REVIEW

CBP/p300 in cell growth, transformation, and development

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CREB binding protein (CBP) and p300 were both identified initially in protein interaction assays–the former through its association with the transcription factor CREB (Chrivia et al. 1993) and the latter through its interaction with the adenoviral-transforming protein E1A (Stein et al. 1990; Eckner et al. 1994). The recognition that these two proteins, one involved in transcription and the other in cell transformation, had highly conserved sequences suggested that they had the potential to participate in a variety of cellular functions (Fig. 1). Several excellent reviews (Janknecht and Hunter 1996; Shikama et al. 1997; Giles et al. 1998) have addressed the transcriptional coactivator functions of CBP/p300; this review focuses on the involvement of these proteins in the complex biological processes that affect cell growth, transformation, and development.

Involvement of CBP/p300 in cell growth and transformation

CBP/p300 as tumor suppressors

One of the major paradoxes in CBP/p300 function is that these proteins appear to be capable of contributing to diametrically opposed cellular processes. This section begins with a review of the evidence that CBP and p300 participate in various tumor-suppressor pathways. It ends with the demonstration that these coactivators are essential for the actions of many oncogenes. Whether CBP and p300 promote apoptosis or cell proliferation appears to be highly context dependent.

Several characteristics of CBP and p300 suggested that these proteins might serve as tumor suppressors, but clear evidence for this function awaited the studies of Kung et al. (2000). Mice engineered to contain a null mutation in one CBP allele developed a variety of hematological abnormalities, including extramedullary myelopoiesis and erythropoiesis, lymph node hyperplasia, and splenomegaly. Most of these effects were attributed to an underlying bone marrow failure. As the mutant animals aged, they developed a strikingly high incidence of hematological malignancies, including histiocytic sarcomas, monomyelocytic leukemia, and lymphocytic leukemia. More aggressive forms of leukemia developed in sublethally irradiated recipients transplanted with bone marrow or splenocytes from the CBP heterozygotes. Overall, nearly 40% of the CBP heterozygotes either developed tumors or harbored tumorigenic cells that were revealed by the transplantation assay. Analysis of tumors isolated from the irradiated recipients revealed that the wild-type CBP allele was typically lost as well. This loss of heterozygosity in a population of cells that undergoes transformation is a hallmark feature of a tumor-suppressor gene. As discussed below, human patients with the Rubinstein-Taybi syndrome (RTS), due to CBP heterozygosity, also have an increased incidence of malignancy. Surprisingly, the hematological defects and cancer predisposition were not seen in mice that contained the identical mutation in one p300 allele. However, a tumor-suppressor role for p300 in humans is not inconceivable. Muroaka et al. (1996) identified p300 missense mutations associated with loss of heterozygosity in tumors from two patients, one with colorectal and the other with gastric carcinoma, and Gayther et al. (2000) recently reported five additional examples.

The growth suppression functions of CBP and p300 are also exemplified by their interactions with the tumor suppressor p53. Most of the critical functions of p53 are believed to occur through its ability to activate genes involved in the response to DNA damage, such as murine double minute (mdm-2), p21, cyclin G, and bax. Studies have shown that p53 interacts with a carboxy-terminal region of CBP/p300 and that this interaction contributes to transcriptional activation of the p53-responsive mdm-2, p21, and bax promoters (Avantaggiati et al. 1997; Gu et al. 1997; Lill et al. 1997). Because adenovirus E1A blocks CBP/p300 function (see below), it has been suggested that at least some of its effects on cell transformation might occur by inhibiting the actions of p53. Conversely, the growth suppression activities of CBP and p300 have been attributed to their ability to augment p53-mediated transcription. In addition to its transcriptional activation functions, p53 negatively regulates genes whose promoters do not contain a suitable binding site. Avanaggiati et al. (1997) have suggested that the association of p53 and p300 might account for

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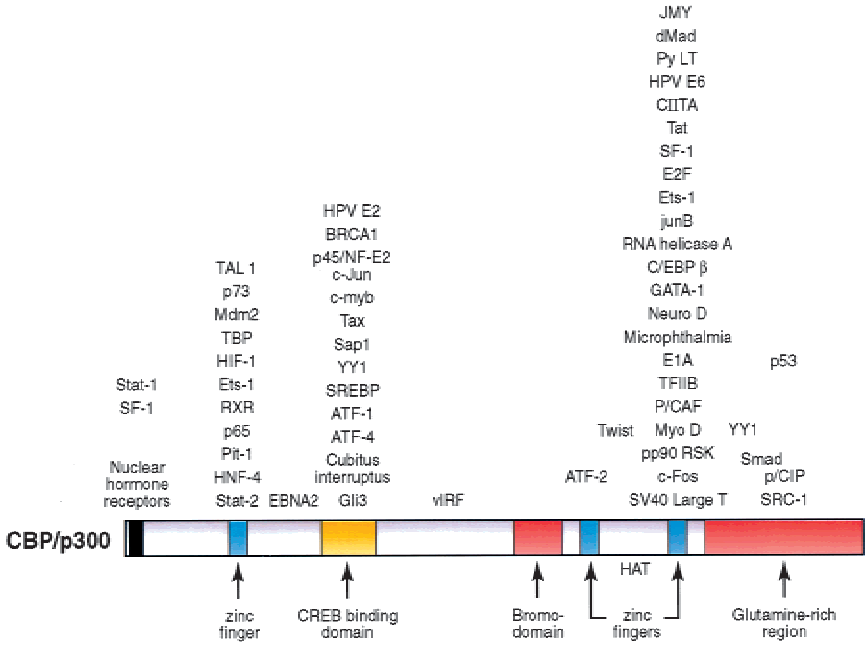

Figure 1. CBP/p300 organization and interactions. Interacting proteins are shown at the top of the figure; functional domains are depicted below. Many known interactions are not included due to space limitations.

this effect, presumably by limiting access of the coactivator to promoter-bound transcription factors such as AP-1. p53 has also been shown to bind to the first zinc finger region of CBP/p300 (Grossman et al. 1998; Wadgaonkar and Collins 1999), and this interaction has been proposed to participate in both p53 transcriptional activation and in targeting the tumor suppressor protein toward a degradation pathway (see below). A p300-interacting factor called JMY has been identified and proposed to augment p53-dependent apoptosis (Shikama et al. 1999).

The finding by Gu and Roeder (1997) that p53 could be acetylated by p300 uncovered a previously unrecognized aspect of CBP/p300 function. Acetylation of specific lysine residues in the carboxyl terminus of p53 was found to increase DNA binding dramatically, presumably by altering the conformation of an inhibitory regulatory domain. One model for how this might occur is that the neutralization of positively charged lysine residues through acetylation might disrupt interactions between the p53 carboxyl terminus and the core DNA-binding domain. The ability of p53 to be acetylated in vivo was confirmed subsequently by using acetylation-specific antibodies (Sakaguchi et al. 1998; Liu et al. 1999a). Lambert et al. (1998) have recently reported that ionizing irradiation promotes the amino-terminal phosphorylation of p53, resulting in an increased affinity for CBP/p300 and an accompanying stimulation of p53 acetylation. Amino-terminal phosphorylation also decreases the affinity of p53 for mdm-2 (Chehab et al. 1999; Unger et al. 1999), a protein that inhibits p53 function through a variety of mechanisms (for review, see Lozano and Oca Luna 1998).

