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Mechanisms of transcriptional activation of cAMP-responsive element-binding protein CREB

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Abstract

The CREB-CREM transcription factors are the main gene regulatory effectors of the cAMP signaling pathway. The investigations of this family of transcription factors had a profound impact on the understanding of signaling-induced gene transcription. Here we discuss some key aspects of the underlying biology, review transcriptional activation by CREB proteins through transcription cofactors and present novel insights into the context- and position-specific function of CREB on complex genes. (Mol Cell Biochem **212**: 5–9, 2000)

Key words: transcriptional regulation, gene expression, coactivator, repressor

Biology

The cAMP-pathway is a widely used signaling process that senses and amplifies the response of cells to hormones, growth factors and neurotransmitters. Among the target proteins of protein kinase A are the transcription factors CREB and CREM. Gene regulatory programs induced by CREB control different biological processes (for review see [1]) such as T cell development, spermatogenesis, long term memory but also the regulation of the blood pressure through angiotensin. The latter is the focus of this issue.

Angiotensins

There is increasing evidence, that the protein CREB is involved in the biology of the renin-angiotensin system (RAS), which plays an important role in the regulation of the blood pressure. Expression of the rat angiotensinogen gene is positively influenced by CREB in a cAMP-dependent manner [2]. The stimulating effect of CREB depends on a functional cAMP-responsive element (CRE), located in the 5′-flanking region of the angiotensinogen gene [3]. The expression of testis angiotensin converting enzyme depends on a functional CRE although it does not appear to be responsible for its testis-specific expression [4]. CREB seems to also function as an effector of angiotensin II signaling. The stimulatory effect of angiotensin II depends on a cAMP-responsive element within the fibronectin promoter [5]. Angiotensin II stimulation leads to moderate induction of the CREB protein in human mesangial cells. Angiotensin II increases interleukin-6 expression in a dose-dependent manner, which is mediated through a cAMP-responsive element in the interleukin-6 promoter [6]. As one other example a cAMP-responsive element is found in the tyrosine hydroxylase gene promoter [7]. In this study the CRE proves to be one critical angiotensin II-responsive element in cultured bone adrenal medullary cells.

Regulation of long term memory

A correlation between memory and cAMP pathways became first evident in genetic studies. Flies were trained to discriminate between two different odors, one accompanied with an electric shock, and the other not associated with a shock. Chemically mutagenized flies were used to find mutants which failed to learn the discrimination between the two odors without being affected in other characteristics like lo-

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comotion or odor detection [8]. Four mutants were found and the corresponding genes were identified. Remarkably, three out of four mutants affect molecules that are involved in cAMP signaling [9]. Long term – in contrast to short term memory storage depends on transcriptional activity and the synthesis of new proteins [10]. Studies in Drosophila and Aplysia demonstrated that CREB is critically involved in this process [11]. One model implies that CREB-mediated transcription activates a gene expression program that ultimately leads to the production of new synapses between neurons and a prolonged stabilization of the synaptic facilitation (see review [12]).

CREB function in the immune system

ATF/CREB proteins are involved in the development and function of T lymphocytes. The signal transduction pathways in T cells after T-cell receptor engagement, which lead to phosphorylation and activation of CREB, include protein kinases C, RAS, RAF-1, MEK and RSK2 [13]. Functional binding sites for members of the ATF/CREB family were identified in the promoters and enhancers of many T-cell specific genes, including the TCR-α and -β enhancers [14, 15], the CD3δ enhancer [16] and the TCR Vβ promoters [17]. Transgenic models provided strong *in vivo* evidence for the importance of CREB/ATF proteins in the immune system. CREB knock-out mice show defects in the development of specific T-cell lineages [18]. A dominant negative form of CREB under the control of the T-cell specific CD2 promoter/enhancer leads to a profound defect in T cell proliferation after stimulation of the T-cell receptor pathways [19].

