Transcriptional control: **Versatile molecular glue**

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CBP and p300 are versatile coactivators that physically connect many DNA-binding factors to the basal transcription machinery. Phosphorylation by cyclin-dependent or signal-induced protein kinases may regulate their function.

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Initiation of transcription by RNA polymerase II commonly requires the cooperation of two different sets of proteins, the basal transcription machinery and sequence-specific factors that bind to promoter/enhancer elements. How do the latter transcription factors relay their activating or repressing function to the basal transcription machinery? One way would be by direct interaction of the sequence-specific transcription factors with components of the basal transcription machinery, such as the TATA-box binding protein (TBP) or its associated factors (TAFs). Alternatively, bridging proteins may establish an indirect contact. During the last three years, a family of mammalian bridging proteins has been identified, consisting of CREB-binding protein (CBP) and p300. These very similar proteins are coactivators of transcription that themselves do not specifically interact with promoter elements but rather are recruited to promoters by protein–protein interactions [1,2]. CBP and p300 act as a crucial scaffold for the formation of transcriptional initiation complexes and their activity might be regulated by phosphorylation.

Coactivation of cAMP-stimulated transcription

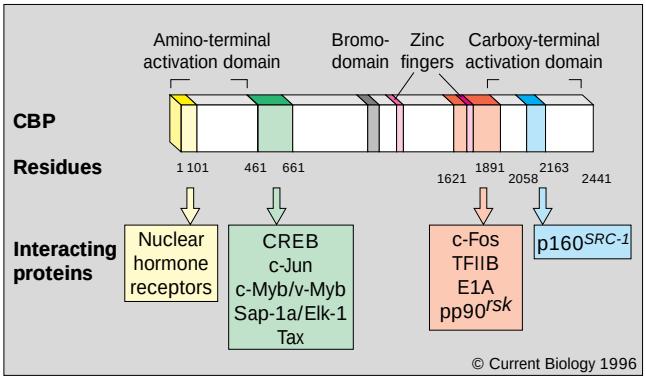
CBP was originally identified by its ability to interact with the cAMP-response element binding protein (CREB). CREB itself requires phosphorylation by cAMP-dependent protein kinase (PKA) at serine 133 to be activated. The mechanism by which this enhances the transactivation function of CREB has now been resolved: this phosphorylation is necessary for interaction with the coactivator CBP, or alternatively p300 [1,2]. CBP interacts directly with phosphoserine 133 through residues 590–669 [3], making it one of the first proven phosphoserine-recognizing proteins.

In addition to binding to phospho-CREB, CBP can bind to the basal transcription factor TFIIB (Fig. 1) and thereby act as a bridging molecule between phospho-CREB and the basal transcription machinery [1]. Functionally, CBP and p300 enhance CREB-mediated transcription upon PKA activation [1,2,4]. This coactivation can be suppressed by expressing the adenoviral protein E1A, which sequesters CBP and p300 by directly binding to them [2]. As CREB's E1A-binding domain overlaps its TFIIB-binding domain (Fig. 1), binding of E1A could preclude interaction of TFIIB with CBP or p300 and thereby inhibit CREB function.

Phosphorylation of CREB solely at serine 133 is also elicited upon T-cell receptor activation independently of PKA, and protein kinase C appears to be responsible [5]. Unexpectedly, this does not lead to transcriptional coactivation by CREB and CBP. Costimulation with a cAMP agonist at a suboptimal dose, however, which does not induce CREB phosphorylation, is able to achieve this. These data imply that phosphorylation by PKA of a factor other than CREB, which might be CBP itself (see below), is required for CREB and CBP to coactivate transcription.

CREB and CBP may also form a complex independently of PKA activation at the human T-cell leukaemia virus-1 (HTLV-1) long terminal repeat [6]. This interaction is mediated by the viral Tax protein, which appears to be an adaptor between CREB and CBP. Surprisingly, formation of a tripartite CREB–CBP–Tax complex depends on PKA phosphorylation of CREB when CREB is bound to the somatostatin cAMP-response element. This suggests that the nature of the recognition sequence bound by CREB may dictate different modes of protein–protein interaction within the tripartite complexes.

Figure 1



Mouse CBP. Regions 1–101, 461–661, 1621–1891 and 2058–2163 contain binding sites for the proteins indicated below. The bromodomain comprises residues 1107–1171; the two zinc fingers comprise residues 1284–1312 and 1708–1733. The domain structure of p300 appears to be basically similar.

CBP and p300: promiscuous molecules

Injection of anti-CBP antibodies into cells inhibits not only cAMP-stimulated gene activation but also E1A-sensitive gene activation by mitogens or hormones ([4,7] and D. Chakravarti and R. Evans, personal communication), indicating that CBP has various interaction partners. Indeed, CREB [3], c-Jun [4,8], c-Myb and v-Myb [9,10], and Sap-1a and Elk-1 ([11] and R.J. and A. Nordheim, unpublished data) all interact with CBP residues 461–661. In addition, c-Fos [12] and the nuclear receptors for steroids, thyroids and retinoids [7] interact with CBP residues 1621–1877 or 1–101, respectively. Unlike CREB, however, most or all of these proteins do not require phosphorylation to bind to CBP (the situation with c-Jun remains controversial).