The correlation between histone acetylation and gene activation has been reviewed extensively (for example, see Strahl and Allis 2000). The studies of Gu and Roeder (1997) provided the first demonstration that acetylation can regulate the transcriptional functions of non-histone proteins as well. Several additional transcriptional regulators were shown subsequently shown to be modified by acetylation, including EKLF, GATA-1, dTCF, NF-Y, TFIIE, TFIIF, HMGI(Y), c-*myb*, myo-D, HIV-1 Tat, and E2F (Imhof et al. 1997; Boyes et al. 1998; Li et al. 1998; Munshi et al. 1998; Waltzer and Bienz 1998; Zhang and Bieker 1998; Ott et al. 1999; Sartorelli et al. 1999; Martinez-Balbas et al. 2000; Tomita et al. 2000). However, in most instances, precisely how acetylation affects the transcriptional properties of these other proteins is not completely understood.

CBP/p300 may also contribute to the mechanisms that regulate p53 degradation. For example, the p53 protein is known to be stabilized in cells that express adenovirus E1A. As described above, E1A blocks the ability of p53 to induce its target genes, such as mdm2. Because mdm2 is implicated in p53 degradation (Haupt et al. 1997; Kubbutat et al. 1997), the block of mdm-2 expression by E1A would be expected to suppress a negative feedback loop (Wu et al. 1993), wherein the p53-mediated induction of mdm-2 promotes p53 degradation. Grossman et al. (1998) have shown that both p53 and mdm-2 bind to the first zinc finger domain of p300. mdm-2 mutants incapable of interacting with p300 (but still able to bind to p53) are defective for inducing p53 degradation. Similarly, p53 mutants rendered incapable of interacting with p300 escape mdm-2-mediated degradation. These findings led to the suggestion that interaction of mdm-2 and p53 with p300 is required for p53 turnover. One possible problem with this model is that

the degradation of p53 is believed to occur in a cytoplasmic compartment (Freedman and Levine 1998). How the nuclear p300 might assist mdm-2 in this cytoplasmic p53 degradation process is unclear. In contrast, Yuan et al. (1999a) suggested that p300 might actually stabilize p53. Using cells engineered to express ribozymes directed against either CBP or p300, these researchers also argued that p53 interacts specifically with p300, and not with CBP, to mediate an apoptotic response (Yuan et al. 1999b). These contrasting models reveal an unresolved question—if p300 assists mdm-2 in degrading p53, how does it also function as a tumor suppressor? Further studies confirming the tumor suppressor role of CBP, and not of p300, and confirmation of the specificity of the p53–p300 interaction, will be required to address this issue.

mdm-2 may also block the function of p53 (and related proteins) by competing for CBP/p300 binding. For example, the p53 homolog p73 binds to the first zinc finger domain of CBP/p300 and mdm-2 may function in part by preventing this interaction (Zeng et al. 1999). A related mechanism was proposed for p53 (Wadgaonkar and Collins 1999), whereby binding of mdm-2 to the p53 activation domain was proposed to prevent its ability to associate with CBP/p300.

Another tumor suppressor that uses CBP/p300 is BRCA-1, mutations in which cause breast and ovarian cancers. Although BRCA-1 is believed to mediate most of its key functions by participating in the maintenance of genomic integrity and response to DNA damage, it has also been proposed to serve as a transcriptional regulator (Chapman and Verma 1996). Studies have shown that Gal–BRCA-1 fusion proteins can activate Gal reporter genes, that BRCA-1 associates with the RNA polymerase II holoenzyme complex, and that BRCA-1, in concert with p53, can activate the mdm-2 promoter (Scully et al. 1997; Ouchi et al. 1998). The ability of BRCA-1 to activate expression of the p21 gene may contribute to its tumor suppressor activity (Missero et al. 1995). The transcriptional activation mediated by BRCA-1 is augmented by CBP/p300 and p300 has been shown to interact with BRCA-1 biochemically (Pao et al. 2000). This interaction appears to occur through the "CREB binding domain" of CBP/p300 but does not require BRCA-1 phosphorylation.

CBP and p300 may serve as tumor suppressors through more indirect mechanisms as well. For example, CBP has been shown to be a negative regulator of the Wnt pathway in *Drosophila* (Waltzer and Bienz 1998; see below). Because activation of the Wnt pathway induces c-*myc* and cyclin D1 (at least in mammalian cells) (He et al. 1998; Tetsu and McCormick 1999), loss of CBP would be expected to activate these Wnt targets, with a resultant induction of tumor formation (Fig. 2).

Figure 2. Model for the suppressive effect of CBP/p300 on expression of c-*myc*, cyclin D1.

Chromosomal translocations

The participation of CBP/p300 in cell transformation is perhaps most evident from the study of various forms of leukemia that are associated with CBP-disrupting chromosomal translocations (Fig. 3). Unlike the constitutional CBP mutations in the RTS (see below), the translocations that lead to leukemia fuse CBP sequences onto other genes and are invariably somatic. The first of these translocations to be characterized in detail was the t(8;16)(p11;p13) associated with the M4/M5 subtype of acute myeloid leukemia (AML). This translocation fuses the gene encoding MOZ (monocytic leukemia zinc finger protein) onto the amino terminus of CBP (Borrow et al. 1996). The t(8;16) translocation is fairly rare, accounting for only ~4 in 1000 patients with AML. The reading frame of the MOZ–CBP fusion is maintained by a 2-bp insertion of unknown origin, whereas the reciprocal CBP–MOZ fusion is out of frame beyond the CBP amino terminus. Thus, it is believed that the 5′-MOZ–CBP-3′ protein is responsible for transformation. MOZ is related to the SAS proteins of yeast, which have been proposed to encode histone acetyltransferases (HATs) (Reifsnyder et al. 1996; Roth 1996). Although neither MOZ nor SAS2 has actually been shown to contain HAT activity, this activity has been detected in the structurally related mammalian protein Tip60 (human immunodeficiency virus (HIV) tat-interacting protein; Yamamoto and Horikoshi 1997), the yeast Esa-1 (Smith et al. 1998), and the *Drosophila* dosage compensation gene *Mof* (Akhtar and Becker 2000). It has been hypothesized that the addition of the MOZ acetyltransferase domain onto CBP results in aberrant chromatin acetylation, but precisely how this occurs is not clear. Acetyltransferase function is typically associated with gene activation, whereas the phenotypes of yeast SAS2 mutants suggest that this protein is involved in silencing (thus the name, something about silencing). However, the specific acetylation targets of SAS2/MOZ have not been determined; therefore, at this point it is unknown whether the MOZ component of the fusion gene confers inappropriate activation or silencing functions. Other translocations involving MOZ sequences have also been linked to myeloproliferative disorders. One particularly interesting example fuses MOZ onto the amino terminus of the nuclear receptor transcriptional coactivator TIF-2/GRIP-1/NCoA-2. This fusion places the zinc finger domains, nuclear localization signal, HAT, and acidic domains of MOZ adjacent to a region of TIF-2 that has been shown to interact biochemically with CBP (Carapeti et al. 1998). Although the most compelling explanation for the transforming effects of the MOZ–CBP (or MOZ–TIF-2) fusions is that the MOZ component contributes a deleterious property to CBP, it is also possible that the transforming activity could result from the deletion of the CBP amino terminus. W. Yuan and R.H. Goodman (unpubl. observations) have shown that this portion of CBP/p300 contains a phosphorylation site for protein kinase C, that downregulates coactivator function in a cell-cycle dependent manner.

