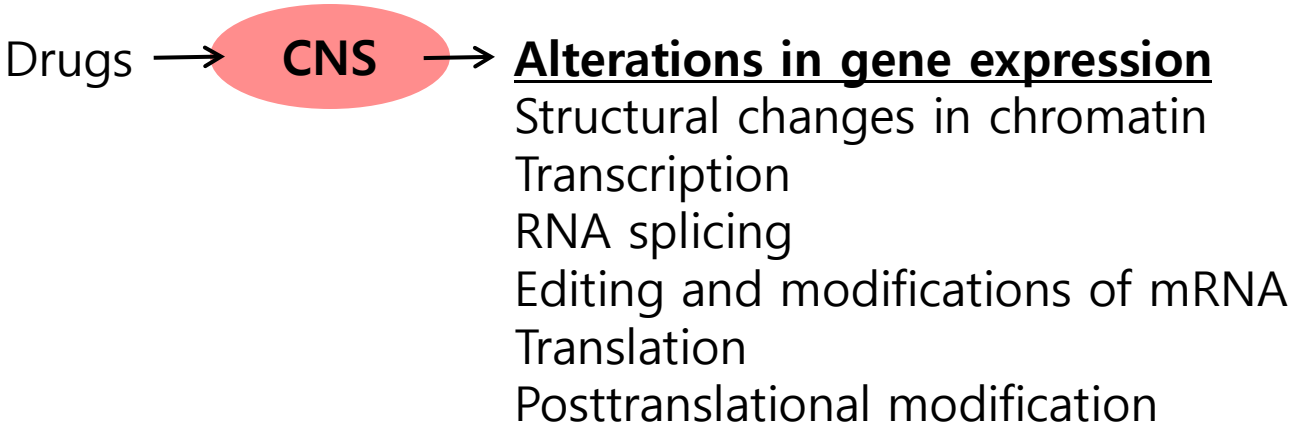
Chapter 6. Signaling to the nucleus



1. Control of gene expression

DNA replication and transcription

Regulation of gene expression by chromatin

Genes and the genome

Regulated steps of transcription

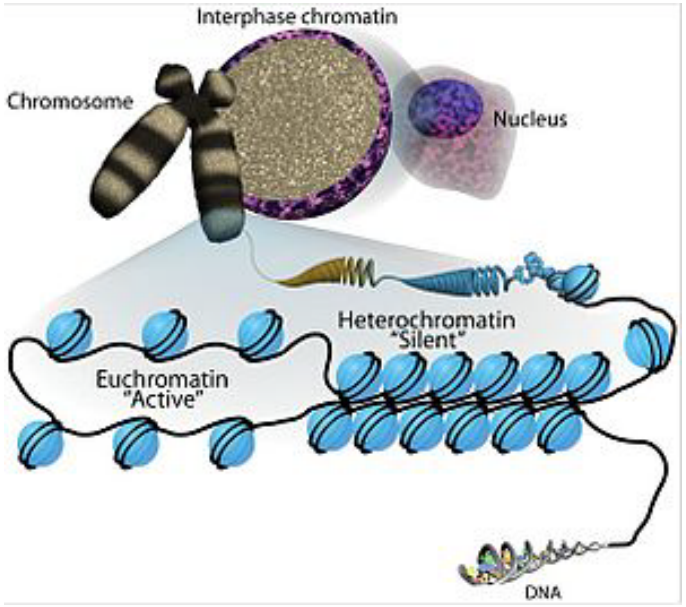
Alternative splicing of primary RNA transcripts

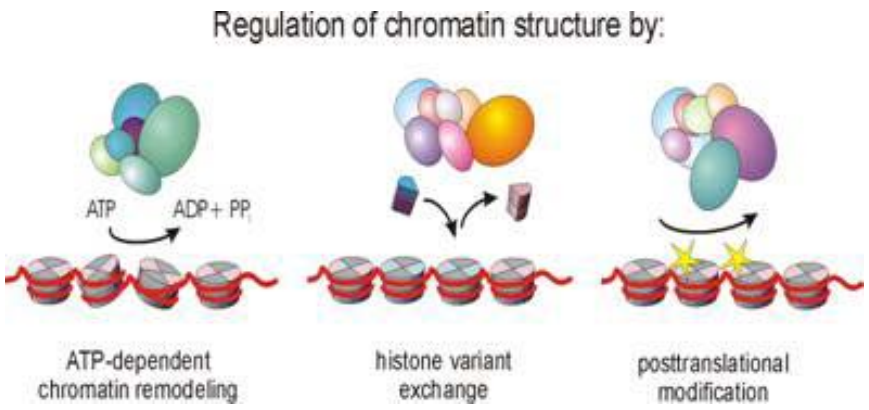
Regulation of mRNA stability and translatability

