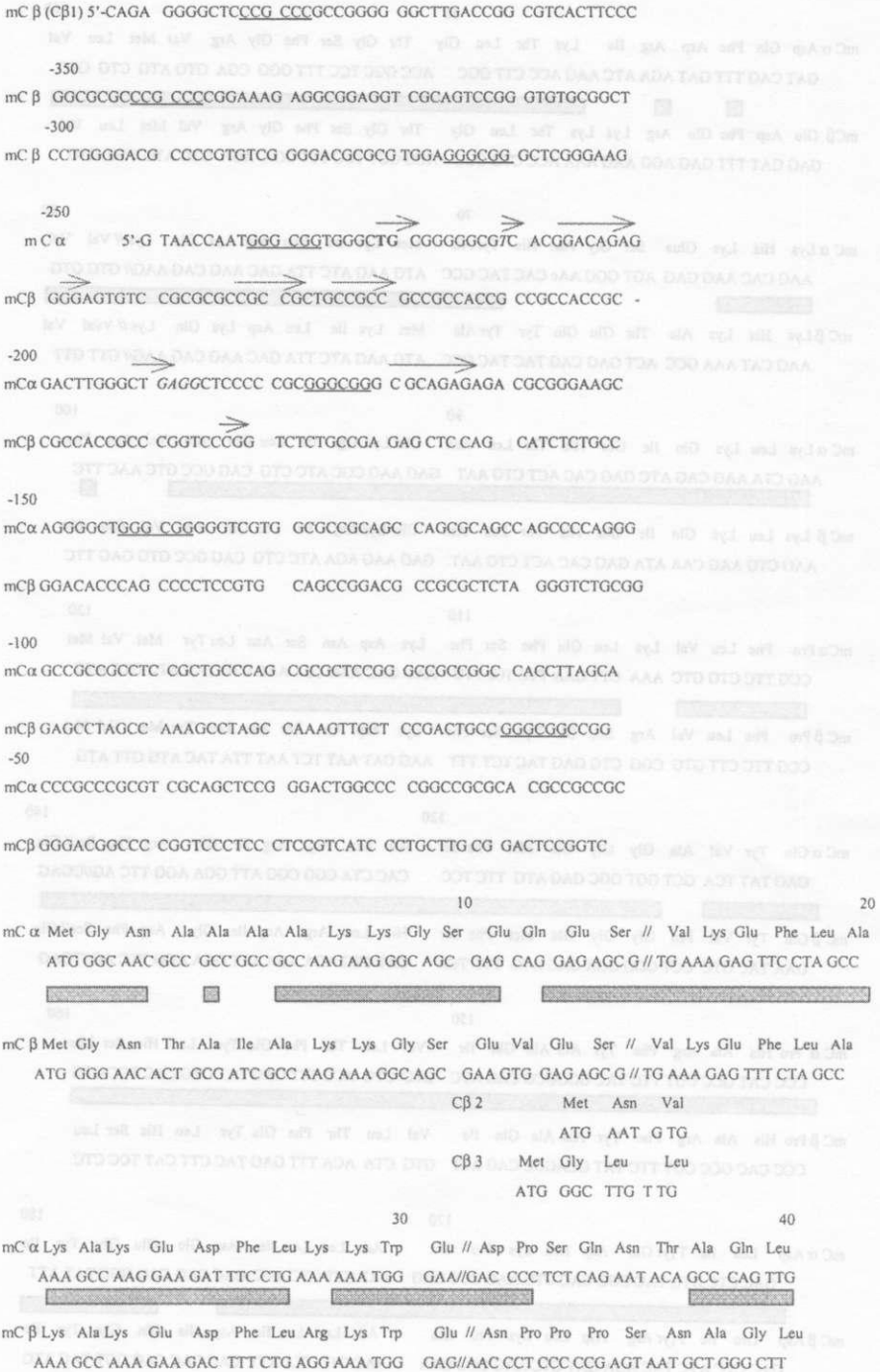
Two polyadenylation AATAAA signals have been found in the 3'-untranslated region at 2057-2062 and 2076 -2081 nucleotides in the C α subunit gene (Chrivia et al., 1988), however, the polyadenylation signals are absent in the 3'-untranslated region of the C β subunit gene (Uhler et al., 1986a).

The amino acid homology between C α and C β is 91%, the weakest homology is in the N-terminal part of the molecule, 70% identity over 70 N-terminal amino acids (Uhler et al., 1986a,b). Mouse brain C β catalytic isoforms C β2 and C β3

TABLE I

The exon/intron structure of catalytic C α subunit of protein kinase A (according to Chrivia et al., 1988)

Exon/intron number	Exon size nt	Intron size nt
I	330 + 45	8900
II	62	500
III	132	5200
IV	83	1500
V	83	2400
VI	127	200
VII	96	300
VIII	123	3900
IX	165	400
X	123	


mC β (Cβ1) 5'-CAGA GGGGCTCCCG CCCGCCGGGG GGCTTGACCGG CGTCACTTCCC
-350
mC β GGCGCGCCCG CCCCGGAAAG AGGCGGAGGT CGCAGTCCGG GTGTGCGGCT
-300
mC β CCTGGGGACG CCCCGTGTCG GGGACGCGCG TGGAGGGCGG GCTCGGGAAG
-250
mC α 5'-G TAACCAATGGG CGGTGGGCTG CGGGGGCGTC ACGGACAGAG
mCβ GGGAGTGTC CGCGCGCCGC CGCTGCCGCC GCCGCCACCG CCGCCACCGC -
-200
mCα GACTTGGGCT GAGGCTCCCC CGCGGGCGGG C GCAGAGAGA CGCGGGAAGC
mCβ CGCCACCGCC CGGTCCCGG TCTCTGCCGA GAG CTC CAG CATCTCTGCC
-150
mCα AGGGGCTGGG CGGGGGTCGTG GCGCCGCAGC CAGCGCAGCC AGCCCCAGGG
mCβ GGACACCCAG CCCCTCCGTG CAGCCGGACG CCGCGCTCTA GGGTCTGCGG
-100
mCα GCCGCCGCCTC CGCTGCCCAG CGCGCTCCGG GCCGCCGGC CACCTTAGCA
mCβ GAGCCTAGCC AAAGCCTAGC CAAAGTTGCT CCGACTGCC GGGCGGCCGG
-50
mCα CCCGCCCGCGT CGCAGCTCCG GGACTGGCCC CGGCCGCGCA CGCCGCCGC
mCβ GGGACGGCCC CGGTCCCTCC CTCCGTCATC CCTGCTTG CG GACTCCGGTC
10
20
mC α Met Gly Asn Ala Ala Ala Ala Lys Lys Gly Ser Glu Gln Glu Ser // Val Lys Glu Phe Leu Ala
ATG GGC AAC GCC GCC GCC GCC AAG AAG GGC AGC GAG CAG GAG AGC G // TG AAA GAG TTC CTA GCC
mC β Met Gly Asn Thr Ala Ile Ala Lys Lys Gly Ser Glu Val Glu Ser // Val Lys Glu Phe Leu Ala
ATG GGG AAC ACT GCG ATC GCC AAG AAA GGC AGC GAA GTG GAG AGC G // TG AAA GAG TTT CTA GCC
Cβ 2 Met Asn Val
ATG AAT G TG
Cβ 3 Met Gly Leu Leu
ATG GGC TTG T TG
30
40
mC α Lys Ala Lys Glu Asp Phe Leu Lys Lys Trp Glu // Asp Pro Ser Gln Asn Thr Ala Gln Leu
AAA GCC AAG GAA GAT TTC CTG AAA AAA TGG GAA//GAC CCC TCT CAG AAT ACA GCC CAG TTG
mC β Lys Ala Lys Glu Asp Phe Leu Arg Lys Trp Glu // Asn Pro Pro Pro Ser Asn Ala Gly Leu
AAA GCC AAA GAA GAC TTT CTG AGG AAA TGG GAG//AAC CCT CCC CCG AGT AAT GCT GGG CTT


