Eukaryotic Gene Expression: Basics & Benefits

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Lecture 16

Regulation of gene expression by cyclicAMP

In the previous lecture……

Introduction to signal transduction

Lipophilic molecules transduce signals through intracellular receptors

Water soluble molecules transduce signals through membrane receptors

GPCRs, activation of adenylate cyclase and synthesis of cAMP involving trimeric G proteins

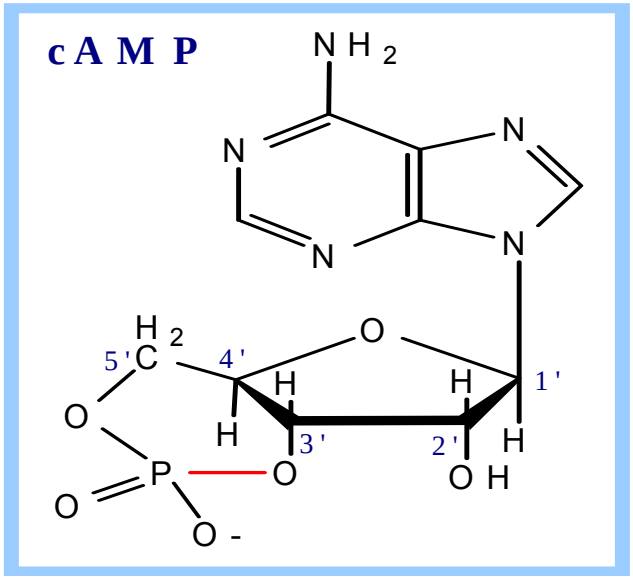
In this lecture……

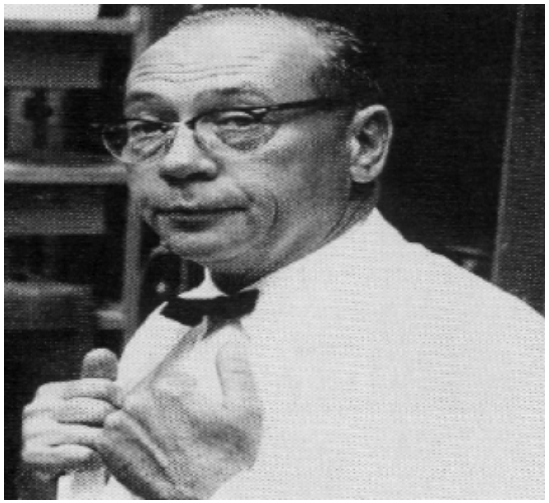
Regulation of gene expression by cAMP and Protein kinase A

cAMP (Cyclic Adenosine 3',5'-monophosphate) is the first second messenger to have been identified and it has a fundamental role in the cellular response to many extracellular stimuli.

The cAMP signaling pathway controls a diverse range of cellular processes.

cAMP not only provided the paradigm for the second messenger concept, but also provided the paradigm for signaling compartmentalization.





Earl Wilbur Sutherland, Jr., discovered cAMP for which he was awarded the Nobel Prize in Physiology or Medicine in the year 1971.

Sutherland observed that epinephrine would stimulate the liver to convert glycogen to glucose in liver cells. However, epinephrine by itself would not convert glycogen to glucose.

He discovered that epinephrine had to trigger the synthesis of a second messenger, cyclic AMP, for the liver to convert glycogen to glucose.

Formation of a Cyclic Adenine Ribonucleotide by Tissue Particles (Rall, T. W., and Sutherland, E. W. (1958) *J. Biol. Chem.* 232, 1065–1076)

Fractionation and Characterization of a Cyclic Adenine Ribonucleotide Formed by Tissue Particles (Sutherland, E. W., and Rall, T. W. (1958) *J. Biol. Chem.* 232, 1077–1092)

JBC Centennial
1905–2005
100 Years of Biochemistry and Molecular Biology

Earl W. Sutherland's Discovery of Cyclic Adenine Monophosphate and the Second Messenger System

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 42, Issue of October 21, p. e39, 2005

cyclicAMP is an important second messenger in biological systems.

Other water soluble molecules such as cGMP, Inositol triphosphate (IP_3), and Ca^{2+} also act as second messengers. All these are localized in the cytoplasm.

Certain hydrophobic (water-insoluble) molecules such as diacylglycerol, and phosphatidylinositols, which are membrane-associated also act as second messengers.

They diffuse from the plasma membrane into the intermembrane space where they can interact with membrane-associated effector proteins.

Gaseous molecules such as nitric oxide (NO) and carbon monoxide (CO), which can diffuse both through cytosol and across cellular membranes also act as second messengers and regulate important biological processes.

Hormones that transduce signals through cAMP

Epinephrine
Norepinephrine
Glucagon
ACTH
Vasopressin
Luteinizing hormone (LH)
Thyroid stimulating hormone (TSH)

Terminologies used in cAMP signalling pathways

Receptor	Membrane protein with extracellular and intracellular domains that interacts with the hydrophobic hormone/ligand and the transducer
Transducer	G protein
Primary effector	Adenylyl cyclase
Secondary messenger	cAMP (cyclic adenosine monophosphate)
Secondary effector	protein kinase A

In eukaryotic cells, cAMP is produced by a number of signalling pathways involving:

GPCRs (G-Protein Coupled Receptors),
Alpha and Beta-ADRs (Adrenergic Receptors),
Growth Factor receptors,
CRHR (Corticotropin Releasing Hormone Receptor),
GcgR (Glucagon Receptor),
DCC (Deleted in Colorectal Carcinoma)

Neurotransmitters, hormones, inflammatory stimuli, stress, epinephrine, norepinephrine, etc., activate the G-proteins through receptors such as GPCRs (G-Protein Coupled Receptors) and Alpha and Beta-ADRs (Adrenergic Receptors).

Ligands such as Glucagon, Urocortins and Netrin-1, either directly regulate activity of ACs

or

via G-protein activation through their respective receptors like GcgR, Glucagon Receptor, Deleted in Colorectal Carcinoma and Corticotropin Releasing Hormone Receptor

Adenylate cyclase are of two types:

Soluble and trans-membrane adenylate cyclases

In mammals, cAMP is synthesized from ATP by members of the Class-III AC (Adenylyl Cyclase) / ADCY (Adenylate Cyclase) family.

In humans, this family comprises of :

nine trans-membrane AC enzymes or tmACs
and
one soluble AC or sAC

tmACs are regulated by heterotrimeric G-proteins in response to the stimulation of various types of GPCRs and therefore play a key role in the cellular response to extracellular signals.

sAC, which predominantly occurs in mature spermatozoa, is insensitive to G-proteins. It is directly activated by Ca^{2+} and HCO^{3-}, rendering the enzyme an intracellular metabolic sensor.

Together, tmACs and sAC regulate a diverse set of essential biological processes, such as differentiation and gene transcription, and this makes cAMP signaling, an important mediator of intra- and extracellular signals in eukaryotes as well as prokaryotes.

The main targets of cAMP are:

PKA, ←
the GTP-exchange protein,
EPACs (Exchange Protein Activated by cAMP) and
the CNG (Cyclic-Nucleotide Gated Ion Channel).

PKA is the major 'read-out' for cAMP and is the predominant cellular effector of cAMP.

PKA is tethered to specific cellular locations by a class of proteins called AKAPs (A-Kinase Anchor Proteins).

Targeting of PKA isozymes by AKAPs is important for a number of physiological processes such as cAMP regulation of ion channels in the nervous system, regulation of the cell cycle, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, steroidogenesis, reproductive function, immune responses and numerous intracellular transport mechanisms.

Protein Kinase A (cAMP-Dependent Protein Kinase) transfers P_i from ATP to OH of a Ser or Thr in a particular amino acid sequence (RRPS).

Protein Kinase A in the resting state is a complex of:

- 2 catalytic subunits (**C**)
- 2 regulatory subunits (**R**).