Translation of mature mRNA in the cytoplasm

Posttranslational processing of proteins

Chromatin remodeling



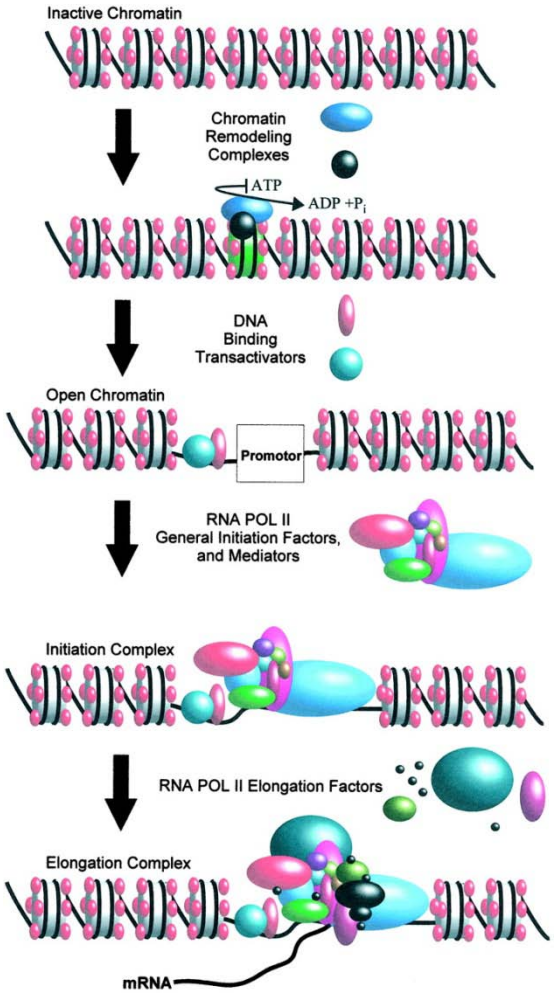


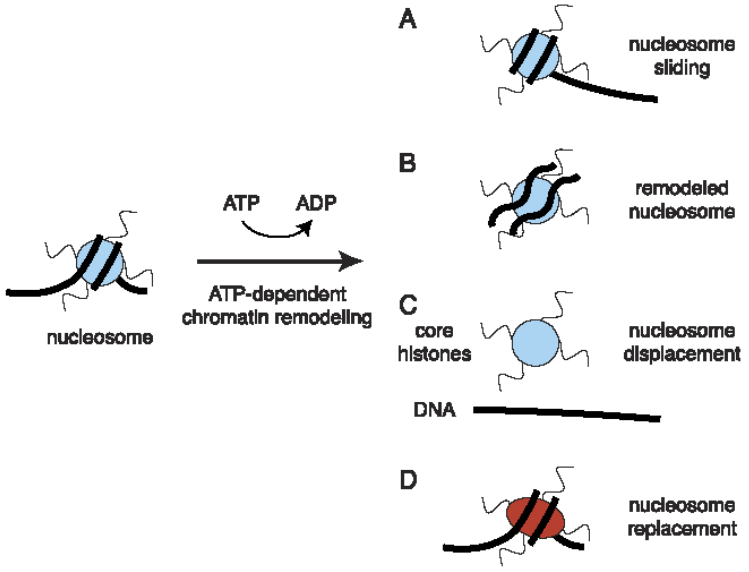
Altered histone-DNA interactions

↓

Altered transcription, DNA replication, recombination and repair

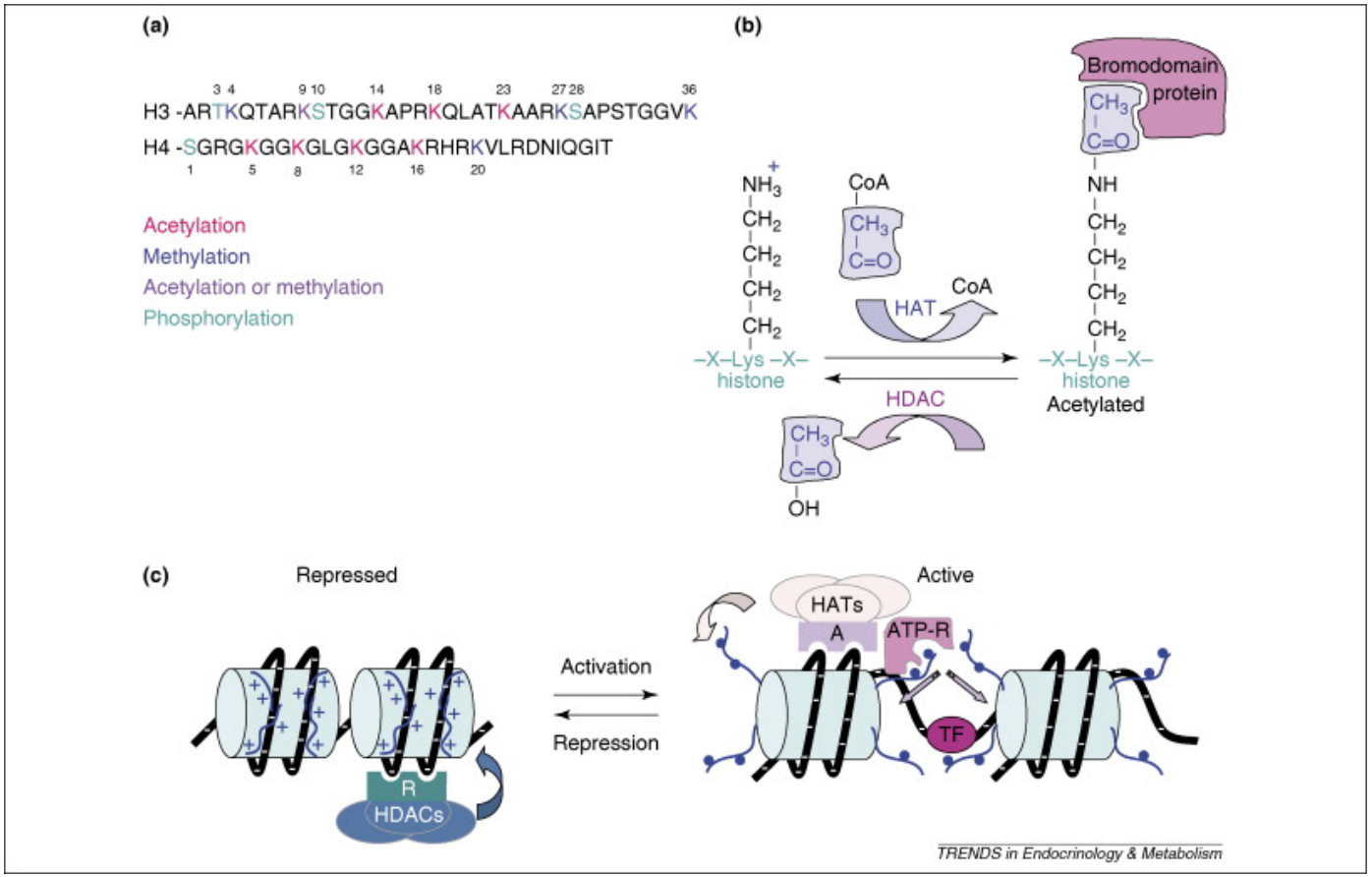
Chromatin remodeling complex



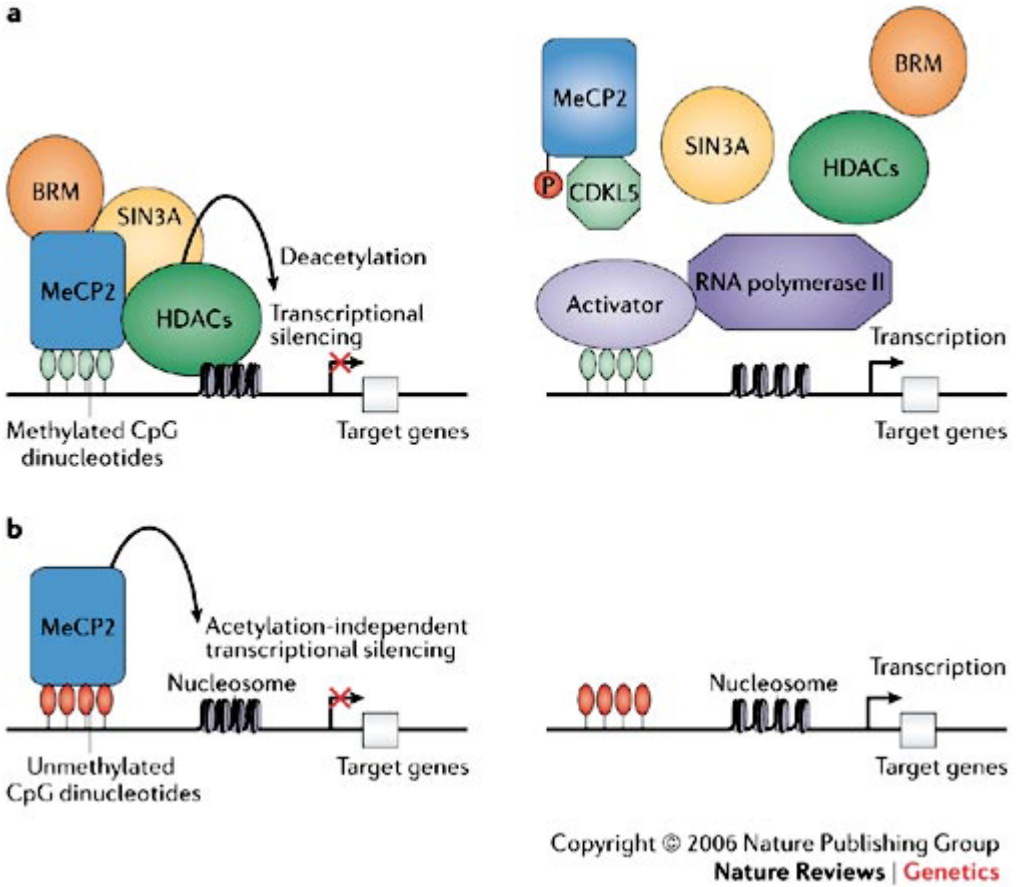


Formation of the multiprotein complex at the promoter of a gene. The depicted model shows acetylated chromatin (acetylatio n = red spheres) that is selectively remodeled by ATP-dependent chromatin remodeling complexes (blue sphere and black sp here) and thus allowing the binding of DNA-binding transactivators close to the promoter of the gene. Then the RNA polyme rase II, general initiation factors, and mediators bind at the promoter followed by the binding of RNA polymerase II elongati on factors to the multiprotein complex, finally leading to mRNA transcription. The proteins that form the multiprotein compl ex are represented by spheres in different shapes and colors. *Pharmacological Reviews March 1, 2002 vol. 54 no. 1 12 9-158*

Histone acetylase (HAT)/Histone deacetylase (HDAC)