50
60
mC α Asp Gln Phe Asp Arg Ile Lys Thr Leu Gly Thr Gly Ser Phe Gly Arg Val Met Leu Val
GAT CAG TTT GAT AGA ATC AAG ACC CTT GGC ACC GGC TCC TTT GGG CGA GTG ATG CTG GTG
mCβ Glu Asp Phe Glu Arg Lys Lys Thr Leu Gly Thr Gly Ser Phe Gly Arg Val Met Leu Val
GAG GAT TTT GAG AGG AAG AAA ACC CTC GGG ACG GGT TCC TTT GGA AGA GTC ATG TTG GTG
70
80
mC α Lys His Lys Glua Ser Gly Asn His Tyr Ala Met Lys Ile Leu Asp Lys Gln Lys // Val Val
AAG CAC AAG GAG AGT GGG AAc CAC TAC GCC ATG AAG ATC TTA GAC AAG CAG AAG// GTG GTG
mC β Lys His Lys Ala Thr Glu Gln Tyr Tyr Ala Met Lys Ile Leu Asp Lys Gln Lys // Vval Val
AAG CAT AAA GCC ACT GAG CAG TAC TAC GCC ATG AAG ATC TTA GAC AAG CAG AAG// GTT GTT
90
100
mC α Lys Leu Lys Gln Ile Glu His Thr Leu Asn Glu Lys Arg Ile Leu Gln Ala Val Asn Phe
AAG CTA AAG CAG ATC GAG CAC ACT CTG AAT GAG AAG CGC ATC CTG CAG GCC GTC AAC TTC
mC β Lys Leu Lys Gln Ile Glu His Thr Leu Asn Glu Lys Arg Ile Leu Gln Ala Val Glu Phe
AAG CTG AAG CAA ATA GAG CAC ACT CTG AAT GAG AAG AGA ATC CTG CAG GCC GTG GAG TTC
110
120
mCα Pro Phe Leu Val Lys Leu Glu Phe Ser Phe Lys Asp Asn Ser Asn Leu Tyr Met Val Met
CCG TTC CTG GTC AAA CTT GAA TTC TCc TTC AAG GAC AAC TCA AAC CTG TAC ATG GTC ATG
mC β Pro Phe Leu Val Arg Leu Glu Tyr Ser Phe Lys Asp Asn Ser Asn Leu Tyr Met Val Met
CCG TTC CTT GTG CGG CTG GAG TAC TCT TTT AAG GAT AAT TCT AAT TTA TAC ATG GTT ATG
130
140
mC α Glu Tyr Val Ala Gly Gly Glu Met Phe Ser His Leu Arg Arg Ile Gly Arg Phe Ser// Glu
GAG TAT TCA GCT GGT GGC GAG ATG TTC TCC CAC CTA CGG CGG ATT GGA AGG TTC AG//CGAG
mC β Glu Tyr Val Pro Gly Gly Glu Met Phe Ser His Leu Arg Arg Ile Gly Arg Phe Ser// Glu
GAA TAC GTC CCT GGG GGA GAG ATG TTC TCA CAT CTG AGA AGA ATT GGA AGG TTC AG//TGAG
150
160
mC α Pro His Ala Arg Phe Tyr Ala Ala Gln Ile Val Leu Thr Phe Glu Tyr Leu His Ser Leu
CCC CAT GCC CGT TTC TAC GCGGCG CAG ATC GTC CTG ACC TTT GAG TAT CTG CAC TCC CTG
mC β Pro His Ala Arg Phe Tyr Ala Ala Gln Ile Val Leu Thr Phe Glu Tyr Leu His Ser Leu
CCC CAC GCC CGT TTC TAT GCAGCC CAG ATT GTG CTA ACA TTT GAG TAC CTT CAT TCC CTC
170
180
mC α Asp Leu Ile T{yr Gln Asp Leu Lys Pro Glu Asn Leu Leu Ile Asp Glu Glu Gly Tyr Ile
GAC CTC ATC TAC CGG GAC CTG AAG CCC GAG AAT CTT CTC ATC GAC CAG CAG GGC TAT ATT
mC β Asp Leu Ile T{yr Arg Asp Leu Lys Pro Glu Asn Leu Leu Ile Asp His Gln Gly Tyr Ile
GAC CTC ATC TAC AGA GAT CTC AAG CCG GAA AAC CTC TTA ATT GAC CAC CAG GGT TAC ATC


190
200
mC α Gln // Val Thr Asp Phe Gly Phe Ala Lys Arg Val Lys Gly Arg Thr Trp Thr Leu Cys Gly
CAG //GTG ACA GAC TTT GGG TTT GCC AAG CGT GTG AAA GGC CGT ACT TGG ACC TTG TGT GGG
mC β Gln // Val Thr Asp Phe Gly Phe Ala Lys Arg Val Lys Gly Arg Thr Trp Thr Leu Cys Gly
CAG //GTC ACA GAT TTC GGG TTC GCC AAA AGA GTC AAG GGC AGG ACA TGG ACA TTG TGT GGC
210
220
mC αThr Pro Glu Tyr Leu Ala Pro Glu Ile Ile Leu Ser Lys// Gly Tyr Asn Lys Ala Val Asp
AAC CCT GAG TAC TTG GCC CCC GAG ATT ATC CTG AGC AAA //GGC TAC AAC AAG GCT GTG GAC
mC β Thr Pro Glu Tyr Leu Ala Pro Glu Ile Ile Leu Ser Lys// Gly Tyr Asn Lys Ala Val Asp
AAC CCA GAG TAC CTG GCCCCG GAG ATC ATC CTC AGC AAG //GGT TAC AAT AAG GCG GTG GAC
230
240
mCα Trp Trp Ala Leu Gly Val Leu Ile Tyr Glu Met Ala Ala Gly Tyr Pro Pro Phe Phe Ala
TGG TGG GCT CTC GGA TTC CTC ATC TAC GAG ATG GCT GCT GGT TAC CCA CCC TTC TTC GCT
mCβ Trp Trp Ala Leu Gly Val Leu Ile Tyr Glu Met Ala Ala Gly Tyr Pro Pro Phe Phe Ala
TGG TGG GCA CTG GGC TTG CTG ATC TAT GAG ATG GCT GCT GGC TAC CCT CCA TTC TTT GCT
250
260
mCα Asp Gln Pro Ile Gln Ile Tye Glu Lys Ile Val Ser Gly Lys// Val Arg Phe Pro Ser His
GAC CAG CCT ATC CAG ATC TAT GAG AAA ATC GTC TCT GGG AAG///GTG CGG TTC CCA TCC CAC
mC β Asp Gln Pro Ile Gln Ile Tye Glu Lys Ile Val Ser Gly Lys// Val Arg Phe Pro Ser His
GAC CAG CCA ATT CAG ATC TAT GAG AAG ATT GTC TCT GGA AAG///GTC CGG TTC CCA TCA CAC
270
280
mCα Phe Ser Ser Asp Leu Lys Asp Leu Leu Arg Asn Leu Leu Glu Vsl Asp Leu Thr Lys Arg
CTT AGC TCT GAC TTG AAG GAC CTG CTG CGG AAC CTT CTG CAG GTG GAT CTA ACC AAG CGC
mCβ Phe Ser Ser Asp Leu Lys Asp Leu Leu Arg Asn Leu Leu Glu Vsl Asp Leu Thr Lys Arg
TTC AGC TCG GAT CTC AAG GAC CTT CTG CGG AAC CTG CTG CAG GTG GAT CTG ACA AAG CGA
290
300
mCα Phe Gly Asn Leu Lys Asp Gly Val Asn Asp Ile Lys Asn His Lys Trp Phe Ala Thr Thr
TTT GGA AAC CTC AAG GAC GGG GTC AAT GAC ATC AAG AAC CAC AAG TGG TTT GCC ACG ACT
mCβ Phe Gly Asn Leu Lys Asn Gly Val Ser Asp Ile Lys Thr His Lys Trp Phe Ala Thr Thr
TTC GGG AAC CTG AAG AAC GGC GTG AGT GAC ATA AAG ACC CAC AAG TGG TTT GCC ACA ACT