$\mathbf{R_2C_2}$

R_2C_2

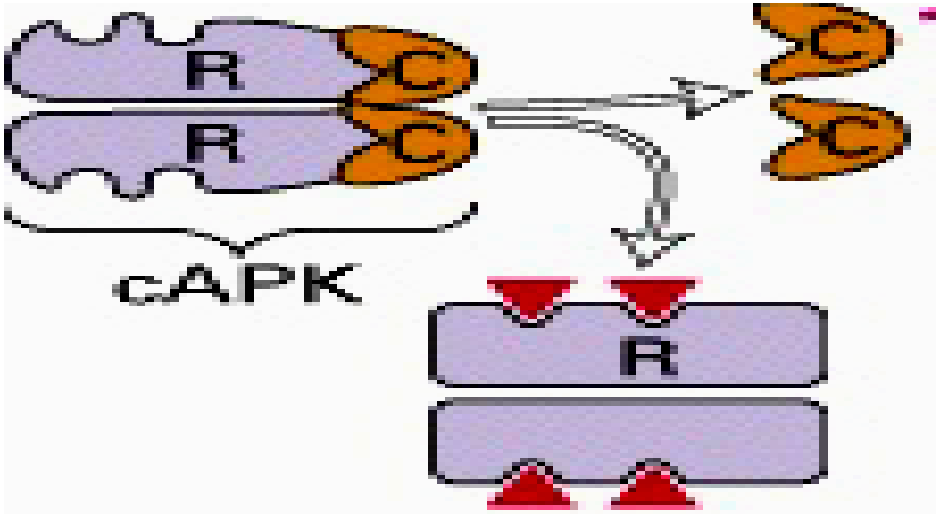
Each regulatory subunit (**R**) of Protein Kinase A contains a **pseudosubstrate** sequence, like the substrate domain of a target protein but with Ala substituting for the Ser/Thr.

The pseudosubstrate domain of (**R**), which **lacks** a **hydroxyl** that can be phosphorylated, binds to the active site of (**C**), blocking its activity.

$$R_2C_2 + 4\ cAMP \rightarrow R_2cAMP_4 + 2\ C$$

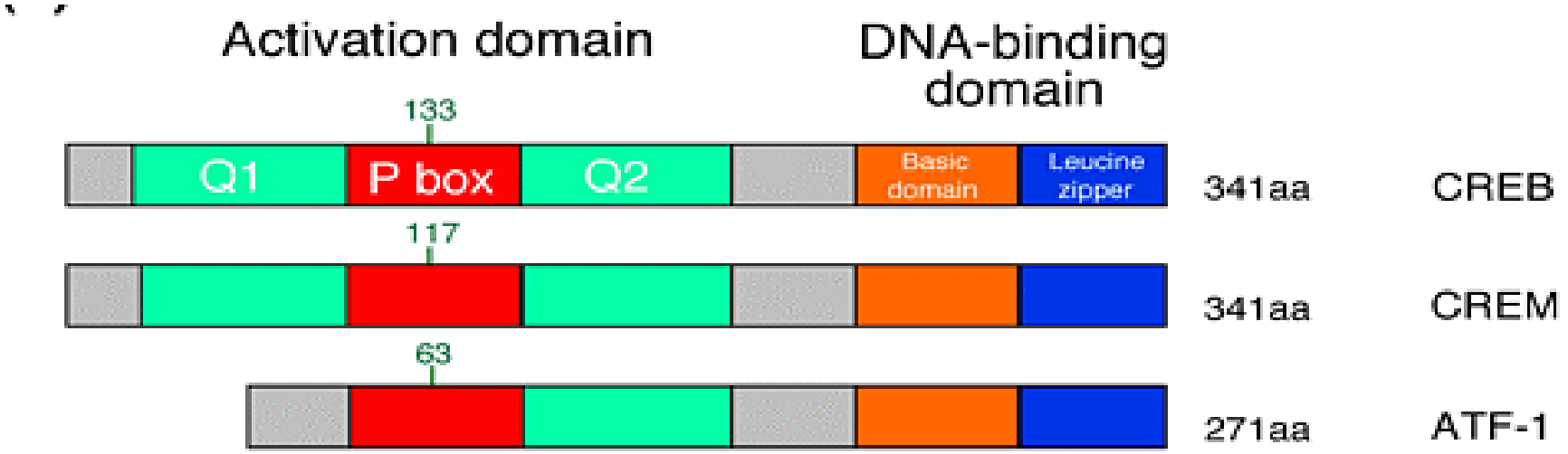
When each (**R**) binds 2 cAMP, a conformational change causes (**R**) to release (**C**).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.



Following activation by cAMP, the catalytic subunit of PKA enters the nucleus and regulates the function of atleast three transcription factors belonging to the ATF-CREB family:

CREB (cAMP Response Element-Binding Protein),
CREM (cAMP Response Element Modulator) and
ATF1 (Activating Transcription Factor-1)

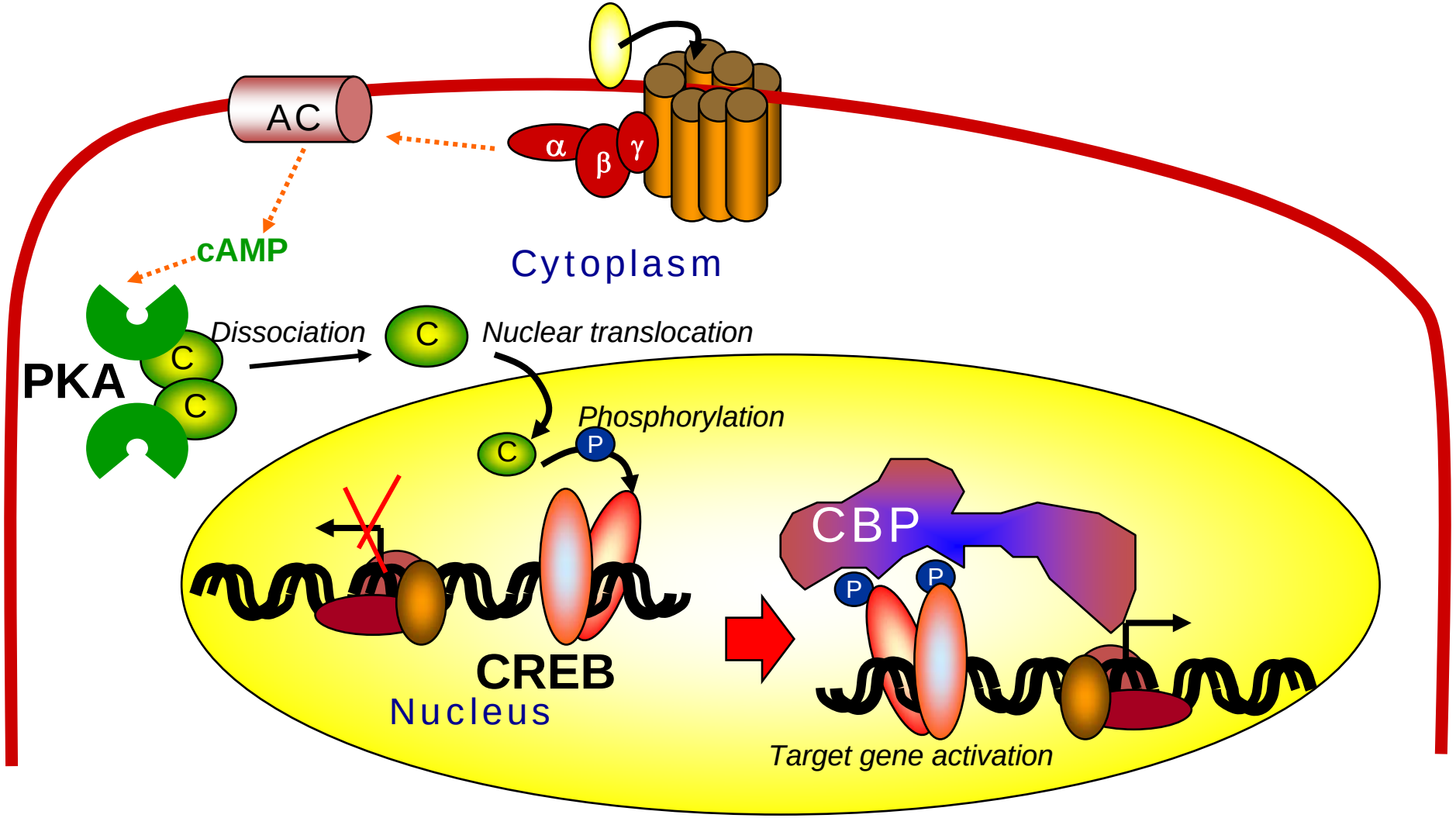

Activation domain
DNA-binding domain
133
Q1
P box
Q2
Basic domain
Leucine zipper
341aa
CREB
117
341aa
CREM
63
271aa
ATF-1

CREB

CREB specifically binds to the CRE (cAMP responsive element), TGACGTCA in the promoters of cAMP responsive genes. CREB binds DNA as a homodimer.