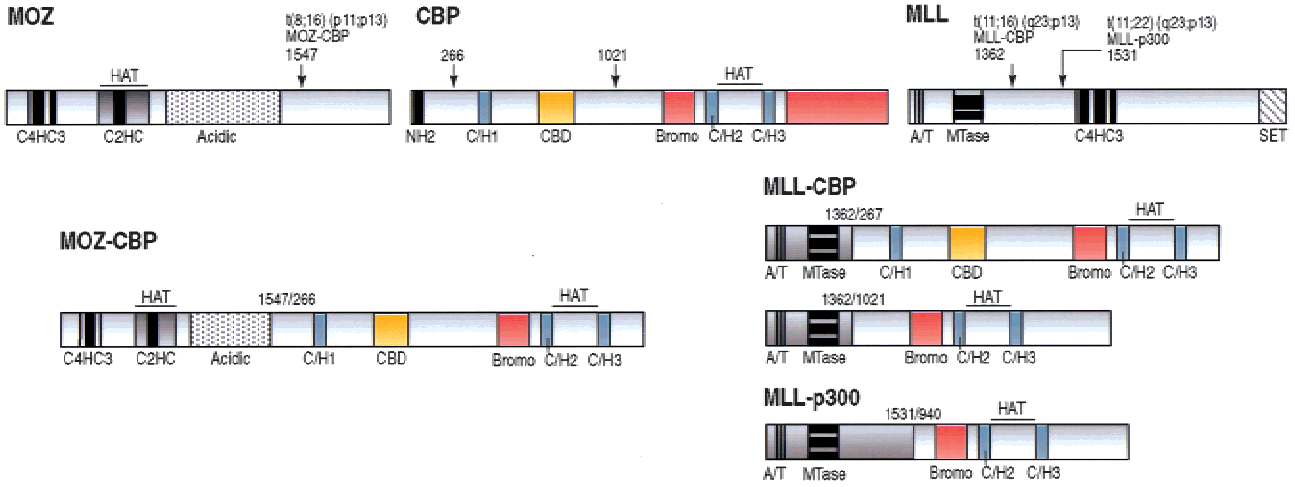

Figure 3. Chromosomal translocations involving CBP/p300. On the top of the figure are depictions of the intact MOZ, CBP, and MLL genes. Below are MOZ–CBP, MLL–CBP, and MLL–p300 translocations associated with human malignancies. Functional domains in CBP/p300 are as described in Fig. 1. The zinc finger HAT and acidic domains of MOZ, as well as the AT hook, methyltransferase, and SET domains of MLL, are depicted.

Translocations involving the MLL gene (mixed lineage leukemia, also called ALL and HRX) account for up to 80% of infant leukemias and the majority of leukemias and myelodysplasias that occur after treatment with topoisomerase II inhibitors, such as the etoposides. These drugs are used as chemotherapeutic agents for treatment of a variety of malignancies. In one such translocation, t(11:16)(q23;p13.3), a large portion of CBP, containing all but the most amino-terminal sequences, is fused onto the 3′ end of the gene encoding MLL (Rowley et al. 1997; Sobulo et al. 1997). MLL is highly related to the *Drosophila* trithorax group genes, which are believed to function during development by maintaining specific chromatin domains in their open configuration. A similar role has been proposed for the mammalian MLL (Yu et al. 1998). It has been suggested that this action might be mediated through the SET domain of MLL, which interacts with components of the Swi/Snf complex (Rozenblatt-Rosen et al. 1998). Other studies have argued that the trithorax group and Swi/Snf proteins exist in separate complexes, at least in *Drosophila* (Papoulas et al. 1998). The portion of MLL included in the MLL–CBP fusion protein does not include the PHD, transcriptional activation, or SET domains, but does contain other motifs that may contribute to its oncogenic potential, such as the AT hooks and a methyltransferase homology region that acts functionally as a repression domain. Presumably, both the CBP and MLL genes contain sequences that are particularly susceptible to rearrangement after exposure to chemotherapeutic drugs that target topoisomerase II. The underlying genomic instability of sequences within the CBP gene may also be reflected by the prevalence of translocations and deletions that result in the RTS (see below). For example, six of the seven translocations associated with this syndrome, as well as six of the microdeletion breakpoints, fall within a 13-kb region (Petrij et al. 2000). Although much less common, translocations involving MLL and p300 have also been detected and are associated with AML (Ida et al. 1997).

In contrast to the examples discussed above, CBP and p300 are involved with the products of some leukemogenic chromosomal translocations through protein–protein interactions. One such example involves the translocation t(7;11)(p15;p15), which is also associated with AMC (Kasper et al. 1999). In this translocation, the amino terminus of the nuclear pore complex (NPC) protein NUP98 is fused onto the carboxyl terminus of the homeotic transcription factor HOXA9. DNA binding of the fusion protein is mediated by the HOXA9 component and is required for transformation. The NUP98 segment contributes a transcriptional activation function that is also required for transformation. The ability of the NUP98 portion to activate transcription depends on a region termed the FG (Phe–Gly) repeat, which is found in several other NPC proteins as well. The FG repeat reportedly activates transcription by interacting with CBP. Moreover, mutations that block the interaction of the FG repeats with CBP prevent both transactivation and transformation. Interestingly, chromosomal translocations involving other NPC genes have also been associated with leukemia and, in these instances, the FG repeats similarly appear to be essential for transformation. Thus, CBP recruitment through FG repeat elements might provide a general mechanism for the actions of a family of leukemia-associated translocations.

Similar CBP/p300 interactions may contribute to disorders caused by the leukemia-associated transcription factors AML-1 and TAL-1. The AML-1 gene is the most common target of translocations involved in human leukemia. Kitabayashi et al. (1998) reported that AML-1 interacts with an amino-terminal domain in CBP/p300 and that p300 stimulates AML-1-dependent transcription and cell differentiation. p300 was also reported to serve as a transcriptional coactivator for TAL-1, which was originally identified through its involvement in a

chromosomal translocation associated with T-cell acute lymphoblastic leukemia (T-ALL; Huang et al. 1999). T-ALL is occasionally caused by chromosomal rearrangements that increase TAL-1 expression without forming a novel fusion protein, therefore it is likely that the inappropriate expression of TAL-1, rather than the addition or deletion of extraneous functional domains, is responsible for transformation. Huang et al. (1999) showed that the basic helix–loop–helix (bHLH) domain of TAL-1 interacts with the first zinc finger domain of p300 and that this interaction augments TAL-1-mediated transcriptional activation. Interestingly, interaction of the two proteins in erythroleukemia cells occurred only after treatment with dimethylsulfoxide, which induces differentiation in this cell line.

Involvement of CBP/p300 in the actions of cellular oncogenes

Not surprisingly, many other oncogenic transcription factors also mediate their effects through CBP/p300. One such example is the AP-1 complex (Arias et al. 1994). Both components of AP-1, c-*jun* and c-*fos*, appear to interact with CBP/p300, but the mechanisms underlying these interactions remain poorly defined. Initial studies indicated that binding of CBP required c-*jun* to be phosphorylated by the jun kinase JNK (Arias et al. 1994; Bannister et al. 1995). Subsequently, a phosphorylation independent interaction between CBP and c-*jun* was detected (Lee et al. 1996). An interaction between the c-*fos* activation domain and the third zinc finger domain of CBP also occurs independently of phosphorylation (Bannister and Kouzarides 1995). Whether the two components of the AP-1 complex interact simultaneously with a single CBP molecule has not been determined.