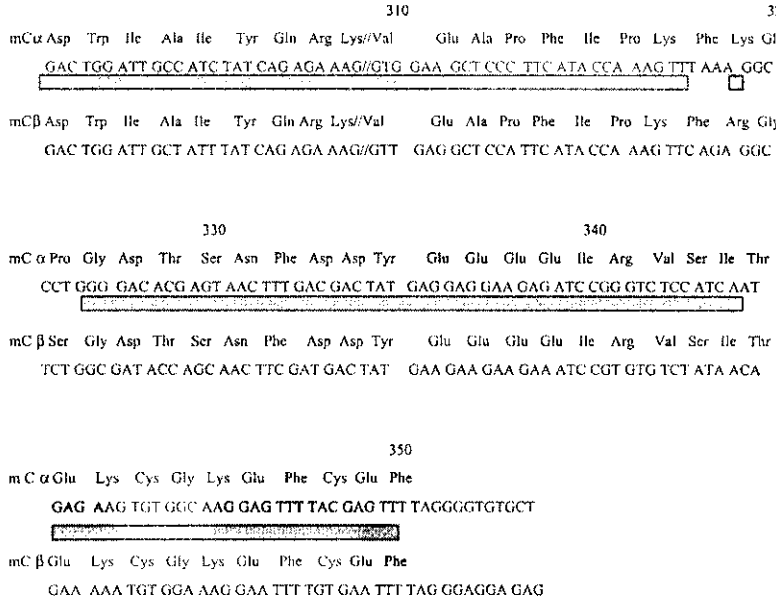


Figure 6. The nucleotide sequence and primary structure of the mouse catalytic C α and C β (Uhler et al., 1986 A and B; Chrivia et al., 1988) subunits and C β2 and C β3 subunit isoforms (Guthrie et al., 1997) of protein kinase A. The conserved amino acid sequence is shown by grey filled bars. The Sp1 consensus sequences are underlined. The transcription start sites are indicated by arrows. The gaps indicate the sites of intron insertions

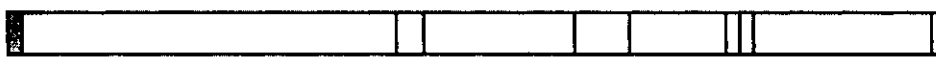


Figure 7. The scheme of mouse catalytic C α subunit gene of protein kinase A. The lines represent the exon localization, the rectangles represent intron sizes (Chrivia et al., 1988)

differ from C β1 (C β) in the length of the protein chain and N-terminal amino acid composition. The C β2 and C β3 proteins are not myristoylated, unlike the other catalytic subunits (Guthrie et al., 1997).

SYNTHESIS OF PROTEIN KINASE A SUBUNITS

The regulatory R I α, R I β, R II α, R II β and catalytic C α, C β and C γ subunits of protein kinase A are expressed from single genes (Uhler and McKnight, 1987; Clegg et al., 1988; McKnight et al., 1988; Oyen et al., 1988; Landmark et al.,

1991; Garrel et al., 1993). The isoforms of mouse C β subunit, C β1, C β2 and C β3 originate from the same gene (Guthrie et al., 1997). The genes of protein kinase A share some common characteristics: they belong to the class of genes whose promoters are GC-rich, lack TATA boxes, and initiate transcription at multiple sites. Most of them are activated by cAMP.

The expression of the R II β regulatory subunit is induced hormonally and limited to specific tissues such as endocrine, neuroendocrine, neural and leukemic. The DNA for the R II β subunit was isolated from genomic libraries of the mouse (Singh et al., 1991) and rat (Kurten et al., 1992) and sequenced. The 5'-flanking, 4530 nucleotide non-coding region of the rat liver R II β gene has been sequenced and characterized. The core of the promoter region starting from nucleotide -400 is presented below (Kurten et al., 1992).

```
        -390       -380       -370       -360       -350       -340       -330
GAGCTCAGGT GGAGCGCGCG GCCTCGCCCC CACGCCCCAG GCGCCCGCTG CCTCCACCCT TAGGCCACTT
        -320       -310       -300       -290       -280       -270       -260
GAGAGGCGGC AGCGCTCCCG CCCGCCGGGT TGCCATGGTT TCCGGGGATC ACGTGGGCGC GCGGGCGGGG
        -250       -240       -230       -220       -210       -200       -190
GCGGGGCGCC GGGCGGGGGG GGGGGCGGTG CAGCGGCGGC GGGAGCGGGA GCGGGAGGAG CTGGAGAGTC
        -180       -170       -160       -150       -140       -130       -120
TGCCAACCCT CCCCGGGTTG TGCTCGCTCT GCTGCCGCCG CCGCACGGAG CAGCCTCGCC GGGGGCCCAG
        -110       -100        -90        -80        -70        -60        -50
TGCGCCGCGC TCGCAGCCCG TAGCGCCCGG GGCGCTCGCT CGGGGGCCGC GCAGCCCAAG ACCCGACCGC
         -40        -30        -20        -10
GGATAGGAGG CGAGGGCGGC GTCCAGGCGC CTCGGCGTGC ACAGGCAGG ATG
```

The promoter region is GC-rich and does not contain TATA nor CAAT boxes. Nucleotide sequence analysis shows two tandem AP2 consensus sequences (boxed) and five Sp1 binding sites (underlined). RNase protection experiments indicated the presence of 11 transcription initiation sites, indicated by arrows. A transfection study of rat granulosa cells using different constructs of R II β promoter and mobility shift assay carried out on nuclear extracts of rat brain, ovary and liver showed that the region induced by cAMP was localized between 394 and 176 nucleotides of the promoter region. The sequences distal and proximal to this region are involved in the basal gene expression (Kurten et al., 1992). The AP2 binding sites confers cAMP inducibility. The GC-rich sequences are activated by Sp1 protein and may be activated by cAMP (Ahlgren et al., 1999). In the mouse, the AP-1 sequence located 1180 bases upstream from the ATG initiation codon has been

identified. This site may be additionally regulated by cAMP (Singh et al., 1991). The polyadenylation signals AATAAA were found in the regions: 1508-1513; 1761-1766; 3041-3046; and 3058-3064 nucleotides in the 3'-end of the rat ovary R II β subunit cDNA (Jahnsen et al., 1986).