Signalling pathway leading to activation of CREB

Hormone or ligand binds to membrane receptor
G-protein stimulates adenylate cyclase
cAMP is synthesized
cAMP binds R-subunits of PKA
Active catalytic C-subunit liberated
PKA-c migrates to the nucleus
PKA phosphorylates the serine residue in the RRxS* sequence of CREB´s TAD
Phoshorylated CREB recruits the coactivator CBP
Genes having CREs are activated

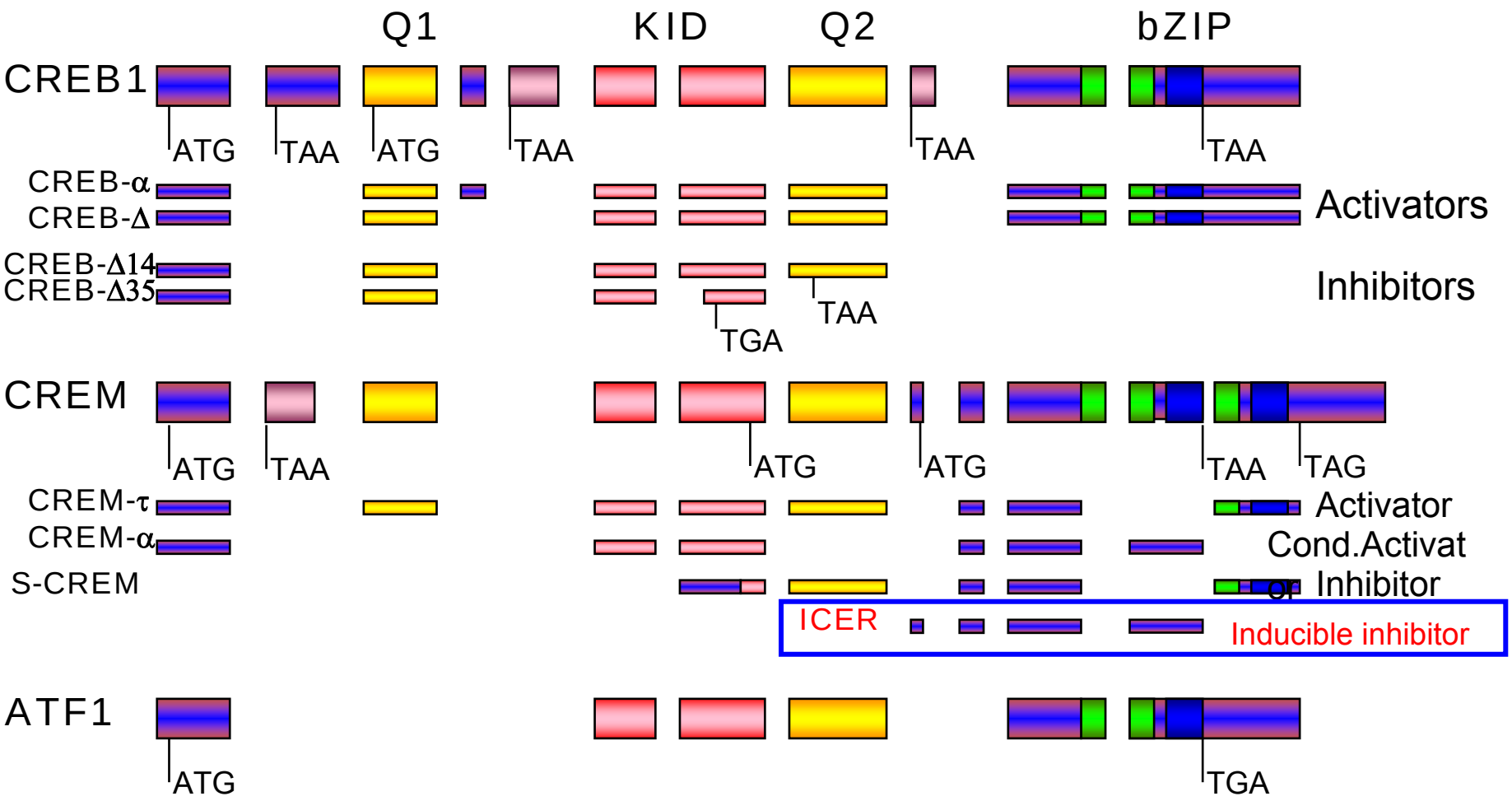

AC
α
β
γ
cAMP
Cytoplasm
PKA
C
C
Dissociation
C
Nuclear translocation
Phosphorylation
C
P
CBP
P
P
CREB
Nucleus
Target gene activation

The CREB protein has a modular structure with distinct domains exerting different functions.

The basic leucine zipper motif mediates dimerization and DNA binding, while the glutamine-rich domains Q1 and Q2, and the kinaseinducible domain (KID) constitute the transcription activation domains of CREB.

These different domains can recruit distinct proteins that can modify the transcriptional activity of CREB.

Alternative splicing produces both activators and repressors



The *CREM* gene contains two alternatives promoters termed P1 and P2.

P1 is a housekeeping promoter that directs the expression of the several transcriptional activators (CREMτ, CREMτ1, CREMτ2, CREMτα) as well as the expression of several transcriptional repressor (CREMα, -β, -γ, etc.).

Most of the CREM isoforms emanating from P1 are regulated by PKA phosphorylation.

The P2 promoter is strongly induced by cAMP by virtue of four CREs in tandem.

The induced isoform, termed ICER, essentially consists of a DNA-binding domain, functions as a powerful repressor of cAMP-mediated gene expression, and is not directly regulated by PKA phosphorylation.

ICER constitutes a negative autoregulatory control mechanism by repressing its own production.

A family of four isoforms named ICER-I, ICER-Iγ, ICER-II, and ICER-IIγ are collectively referred to as ICER (Inducible cAMP early repressor).

ICER comprises the bZIP domain of CREM, and its increased expression following cAMP elevation is driven by multiple CRE elements within an intronic promoter termed CARE.