Somewhat better characterized is the interaction between CBP and the proto-oncogene c-*myb*. c-*myb* is the cellular progenitor of the v-*myb* oncogene, which is carried by the avian myeloblastosis virus (AMV). Dai et al. (1996) showed that the ability of c-*myb* to activate transcription depended on its interaction with the CREB-binding domain (also called the KIX domain) of CBP. CBP interacts with v-*myb* as well and potentiates the abilities of both the cellular and viral proteins to activate their target genes, such as mim-1 (Oelgeschlager et al. 1996). The activation of mim-1 by c-*myb* was shown to be markedly potentiated by addition of another transcription factor NF-M, a member of the CCAAT/enhancer-binding protein (C/EBP) family. Although direct binding between NF-M and CBP could not be detected, CBP was found to increase NF-M-mediated transcription (Oelgeschlager et al. 1996). Thus, the synergy between c-*myb* and NF-M was proposed to occur through their simultaneous interactions with CBP. In support of this model, Mink et al. (1997) showed that another C/EBP family member, C/EBPβ, binds to the third zinc finger region of CBP/p300 and that this interaction augmented C/EBPβ-mediated transcriptional activation.

A similar mode of cooperative binding to CBP might contribute to the activity of the AMV–E26-transforming protein. AMV–E26 encodes a tripartite protein that contains, in addition to myb, portions of the proteins gag and ets-1. The region of ets-1 retained in the fusion protein binds to the first zinc finger domain of CBP/p300 (Yang et al. 1998), therefore it is possible that CBP/p300 could interact with two components of the E26-transforming protein simultaneously. Although relatively little is known about the interactions between C/EBP, ets-1, and CBP/p300, the interaction of CBP with c-*myb* has been studied in some detail. For example, Parker et al. (1999) have determined that the domain of c-*myb* that interacts with CBP is highly related structurally to the interaction domain of phosphorylated CREB. However, the affinity of c-*myb* for CBP is much lower than that of CREB. It is possible that the cooperativity provided by NF-M and ets-1 are required for c-*myb* to associate effectively with CBP.

The E2F1 transcription factor controls a variety of promoters involved in cell cycle progression, including those in the c-*myc*, dihydrofolate reductase (DHFR), and cdc-2 genes. Recent evidence suggests that interactions with CBP/p300 are in part responsible for some of these actions. During G_1, E2F1 activity is repressed by the hypophosphorylated form of Rb and this repression must be overcome for cells to progress through the cell cycle. Trouche et al. (1996) reported that the transcriptional activity of E2F1 depends on an amino-terminal sequence that interacts in vitro with the third zinc finger domain of CBP. These workers also found that CBP increases E2F1-mediated transcriptional activation and proposed that the ability of E1A to stimulate E2F1 directed transcription might be due, at least partially, to its ability to deliver CBP to E2F1-responsive promoters, while at the same time removing Rb. However, the biochemical association of CBP and E2F1 could not be demonstrated in vivo. This group has recently extended this model by showing that E2F1 can be acetylated by P/CAF, a CBP/p300-associated factor, and to a lessor degree by CBP/p300 itself (Martinez-Balbas et al. 2000). Acetylation of E2F1 was reported to increase its ability to bind DNA, stimulate its transcriptional activity, and prolong its half-life. These acetylation-induced changes were shown to be reversed by Rb, which is known to associate with histone deacetylases (Brehm et al. 1998; Luo et al. 1998).

The causal relationships between CBP/p300 and other proteins associated with malignancy are more tenuous. For example, CBP/p300 has been reported to associate with the promyelocytic oncoprotein in nuclear bodies (also called PML oncogenic domains or PODs; LaMorte et al. 1998; Doucas et al. 1999), but how these associations relate to cell transformation is unclear. It is possible that the colocalization of CBP/p300 with PML contributes to the link between RAR signaling and certain forms of leukemia.

Association of CBP/p300 with viral oncoproteins

Some viral oncoproteins, such as v-*myb* and the human T-cell leukemia virus (HTLV-1)–Tax, require CBP/p300

for their transcriptional activities, whereas others, such as adenovirus E1A, simian virus (SV) 40 T antigen, and human papillomavirus E6, appear to block CBP/p300 functions. The viral oncoproteins that use CBP/p300 as a coactivator appear to do so by interacting with the CREB binding domain. These viral transactivators must also be capable of binding, either directly or indirectly, to appropriate sites in gene promoters. Viral oncoproteins that block CBP/p300 function typically do not bind DNA. These proteins tend to interact with the third zinc finger domain, a region that has also been shown to mediate interactions with positive effectors of CBP/p300 action, such as RNA helicase A, TFIIB, and the histone acetylase P/CAF.

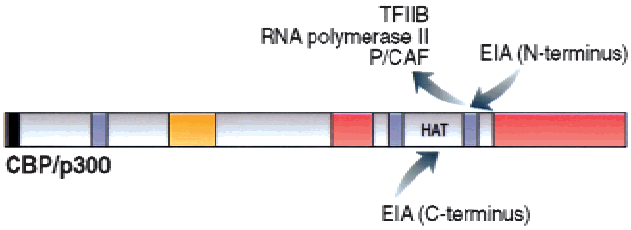

Figure 4. Models for the E1A inhibition of CBP/p300. Interaction of the E1A amino terminus with the third zinc finger domain of CBP/p300 is proposed to displace TFIIB, the RNA polymerase II holoenzyme, and P/CAF. Interactions involving other associated factors may be disrupted as well. The carboxyl terminus of E1A is proposed to inhibit CBP/p300 HAT function directly.

DNA tumor viruses

Adenovirus E1A

Of all the CBP/p300-interacting viral oncoproteins, perhaps the best studied is the adenoviral E1A protein. The early region of adenovirus gives rise to two proteins, designated 12S and 13S E1A. 12S E1A contains four principal protein interaction domains that have been shown to be important for transformation. The carboxyl terminus encodes conserved region 2 (CR2), which associates with Rb and related proteins, and the carboxy-terminal binding protein (CtBP) interaction motif (for review, see Moran 1993). The latter region has been reported to negatively regulate transformation, as deletion of this region increases the number of transformants in cells transfected with E1A and ras (Boyd et al. 1993). This effect has been attributed to the ability of CtBP to interact with histone deacetylases, although other mechanisms have been proposed (Sundqvist et al. 1998). The amino terminus of E1A, along with a region designated conserved region 1 (CR1), interacts with CBP/p300 (Stein et al. 1990; Arany et al. 1995; Lundblad et al. 1995) and is believed to mediate cell immortalization. The importance of the amino-terminal interaction for transformation is apparent from the numerous studies showing that E1A-mediated transformation is blocked by overexpression of CBP/p300 (for example, see Smits et al. 1996). Similarly, overexpression of the p300/CBP-associated factor P/CAF suppresses the cell cycle progressive effects of E1A (Yang et al. 1996). Recently, it has been reported that the E1A proteins of oncogenic (adenovirus 12) and nononcogenic (adenovirus 2/5) viruses differ in their mechanisms of binding to CBP/p300 (Lipinski et al. 1999). Further studies are required to determine whether these differences contribute to oncogenicity.