Like CREB, the nuclear hormone receptors require activation — in their case by binding cognate hormone — to interact with CBP [7]. Thus, the observed inhibition of the transcription factor AP-1 — composed of c-Jun, c-Fos and related factors — by nuclear receptors upon hormone binding can be explained by the competition for a limiting cofactor, namely CBP. Furthermore, the nuclear hormone receptor coactivator p160^{SRC-1} can interact with residues 2058–2163 of CBP, and may stabilize the interaction between nuclear hormone receptors and CBP ([7] and D. Livingston, personal communication).

Interestingly, c-Fos binds to the same CBP region as E1A [12]. E1A-mediated inhibition of c-Fos activity could therefore be due to competition between E1A and c-Fos for the same binding site in CBP. This also suggests that, like E1A, binding of c-Fos precludes TFIIB binding to CBP. If so, CBP must be capable of contacting another component of the basal transcription machinery to coactivate c-Fos-mediated transcription. This may be TBP, as TBP binds to both the amino- and carboxy-terminal regions of p300 *in vitro*, implying that p300 can act as a TAF [13]. Like c-Fos and CBP, the basic helix–loop–helix protein MyoD appears to interact with p300 through the region that is also bound by E1A, and this interaction is critical for muscle differentiation ([13] and D. Livingston, personal communication).

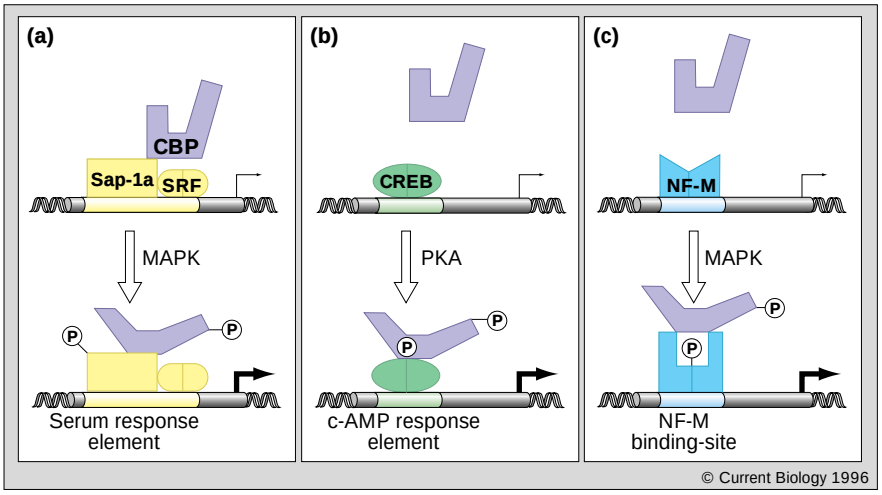
Surprisingly, E1A acts positively in combination with the transcription factor YY1 [14]. No direct interaction between E1A and YY1 was reported, but rather p300 can bind to both proteins at the same time, resulting in a tripartite complex. The YY1-binding domain was coarsely mapped to the last 800 residues of p300, but apparently it does not overlap with the E1A-binding domain [14]. That the YY1–p300–E1A complex, but not the CREB–p300–E1A complex, can facilitate transcriptional activation may be because the two tripartite complexes have different geometries.

Phosphorylation of p300 and CBP

Although p300 is phosphorylated in both quiescent and proliferating cells, the phosphorylation levels change during the cell cycle and hyperphosphorylation occurs during mitosis [15]. This suggests that p300 could be a substrate for cyclin-dependent protein kinases (Cdks). Consistent with this, Cdk2 and Cdc2 are capable of phosphorylating p300 *in vitro* [16]. E1A expression in adenovirus-infected cells might promote phosphorylation of p300, as E1A can bind simultaneously to p300 and either pRB, p107 or p130, all of which can recruit cyclin–Cdk2 *via* cyclin interactions into a multiprotein complex [17,18].

Cdc2-related protein kinases are activated during retinoic acid-triggered differentiation of F9 cells. Like retinoic acid, adenoviral infection elicits differentiation of F9 cells and both stimuli result in hyperphosphorylation of p300

Figure 2



Models for the phosphorylation-dependent cooperation between CBP and Sap-1a (**a**), CREB (**b**) or NF-M (**c**). Note that it is unclear whether two molecules of CBP can be recruited by a single CREB or NF-M dimer.