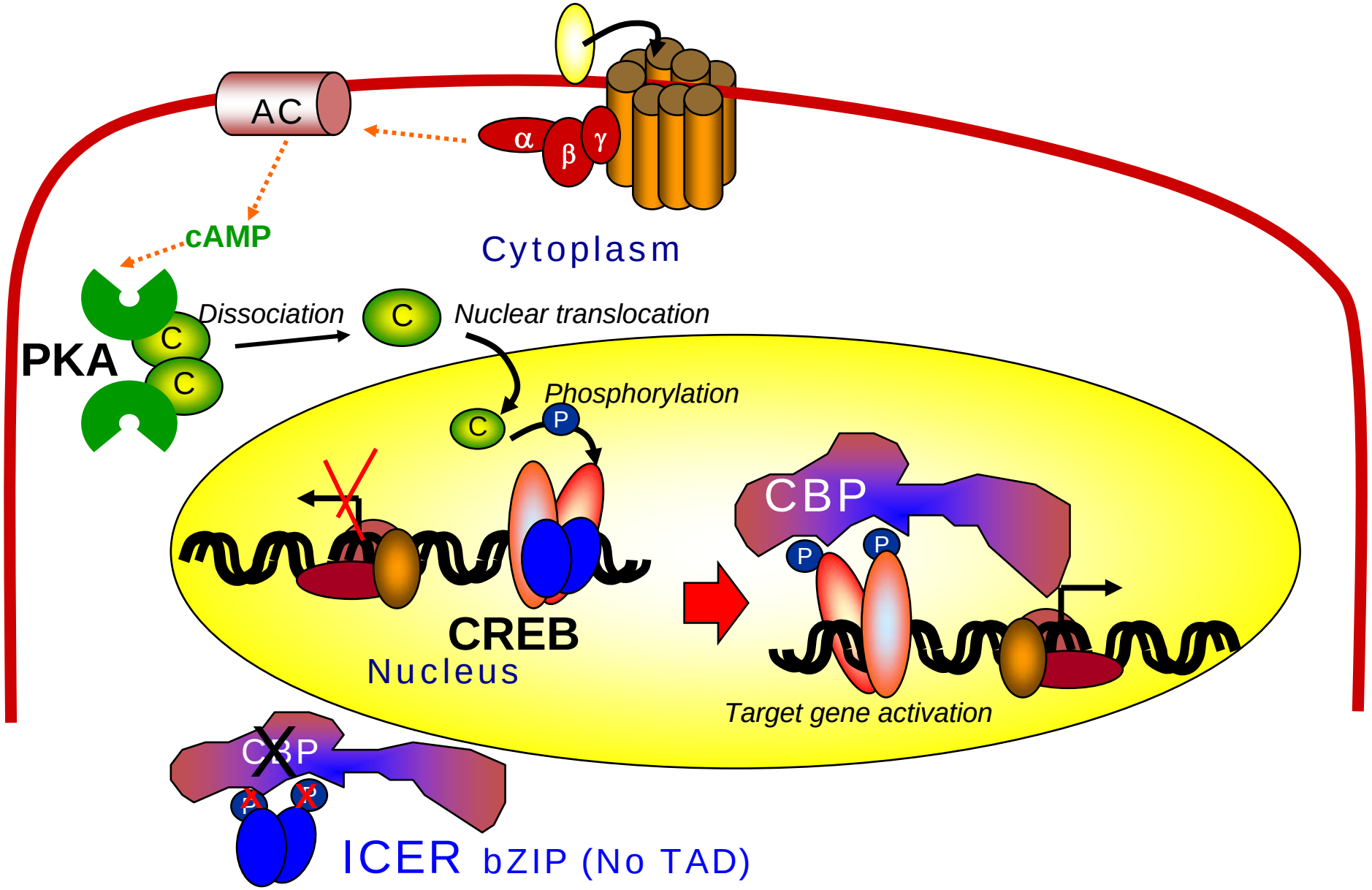
Once induced, ICER localises to the nucleus where it binds target DNA and, since it lacks an activation domain, functions as a powerful repressor of CRE-driven transcription.

However, its effects are transient since it can itself bind to CARE to repress its own transcription.

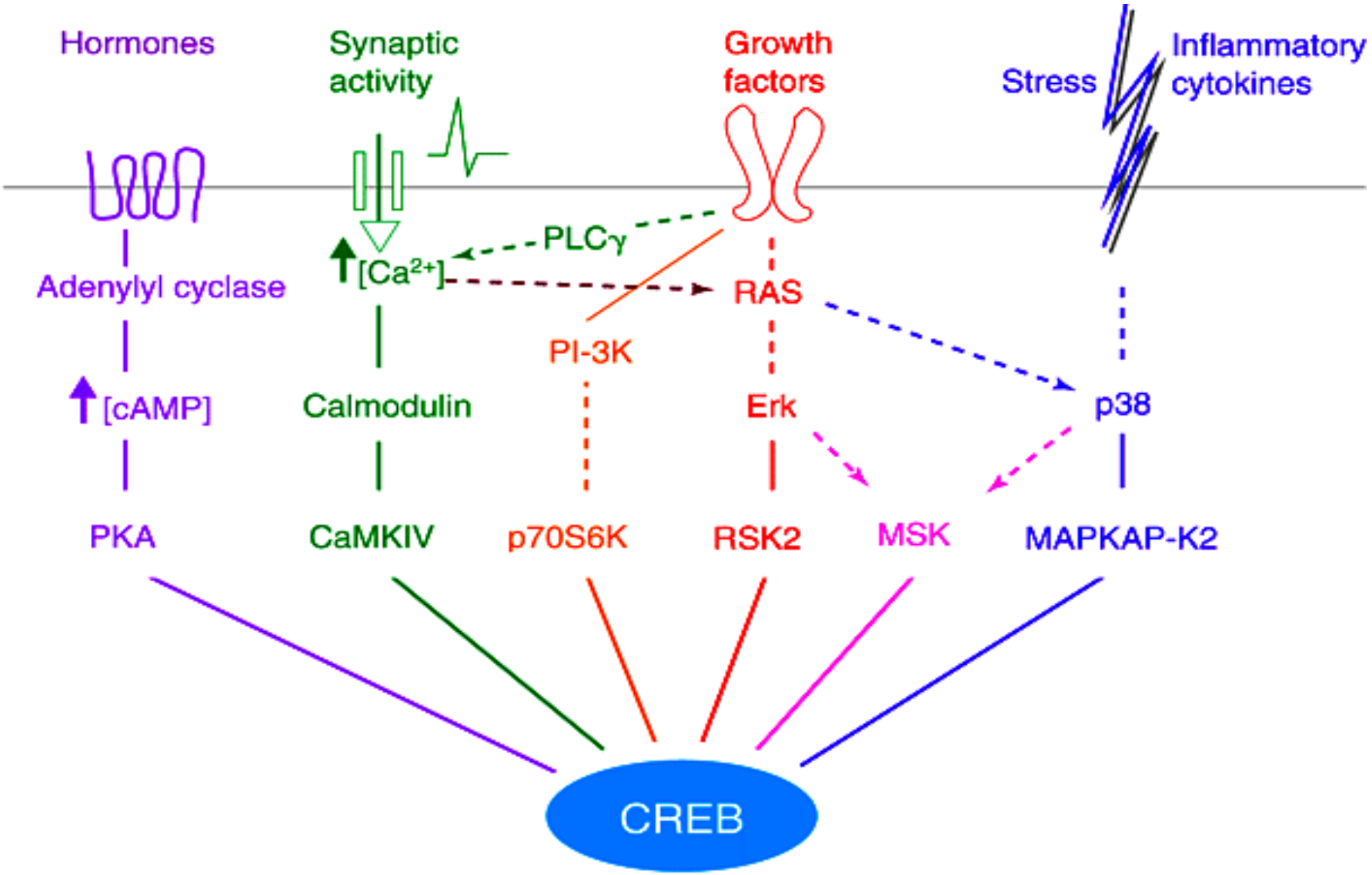
ICER plays a key role in $CD4^+$ T cells, where it attenuates chemokine and cytokine induction following antigen stimulation, andcardiac myocytes, where blockade of CRE-mediated transcription attenuates the hypertrophic response

Molina C. A., Foulkes N. S., Lalli E., Sassone-Corsi P. Inducibility, and negative autoregulation of CREM (1993). An alternative promoter directs the expression of ICER, an early response repressor. Cell, 75: 875-886.

ICER is a negative regulator of cAMP responsive genes



CREB – end-point of several signaling pathways



CREB is activated by multiple signalling pathways.