In the anterior pituitary gland, mRNAs of all known regulatory subunits, R I α, R I β, R II α and R II β and two catalytic subunits, C α and C β of protein kinase A are expressed. The expression of mRNAs of R II β and C α subunits is activated by cAMP. This activation is supposed to be mediated via cAMP induced protein(s) that are involved in the induction of R II β and C α genes (Garrel et al., 1993).

ACTIVATION AND REGULATION OF PROTEIN KINASE A ACTIVITY

Protein kinase A is activated by cAMP binding to its R subunit followed by dissociation of the C subunit. Since the protein kinase A complex is composed of two regulatory and two catalytic subunits and each of them contains two cAMP binding sites, the activation of one molecule of the enzyme requires four molecules of cAMP. The kinetic study of cAMP binding to regulatory subunits mutated in the A or B cAMP-binding sites has shown that the cyclic nucleotide binds first to site B, causing conformational changes that make site A accessible to cAMP. The liberation of the catalytic subunit results in uncovering its active center that had been blocked by an auto-inhibitory region of the regulatory subunit, which has an amino acid sequence similar to the substrate sequence recognized by catalytic subunit (McKnight et al., 1988; Taylor et al., 1990).

Protein kinase A anchoring proteins

The activity of protein kinase A is compartmentalized to specific regions of the cell; compartmentalization is determined by subcellular localization of the regulatory subunit. The regulatory R I subunit is found primarily in cytoplasm, while the regulatory R II subunit binds to membrane structures such as plasma membranes, Golgi complexes, centrosomes, mitotic spindle poles and nuclear proteins (Nigg et al., 1985; Meinkoth et al., 1990; Podesta et al., 1991; Coghlan et al., 1994; Faux and Scott, 1996; Dell'Acqua and Scott, 1997; Colledge and Scott, 1999).

Two types of regulatory R II subunit anchoring proteins are known, microtubule-associated protein 2 (MAP-2) and protein kinase A anchor protein (AKAP). The MAP-2 (270-300 kDa) copurifies with brain microtubules. It appears as a projection on the microtubule surface and also has the property of promoting microtubule assembly *in vitro* (Vallee, 1980; Majewska, 1995).

At the beginning, AKAPs were isolated from bovine (p75) and rat (p150) brains and thereafter from other animal tissues and species. These proteins bind calmo-

dulin. More than forty regulatory R II α subunit binding bands, ranging in size from 25 to 300 kDa have been detected electrophoretically in various tissues (Carr et al., 1992). Several AKAPs have been cloned and well characterized, AKAP-75 from bovine brain (Hirsch et al., 1992), Ht31 from human thyroid (Carr et al., 1992), AKAP-84 from hepatic cells (Chen et al., 1997), AKAP-550 from *Drosophila melanogaster* (Han et al., 1997) and AKAP-95 from rat pituitary GH_4C_1 cells (Coghlan et al., 1994). The latter AKAP has been shown to be an ubiquitous nuclear regulatory RII subunit anchoring protein also having a DNA binding domain. It has been suggested that AKAP-95 plays a role in promoting the binding of transcriptional factors to DNA, affecting chromatin structure and activating transcription.

In the regulatory R II subunit, the domain binding MAP-2 and AKAPs is located within the eighty amino acid N-terminal. Dimerization of the regulatory R II β subunit is required for binding anchoring protein (Hausken et al., 1994; Dell'Acqua and Scott, 1997). The MAP-2 protein binds the regulatory R II subunit at a site near its N-terminal and, in AKAPs, the R II binding site is located at the C-terminal part of the molecule (Carr et al., 1992; Hirsch et al., 1992; Coghlan et al., 1994; Chen et al., 1997). The regions binding the R II subunit in AKAPs and MAP-2 do not have a conserved primary structure. Studies of the secondary and tertiary structures of the regulatory R II subunit and its anchoring proteins, and assay of mutated R II subunit and mutated proteins anchoring the R II subunit revealed that hydrophobic surfaces at the tethering sites of AKAPs and complementary surfaces on R II subunit dimers are essential for formation of stable AKAP-protein kinase A complexes (Hausken et al., 1994, 1996; Li and Rubin, 1995; Newlon et al., 1997).

Proportions of regulatory and catalytic subunits

The endogenous activity of protein kinase A depends on the proportions of the regulatory and catalytic subunits and combination of their isoforms. Maintaining cAMP-dependent control of protein kinase A activity is due to a certain excess of the regulatory subunit in relation to the catalytic subunit. In an excess of catalytic subunit, the cell would lose the effect of cAMP on kinase activation and would have a continuously active protein kinase A. The cell is protected against an excess of catalytic subunit via a compensation mechanism of the regulatory subunit. This mechanism was observed in S49 cells overproducing the catalytic subunit. In these cells, the level of regulatory R I α subunit increased exceeding the catalytic subunit level (the level of R II subunit remained constant). It has been postulated that this compensation results from an increase in the stability and rate of translation of regulatory R I α subunit mRNA and stabilization of R I α protein (McKnight et al., 1988; Knutsen et al., 1991). On the

other hand, the associated holoenzyme is protected against proteolysis. The catalytic subunit dissociated from the regulatory subunit easily undergoes proteolysis (Hemmings, 1986). Thus, the excess of regulatory over catalytic subunits provides a threshold for cAMP concentration-dependent protein kinase A activation and establishes protection of protein kinase A activity.

THE ROLE OF PROTEIN KINASE A IN CELLULAR METABOLISM

Protein kinase A is involved in various cellular processes. Protein synthesis is affected by protein kinase A at the transcription level. Genes are regulated via phosphorylation and activation of cAMP response element binding protein (CREB) interacting with cAMP response element (CRE). The CREB is a 45 kDa protein which dimerizes after cAMP dependent phosphorylation and binds CRE located in the promoter regulatory regions of cAMP-activated genes (Montminy and Bilezikijan, 1987; Gonzalez et al., 1989). It recruits basal transcriptional factors TFIIB, TFIID, TATA-box binding protein (TBP) to the promoter (Ferreri et al., 1994; Kwok et al., 1994; Parker et al., 1996; Nakajima et al., 1997a). Phosphorylated CREB dimerizes by a leucine zipper structure, forming a homodimer, or, if it dimerizes with another transcriptional factor, a heterodimer is formed. Phosphorylation and activation of CREB promote binding a large 265 kDa protein, CREB binding protein (CBP). The CBP binds histone acetyltransferase and itself possesses intrinsic histone acetyltransferase and helicase activity (Bannister and Kouzarides, 1996; Nakajima et al., 1997b). This complex recruits RNA polymerase II to the promoter and activates transcription (Ferreri et al., 1994; Kwok et al., 1994; Liang and Hai, 1997; Nakajima et al., 1997a,b). It is suggested that CRE is also activated by the R II β regulatory subunit of protein kinase A (Srivastava et al., 1998). Another cAMP-regulated element is the AP-2 sequence (CCCCAGGC consensus) activated by AP-2 protein (Johnson et al., 1997; Wang et al., 1997).

Protein kinase A is involved in the synthesis and release of hormones (Roesler et al., 1988; Counis, 1990; Kato et al., 1992; Puttagunta et al., 1992; Inukai et al., 1993; Counis et al., 1993; Sladek and Gallagher, 1993; Jacob and Stanley, 1994; Johnson et al., 1997; Spiro and McMurray, 1997; Klemm et al., 1998), synthesis of adrenergic receptors (Thomas et al., 1992; Razik et al., 1997), enzymes synthesizing neurotransmitters (Nagamoto-Combs et al., 1997; Swanson et al., 1997) and melanin (Bertollotto et al., 1998), ferritin (Bevilacqua et al., 1997), it participates in the activation of the immediate-response genes (Ramirez et al., 1997), and the expression of interleukine-2 gene in T-lymphocytes (Butscher et al., 1998).