Several studies have shown that 12S E1A blocks CBP/p300 coactivator functions, but precisely how this occurs is not clear (Fig. 4). As discussed above, E1A binds to the same region of CBP/p300 that interacts with TFIIB and components of the RNA polymerase II holoenzyme complex (Kwok et al. 1994; Nakajima et al. 1997; Kim et al. 1998; Felzien et al. 1999). Thus, it is likely that E1A functions in part by preventing these factors from associating with the coactivator. Similarly, E1A has been reported to block the association of HATs, such as P/CAF, which bind to the same region (Yang et al. 1996). Both of these models are supported by the recent studies of Kraus et al. (1999), which indicated that a GST–E1A fusion protein containing only the amino-terminal CBP/p300-interacting sequences of E1A blocked p300-directed transcription in the context of reconstituted chromatin templates. In addition, direct binding of E1A to the HAT or third zinc finger domains of CBP/p300 has been detected and shown to inhibit acetyltransferase activity (Chakravarti et al. 1999; Hamamori et al. 1999; Perissi et al. 1999). This effect was shown to be mediated primarily through the carboxy-terminal region of E1A (Chakravarti et al. 1999). The level of E1A required for this direct block is fairly high, but perhaps not unreasonable when compared to that seen after acute viral infection. Interactions of E1A with other domains of CBP/p300 have been reported (Kurokawa et al. 1998; Lipinski et al. 1999), and one group has found that E1A increases CBP/p300 HAT activity (Ait-Si-Ali et al. 1998).

Although there is no dearth of explanations for why E1A might block the various CBP/p300 activities, several important issues remain unsettled. First, despite the high levels of E1A present in adenovirally infected 293 cells, CBP/p300-mediated gene expression appears normal (Nakajima et al. 1997). Thus, it is conceivable that the block of CBP/p300 caused by E1A could occur only under the artificial conditions inherent in transfection or in vitro transcription assays. Second, the mechanism through which CBP/p300 overcomes E1A-mediated gene repression and cell transformation (Smits et al. 1996) may not be as simple as it appears. Although this finding has been used to argue that the actions of E1A occur through inhibition of CBP/p300, it is also possible that excess CBP/p300 simply titrates the adenoviral protein away from other targets. Finally, it is not clear why a block by E1A is conclusive proof of the involvement of CBP/p300 in any specific transcriptional or cellular event (for example, see discussion by Li et al. 1999). E1A has been shown to inhibit the actions of general transcription factors (Kraus et al. 1994; Song et al. 1995), interfere with components of the mediator complex (Boyer et al. 1999), and, in some systems, block function

of the chromatin remodeling machinery (Miller et al. 1996; Li et al. 1999). This plethora of targets raises the concern that E1A may not be very useful as a specific probe of CBP/p300 function.

SV40 T antigen

The early region of SV40 encodes two proteins, the large T and small t antigens. Large T is the primary transforming agent of SV40 and its ability to transform cells depends on three domains comprising residues 1–82, 105–114, and 351–626. The latter two regions interact with Rb and p53, respectively. Several lines of evidence have suggested that the 1–82 region interacts with CBP/p300. For example, early studies had shown that T-antigen proteins containing mutations in the 1–82 region could not mediate transformation (Yaciuk et al. 1991). Coexpression of adenovirus E1A restored the transformation capability of these mutants, but E1A proteins rendered incapable of binding p300 were ineffective. Similarly, transformation-defective forms of T-antigen that bound to p300 were found to restore the transforming properties of E1A mutants lacking their p300 binding sites (Quartin et al. 1994). These and other studies led to the suggestion that transformation by T-antigen might require interactions with p300.

Biochemical confirmation of the p300/T-antigen interaction was provided by Avantaggiati et al. (1996) and Eckner et al. (1996a). The former group showed that T-antigen interacts exclusively with the unphosphorylated form of p300. Because p300 is differentially phosphorylated during the cell cycle, this property could have implications for T-antigen-mediated transformation. T-antigen was found to block activation of a CRE reporter gene through the CREB/protein kinase A pathway only in cells that contained unphosphorylated p300. (In contrast, the E1A block was not influenced by the phosphorylation state of CBP/p300; see also Kitabayashi et al. 1995.) The inability of SV40-transformed rat embryo fibroblasts to express a CRE reporter gene in response to forskolin, an activator of adenylyl cyclase, is consistent with the idea that T-antigen blocks p300 function. Both groups found that T-antigen binds primarily to the third zinc finger domain of CBP/p300, a region that mediates interactions with many effectors of coactivator function.

Human papillomavirus

Like adenovirus E1A and SV40 T-antigen, the E6 protein of human papillomavirus (HPV) achieves its transformation capability by interacting with critical cellular transcriptional regulators. The best characterized action of E6 involves its ability to direct the tumor suppressor p53 toward degradation. In this process, E6 recruits p53 into a complex that contains the ubiquitin ligase E6AP (Scheffner et al. 1993) and the subsequent ubiquitination of p53 leads to its rapid degradation. Zimmermann et al. (1999) have recently shown that the E6 proteins from "high risk" (associated with invasive cervical carcinoma), but not "low risk," HPV bind to the third zinc finger region of CBP/p300 (and possibly additional regions; see Patel et al. 1999). This interaction displaces p53 from its binding site on CBP/p300, resulting in a block of p53-mediated transcriptional activation. Whether E6 also blocks the ability of CBP/p300 to acetylate p53 is unknown.

The E2 gene product of HPV, in contrast to E6, appears to play an inhibitory role in carcinogenesis. Indeed, studies have shown that the E2 gene is frequently disrupted in HPV isolates from cervical carcinomas (Baker et al. 1987). Lee et al. (2000) have shown that the HPV E2 protein interacts with the CREB-binding domain of CBP and that this interaction activates E2-dependent transcription. Presumably, activation of some of these E2-dependent genes contribute to the growth arrest of cells infected with low risk HPV.

Other viruses

Other viral-transforming proteins appear to use CBP/p300 through somewhat more obscure mechanisms. For example, the Kaposi sarcoma-associated herpesvirus protein vIRF is believed to transform NIH 3T3 cells by inducing expression of c-*myc*. vIRF does not bind DNA directly, but rather activates an element termed the PRF (plasmacytoma repressor factor) in the c-*myc* promoter by interacting with an as yet undefined member of the interferon-stimulated response element (ISRE)-binding factor family. vIRF binds to a region of CBP/p300 located between the CREB binding domain and the second zinc finger, an unusual site for transcription factor interaction (Jayachandra et al. 1999). Remarkably, vIRF function appears to be stimulated by CBP and repressed by p300.

The large T antigen of polyomavirus (PyLT) is similar to E1A and SV40 large T antigen in its ability to affect cell function through interactions with Rb. PyLT (like E1A and SV40 T antigen) binds to the third zinc finger region of CBP/p300 and this interaction suppresses p300-mediated transcriptional activation (Nemethova and Wintersberger 1999). Interestingly, PyLT has been reported to interact with a CBP/p300 fraction that has elevated HAT activity. Whether this fraction has a specifically altered phosphorylation pattern has not been determined.

The Epstein-Barr virus (EBV)-induced nuclear antigen 2 (EBNA2), associated with EBV-related lymphoproliferative disorders, is also believed to transform cells by inducting the PRF element in c-*myc*. EBNA2 binds CBP/p300 through a region between the first zinc finger and the CREB binding domain and, as with vIRF, CBP augments its ability to activate transcription, whereas p300 functions as a repressor (Jayachandra et al. 1999). How the different effects of CBP and p300 in these experiments pertain to their actions in normal cells (or whether they are simply a function of coactivator overexpression) is uncertain. A recent study suggested that the ability of EBNA2 to activate the LMP (latent mem-

brane protein) 1 promoter additionally depends on interactions with CBP/p300 (Wang et al. 2000b).