[19]. In mouse primary keratinocytes or myoblasts, transcription of the *p21* gene, which encodes a Cdk-inhibitor, is stimulated upon Ca^{2+}-induced differentiation. Normal E1A, but not mutant forms incapable of binding to p300, suppresses *p21* activation, suggesting that p300 is involved. After *p21* activation, which leads to reduced Cdk2 activity, the phosphorylation level of p300 appears to decrease [20], supporting the notion that p300 phosphorylation is in part due to Cdks. Thus, differentiation of cells can be associated with both hyperphosphorylation or hypophosphorylation of p300. The extent and sites of p300 phosphorylation may determine which interaction partners bind to p300, as SV40 large T antigen coimmunoprecipitates only with hypophosphorylated p300 [21], an interaction which, like that with E1A, suppresses p300-dependent transcription ([21] and R. Eckner, personal communication).

CBP is phosphorylated *in vivo* within its carboxy-terminal glutamine-rich region. *In vitro*, CBP can be phosphorylated within this region by ERK-subclass mitogen-activated protein kinases (MAPKs) or PKA, both of which can enhance CBP's transactivation potential ([11] and R.J. and A. Nordheim, unpublished data). This raises the possibility that CBP is a target of signal transduction pathways funnelling through ERK-MAPK or PKA. ERK-MAPK and PKA together do not have a cooperative effect on CBP, rather PKA reduces the activation by ERK-MAPK [11]. Conversely, Ras activation, which leads to ERK-MAPK-mediated phosphorylation, activation and nuclear translocation of the $pp90^{rsk}$ protein kinase, induces association of $pp90^{rsk}$ with CBP and thereby inhibits cAMP-mediated gene transcription in a manner similar to E1A (M. Montminy, personal communication). Thus, CBP may be a dampening integrator of ERK-MAPK and PKA signalling pathways.

Sap-1a and Elk-1, which are recruited by the serum response factor (SRF) to the serum response element (SRE) of c-*fos*, activate transcription upon MAPK stimulation. Sap-1a and Elk-1 interact with CBP in a phosphorylation-independent manner, yet this does not lead to an enhancement of transcription. Rather, Sap-1a and Elk-1 have to be phosphorylated by MAPK in order to stimulate the c-*fos* SRE together with CBP ([11] and R.J. and A. Nordheim, unpublished data). As ERK-MAPK also phosphorylates and thereby stimulates CBP — perhaps by inducing a conformational change that unmasks CBP's activation domains — ERK-MAPK plays a dual role in the activation of the SRE (Fig. 2a).

The cAMP–PKA pathway of gene activation may similarly involve phosphorylation of both CREB, allowing it to recruit CBP, and of CBP, increasing its coactivation potential (Fig. 2b). Phosphorylation of CREB at serine 142 by Ca^{2+}/calmodulin-dependent protein kinase II blocks the activation of CREB by cAMP. This phosphorylation does not interfere with the ability of CREB — also phosphorylated at serine 133 — to interact with CBP, demonstrating that interaction of CREB and CBP is not sufficient for coactivation. Functional cooperation may require an induced conformational change in CBP that is precluded by phosphorylation of CREB at serine 142 [22].

Coactivation by the CAAT/enhancer-binding protein NF-M and CBP is detectable in the presence of oncogenic Ras [10]. Oncogenic Ras triggers activation of ERK-MAPK, which phosphorylates both NF-M and CBP; the latter phosphorylation increases CBP's coactivation potential, and the former may induce a conformational changes that leads to an interaction between NF-M and CBP (Fig. 2c). Other hypotheses cannot be ruled out — the phosphorylation of CBP may be required for the NF-M–CBP interaction, or NF-M and CBP may cooperate less directly (the two proteins have not yet been shown to interact physically).

Concluding remarks

How many more CBP/p300 interaction partners — either sequence-specific transcription factors, coactivators or components of the basal transcription machinery — will be discovered in the near future? There are probably a plethora of them. And mammals may have more members of the CBP/p300 family. Will all members of this family turn out to act similarly? Although CBP and p300 can substitute for each other in their interactions with CREB, nuclear hormone receptors and c-Myb [2,7,10], the DRF1 and DRF2 transcription factor complexes that regulate c-*jun* induction by retinoic acid appear to contain p300 but not CBP [19]. Whether CBP or p300 is present, the precise three-dimensional geometry of the multiple CBP/p300 contacts with such complexes is presumably critical for efficient stimulation (or inhibition) or transcriptional initiation.

What phenotype would CBP or p300 knockout mice be expected to have? If CBP and p300 can widely substitute for each other, little effect may be noticeable. If CBP and p300 perform different functions, however, given their promiscuity one might expect the knockout mice to have pleiotropic defects, some of which may be exhibited even in a hemizygous animal. In this regard, the autosomal dominant Rubinstein-Taybi syndrome is associated with the mutation of one CBP allele [23], but it is still possible that another, unknown gene causes this disease. Analysis of a small number of colorectal and gastric carcinomas revealed that two of them displayed the loss of one p300 allele and a somatic point mutation in the other one [24]. This, together with the ability of p300 to suppress cell transformation by E1A [25], points to the possibility that p300 is a tumor suppressor, as had originally been suspected from its discovery as an E1A-associated protein.

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