Recently, a role for protein kinase A in cell cycle regulation has been reported. Protein kinase A functions as a positive regulator of the cycline A gene

promoter. Cycline A associates with cycline-dependent protein kinases (cdks) during phases of the cell cycle and is required for DNA replication in the S phase (Desdouets et al., 1995). Protein kinase A-dependent phosphorylation interferes with the tyrosine kinase phosphorylation of growth receptors and Ras-stimulated activation of Raf-1 kinase, resulting in the inhibition of MAP kinase and alteration of cell proliferation (Giasson et al., 1997; York et al., 1998). Protein kinase A downregulates oncoprotein Op 18 (metablastin or stathmin), the protein destabilizing microtubules, which diminishes oncoprotein activity (Gradin et al., 1998). Furthermore, protein kinase A is involved in the arrest of virus multiplication and cell transformation by induction of p27 and p21, proteins that inhibit viral promoter activation and tumor development (Chinery et al., 1997; Deleu et al., 1998). Protein kinase A phosphorylates cytoplasmic and membrane protein substrates that influence the movement of proteins in the cytoplasm and ion channel permeability, and is involved in the secretion processes (Vallee, 1980; Carr et al., 1992; Hirsch et al., 1992; Kurashima et al., 1997).

Changes in the phosphorylation of key enzymes of metabolic pathways affect basal cellular metabolism leading to intensification of catabolic processes (Cohen and Hardie, 1991; Jakubowicz and Gąsior, 1993). Phosphorylation of glycogen phosphorylase activates the enzyme, while phosphorylation of glycogen synthase inhibits it, leading to a decrease in glycogen synthesis. The phosphorylation of acetyl CoA carboxylase and 3-hydroxy-3-methyl-glutamyl CoA reductase diminishes the synthesis of fatty acids and cholesterol, respectively (Cohen and Hardie, 1991; Mounier et al., 1997). Increased phosphorylation of these enzymes is not associated with their direct enzymatic phosphorylation, but is a consequence of the inhibition of the activity of phosphoprotein phosphatases by cAMP-dependent phosphorylation (Cohen and Hardie, 1991). The activity of proteases is regulated by protein kinase A through phosphorylation and activation of calpastatin, a protease inhibitor, and cAMP-dependent activation of the calpastatin gene (Salamino et al., 1994; Cong et al., 1998).

CONCLUSIONS

Protein kinase A mediates the regulation of a great variety of cAMP-dependent processes. The enzyme is involved in growth and development, cell proliferation and basal cellular metabolism. Protein kinase A regulates transcription factors by direct phosphorylation or by stimulation of its synthesis or synthesis of proteins activating the expression of transcription factors, influences the activity of cycline-dependent protein kinases by activating cycline A and cdk inhibitor genes, phosphorylates proteins that are involved in microtubule formation, and affects the activity of mitogen-activated protein kinase.

Protein kinase A occurs as several isoenzymes due to the presence of diverse isoforms of its subunits. The isoenzymes of protein kinase A differ in the substrate specificity and activity. The expression of protein kinase A subunits is tissue- and hormone-specific. The tissue specificity of protein kinase A isoforms and compartmentalization in the cell, which determine access to cAMP and substrate, is the reason why protein kinase A may be involved in many cellular processes and its activation leads to various cellular responses to stimulation mediated by cAMP .

REFERENCES

Ahlgren R., Suske G., Waterman M.R., Lund J., 1999. Role of Sp1 in cAMP -dependent transcriptional regulation of the bovine CYP 11A gene. J. Biol. Chem. 274, 19422-19426

Bannister A.J., Kouzarides T., 1996. The CBP co-activator is a histone acetyltransferase. Nature (London) 384, 641-643

Bayewitch M.L., Avidor-Reiss T., Levy R., Pfeuffer T., Nevo I., Simonds W.F., Vogel Z., 1998. Differential modulation of adenylyl cyclases I and II by various Gβ subunits. J. Biol. Chem. 273, 2273-2276

Bertolotto C., Busca R., Abbe P., Bille K., Aberdam E., Ortonne J.P., Ballotti R., 1998. Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP: pivotal role of M boxes (GTCATGTGCT) and microphthlamia. Mol. Cell. Biol. 18, 694-702

Bevilacqua M.A., Faniello M.C., Quaresima B., Tiano M.T., Giuliano P., Feliciello A., Avvedimento V.E., Cimino F., Costanzo F., 1997. A common mechanism underlying the E1A repression and the cAMP stimulation of the H ferritin transcription. J. Biol. Chem. 272, 20736-20741

Bubis J., Vedvick T.S., Taylor S.S., 1987. Antiparallel alignment of the two promoters of the regulatory subunit dimer of cAMP-dependent protein kinase I. J. Biol. Chem. 262, 14961-14966

Butscher W.G., Powers C., Olive M., Vinson C., Gardner K., 1998. Coordinate transactivation of the interleukin-2 CD28 response element by c-Rel and ATF-1/CREB2. J.Biol. Chem. 273, 552-560

Carr D.W., Hausken Z.E., Fraser J.D.C., Stofko-Hahn R.E., Scott J.D., 1992. Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein. J. Biol. Chem. 267, 13 376-13 392

Chen Q., Liu R.Y., Rubin C.S., 1997. Organelle-specific targeting of protein kinase A II (PKA II). Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKA II to the cytoplasmic surface of mitochondria. J. Biol. Chem. 272, 15247-15257

Chinery R., Brockman J.A., Dransfield D.T., Coffey R.J., 1997. Antioxidant-induced nuclear translocation of CCAAT/enhancer-binding protein. J. Biol. Chem. 272, 30356-30361

Chrivia J.C., Uhler M.D., McKnight G.S., 1988. Characterization of genomic clones coding for the C α and C β subunits of mouse cAMP-dependent protein kinase. J. Biol. Chem. 263, 5739-5744

Clegg C.H., Cadd G.G., McKnight G.S., 1988. Genetic characterization of a brain-specific form of the type I regulatory subunit of cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA 85, 3703-3707

Coghlan V.M., Langeberg L.K., Fernandez A., Lamb N.J.C., Scott J.D., 1994. 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, 7658-7665

Cohen P., Hardie D.G., 1991. The actions of cyclic AMP on biosynthetic processes are mediated indirectly by cyclic AMP-dependent protein kinase. Biochim. Biophys. Acta 1094, 292-299

Colledge M., Scott J.D., 1999. AKAPs: from structure to function. Trends Cell. Biol. 8, 216-222

Cong M., Thompson V.F., Goll D.E., Antin P.B., 1998 The bovine calpastatin gene promoter and a new N-terminal region of the protein are targets for cAMP-dependent protein kinase activity. J. Biol. Chem. 273, 660-666

Counis R., 1990. Les genes des hormones gonadotropes hypophysaires and leur regulation. Annal. Endocrinol. (Paris). 51, 72-81

Counis R., Bouamond N., Lerrant Y., Starzec A., Moumni M., Kottler M.L., Garrel G., Berault A., Bergametti F., 1993. Donnees recent sur le recepteur de la gonadoliberine et les mechanismes de controle par le neuropeptide de l'expression des genes des hormones gonadotropes. Contracept. Fertil. Sex. 21, 773-779