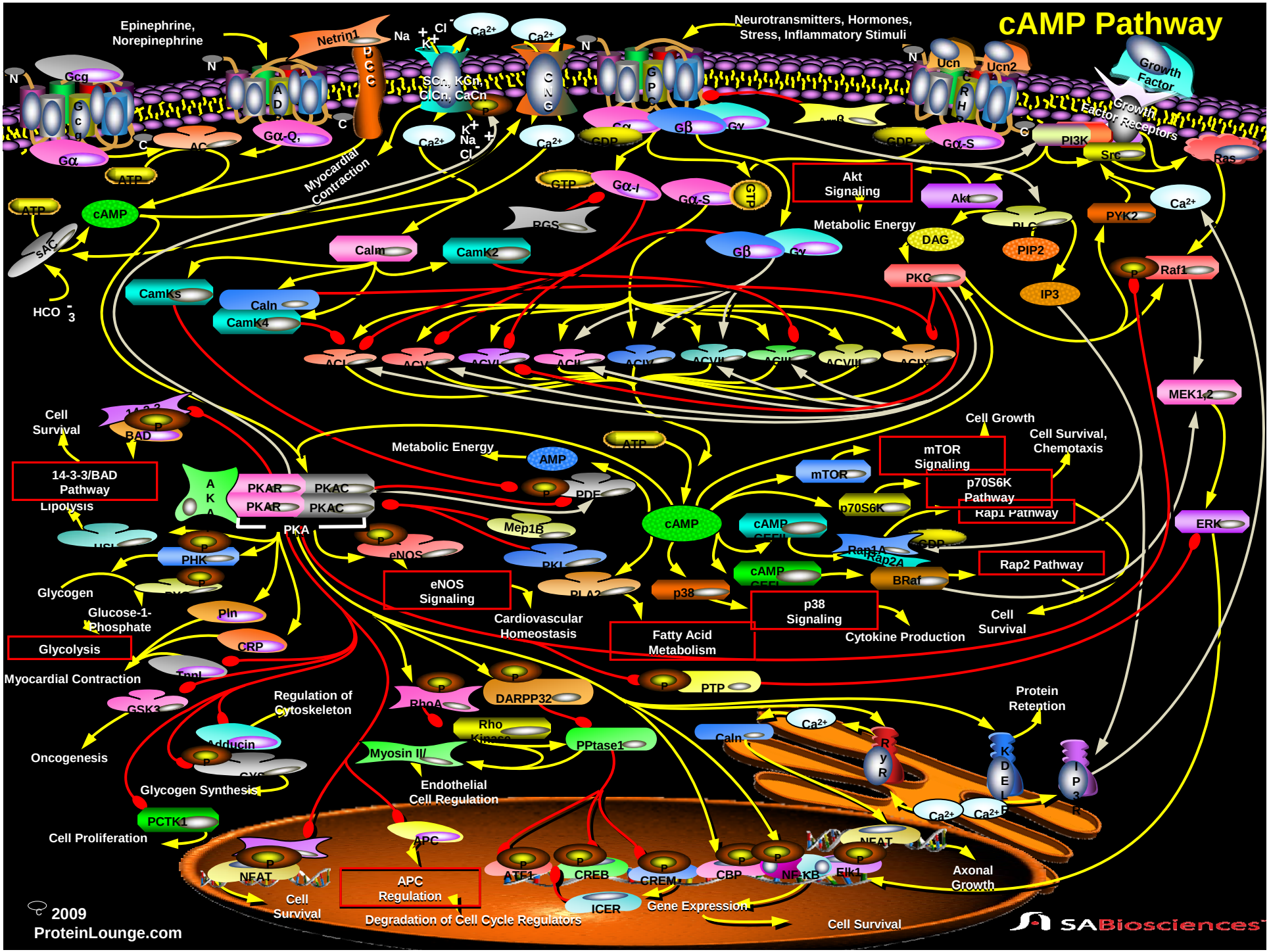
Normally, the level of intracellular cAMP is regulated by the balance between the activities of two types of enzymes; ACs and the cyclic nucleotide phosphodiesterases (PDEs) chiefly in response to hormones and neurotransmitters.

The cAMP signaling is involved in controlling exocytotic events in polarized epithelial cells with implication for Diabetes Insipidus, Hypertension, Gastric ulcers, Thyroid disease, Diabetes Mellitus, and Asthma.

Heterologous sensitization of cAMP signaling contributes to fundamental physiological processes such as the timing of circadian rhythms, sexual behavior, and neurotransmitter crosstalk, and also to neurological disorders such as substance abuse and drug-induced Dyskinesias.

Thus, cAMP has several important regulatory roles in a number of physioloigcal processes.

In this lecture, we have confined ourselves to the role of cAMP and PKA in the regulation of gene expression.


cAMP Pathway
Epinephrine, Norepinephrine
Neurotransmitters, Hormones, Stress, Inflammatory Stimuli
Netrin1
Growth Factor
Growth Factor Receptors
Gcg
Ucn
Ucn2
Gα-Q,
Gα-S
Gα-I
Gβ
Gγ
GDP
GTP
ATP
AC
cAMP
sAC
HCO3-
PI3K
Src
Ras
Akt
Akt Signaling
Metabolic Energy
PLC
DAG
PIP2
IP3
PKC
PYK2
Ca2+
Raf1
MEK1,2
ERK
Calm
CamK2
CamKs
Caln
CamK4
RGS
ACI
ACV
ACVI
ACII
ACIV
ACVII
ACIII
ACVIII
ACIX
Cell Survival
14-3-3
BAD
14-3-3/BAD Pathway
Lipolysis
HSL
PHK
Glycogen
Glucose-1-Phosphate
Pln
CRP
Glycolysis
Myocardial Contraction
TnnI
GSK3
Oncogenesis
Regulation of Cytoskeleton
Adducin
Glycogen Synthesis
PCTK1
Cell Proliferation
AKA
PKAR
PKAC
PKA
AMP
PDE
Mep1β
eNOS
PKI
PLA2
eNOS Signaling
Cardiovascular Homeostasis
Fatty Acid Metabolism
p38
p38 Signaling
Cytokine Production
Cell Survival
mTOR
mTOR Signaling
Cell Growth
Cell Survival, Chemotaxis
p70S6K
p70S6K Pathway
Rap1
Rap1 Pathway
Rap2A
Rap2 Pathway
BRaf
cAMP GEFI
cAMP GEFII
GDP
PTP
RhoA
DARPP32
Rho Kinase
PPtase1
Myosin II
Endothelial Cell Regulation
Protein Retention
RyR
IP3R
KDEL R
NFAT
APC
APC Regulation
Degradation of Cell Cycle Regulators
ATF1
CREB
CREM
CBP
NF-KB
Elk1
ICER
Gene Expression
Axonal Growth
Cell Survival
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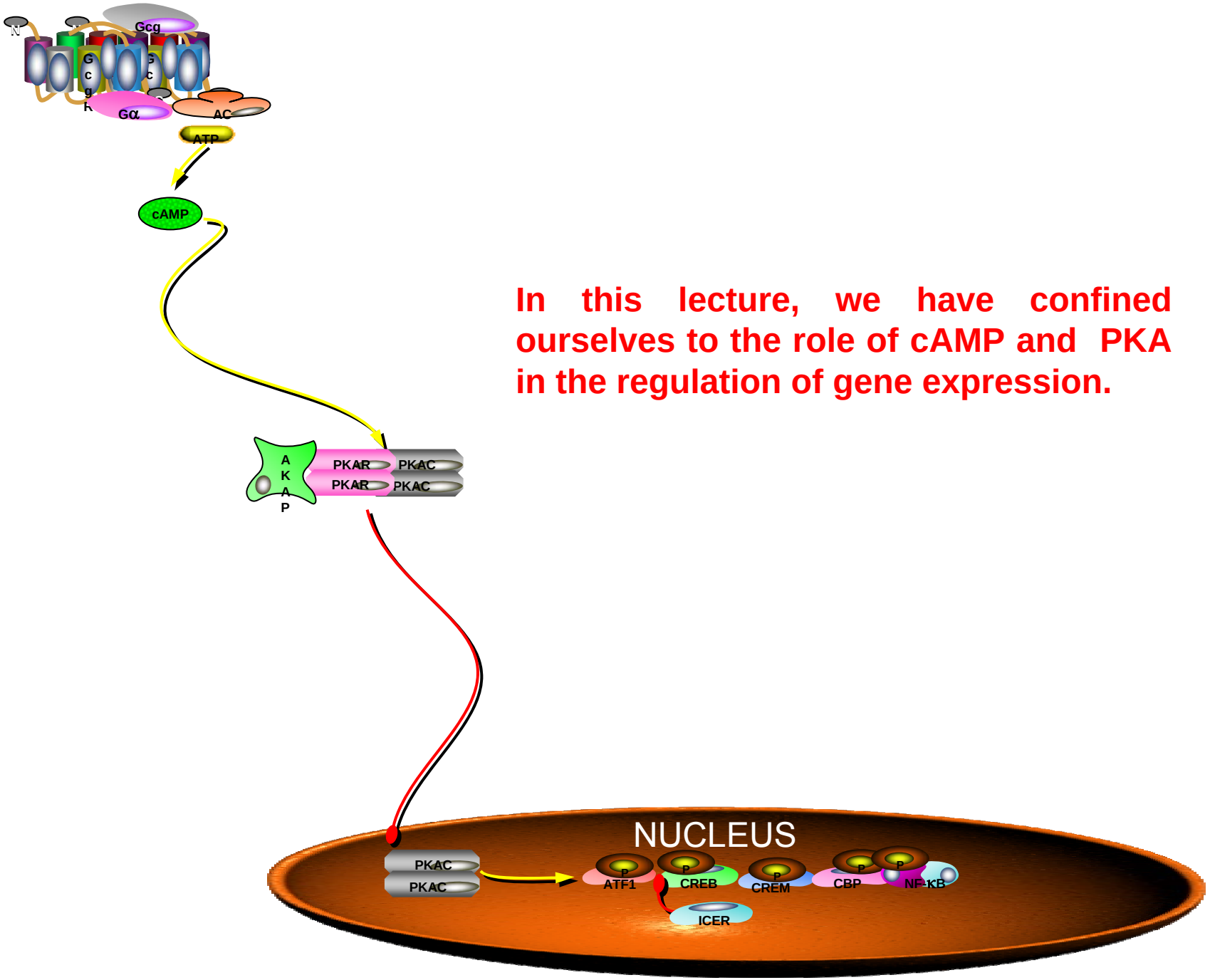