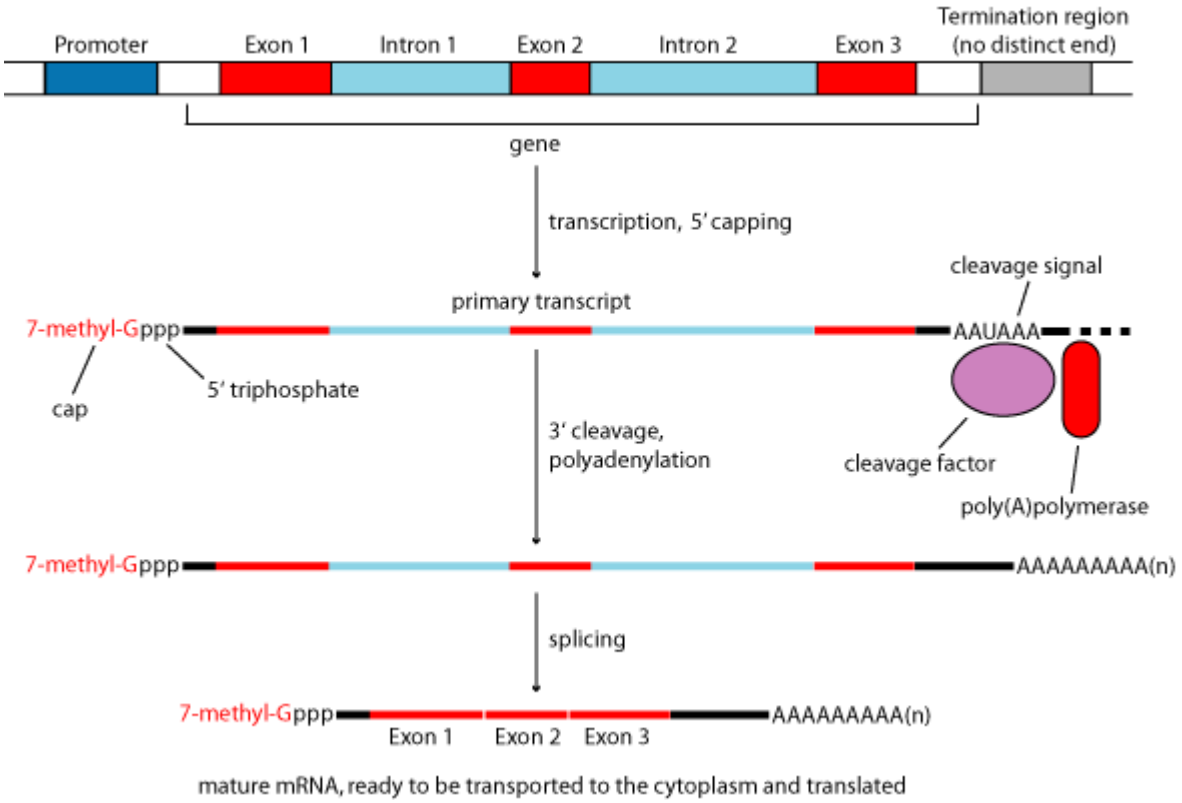


Rett syndrome: MeCP2 regulation of chromatin remodeling and transcription



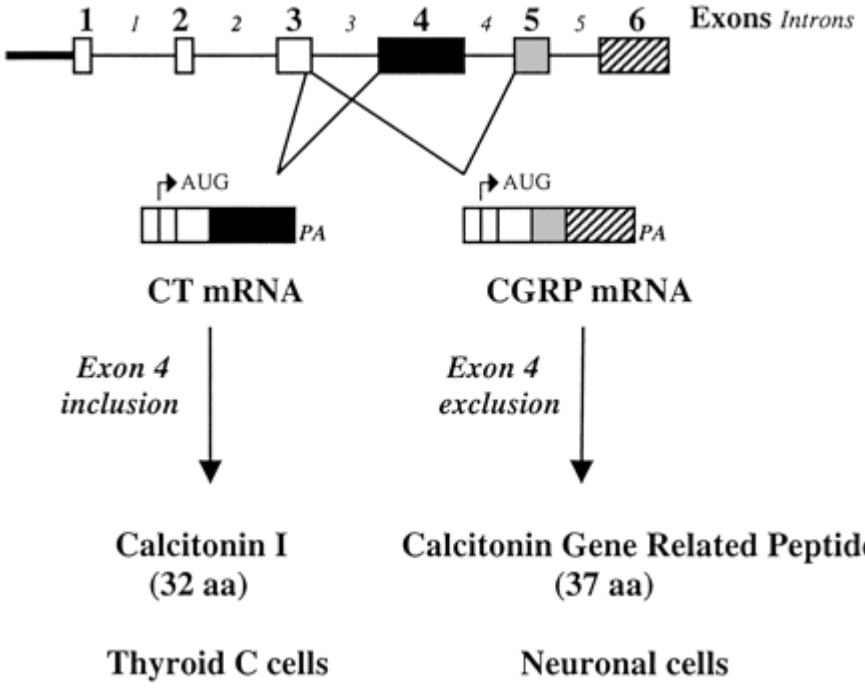
a | Transcription is suppressed in promoter regions containing methylated CpGs that are bound by **MeCP2 (methyl-CpG-binding protein 2).** MeCP2 binds methylated DNA and recruits chromatin-remodelling complexes that contain SIN3A (a transcriptional co-repressor), BRM (a SWI/SNF-related chromatin-remodelling protein) and histone deacetylases (HDACs). This leads to chromatin condensation owing to histone deacetylation, which results in a limited accessibility of the transcriptional machinery to promoter regions. When MeCP2 is not bound to methylated DNA (right panel), the complex that usually contains MeCP2, BRM, SIN3A and HDACs is not recruited. This lack of MeCP2 binding to DNA could be due to the activity of CDKL5 (cyclin-dependent kinase-like 5), which is thought to bind and contribute to the phosphorylation of MeCP2, resulting in the inability of MeCP2 to bind its methylated binding site (as shown). Alternatively, a similar effect could result from a missense mutation in the methyl-CpG-binding domain or loss of expression of MeCP2, In each of these cases, histones remain acetylated and the DNA at the promoter remains in an open conformation, allowing transcription factors to bind DNA and initiate transcription. b | MeCP2 is also a potent chromatin-condensing protein and can repress gene expression independently of DNA methylation, at least in vitro (left panel). At promoters where this DNA-methylation-independent function of MeCP2 is involved in regulating expression, a deficiency or absence of MeCP2 leads to a disorganization of chromatin structure (indicated here by increased spacing between nucleosomes), making transcription more likely to occur (right panel). Nature Reviews Genetics 7, 415-426 (June 2006)

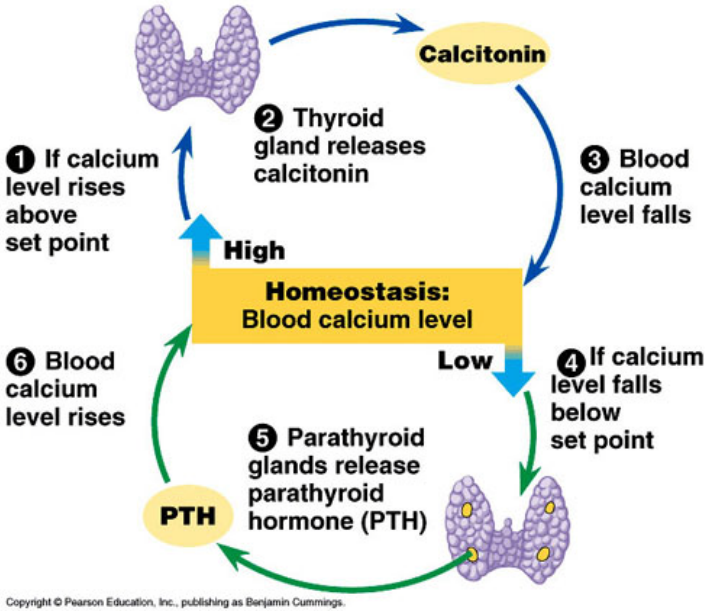
RNA processing in eukaryotes



Alternative splicing

Schematic diagram of the CT/CGRP gene and its alternative RNA processing in thyroid and neuronal cells. *The Journal of Clinical Endocrinology & Metabolism March 1, 2003 vol. 88 no. 3 1310-1318*