CREB structure

The CREB/ATF family consists of a large number of genes that include the factors CREB, CREM, ATF-1, ATF-2, ATF-3 and ATF-4 (also known as CREB2). Various splice variants of each of these proteins have been identified which activate or repress transcription (see review [20]). CREB, first identified [21], is probably one of the most meticulously characterized transcription factors in eukaryotes.

A common feature of all the family members is a basic region leucine zipper (bZIP) domain (Fig. 1). The leucine zipper consists of an α-helical coiled-coil structure, which forms homo- and heterodimers. A particular 'dimerization code' determines which heterodimers are possible [22]. The basic region is responsible for the sequence-specific DNA-binding of the CREB transcription factors to cyclic AMP-response element (CRE). The cognate DNA-recognition motif for the CREB homodimer is a symmetric palindromic motif with sequence 5′-TGACGTCA-3′ [23]. In addition to the core sequence, flanking bases are important for binding of CREB [24].

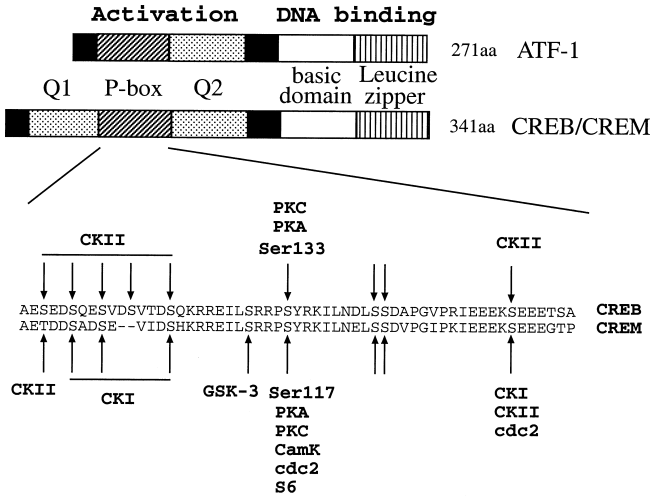

Fig. 1. Structure of ATF-1, CREB and CREM proteins. The P-box, the glutamine-rich domains (Q1 and Q2) and the DNA binding region (leucine zipper and basic domain) are indicated. Sequence alignment of the P-boxes of CREB and CREM. Serine and threonine residues can be phosphorylated by the indicated kinases as marked by arrows.

The members of the CREB family of transcription factors share structural features within their transactivation domains. Transcriptional activation is mediated through two regions (compare Fig. 1). One region contains several recognition motifs for protein kinases. It is therefore called kinase inducible domain (KID) or phosphorylation box (P-box). The transactivation potential of CREB proteins critically depends on their phosphorylation status [25, 26]. The other constitutive activation region, contained in CREB and CREM, consists of two glutamine-rich motifs, called Q1 and Q2, which flank the kinase inducible domain [27]. Mammalian ATF-1 lacks Q1 but contains Q2 [28]. Glutamine-rich regions can be found in many regulatory, coactivator and basal transcription factors and serve as interaction surfaces for other transcription factors. It has been suggested that CREB and CREM require the P-box and at least one glutamine-rich domain [27, 29] to activate transcription.

Several isoforms of each member of the CREB family of transcription factors were identified. Glutamine-rich regions can be removed via alternative splicing, either partially (in Drosophila CREB2b) or completely (in mammalian CREMα, CREMβ and CREMγ), with the consequence that these proteins display repressor function [29]. The insertion of premature stop codons in the CREB gene results in truncated proteins that lack the DNA-binding region and that also function as repressors. In Aplysia, a CREB isoform was identified, which lacks the nuclear localisation signal [30]. This cytoplasmic form (CREB1c) regulates the activity of kinases that phosphorylate nuclear CREB.