In this lecture, we have confined ourselves to the role of cAMP and PKA in the regulation of gene expression.

Although ser133 phosphorylation of CREB by PKA is a key event in cAMP signalling, it induces expression of only a small fraction (2%) of target genes, suggesting that additional CREB-regulatory partners play a key role in recruiting the transcriptional machinery.

CREB activity is also regulated by a family of latent cytoplasmic coactivators, called TORCs, which translocate to the nucleus and bind to CREB in response to extracellular stimuli.

The regulatory effects of these and other CREB family members such as ATF1 and CREM on cellular gene expression are not insignificant

Genome-wide studies put the number of putative CREB target genes at ~5000.

A major point to remember is that the CREB family consists not only activators but also repressors.

For example, the CREM gene codes for at least four different factors that block CRE-dependent transcription, the CREM α, β and γ proteins and ICER.

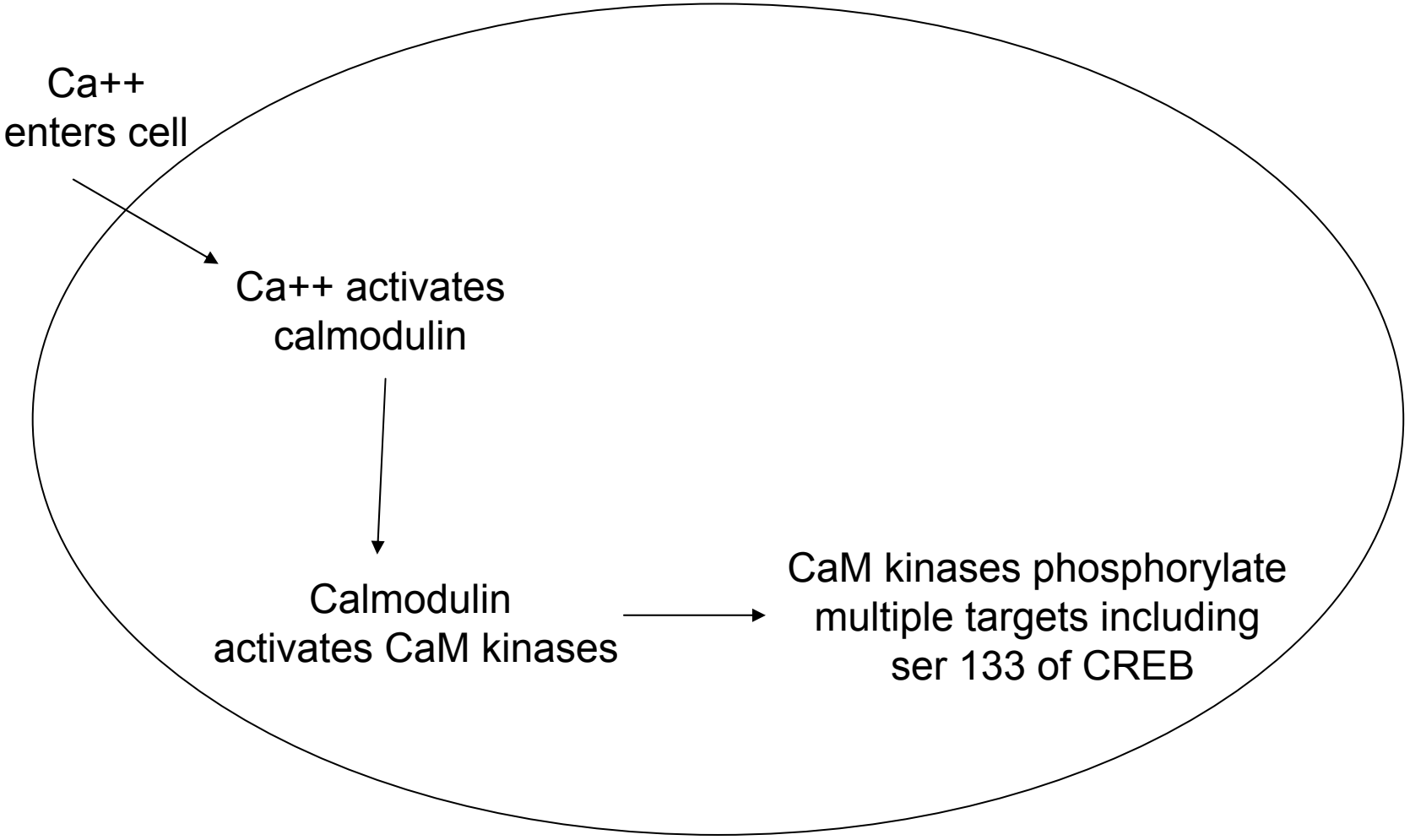
Most of these isoforms are generated by alternative splicing, another common feature of many CREB family genes and these alternatively spliced isoforms have different properties, and their expression can be developmentally regulated.

A second pathway that leads to CREB activation is mediated by increases in intracellular calcium.

Driven by the activation of synaptic *N*-methyl-D-aspartate receptors (NMDARs) and L-type Ca2+ channels, the increase in intracellular Ca2+ may lead to phosphorylation of CREB via the CaMK family of serine/ threonine kinases.