Daaka Y., Luttrell L.M., Lefkowitz R.J., 1997. Switching of the coupling of the β_2-adrenergic receptor to different G proteins by protein kinase A. Nature (London) 390, 88-91

Deleu L., Fuks F., Spitkovsky D., Horlein R., Faisst S., Rommelaere J., 1998. Opposite transcriptional effects of cyclic AMP-responsive elements in confluent or p27 ^{KIP}-overexpressing cells versus serum-starved or growing cells. Mol. Cell. Biol. 18, 409-419

Dell'Acqua M.L., Scott J.M., 1997. Protein kinase A anchoring. J. Biol. Chem. 272, 12881-12884

Desdouets C., Matesik G., Molina C.A., Foulkes N.S., Sassone-Corsi P., Brechot T., Sobczak-Thepot J., 1995. Cell cycle regulation of cyclin A gene gene expression by cAMP-responsive transription factors CREB and CREM. Moll. Cell. Biol. 15, 3300 - 3309

Dessauer C.W., Gilman A.G., 1997. The catalytic mechanism of mammalian adenylyl cyclase. J. Biol. Chem. 272, 27787-27795

Dessauer C.W., Scully T.T., Gilman A.G., 1997. Interactions of forskolin and ATP with the cytosolic domains of mammalian adenylyl cyclase. J. Biol. Chem. 272, 22272-22277

Faux M.C., Scott J.D., 1996. Molecular glue: kinase anchoring protein and scaffold protein. Cell 85, 9-12

Ferreri K., Gill G., Montminy M., 1994. The cAMP-regulated transcription factor CREB interacts with a component of the TFII D complex. Proc. Natl. Acad. Sci. USA 91, 1210-1213

Garrel G., Delahaye R., Hemmings B.A., Counis R., 1995. Modulation of regulatory and catalytic subunit levels of cAMP-dependent protein kinase A in anterior pituitary celss in response to direct activation of protein kinases A and C or after GnRH stimulation. Neuroendocrinol. 62, 514-522

Garrel G., Lerrant Y., Ribot G., Counis R., 1993. Messenger ribonucleic acids for α- and β-isoforms of cyclic adenosine 3',5'-monophosphate-dependent protein kinase subunits present in the anterior pituitary: regulation of RIIβ and Cα gene expression by the cyclic nucleotide and phorbol ester. Endocrinology 133, 1010-1019

Garrel G., McArdle C.A., Hemmings B.A., Counis R., 1997. Gonadotropin-releasing hormone and pituitary adenylate- cyclase activatis polypeptide affect levels of cyclic adenosine 3', 5'-monophosphate-dependent protein kinase A (PKA) subunits in the clonal gonadotrope aT3-i cells: evidence for cross-talk between PKA and protein kinase C pathways. Endocrinology 138, 2259-2266

Giasson E., Servant M.J., Meloche S., 1997. Cyclic AMP-mediated inhibition of angiotensin II - induced protein synthesis is associated with suppression of tyrosine phosphorylation signaling in vascular smooth muscle cells. J. Biol. Chem. 272, 26879-26886

Gibson R.M., Buechler V.J., Taylor S.S., 1997. Interaction of the regulatory and catalytic subunits of cAMP-dependent protein kinase. Electrostatic sites on the type I α regulatory subunit. J. Biol. Chem. 272, 16343-16350

Gibson R.M., Taylor S.S., 1997. Dissecting the cooperative reassociation of the regulatory and catalytic subunits of cAMP-dependent protein kinase. 272, 31998-32005

Gill G.N., Garren L.D., 1970. A cyclic -3',5'-adenosine monophosphate dependent protein kinase from the adrenal cortex: comparison with a cyclic AMP binding protein. Biochim. Biophys. Res. Commun. 39, 335-343

Gonzalez G.A., Yamamoto K.K., Fischer W.H., Karr D., Menzel P., Biggs III W., Vale W.W., Montminy M.R., 1989. A cluster of phosphorylation sites on the cyclic AMP-regulated nuclear factor CREB predicted by its sequence. Nature (London) 337, 749-752

Gradin H.M., Larsson N., Marklund U., Gullberg M., 1998. Regulation of mikrotubule dynamics by extracellular signals: cAMP-dependent protein kinase switches off the activity of oncoprotein 18 in intact cells. J. Cell. Biol. 140, 131-141

Guthrie C.R., Skalhegg B.S., McKnight G.S., 1997. Two novel brain-specific splice variants of the murine C β gene of cAMP-dependent protein kinase. J. Biol. Chem. 272, 29560-29565

Han J.D., Baker N.E., Rubin C.S., 1997. Molecular characterization of a novel A kinase anchor protein from Drosophila melanogaster. J. Biol. Chem. 272, 26611-26619

Hausken Z.E., Coghlan V.M., Schafer-Hastings C.A., Reimann E.M., Scott J.D., 1994. Type II regulatory subunit (R II) of the cAMP-dependent protein kinase interaction with A-kinase anchor proteins requires isoleucines 3 and 5. J. Biol. Chem. 269, 24245-24251

Hausken Z.E., Dell'Acqua M.L., Coghlan V.M., Scott J.D., 1996. Mutational analysis of the kinase A anchoring protein (AKAP)-binding site on R II. J. Biol. Chem. 271, 29016-29022

Hemmings B.A., 1986. cAMP mediated proteolysis of the catalytic subunit of cAMP-dependent protein kinase. FEBS Lett. 196, 126-130

Hirsch A.H., Glantz S.B., Li Y., You Y., Rubin C.S., 1992. Cloning and expression of an intron-less gene for AKAP-75, an anchor protein for the regulatory subunit of cAMP-dependent protein kinase II b. J. Biol. Chem. 267, 2131-2134

Hofmann F., Beavo J.A., Bechtel P.J., Krebs E.G., 1975. Comparison of adenosine 3':5'-monophosphate-dependent protein kinase from rabbit skeletal and bovine heart muscle. J. Biol. Chem. 250, 7795-7801

Inukai T., Wang X., Greer S.E., Greer M.A., 1993. Adenosine 3',5'-cyclic monophosphate-mediated prolactin secretion in GH_4C_1 cells involves Ca^{2+} influx through L-type Ca^{2+} channels. Cell Calcium 14, 219-226

Jacob K.K., Stanley F.M., 1994. The insulin and cAMP response elements of the prolactin gene are overlapping sequences. J. Biol. Chem. 269, 25515-25520

Jahnsen T., Hedin L., Kidd V.J., Beattie W.G., Lohmann S.M., Walter U., Durica J., Schultz T.Z., Schiltz E., Browner M., Lawrence C.B., Goldman D., Ratoosh S.L., Richards J.A.S., 1986. 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, 12352-12361

Jakubowicz T., Gąsior E., 1993. From phosphorylase kinase ... to a thousend and one protein kinases? (in Polish). Post. Biochem. 39, 2-7

Johnson W., Albanese C., Handwerger S., Williams T., Pestell R.G., Jameson J.L., 1997. Regulation of the human chorionic gonadotropin α and β-subunit promoters by AP-2. J. Biol. Chem. 272, 15405-15412

Kato A., Gores G.J., LaRusso N.F., 1992. Secretin stimulates exocytosis in isolated bile duct epithelial cells by a cyclic AMP-mediated mechanism. J. Biol. Chem. 267, 15523-15529

Klemm D.J., Roesler W.J., Boras T., Colton L.A., Felder K., Reusch J.E.B., 1998. Insulin stimulates cAMP-response element binding protein activity in HepG2 and 3T3-L1 cell lines. J. Biol. Chem. 273, 917-923