Control blood Ca^{2+}

Vasodilator

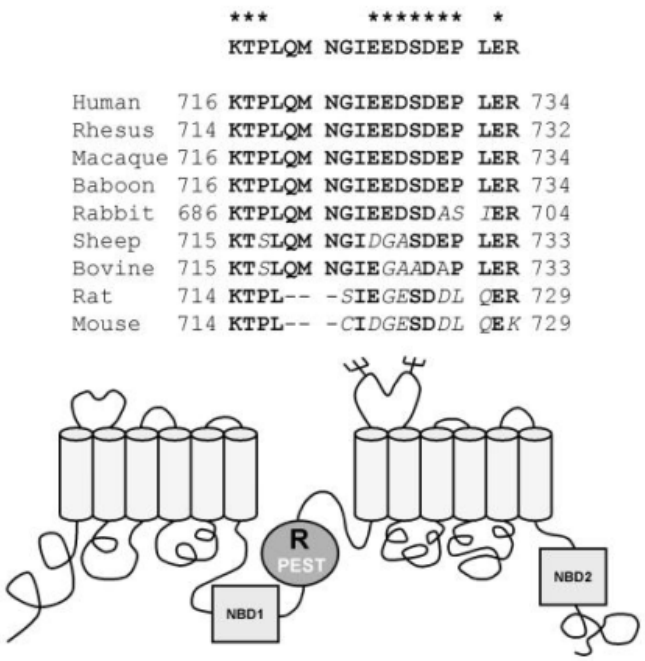
Function in the transmission of pain via GPCR

Protein stability

PEST sequence: rich in P,E,S,T
short life-span via proteasome or calpain

Association with other proteins

Ubiquitin-proteasome pathway



2. Regulation of transcription

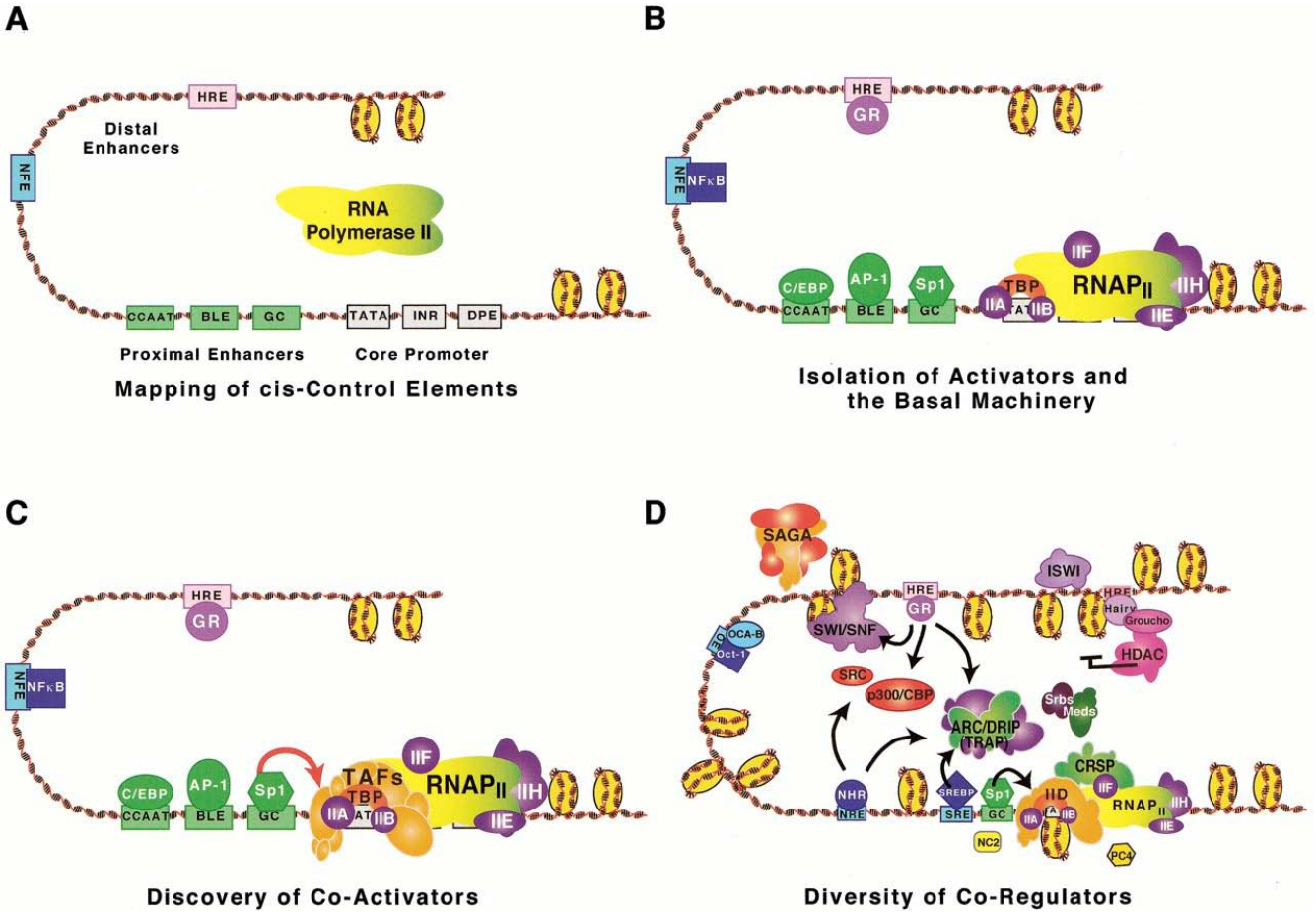
Transcription initiation: a critical biological control point
- positioning of RNA pol: cis-element
- control of the transcription rate

Core (basal) promoters:
- TATA box/initiator
- basal transcription complex: TBP, TBP-associated proteins (TAFs)

Transcription factors: key regulators of gene expression
- cis-elements (promoter, enhancer elements)
- Basal expression/enhanced or suppressed expression
- combination of cis-elements: combinatorial transcriptional regulation

Orchestrated response: a symphony of transcription factors for gene control

Genes & Dev. 2000. 14: 2551-2569

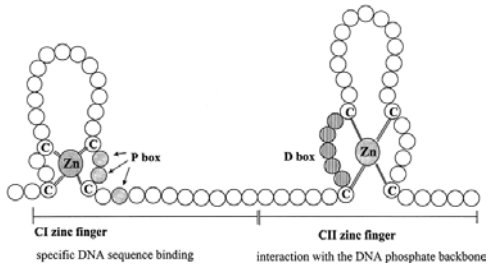


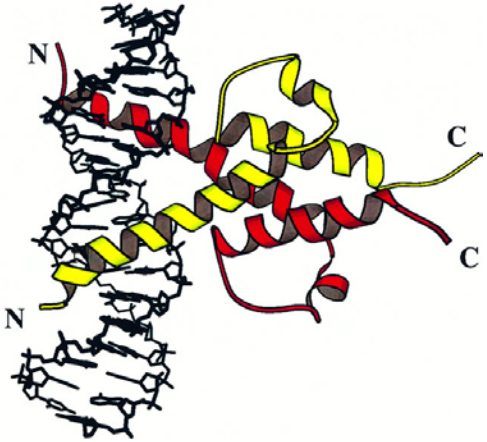
Transcription factor: multiple domains

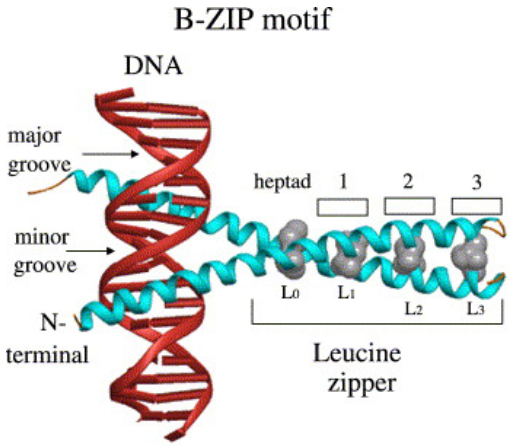
- DNA binding domain
- transcription activation domain
- multimerization domain