CaMKI, CaMKII and CaMKIV all phosphorylate CREB at Ser133 in vitro However, CaMKII, in addition to phosphorylating CREB at Ser133, also phosphorylates CREB at Ser142, which inactivates the transcriptional activating properties of CREB by preventing dimerization but not DNA binding.

CREB is activated not only by cAMP but also by calcium ions



A third pathway by which CREB may be activated is via a cascade of kinase activity initiated by nerve growth factor (NGF) involving the Ras pathway.

One downstream substrate of the Ras/ERK *pathway is a 90 kDa ribosomal S-6 kinase-2 (RSK-2).

Upon activation, both ERKs and RSKs translocate to the nucleus where they may phosphorylate CREB at Ser133

* ERKs - Extracellular signal-regulated protein kinases

Just as phosphorylation of Ser133 seems to be critical for activation of CREB, dephosphorylation of this residue is important for inactivation of CREB. The level of CREB phosphorylation at Ser133 reflects a balance between the oppositional actions of kinases and phosphatases, such as protein phosphatase 1 (PP-1 and PP-2).

For example, dephosphorylation of CREB at Ser133 may be initiated by the activation of calcineurin (PP-2B) by the Ca2+–CaM pathway.

Calcineurin may then activate the nuclear phosphatase PP-1 that goes on to dephosphorylate CREB

CREB AND LONG TERM MEMORY

CREB is thought to be one of the factors necessary for initiating the transcription of proteins required for Long Term Memory in a variety of species.

The first study to suggest that CREB is required for memory formation or plasticity was performed in *Aplysia* cultured neurons.

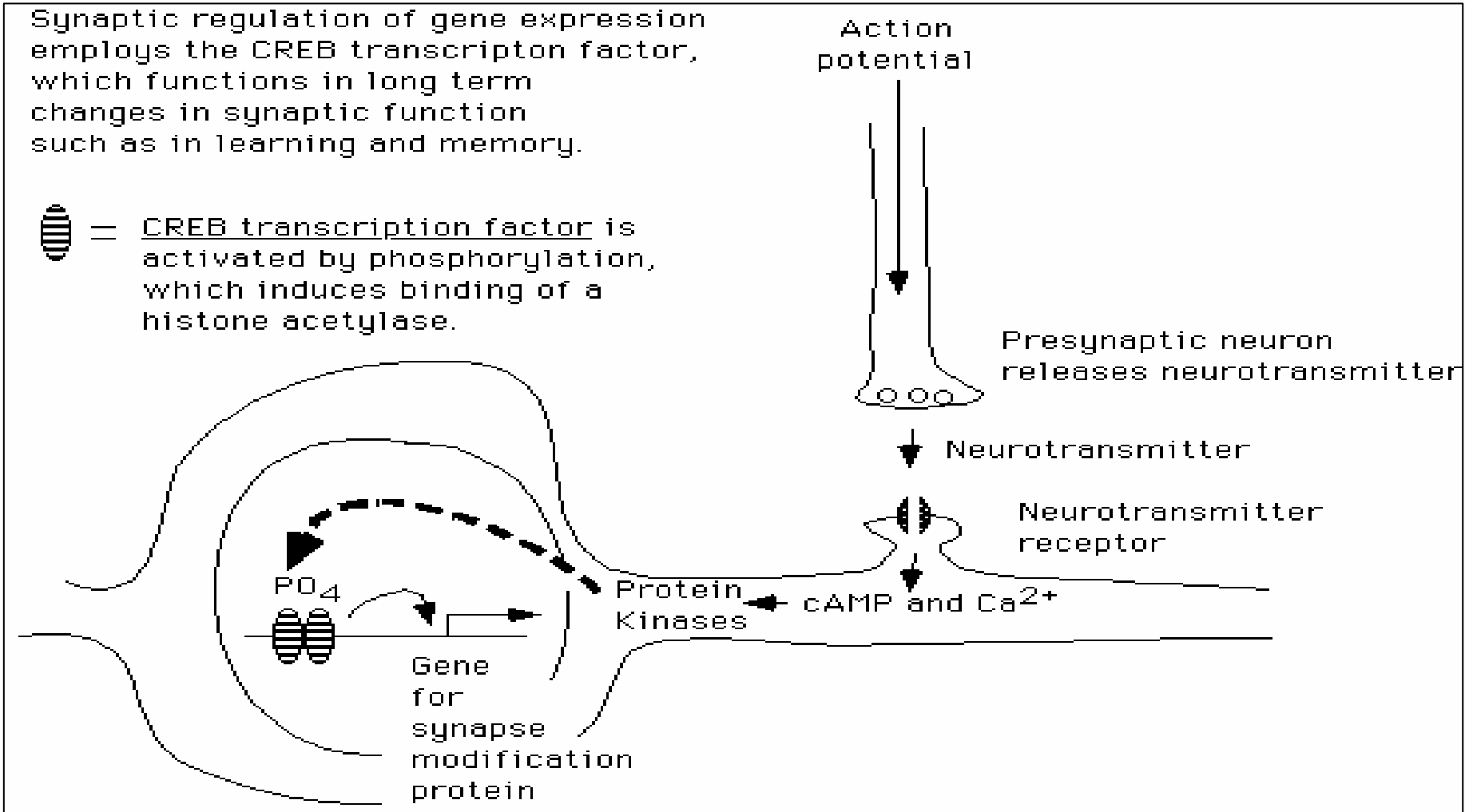
Long term facilitation (LTF) was blocked by injection of oligonucleotides with CRE sequences into cultured sensory neurons. Presumably, the CRE–oligonucleotides trap the CREB proteins needed for the transcriptional activation of genes that ultimately mediate LTF.

Moreover, injection of a reporter gene driven by a CRE-containing promoter shows that repeated pulses of serotonin that produce LTF also trigger CREB activation, while a single pulse of serotonin that does not produce LTF similarly does not trigger CREB activation.

CREB and Memory

Synaptic regulation of gene expression employs the CREB transcripton factor, which functions in long term changes in synaptic function such as in learning and memory.

= CREB transcription factor is activated by phosphorylation, which induces binding of a histone acetylase.



Receptors for neurotransmtter in the postsynaptic membrane respond to neurotransmitters by admitting Ca2+ and by increasing the level of cyclic AMP. These two second messengers activate two protein kinases: Cam Kinase II and Protein Kinase A. These kinases phosphorylate the CREB protein, activating transcription of genes that encode proteins that strengthen synaptic transmission

- Expression and function of CREB is increased by different types of antidepressant treatments, suggesting that CREB is a common post receptor target for antidepressants

Duman, R.
A Molecular and Cellular Theory of Depression:
Arch Gen Psychiat: July 1997

Molecular Biology of Addiction

Beyond Initial Cellular Targets:

CREB and its target BNDF

- Brain-derived neurotrophic factor is a member of the nerve growth factor family, which also includes the prototype nerve growth factor as well as neurotrophin-3 and neurotrophin-4.
- These growth factors are involved in the differentiation and growth of many types of neurons in the developing brain as well as the maintenance and survival of neurons in the mature brain.
- Ref: Lo DC. Neurotrophic factors and synaptic plasticity. *Neuron.* 1995;15:979-981.