Knighton D.R., Zheng J., Ten Eyck L.F., Ashford V.A., Xuong N.H., Taylor S.S., Sowadski J.M., 1991. Crystal structure of the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. Science 253, 407-420

Knutsen H.K., Tasken K.A., Eskild W., Jahnsen T., Hansson V., 1991. Adenosine 3',5'-monophosphate-dependent stabilization of messenger ribonucleic acids (mRNAs) for protein kinase A (PKA) subunits in rat Sertoli cells. Endocrinology 129, 2496-2502

Kurashima K., Yu F.H., Cabado A.G., Szabo E.Z., Grinstein S. Orlowski J., 1997. Identification of sites required for down regulation of Na+/H+ exhanger NHE3 activity by cAMP-dependent protein kinase. J. Biol. Chem. 272, 28672-28679

Kurten R.C., Levy L.O., Shey J., Durica J.M., Richards J.S., 1992. Identification and characterization of the GC-rich and cyclic adenosine 3',5'-monophosphate (cAMP)-inducible promoter of the type II β cAMP-dependent protein kinase regulatory subunit gene. Mol. Endocrinol. 6, 536-550

Kwok R.P.S., Lundblad J.R., Chrivia J.C., Richards J.P., Bachinger H.P., Brennan R.G., Roberts S.G.E., Green M.R., Goodman R.H., 1994. Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature (London) 370, 223-226

Landmark B.F., Fauske B., Eskild W., Skalhegg B., Lohmann S.M., Hansson V., Jahnsen T., Beebe S.J., 1991. Identification, characterization and hormonal regulation of 3',5'-cyclic adenosine monophosphate-dependent protein kinases in rat Sertoli cells. Endocrinology 129, 2345-2354

Leon D.A., Herberg F.W., Banky P., Taylor S.S., 1997. A stable α-helical domain at the N terminus of the R I α subunits of cAMP-dependent protein kinase is a novel dimerization/docking motif. J. Biol. Chem. 272, 28431-28437

Li Y., Rubin C.S., 1995. Mutagenesis of the regulatory subunit (R II β) of cAMP-dependent protein kinase II β reveals hydrophobic amino acids that are essential for R II β dimerization and/or anchoring R II β protein to the cytoskeleton. J. Biol. Chem. 270, 1935-1944

Liang G., Hai T., 1997. Characterization of human activating transcription factor 4, a transcriptional activator that interacts with multiple domains of cAMP-responsive element-binding protein (CREB)-binding protein (CBP). J. Biol. Chem. 272, 24088-24095

Luo Z., Shafit-Zagardo B., Erlichman J., 1990. Identification of the MAP2- and P75-binding domain in the regulatory subunit (R II β) of type II cAMP-dependent protein kinase. J. Biol. Chem. 265, 21804-21810

Majewska B., 1995. MAP-2 (Microtubule-associated protein – 2) and its role in several aspects of plasticity and pathological states in the central nervous system (in Polish). Post. Biochem. 41, 175-182

McArdle C.A., Counis R., 1996. GnRH and PACAP action in gonadotropes. Trends Endocrinol. Metabolism 7, 168-175

McKnight S.G., Clegg C.H., Uhler M.D., Chrivia J.C., Cadd G.G., Correll. L.A., Otten A.D., 1988. Analysis of the cAMP-dependent protein kinase system using molecular genetic approaches. Rec. Progr. Hormone Res. 44, 307-335

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

Montminy M..R, Bilezikijan L.M., 1987. Binding of a nuclear protein to the cyclic AMP response element of the somatostatin gene. Nature (London) 328, 175-178

Mounier C., Chen W., Klautky S.A., Goodridge A.G., 1997. Cyclic AMP mediated inhibition of transcription of the malic enzyme gene in chick embryo hepatocytes in culture. J. Biol. Chem. 272, 23606-23615

Nagamoto-Combs K., Piech K.M., Bets J.A., Sun B., Tank A.W., 1997. Tyrosine hydroxylase gene promoter activity is regulated by cAMP-responsive element and AP1 sites following calcium influx. J. Biol. Chem. 272, 6051- 6058

Nakajima T., Uchida C., Anderson S.F., Parvin J.D., Montminy M., 1997a. Analysis of a cAMP-responsive activator reveals a two-component mechanism for transcriptional induction via signal-dependent factors. Gen. Develop. 11, 738-747

Nakajima T., Uchida C., Anderson S.F., Lee C.G., Hurwitz J., Parvin J.D., Montminy M., 1997b. DNA helicase A mediates association of CBP with RNA polymerase II. Cell 90, 1107-1112

Newlon M.G., Roy M., Hausken Z.E., Scott J.D., Jennings P.A., 1997. The A-kinase anchoring domain of type IIα cAMP-dependent protein kinase is highly helical. J. Biol. Chem. 272, 23637-23644

Nigg E.A., Schafer G., Hilz H., Eppenberger H.M., 1985. Cyclic AMP-dependent protein kinase type II is associated with Golgi complex and with centrosomes. Cell 41, 1039-1051

Ostrowska A., 1987. The role of protein kinases in transmission of intracellular signals taking into account particularly nervous system (in Polish). Acta Physiol. pol. 38, Suppl. 29, 1-16

Ostrowska A., 1999. Various effects of protein kinase Action (in Polish). Post. Biochem. 45, 202-210

Oyen O., Eskild W., Beebe S.J., Hansson V., Jahnsen T., 1988. Biphasic response to 3',5'-cyclic adenosine monophosphate (cAMP) at the messenger ribonucleic acid level for a regulatory subunit of cAMP-dependent protein kinase. Mol. Endocrinol. 2, 1070-1076

Parker D., Ferreri K., Nakajima T., Lamorte V.J., Evans R., Koerber S.C., Hoeger C., Montminy M.R., 1996. Phosphorylation of CREB at Ser-133 induces complex formation with CREB-binding protein via a direct mechanism. Mol. Cell. Biol. 16, 694-703

Podesta E.J., Solano A.R., Lemos J.R., 1991. Stimulation of an individual cell with peptide hormone in a prescribed region of its plasma membrane results in a compertmentalized cyclic AMP-dependent protein kinase response. J. Mol. Endocrinol. 6, 269-279

Puttagunta A.L., Chik C.L., Girard M., O'Brien L., Ho A.K., 1992. Intracellular pH and growth hormone-releasing factor stimulated adenosine 3',5'-monophosphate, intracellular calcium and growth hormone release from rat anterior pituitary cells. J. Endocrinol. 135, 343-352

Ramirez S., Ali S.A.S., Robin P., Trouche D., Harel-Bellan A., 1997. The CREB-binding protein (CBP) cooperates with the serum response factor for transactivation of the c-fos serum response element. J. Biol. Chem. 272, 31016-31021

Razik M.A., Lee K., Price R.R., Williams M.R., Ongjoco R.R., Dole M.K., Rudner X.L., Kwatra M.M., Schwinn D.A., 1997. Transcriptional regulation of the human α_{1a}-adrenenergic receptor gene. J. Biol. Chem. 272, 28237-28246

Roesler W.J., Vandenbark G.R., Hanson R.W., 1988. Cyclic AMP and the induction of eukaryotic gene transcription. J. Biol. Chem. 263, 9063-9066

Salamino F., De Tulio R., Michetti M., Mengotti P., Melloni E., Pontremoli S., 1994. Modulation of calpastatin specifity in rat tissues by reversible phosphorylation and dephosphorylation. Biochem. Biophys. Res. Commun. 199, 1326-1332