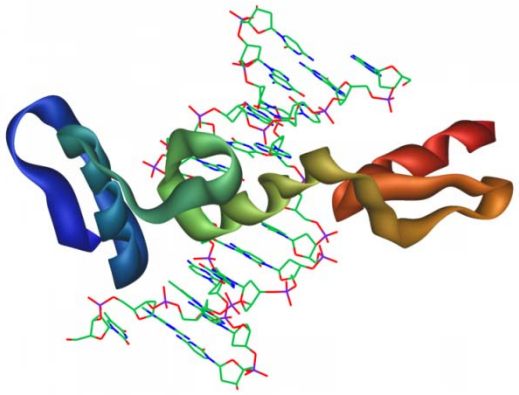
DNA-binding domain

- Basic helix-loop-helix
- Basic leucine zipper
- Zinc fingers



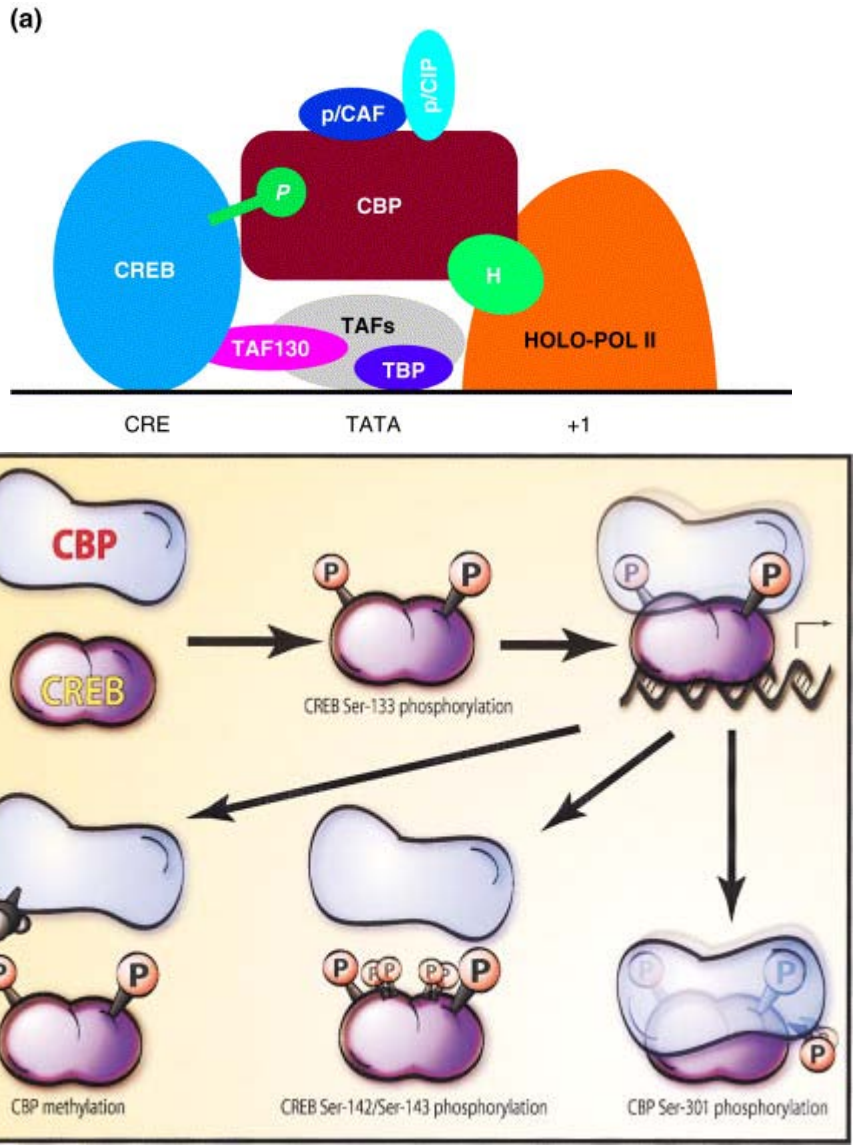




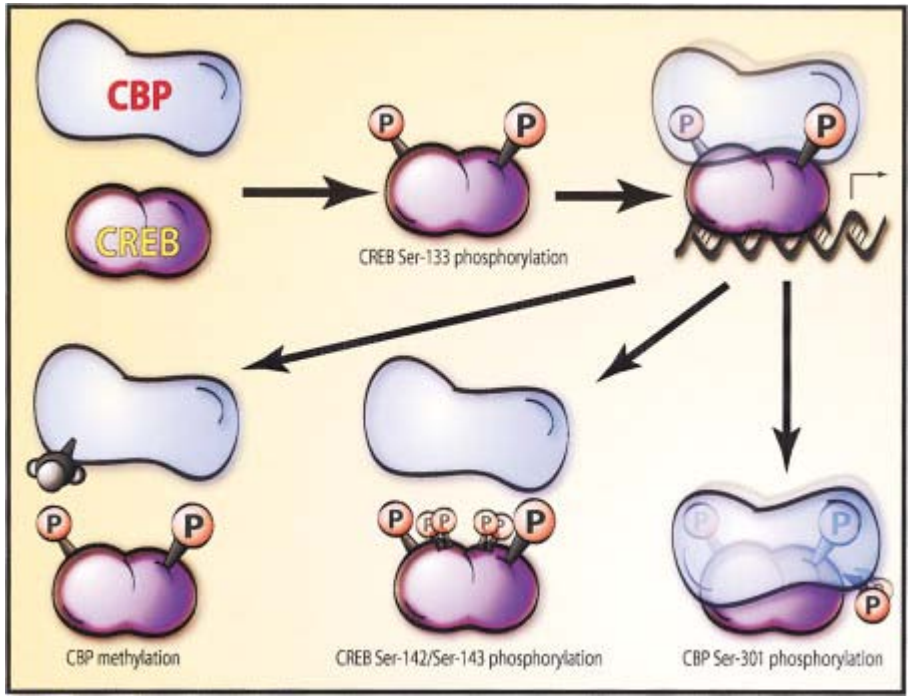


DNA binding as a homo-dimer or hetero-dimer
Dominant negative inhibition, but not all-or-none effect

Phosphorylation of CREB (cAMP response element-binding protein)



Phosphorylation of CREB Ser-133 and Additional Modifications of both CREB and CBP Control CREB-Dependent Gene Transcription. Phosphorylation of CREB Ser-133 serves to recruit the coactivator CBP to the promoter. CBP acetylates histones and other regulatory proteins, and interacts directly with components of the basal transcription machinery, thereby facilitating initiation of transcription. Other stimulus-dependent modifications found to affect CREB-dependent transcription and CBP recruitment include phosphorylation of CREB Ser-142 and Ser-143, phosphorylation of CBP Ser-301, and methylation of Arginine residues within the KIX domain of CBP. Interestingly, evidence indicates that both phosphorylation of CREB Ser-142/Ser-143 and methylation of CBP disrupt CREB-CBP interactions, but these modifications may not in all cases disrupt CREB-mediated gene expression. While the significance of phosphorylation of CREB Ser-133 is well understood, the functions of these additional modifications of CREB and CBP are only now becoming appreciated, and may not be universal.