Signal-induced activation by CREB

CREB binding sites had been identified in a multitude of inducible promoters. Examples are the somatostatin- [31] and the proenkephaline promoter [32] as well as many others (see reviews [1, 20]) The critical region in CREB for the response to cAMP [25] is the phosphorylation box (P-box) or kinase-inducible domain (KID). As depicted in Fig. 1, the P-box contains several consensus phosphorylation sites for kinases such as PKA, PKC, glycogen synthase kinase-3 and casein kinases (CK) I and II [25] [33]. Upon activation of the adenylate cyclase pathway, the serine at position 133 of CREB (serine 117 in CREM) is phosphorylated by PKA, which enhances the transcriptional activity of the proteins CREB and CREM.

In addition to PKA, other signal transduction pathways target the CREB protein, in order to either increase or decrease its transcriptional activity. For example, the Ca^{2+}-calmodulin-dependent kinase IV (CaMKIV) phosphorylates CREB at Ser133 after membrane depolarization in neuronal cells [34]. Also signal transduction pathways triggered by growth factors and inflammatory cytokines lead to a phosphorylation of CREB (see review [35]). Ca^{2+}-calmodulin-dependent kinase II (CaMKII) phosphorylates CREB at Ser133 and Ser142. Remarkably, phosphorylation of Ser142 by CaMKII neutralizes the activity of CREB [36].

CREB and CREM activate through transcription cofactors

A breakthrough in the understanding of inducible CREB function came from the discovery of the cofactor CBP (CREB binding protein) that interacts specifically with the phosphorylated CREB P-box domain [37]. In the current model, the cofactor CBP and its close relative p300 serve as bridging factors between the activator CREB and the general transcription factors [27, 38]. These cofactors also possess a histone acetyltransferase (HAT) activity, which is thought to play a critical role for gene activation in the chromatin (reviewed in [39]). CBP and p300 bind to other cofactor complexes among them PCAF, SRC-1/NcoA-1, TIF-2/NcoA-2 and pCIP/ACTR which also possess histone acetyltransferase activity (reviewed in [40]). There are indications for the formation of gene- and pathway-specific complexes. For example, binding of CBP to PCAF and pCIP has been reported to be necessary for induced CREB function [41]. The interaction between cofactors and the P-box of CREB and CREM is not always phosphorylation-dependent. A new route for transcriptional activation by CREB and CREM was reported recently, demonstrating the functional interaction between ACT (for activator of CREM in testis) and CREM [42]. ACT appears to be a tissue-specific coactivator for CREM. It possesses an intrinsic activation domain and interacts with CREM in a phosphorylation-independent manner. In addition to the inducible P-box domain, CREB also contains a constitutive activation domain (CAD), which is responsible for the interaction with one or more of the TATA-box associated factors (TAFs), one of which is $TAF_{II}110$ (the drosophila homolog of human $TAF_{II}130$). The constitutive activation domain of CREB can be subdivided into three regions, which are rich in either serine, hydrophobic amino acids, or glutamine. All three regions are necessary for effective interaction with $TAF_{II}110$ in a yeast two-hybrid assay [43].

Transcriptional effects of CREB are context- and position-specific

The biological effects of the cAMP pathway through CREB are entirely based upon a gene expression program initiated by the activator. Hence, unraveling of CREB transcriptional activation is crucial for the understanding of the biological processes. Above we have discussed CREB-structure and -function through cofactors. Additional important parameters for CREB function are the context in which CREs are embedded and the position of CREs relative to the start site of transcription. This has been most clearly demonstrated on the gene encoding the T-cell receptor beta (TCRβ) chain [44]. These studies could have model character for the many other target genes of cAMP-induced CREB proteins and, therefore, will be briefly reviewed here.