Scott J.D., Glaccum M.B., Zoller M.J., Uhler M.D., Helfman D.M., McKnight G.S., Krebs E.G., 1987. The molecular cloning of a type II regulatory subunit of the cAMP-dependent protein kinase from rat sceletal muscle and mouse brain . Proc. Natl. Acad. Sci USA 84, 5192-5196

Scott J.D., Stofko R.E., McDonald J.R., Comer J.D., Vitalis E.A., Mangili J.A., 1990. Type II regulatory subunit dimerization determines the subcellular localiztion of the cAMP-dependent protein kinase. J. Biol. Chem. 265, 21561-21566

Shabb J.B., Corbin J.D., 1992. Cyclic nucleotide-binding domains in proteins having diverse functions. J. Biol. Chem. 267, 5723-5726

Singh I.S., Luo Z., Eng A., Erlichman J., 1991. 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, 221-226

Sladek C.D., Gallagher M.J., 1993. The stimulation of vasopressin gene expression in cultured hypothalamic neurons by cyclic adenosine 3',5'-monophosphate is reversible. Endocrinology 133, 1320-1330

Spiro C., McMurray C.T., 1997. Switching of DNA secondary structure in proenkephalin transcriptional regulation. J. Biol. Chem. 272, 33145-33152

Srivastava R.K., Lee N.Y., Noguchi K., Park Y.G., Ellis M.J.C., Jeong J-S., Kim S.N., Cho-Chung Y.S., 1998. The RIIβ regulatory subunit of protein kinase A binds to cAMP response element: An alternative cAMP signalling pathway. Proc. Natl. Acad. Sci. USA 95, 6687-6692

Swanson D.J., Zellmer E., Lewis E.J., 1997. The homeodomain protein Arix interacts synergistically with cyclic AMP to regulate expression of neurotransmitter biosynthetic genes. J. Biol. Chem. 272, 27382-27392

Takio K., Smith S.B., Krebs E.G., Walsh K.A., Titani K., 1982. Primary structure of the regulatory subunit of type II cAMP-dependent protein kinase from bovine cardiac muscle. Proc. Natl. Acad. Sci. USA 79, 2544-2548

Taussing R., Gilman A.G., 1995. Mammalian membrane-bound adenylyl cyclases. J. Biol. Chem. 270, 1-4

Taylor S.S., Buechler J.A., Yonemoto W., 1990. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. Annu. Rev. Biochem. 59, 971-1005

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

Thomas R.F., Holt B.D., Schwinn D.A., Liggett S.B., 1992. Long-term agonist exposure induces upregulation of β_3-adrenergic receptor expression via multiple cAMP response elements. Proc. Natl. Acad. Sci. USA 89, 4490-4494

Titani K., Sasagawa T., Ericsson L.H., Kumar S., Smith S.B., Krebs E.G., Walsh K.A., 1984. Amino acid sequence of the regulatory subunit of bovine type I adenosine cyclic 3',5'-phosphate dependent protein kinase. Biochemistry 23, 4193-4199

Uhler M.D., Carmichael. D.F., Lee D.C., Chrivia J.C., Krebs E.G., McKnight G.S., 1986a. Isolation of cDNA clones coding for the catalytic subunit of mouse cAMP-dependent protein kinase. Proc. Natl. Acad Sci USA 83, 1300-1304

Uhler M.D., Chrivia J.C., McKnight G.S., 1986 b. Evidence for a second isoform of the catalytic subunit of cAMP-dependent protein kinase. J. Biol. Chem. 261, 15360-15363

Uhler M.D., McKnight G.S., 1987. Expression of cDNAs for two isoforms of the catalytic subunit of cAMP-dependent protein kinase. J. Biol. Chem. 262, 15202-15207

Vallee R., 1980. Structure and phosphorylation of microtubule-associated protein 2 (MAP2). Proc. Natl. Acad. Sci. USA 77, 3206-3210

Wang D., Shin TH., Kudlow J.E., 1997. Transcription factor AP-2 controls transcription of the human transforming growth factor - α gene. J. Biol. Chem. 272, 14244 - 14250

York R.D., Yao H., Dillion T., Ellig C.L., Eckert S.P., McCleskey E.M., Stork P.J.S., 1998. Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. Nature (London) 392, 622-626

Zick S.K., Taylor S.S., 1982. Interchain disulfide bonding in the regulatory subunit of cAMP-dependent protein kinase. J. Biol. Chem. 257, 2287-2293

STRESZCZENIE

Kinaza białkowa A. Struktura i aktywność

W artykule przedstawiono najnowsze wyniki badań dotyczące roli kinazy białkowej A (kinazy białkowej zależnej od cAMP) w ekspresji genów i ogólne informacje dotyczące struktury, syntezy i aktywności kinazy białkowej A. Kinaza białkowa A jest tetramerycznym białkiem, złożonym z dwu podjednostek regulacyjnych i dwu podjednostek katalitycznych. Aktywowanie kinazy białkowej A następuje przez związanie cAMP z podjednostką regulacyjną i uwolnienie aktywnej podjednostki katalitycznej. Cykliczny AMP jest syntetyzowany przez cyklazę adenylanową, aktywowaną przez stymulację dochodzącą z poza komórki. Znane są dwa typy kinazy białkowej A, typ I i typ II, różniące się podjednostkami regulacyjnymi R I i R II, z których każda występuje w dwu izoformach, R I α i R I βb oraz R II α i R II β. Podjednostka katalityczna wystepuje w trzech wariantach, C α, C β i C γ. Istnieją trzy izoformy podjednostki C β: C β1, C β2 i C β3. Podjednostki R I α, R II α i C α występują we wszystkich tkankach, natomiast podjednostki R I β, R II β, C β i C γ są syntetyzowane tylko w tkankach endokrynnej, neuroendokrynej, nerwowej i krwiotwórczej. Aktywność kinazy białkowej A zależy od rozmieszczenia enzymu w komórce, co określa dostęp do cAMP i substratu, a także od proporcji podjednostek regulacyjnych i katalitycznych. Kinaza białkowa A II jest związana ze strukturami komórkowymi przez podjednostkę regulacyjną R II, która wiąże się z białkami zakotwiczającymi (AKAP) lub z białkami powiązanymi z mikrotubulami (MAP-2). Kinaza białkowa A I jest rozpuszczona w cytosolu komórkowym Geny podjednostek kinazy białkowej A posiadają w części promotorowej sekwencje aktywowane przez cAMP, miejsca wiążące białko Sp 1, są pozbawione sekwencji TATA i sekwencji CAAT i posiadają wiele miejsc początku transkrypcji. Kinaza białkowa A wpływa na ekspresję białek poprzez regulowanie aktywności i syntezy czynników transkrypcji, cykliny A, inhibitorów kinaz białkowych zależnych od cyklin, fosforylacje białek związanych z mikrotubulami. Kinaza białkowa A ma wpływ na podstawowy metabolizm komórkowy poprzez fosforylacje innych kinaz białkowych, fosforylaz, fosfataz białkowych, inhibitorów proteaz i regulowanie ich syntezy. Kinaza białkowa A wpływa na syntezę i sekrecję hormonów. Rozmieszszenie kinazy białkowej A w różnych przedziałach komórkowych, określające dostęp enzymu do cAMP i substratu, obecność różnych form izoenzymaty cznych o różnej aktywności i specyficzności substratowej decyduje o różnorodności efektów kinazy białkowej A w odpowiedzi na hormonalne stymulacje.