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3. Regulation of gene expression by extracellular signals

Transcription factors: targets of signaling pathways

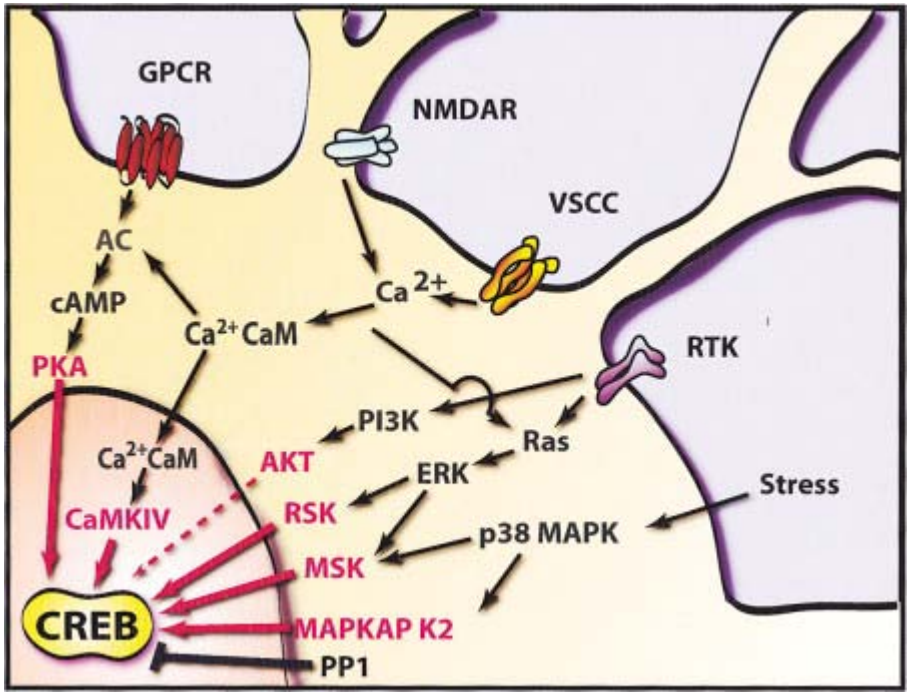
CREB family of transcription factors
- Regulation of CREBs by cAMP, Ca2+, and growth factors
- CREB mediation of neural plasticity
- CREB-like proteins
- The leucine zipper

AP-1 family of transcription factors
- Activation of cellular genes by AP-1
- Activation by multiple signaling pathways
- Regulation by phosphorylation
- Generation of unique AP-1 complexes

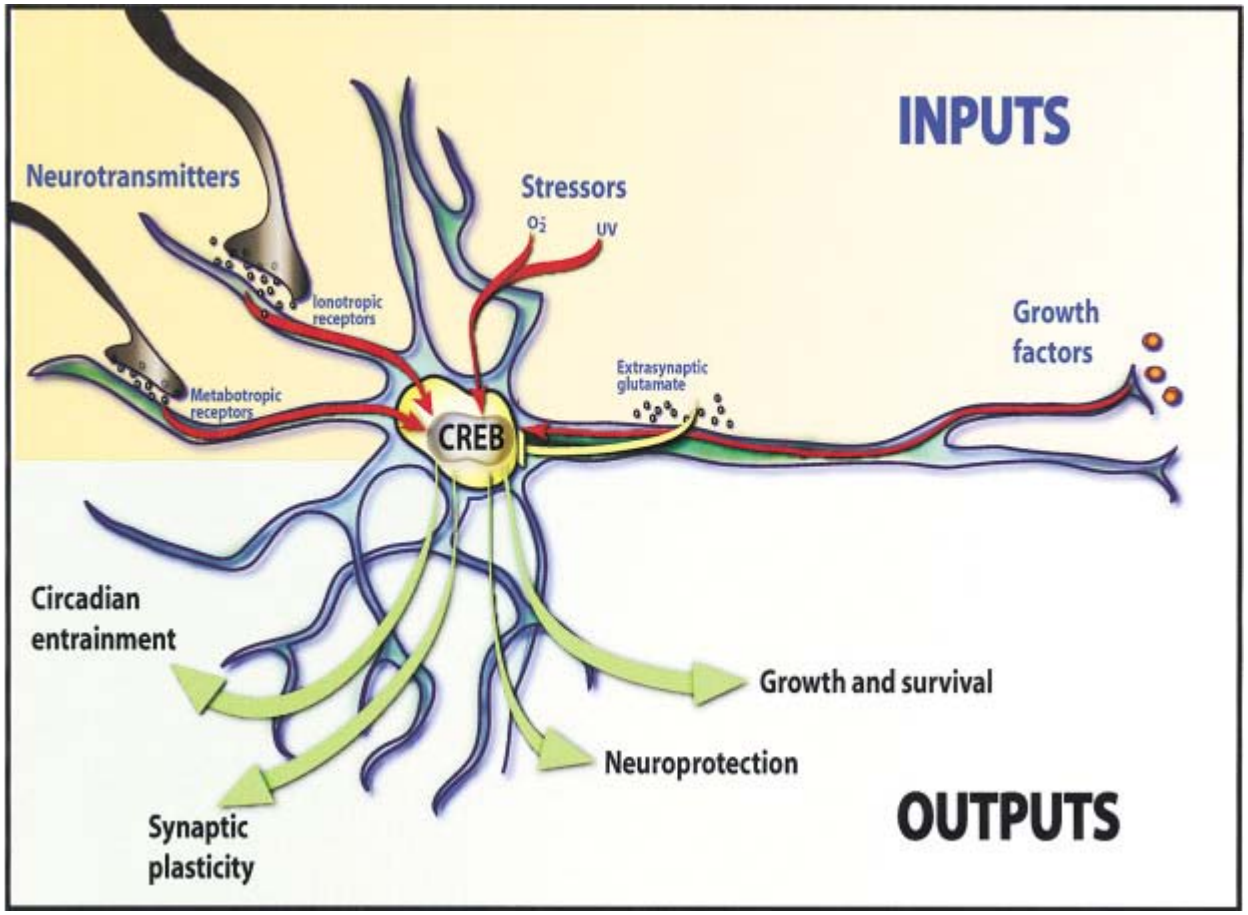
Steroid hormone receptor superfamily

Other transcription factors

Signaling convergence: synergistic effect

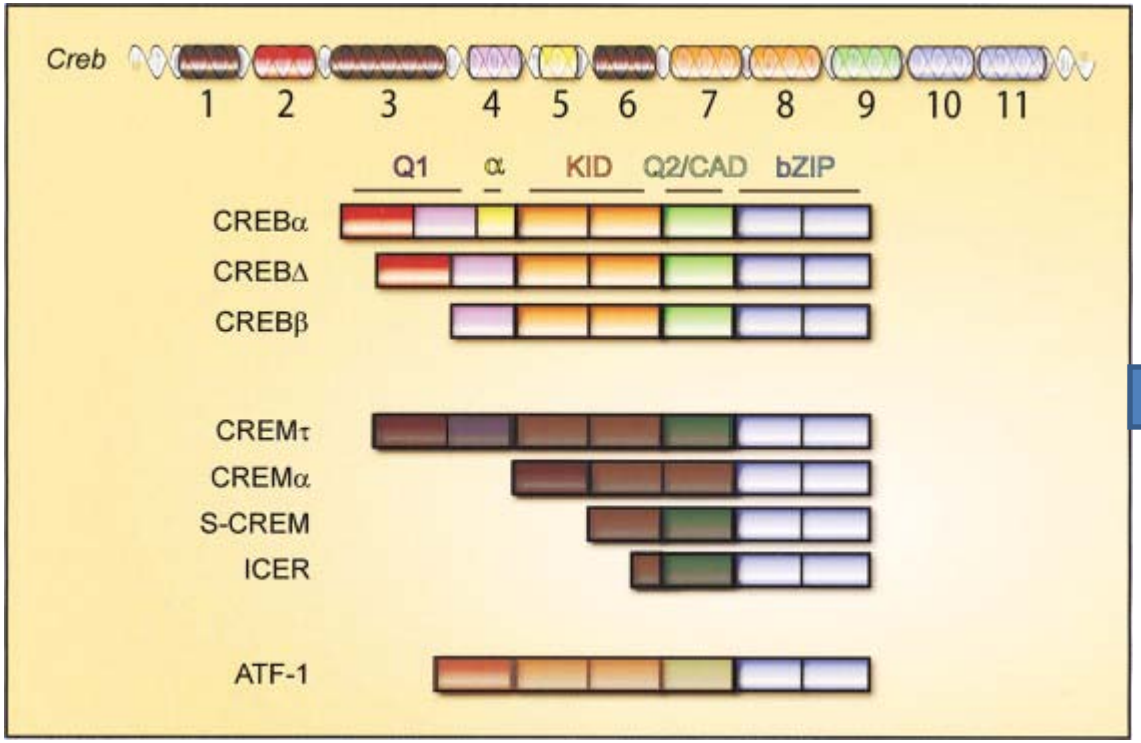


An Overview of Signaling Pathways that Converge on CREB. Excitatory neurotransmitters, ligands for GPCRs, neuronal growth factors, and stress inducers are among the stimuli that activate signaling pathways that converge upon CREB. As described in the text, multiple stimulus-dependent protein kinases have been implicated as CREB kinases in neurons, and a high degree of crosstalk exists between these signaling pathways. Stimulus-dependent CREB kinases include PKA, CaMKIV, MAPKAP K2, and members of the pp90RSK (RSK) and MSK families of protein kinases. Protein phosphatase 1 (PP1) has been implicated as the predominant phospho-CREB phosphatase.