RNA tumor viruses

Many of the genes encoded by HTLV-1 are regulated by the virally encoded transactivator Tax, which binds to a set of degenerate CRE-like sequences in the viral long terminal repeat (LTR). The affinity of either Tax or CREB alone for these sequences is fairly low, but the combination of the two proteins results in a relatively high affinity interaction (Kwok et al. 1996; Yin and Gaynor 1996a). Precisely how this change in binding occurs is not clear, but the evidence suggests that the high affinity interaction requires Tax to interact with minor groove sequences flanking the CRE-like component of the binding site (Lenzmeier et al. 1998; Lundblad et al. 1998).

The basic leucine zipper (bZIP) domain of CREB is required for formation of the Tax–CREB–CRE ternary complex, and CREB mutants containing this domain alone are sufficient to cooperate with Tax for DNA binding (Laurance et al. 1997). The subsequent activation of the LTR occurs through multiple mechanisms and involves interactions with components of the basal transcriptional machinery, such as TFIIA (Clemens et al. 1996), and with the coactivators CBP and p300 (Kwok et al. 1996; Yin and Gaynor 1996b; Giebler et al. 1997; Harrod et al. 1998). The latter interactions have been shown to be essential for activation in in vitro transcription assays (Kashanchi et al. 1998). Because the ability of the Tax–CREB complex to recruit CBP/p300 does not involve the CREB activation domain, it is not surprising that the formation of the ternary complex occurs independently of CREB phosphorylation (Fig. 5). Consequently, unlike the process used by typical CRE-containing cellular genes, Tax directs CBP to the atypical CRE sequences in the viral promoter without requiring CREB to be phosphorylated. Fax et al. (2000) have recently proposed a very similar mechanism for activation of the adenovirus type 12 E2 promoter. In this case, CREB binds to a consensus CRE sequence in the Ad12 promoter and recruits CBP through interactions with 12S E1A.

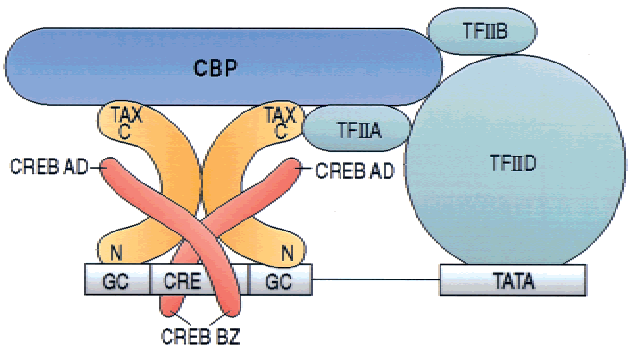

Figure 5. Model for the recruitment of CBP/p300 by HTLV-1 Tax. Binding of Tax to the CRE-like sequences in the HTLV-1 LTR requires the bZip region of CREB. The carboxyl terminus of Tax is proposed to interact with CBP/p300. In this model, the CREB activation domain (AD) is not involved in CBP binding, therefore phosphorylation of CREB is not required.

Tax may also regulate the activities of certain cellular transcription factors by competing for CBP/p300 binding. Examples of factors blocked by such a mechanism include c-*jun*, c-*myb*, p53, and p73 (Colgin and Nyborg 1998; Suzuki et al. 1999; Van Orden et al. 1999a,b; Kaida et al. 2000), all of which have been shown to interact with the CREB-binding domain of CBP/p300. In contrast, Tax and phosphorylated CREB appear to be capable of interacting with this region of CBP/p300 simultaneously (Kwok et al. 1996; Yan et al. 1998).

Regulation of CBP/p300 by phosphorylation

Most proteins involved in the control of cell growth are regulated by phosphorylation, and CBP and p300 do not appear to be exceptions. However, relatively little is known about how phosphorylation affects CBP/p300 functions. Cell cycle-dependent phosphorylation of p300 was first noted by Yaciuk and Moran (1991). Subsequently, Banerjee et al. (1994) showed that p300 could be phosphorylated by cyclin/Cdc2 and cyclin/Cdk2 and that these phosphorylation events were blocked by adenovirus E1A. Kitabayashi et al. (1995) reported that the ability of E1A or retinoic acid (RA) to stimulate the differentiation of F9 teratocarcinoma cells correlated with their abilities to induce p300 phosphorylation. The mechanism of this modulation is unclear but, in the case of E1A, phosphorylation was speculated to depend on the recruitment of cyclin-dependent kinases associated with Rb. They further showed that an E2-p300 fusion gene was transcriptionally inactive in undifferentiated F9 cells but became capable of activating transcription in cells that had been differentiated by treatment with RA. They proposed that the phosphorylation of p300 disrupts a complex termed DRF2 that normally represses c-*jun* expression and thereby blocks differentiation.

Further understanding of the functional implications of CBP/p300 phosphorylation by cell cycle-dependent kinases was provided by Perkins et al. (1997), who showed that p300 was regulated negatively by cyclin E/Cdk2. This negative regulation was shown to be blocked by the cyclin-dependent kinase inhibitor p21. Consistent with this scenario, p21 was found to increase the efficacy of p300 in mediating transcriptional activation through NF-κB. The finding that p21 augments NFκB-mediated transcriptional activation is of interest because many extracellular stimuli, including growth factors and ionizing radiation, induce p21 and NFκB in a concerted fashion. Perkins et al. (1997) proposed that the binding of cyclin E/Cdk2 to p300 provides a checkpoint that regulates the G_1/S transition, possibly allowing cells to integrate extracellular signals with aspects of their internal state before DNA replication. In all likelihood, binding of the cyclin E/Cdk2 complex requires some sort of post-translational modification, because the interaction with p300 is only seen when complexes iso-

lated from mammalian cells are tested (Felzien et al. 1999). In this model, p21 participates in a positive feedback loop, whereby activators such as p53, which depend on CBP/p300, induce p21, which then alleviates the block on CBP/p300 function mediated by cyclin E/Cdk2. Critical to confirmation of this model is the mapping of the p300 phosphorylation site, which has not yet been accomplished. It is also uncertain how these various transcriptional events might be integrated in vivo. For example, other studies have shown that p53 may actually block NFκB-mediated transcription by competing for CBP/p300 binding sites (Webster and Perkins 1999).

Phosphorylation of CBP by cyclin E/Cdk2 was also reported by Ait-Si-Ali et al. (1998). These investigators suggested that the cyclin E/Cdk2-mediated phosphorylation of CBP, which involves an as yet unidentified site near the carboxyl terminus of the protein, increases HAT activity in vitro. They went on to propose that this change might activate expression of S-phase genes that are repressed in early G_1. Surprisingly, E1A was also found to activate the CBP HAT function. This result contradicts the observations of other investigators (Chakravarti et al. 1999; Hamamori et al. 1999; Perissi et al. 1999) and may reflect methodological discrepancies. For example, the E1A and CBP fusion proteins used in the Ait-Si-Ali et al. (1998) paper may dimerize through their attached GST domains, possibly explaining the paradoxical activation.