The TCRβ gene is an attractive model for the study of promoter and enhancer function. This is mainly based upon the fact that the genome contains many different promoters that can be compared. A functional TCRβ gene is generated through recombination events in which the enhancer is brought into the relative vicinity of one of the many Vβ promoters (although it remains a distal element, several kilobases apart from the promoter). The rearranged TCRβ gene contains CREs in three different positions that seem to fullfill alternative tasks (Fig. 2). Firstly, CREs are contained within the distal TCRβ enhancer [15]. Secondly, in many Vβ promoters one CRE is found in a promoter-proximal position [17], in between position –100 to –40 upstream of the start site of transcription. In our studies of the human Vβ 8.1 promoter we could also detect a third cryptic CRE within the core promoter region [44], located in between position –30 and +11 (compare Fig. 2). This is the region where TFIID and the other general transcription factors bind to the promoter.

In the enhancer CREB appears to be part of a multiprotein enhanceosome [15]. The enhancer is efficiently repressed by overexpression of the 12S form of adenovirus encoded E1A, which is known to compete for the CREB-binding proteins

CBP and p300. One simple scenario would imply that CBP binds to and functions via binding to CREB in the enhancer. However, E1A retained its repression potential even after removal of the CRE. Repression by E1A seems to be rather correlated to the overall enhancer activity. This suggests that CBP is part of, or functions through, the multiprotein enhanceosome rather than through individual activators alone such as CREB [45]. Further evidence for this hypothesis came from experiments with multimerized CREs that proved to act as a poor enhancer element at a distance (unpublished observations). The promoter-upstream (UAS) CRE mainly serves as a platform for the enhancer. This is concluded from the fact that it raises relative enhancer activity but displays little influence on the promoter [44, 45]. Activation requires an intact PKA phosphorylation site in CREB. In contrast, the third functional CRE within the core promoter contributes strongly to Vβ 8.1 promoter activity. This low affinity CRE can be activated through overexpression of CREB, but not through a mutant lacking Ser133. Moreover, replacement of the weak CRE by a consensus CRE efficiently raises promoter activity [44]. Thus, the core CRE is critical for promoter function, whereas the two other CREs help to establish a functional enhancer. Related mechanisms could add a new level of complexity to the control of cAMP-induced gene activation in other biological processes.

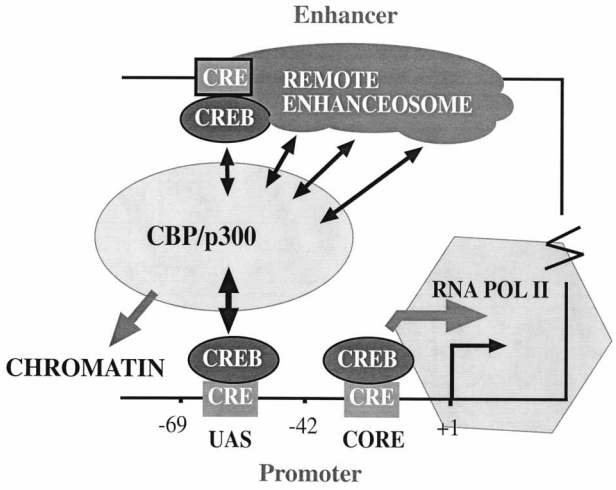

Fig. 2. Position depending function of CREs. The model is based upon the analysis of the TCRβ gene consisting of the distal enhancer and the Vβ 8.1 promoter. CREs (cAMP-responsive elements) are found within the TCRβ enhancer and the Vβ8.1 promoter. The promoter comprises two CREs upstream of the core region (upstream activating sequence (UAS)) and within the core region (positions –30 to +11). Only the core element affects promoter activity (arrow) whereas the two other elements mainly contribute to enhancer-promoter communication. Possible functional interactions between the enhancer and the promoter via the cofactor CBP/p300 are indicated by black arrows. The model implies that upon interaction with the enhanceosome CBP and p300 acetylate histones (grey arrow), which could help to keep the promoter accessible for CREB and other activators that bind to weak interaction sites on the core promoter and subsequently activate transcription.

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