CREB-Dependent Gene Expression Is Critical for a Variety of Functions in the Developing and Mature Nervous System. Shown are some of the processes for which CREB-dependent gene expression has been implicated. Stimuli and conditions that promote CREB phosphorylation and CREB-mediated gene expression are indicated in the upper half of the diagram, while physiological and pathological consequences of CREB activation are depicted in the lower half.

CREB-like proteins



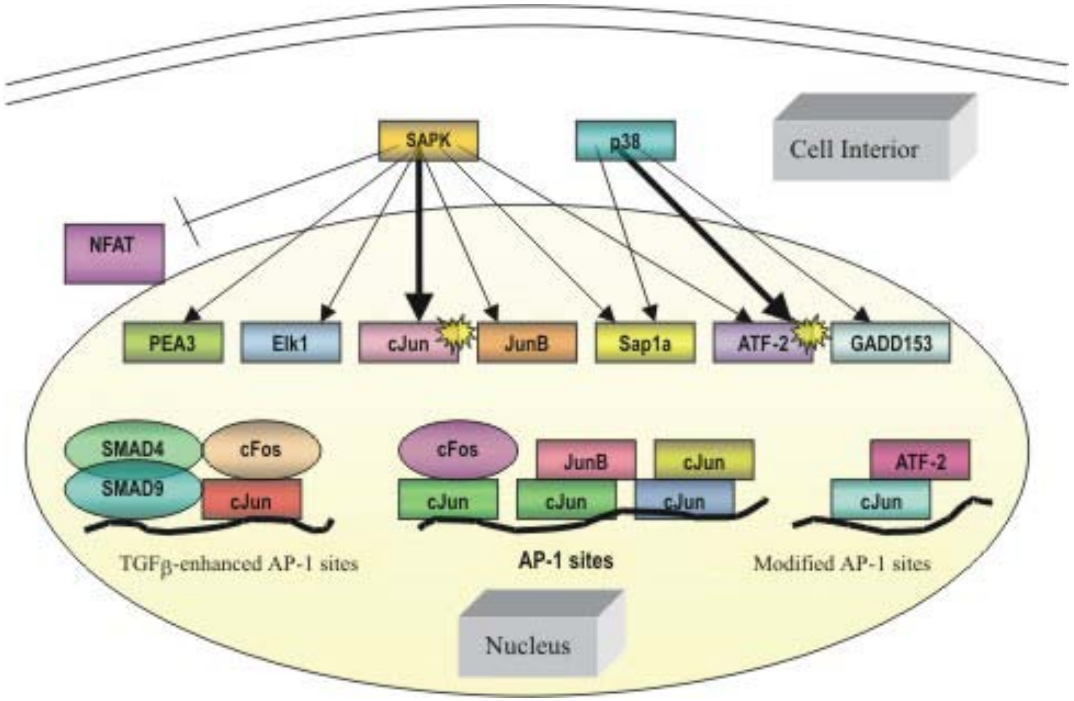
Leucine zipper
Homo- or hetero-dimer

Gene Organization and Domain Structure of CREB Family Transcription Factors. The Creb and Crem genes support expression of multiple splice variants, while the Atf-1 gene encodes one major protein product. CREBα and CREBΔ are the major products of the Creb gene. CREBβ, while a normally minor product, is upregulated in the CREBα/Δ hypomorphic mouse (see text for details). The Crem gene encodes both activators (CREMτ and CREMα) and repressors (S-CREM and ICER) of transcription. An alternate, CRE-driven intronic promoter within the Crem gene drives expression of the repressor ICER in a subset of neuroendocrine tissues. Creb is widely expressed throughout the nervous system and elsewhere, while Atf-1 and Crem exhibit more restricted patterns of expression.

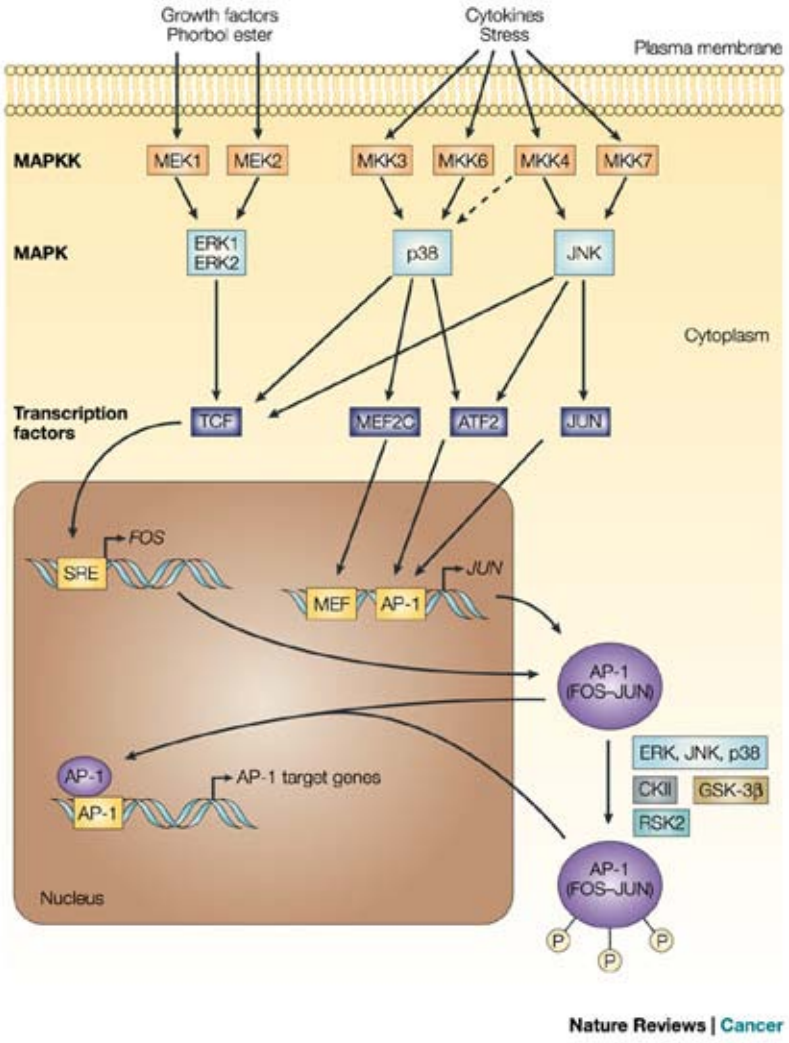
AP(activator protein)-1

In the field of molecular biology, the activator protein 1 (AP-1) is a transcription factor which is a heterodimeric protein composed of proteins belonging to the c-Fos, c-Jun, ATF and JDP families. It regulates gene expression in response to a variety of stimuli, including cytokines, growth factors, stress, and bacterial and viral infections. AP-1 in turn controls a number of cellular processes including differentiation, proliferation, and apoptosis.

AP-1 upregulates transcription of genes containing the TPA DNA response element (TRE; 5'-TGAG/CTCA-3'). AP-1 binds to this DNA sequence via a basic amino acid region, while the dimeric structure is formed by a leucine zipper.