In their initial description of CBP, Chrivia et al. (1993) showed that the transcriptional activity of CBP sequences fused to a Gal4 DNA-binding domain was augmented by the addition of PKA. They proposed that this regulation might be mediated by a consensus PKA phosphorylation site located near the third zinc finger domain. Xu et al. (1998) argued that phosphorylation of this site might also be responsible for the augmentation of Pit-1 function seen in the presence of cAMP. Using microinjection assays, these workers demonstrated that CBP proteins containing a point mutation at the consensus PKA phosphorylation site lost the ability to mediate the cAMP induction of Pit-1 function. In addition, Zanger et al. (1999) observed that cAMP activated a Gal–CBP fusion protein, but found that the consensus PKA site was not essential for this regulation and concluded that PKA might modify a component of the transcriptional machinery that is downstream from CBP. A similar conclusion was reached by Kwok et al. (1994); therefore, the argument has gone full circle. In the meantime, Swope et al. (1996) mapped the PKA responsive element to a domain near the CBP amino terminus that does not contain a consensus PKA site. At this point, it must be concluded that the mechanism underlying PKA activation of CBP/p300 remains totally unknown.

Several papers have suggested that CBP is regulated by the calcium/calmodulin (CaM)-dependent protein kinase CaMKIV (for example, see Chawla et al. 1998), and it has been proposed that this phosphorylation event might even be required for CBP-mediated transcriptional activation (Hardingham et al. 1999; Hu et al. 1999). This is an intriguing model because it suggests that CaMKIV, which is a well-known activator of CREB, might have a second essential target in the CREB–CBP pathway. However, mapping of the critical phosphorylation site on CBP remains to be accomplished. Moreover, by using a form of CREB that has been mutated so that it interacts with CBP/p300 constitutively, Cardinaux et al. (2000) found that CBP/p300 recruitment by itself is sufficient for gene activation. The differences among these various reports may relate to the utilization of Gal–CBP fusion proteins in the studies that demonstrated a dependence on CaMKIV, or the use of different cell lines.

Phosphorylation of CBP by MAPK was first reported by Janknecht and Nordheim (1996) and was proposed to augment the ability of CBP to mediate the transcriptional activity of Elk-1. (The interaction of CBP with Elk-1 occurs independently of phosphorylation.) The precise MAPK phosphorylation site in CBP remains unidentified but was reported to be localized somewhere in the carboxy-terminal domain. Liu et al. (1998) subsequently proposed that CBP was activated by nerve growth factor (NGF) in PC12 cells through a MAPK-dependent pathway. In support of this model, these workers found that the activation of a Gal–CBP fusion protein by NGF was blocked by an inhibitor or dominant negative form of MAPK and was stimulated by a constitutively activated form of MAPKK. Subsequent studies suggested that CBP/p300 and p42MAPK may associate in vivo (Liu et al. 1999b). NGF was also reported to increase phosphate incorporation into CBP, but the phosphorylation site was not identified. Ait-Si-Ali et al. (1999) additionally reported that p44MAPK could phosphorylate CBP in vitro and suggested that this modification increased the CBP HAT activity. The phosphorylation site was again determined to be somewhere near the carboxyl terminus of the protein, outside of the known HAT domain. It was proposed, therefore, that phosphorylation by p44MAPK might induce HAT function by changing the intramolecular conformation of CBP; however, whether this regulation occurs in vivo is uncertain. Serum stimulation did not induce the HAT activity of CBP in intact cells or increase the level of CBP phosphorylation.

Regulation of CBP by pp90Rsk was reported by Nakajima et al. (1996). These investigators suggested that activation of the Ras pathway by insulin or NGF recruited pp90Rsk to the third zinc finger domain of CBP and that this association prevented the binding of essential effectors of CBP function, such as the RNA polymerase II holoenzyme. Thus, this interaction could explain how activation of Ras might block transcriptional signaling through cAMP. Consistent with this model, the catalytic activity of pp90Rsk was found to be dispensable for transcriptional repression. Formation of the CBP–pp90Rsk complex was also proposed to contribute to the ability of NGF to induce neuronal differentiation. However, how pp90Rsk affects CBP signaling in other experimental paradigms remains uncertain. For example, Xing et al. (1996) have argued that the Ras activation of pp90Rsk activates the CREB pathway, which presumably must overcome the CBP block.

Involvement of CBP/p300 in the development of model systems

Many (if not most) developmental pathways in mammals culminate in interactions that involve CBP/p300. As might be expected, abnormalities in the expression of CBP/p300 result in a large number of developmental defects. In humans, these defects are displayed in a developmental disorder called the RTS. Insights into the role of CBP in development from human studies have been limited. Most studies addressing the contribution of CBP/p300 to specific developmental pathways have relied on cell culture models that, although informative, may provide only a narrow view of the complex interactions that underlie developmental events. Consequently, the best evidence for the participation of CBP/p300 in specific developmental processes in vivo may, in the end, come from model systems such as *Drosophila*. The genetic techniques in such systems allow the exploration of CBP/p300 function one pathway at a time and, in this way, make it possible to determine the contributions of CBP/p300 function to the evolution of developmental programs. One example, discussed in detail below, involves the *Drosophila* transcription factor Cubitus interruptus (CI). Although the evidence for CBP/p300 involvement in the developmental actions of the mammalian CI homologs is not quite as compelling, the parallel between CI and at least one of its mammalian homologs, Gli-3, is striking.

RTS

RTS is a human developmental disorder comprised of multiple abnormalities, including mental retardation, an unusual facial appearance, broad thumbs, and broad big toes (Rubinstein and Taybi 1963). Additional characteristic features include growth retardation, microcephaly, other digital abnormalities such as broadening of other fingers and radial deviation of the thumbs, and keloid formation. The syndrome is surprisingly common, accounting for as many as 1 of 300 patients institutionalized for mental retardation (1:100,000 newborns overall; Rubinstein 1969), and has been associated with deletions, translocations, or point mutations in the CBP gene (Petrij et al. 1995). Unfortunately, the recognition that RTS is due to mutations in CBP has not been very useful clinically, as only 3–25% of patients have deletions large enough to be detected by fluorescence in situ hybridization (FISH) or the protein truncation test (Taine et al. 1998; Petrij et al. 2000). Most studies have shown no phenotypic differences between patients with and without detectable deletions, although one report suggests that the presence of a detectable deletion by FISH correlates with a more severe phenotype (Bartsch et al. 1999). Although some of the CBP mutations may generate dominant negative isoforms, most of the evidence suggests that RTS is a haploinsufficiency disorder. Unlike the somatic CBP translocations associated with hematopoietic malignancies (discussed above), the CBP mutations in RTS are constitutional. Immediately adjacent to the CBP gene on the 3′ side is the gene responsible for familial Mediterranean fever (FMF). Although some deletions that cause RTS extend into these sequences, there is no additional symptomatology because FMF is an autosomal recessive disease.