Upregulation of cAMP pathway

Occurs in response to chronic administration of drugs
Resulting activation of CREB

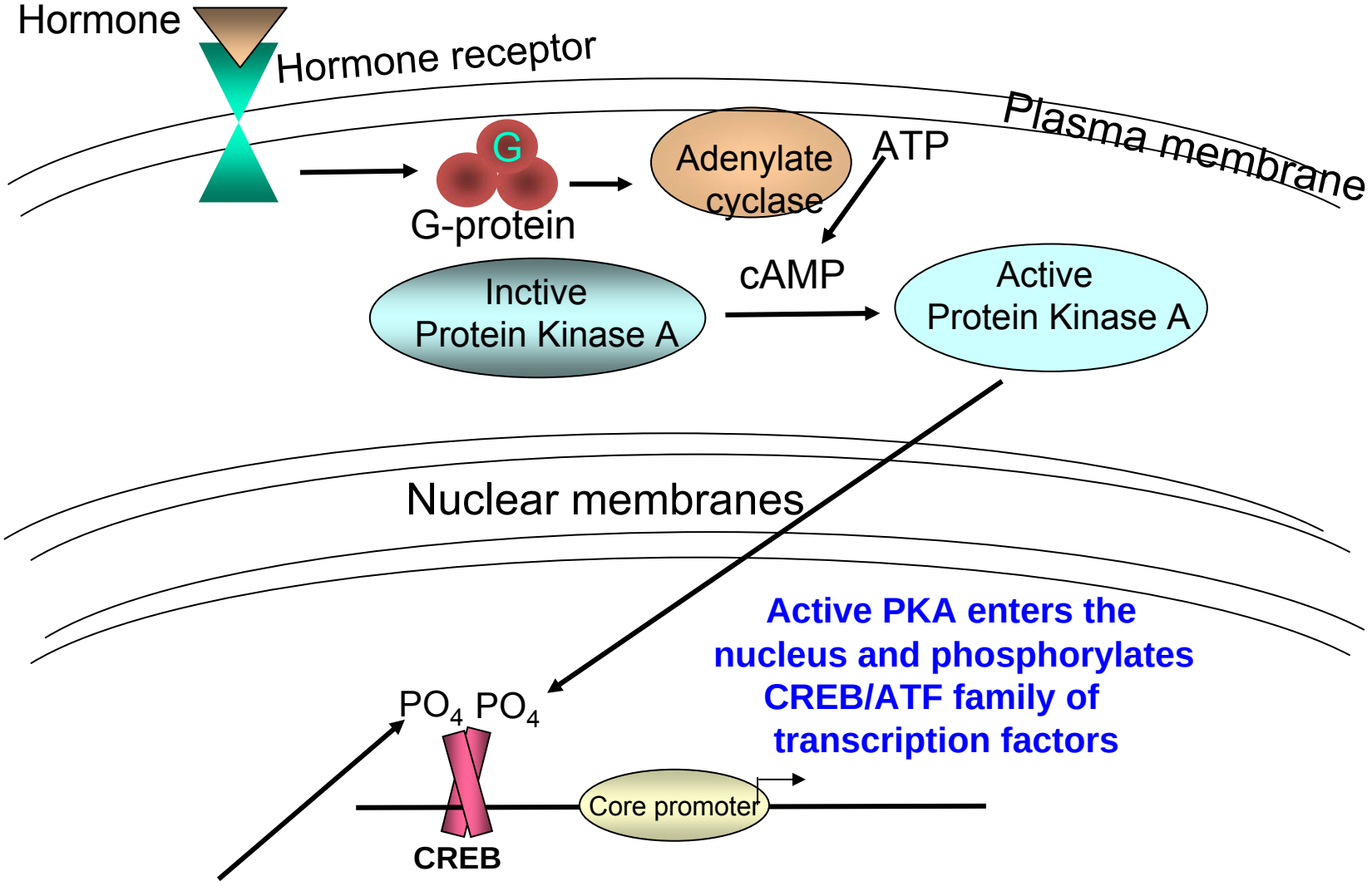
Ref: Nestler, Eric - Molecular Biology of Addiction.
Am J of Addictions 10:201-217, 2001

Regulation of gene expression by cyclicAMP

SUMMARY

POINTS TO REMEMBER

Gene regulation by cAMP and protein kinase A - SUMMARY



Phosphorylation of CREB:

- **stimulates interactions with several core promoter proteins**
- **induces binding of HAT (CBP/P300), acetylation of histones and activation of transcription**

Regulation of gene expression by cyclicAMP

Points to remember

cAMP

cAMP is generated from ATP by adenylyl cyclase

Popular second messenger of GPCRs

cAMP doesn’t function in signaling pathways initiated by receptor tyrosine kinases

cAMP is degraded by phosphodiesterases

Regulation of gene expression by cyclicAMP

Points to remember

Adenylyl cyclase (AC):

Has two catalytic domains
Catalytic domains bind ATP and break it to produce
cAMP and inorganic phosphate

Membrane-bound Acs (tmACs)

These are regulated by heterotrimeric G-proteins in response to the stimulation of various types of GPCRs and play a key role in the cellular response to extracellular signals

Soluble AC(sAC)

This is insensitive to G-proteins and is directly activated by Ca^{2+} and HCO^{3-}, rendering the enzyme an intracellular metabolic sensor.

Regulation of gene expression by cyclicAMP

Points to remember

Targets of cAMP

cAMP-dependent protein kinases (protein kinase A =PKA)

Cyclic nucleotide gated ion channels

Regulation of gene expression by cyclicAMP

Points to remember

Specificity of cAMP signaling

Effects cAMP and PKA are localized to specific regions

cAMP kinase associated proteins (AKAPs) anchor inactive PKAs to specific subcellular localizations

Regulation of gene expression by cyclicAMP

Points to remember

Cellular functions regulated by PKA

Phosphorylation of cellular enzymes and regulate their activity

Ex: lipase, glycogen phosphorylase, cholesteryl esterase - activation

glycogen synthase – inhibition

Regulation of activity of transcription factors such as CREB , CREM, ICER, ATF1

Regulation of gene expression by cyclicAMP

Points to remember

CREB links cAMP signals to transcription

Only genes that have CRE (cAMP Response Element, 5’ TGACGTCA 3’) in their promoters are activated by CREB

CREB needs to be phosphorylated at serine 133 by PKA in order to activate transcription of cAMP responsive genes

Phosphorylated CREB Interacts with a co-activator CBP/P300

CBP/P300 is a Histone Acetyl Transfferase (HAT) and thus acetylates histones, promotes the formation of preinitiation complex and stimulates transcription

Regulation of gene expression by cyclicAMP

Points to remember

CREM gene encodes many different isoforms, some of which have repressive functions.

The repressor ICER (Inducible cAMP Early Repressor), participates in the downregulation of cAMP-induced transcription by competing with the binding of CREB and CREM activators to their DNA binding sites.

Protein Phosphatase1 checks the phosphorylation events in order to inactivate the formation of repressor isoforms like ICER so that CREB, CREM and ATF1 are able to interact with the co-activators like CBP and p300. Hence, under physiological conditions,

Regulation of gene expression by cyclicAMP

Points to remember

Other transcription factors which are phosphorylated by PKA are:

NF$\kappa\beta$
Nuclear receptors
High Mobility Group (HMG) proteins
Gli3 (Gli-Kruppel Family Member-3)

Regulation of gene expression by cyclicAMP

Points to remember

intracellular levels of cAMP is regulated by the equilibrium between the activities of two types of enzymes:

Adenylate cyclases (positve regulators)

and

cyclic nucleotide Phosphodiesterases (Negative regulators)

Regulation of gene expression by cyclicAMP

Points to remember

Physiological processes regulated by cAMP signaling

Exocytotic events in polarized epithelial cells with implication for Diabetes insipidus, Hypertension, Gastric ulcers, Thyroid disease, Diabetes Mellitus, and Asthma.

Physiological processes such as the timing of circadian rhythms, sexual behavior, and neurotransmitter crosstalk, and also to neurological disorders such as substance abuse and drug-induced Dyskinesias

Points to remember

cyclicAMP and Cholera

Cholera toxin, a hexameric protein produced by*Vibrio cholerae causes* Cholera.

It increases cAMP levels in epithelial cells by causing ADP ribosylation of Gsα subunit of the G proteins leading to persistent activation of adenylate cyclase.

As a result, there is a massive flow of water from blood into intestines leading to Cholera

Suggested reading

Montminy M. (1997).Transcriptional regulation by cyclic AMP. Annu. Rev. Biochem., 66: 807-822.

Sands, WA., Palmer, TM. (2008) Regulating gene transcription in response to cyclic AMP elevation. Cellular Signalling 20: 460-466