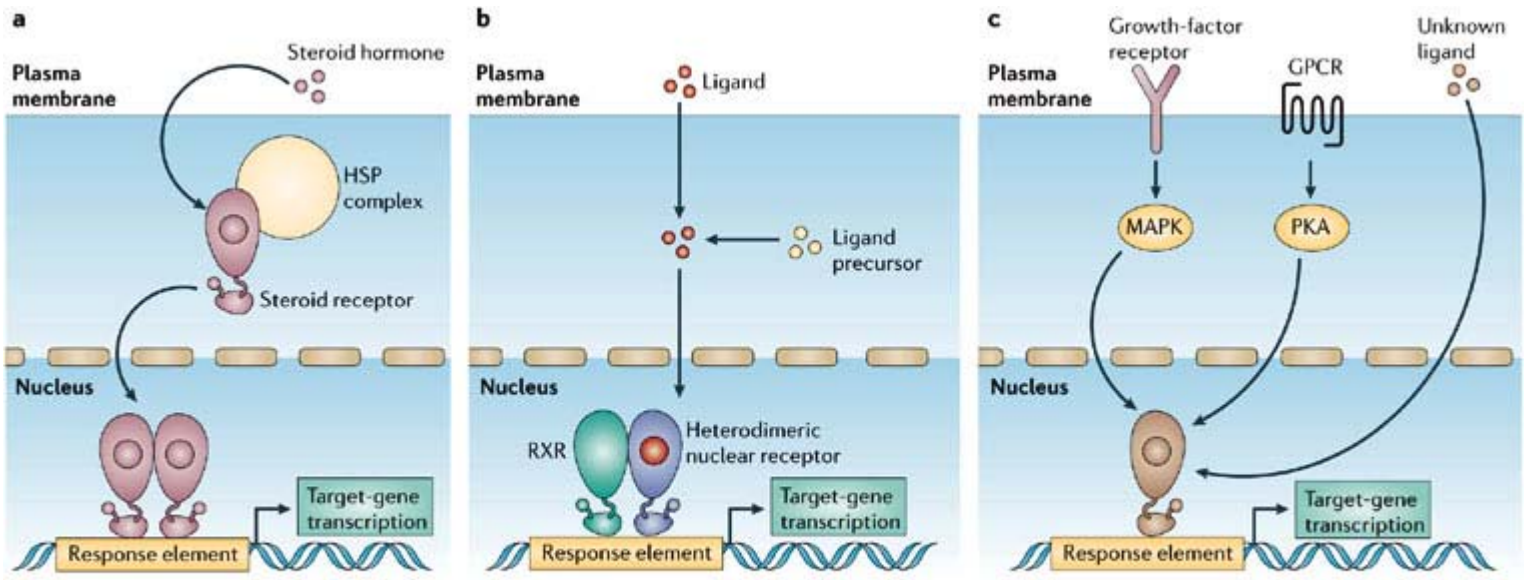


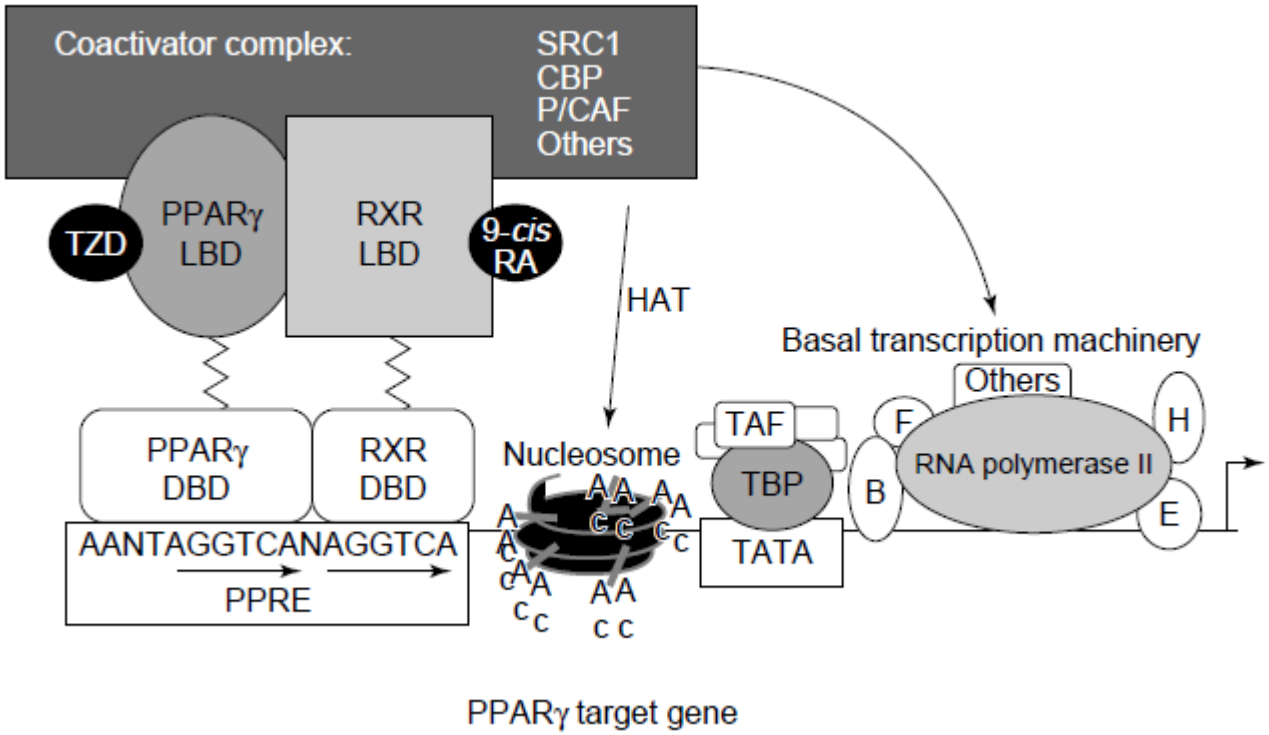
Transcriptional and post-translational a ctivation of AP-1.



AP-1 (activator protein 1) activity is stimulated by a complex network of signalling pathways that involves external signals (for example, growth factors) and mitogen-activated protein kinases (MAPKs) of the extracellular-signal-regulated kinase (ERK), p38 and JUN amino-terminal kinase (JNK) families. The dashed arrow indicates that phosphorylation of p38 by MKK4 is controversial. MAPKs activate various transcription factors (ternary-complex factors (TCFs), myocyte-enhancer factor 2C (MEF2C), activating transcription factor 2 (ATF2) and JUN) that induce the transcription of FOS and JUN genes, thereby increasing the number of AP-1 complexes and activating AP-1 target genes. Post-translational phosphorylation by various kinases regulates AP-1 activity, which includes its transactivating potential, DNA-binding capacity and the stability of AP-1 components. CKII, casein kinase II; GSK-3, glycogen synthase kinase-3; MAPKK, MAPK kinase; RSK2, ribosomal S6 kinase 2; SRE, serum-response element.
Nature Reviews Cancer 3, 859-868 (November 2003)

Nuclear receptor superfamily





Mechanism of thiazolidinedione (TZD) activation of transcription by peroxisome proliferator-activated receptor g (PPARg). PPARg binds to specific DNA sequences in target genes as a heterodimer with retinoid X receptor (RXR). TZDs [and/or an RXR ligand, indicated as 9-*cis retinoic acid (RA)] recruit coactivator complexes to the target gene, resulting in increased* transcription through inherent histone acetylase (HAT) activity or via interactions with the basal transcription machinery. CBP, CREB-binding protein; CREB, cyclic AMP response element-binding protein; DBD, DNA-binding domain; LBD, ligand-binding domain; PPRE, PPAR-response element; P/CAF, p300/CBP-associated factor; SRC1, steroid receptor coactivator 1; TAF, TBP-associated factor; TBP, TATA-binding protein. *TEM Vol. 10, No. 1, 1999*