Mouse models of RTS, generated by embryonic stem (ES) cell gene knockout technology, have provided some insights into the pathogenesis of the developmental defects, but which specific pathways account for the hallmark abnormalities of RTS remain unknown. Tanaka et al. (1997) have proposed that defects in BMP-7 signaling are responsible for some of the skeletal abnormalities in mouse CBP heterozygotes. However, whether the defects in heterozygous CBP mutant mice really resemble those in human RTS patients is questionable (see also Oike et al. 1999a). In addition, the strong contribution of genetic background effects makes it difficult to compare the mouse and human phenotypes. The phenotypic characteristics of other mouse and human mutations may actually be more informative. For example, the fact that the broad thumbs and big toes of RTS patients resemble the "extra toes" phenotype of Gli-3 mutant mice (Hui and Joyner 1993) suggests that the characteristic digital abnormalities in RTS might be due to defects in signaling of the Gli proteins. Gli-3 was shown in subsequent studies to interact biochemically with CBP (Dai et al. 1999). Thus, the idea that the digital abnormalities in RTS could relate to defective Gli-3 signaling, due to a deficiency of CBP, is not unreasonable. Indeed, patients with Greig's cephalopolysyndactyly, due to mutations in the human Gli-3 gene, have digital abnormalities that are very similar to those in RTS. Some similarities also exist between the phenotypes of RTS patients and patients with mutations in the bHLH protein TWIST, which suggests that defects in TWIST signaling could additionally contribute to the RTS symptomatology (Lowry 1990, see below). Because CBP interacts with so many different transcription factors, possibly at the same time, it is difficult to dissect the complex components of the RTS phenotype into distinct contributions from various individual transcriptional pathways. Moreover, CBP haploinsufficiency appears to affect some transcription factor pathways more than others. For example, despite the well-known dependence of the thyroid hormone receptors on CBP/p300 for function, RTS patients do not appear to have defects in thyroid function (Olson and Koenig 1997). It is possible that other nuclear hormone receptor coactivators mask the effects of CBP deficiency, or that the affinity of CBP for the activated thyroid hormone receptor is sufficiently high that a decrease in CBP level is without consequence.

As previously discussed, CPB and p300 have been proposed to be tumor suppressors and studies of RTS patients have suggested that these individuals may be at risk for developing certain types of cancer (Miller and Rubinstein 1995). The precise incidence of tumors in RTS is not known, but estimates have been in the range of 5%. Most of these tumors occur in childhood and are of neural crest origin. (This contrasts with the situation in mouse models, where CBP heterozygosity is associ-

ated primarily with hematological malignancies.) In comparison, patients with the Li-Fraumeni syndrome, due to mutations in p53, have a 50% incidence of malignancy. No systematic studies have attempted to correlate specific CBP mutations with particular types of malignancy. Moreover, it has not been determined whether the remaining CBP allele is somatically mutated in the tumors that arise in these patients.

Role of CBP/p300 during murine development

Despite clear evidence for the participation of CBP and p300 in many critical developmental processes, few studies have examined how these coactivators are regulated during embryogenesis. CBP and p300 appear to be expressed in almost identical patterns in the mouse, with a few notable exceptions (Partanen et al. 1999). For example, within the developing lung, CBP is found in the epithelium and p300 predominates in the mesenchyme. More surprisingly, given that CBP and p300 are localized exclusively in the nuclei of cultured cells (Chrivia et al. 1993), is the finding that the subcellular distribution of CBP/p300 changes during different stages of development. In the developing notochord and foregut, CBP can be detected in both cytoplasmic and nuclear compartments. p300 moves from the cytoplasm to the nucleus of notochord cells between embryonic day (E)11.5 and E12.5, but remains primarily cytoplasmic at E18.5 in the basal cells of the epidermis. Cytoplasmic localization of CBP has also been detected in developing *Drosophila* embryos (S. Smolik, unpubl.). The functional significance of the cytoplasmic CBP/p300 remains uncertain. One possibility is that this localization serves as some sort of checkpoint to ensure that cells reach a critical point of differentiation before they become sensitive to extracellular signals. The overlapping patterns of CBP and p300 expression may explain why many of the functions of these proteins are redundant.

Further insights into the developmental importance of CBP and p300 have been gained from studies of gene knockouts (Yao et al. 1998). Homozygous p300 knockouts are embryonically lethal, with the lethality occurring between E9 and E11.5. The causes of lethality appear to be multifactorial, as the knockout mice display defects in neural tube closure, cell proliferation, and cardiac development. Of note, these animals presumably express normal levels of CBP, indicating either that CBP and p300 have nonoverlapping functions, such that both coactivators are required, or that the total level of CBP and p300 is critical for normal development. Possibly both explanations are correct. Fibroblasts derived from homozygous p300 knockouts are defective for retinoic acid receptor (RAR) but not CREB signaling. It is possible that the specific defect in RAR signaling in the p300 knockout cells could result from the manner in which the p300 gene was disrupted. Insertion of a tK-NEO cassette into the first zinc finger domain of p300, as reported by Yao et al. (1998), should preserve the amino-terminal RAR interaction region that, if expressed, could specifically disrupt RAR-mediated gene activation. However, the observation by Kawasaki et al. (1998) that ribozyme-mediated ablation of p300 mRNA, but not CBP, blocks the RAR response makes this possibility unlikely.

Other studies suggest that the developmental defects result from a reduction in total CBP/p300 level. For example, homozygous CBP mutations in mice are also lethal and these animals display the same constellation of defects as mice lacking p300 (Yao et al. 1998; Oike et al. 1999b; Kung et al. 2000). The finding that CBP/p300 double heterozygotes are invariably lethal also supports the idea that critical developmental events are sensitive to the overall CBP/p300 gene dosage. On the other hand, p300 heterozygotes have an increased incidence of lethality as compared to normals, whereas CBP heterozygotes do not. However, interpretation of this result is complicated somewhat by the effects of genetic background.

As in the situation in RTS, it has not been possible to definitively link defects in specific transcriptional pathways in the knockout mice to particular developmental abnormalities. In part, this is because the lethality occurs so early in embryogenesis. Yao et al. (1998) have suggested that the cardiac abnormalities in homozygous p300 knockout mice may result from defective MEF-2 signaling, as this pathway has been shown in other studies to be important for heart development. Evaluation of the defects in hematopoiesis and vascular development observed by Oike et al. (1999b) in homozygous CBP mutants is complicated by the fact that these animals continue to express the amino-terminal half of the protein, which might function as a dominant negative. The delay in neural tube closure in CBP and p300 homozygous mutants is somewhat reminiscent of what is seen in *TWIST* mutants, which have similar abnormalities (Chen and Behringer 1995). As discussed below, mutations in *Drosophila* CBP (dCBP) cause phenotypes in *Drosophila* that are very similar to mutations in *twist* (Akimaru et al. 1997b), probably because dCBP is required for dorsal function.

The role of CBP in Drosophila *development*

As for the CBP/p300 knockout mice, flies mutant for dCBP have highly pleiotropic phenotypes, suggesting that dCBP is required for multiple developmental processes (Akimaru et al. 1997a,b). Both oogenesis and embryogenesis require dCBP function. Thus, mothers that do not express dCBP in their eggs produce embryos with the most severe dCBP mutant phenotypes. These include the loss of head and thorax structures as well as loss of both dorsal and ventral cuticular structures (Akimaru et al. 1997b). The maternal dCBP contribution can partially rescue embryos that do not express dCBP but these embryos also have a range of phenotypes, including a twisted germ band in which both mesoderm and ectoderm cells are missing, loss of naked cuticle, reduction in the hairs and bristles in the denticle belt, and occasionally, defects in the terminal structures. It is notable that the denticle belt defects do not include homeotic transformations, suggesting that dCBP does not play a

major role in the regulation of homeotic gene function, at least as it affects segmentation.

Two-hybrid screens using the CREB-binding domain of dCBP identified the transcription factor CI as a dCBP-interacting protein (Akimaru et al. 1997a). CI is the transcription factor that transduces the *hedgehog (hh)* signaling cascade into the nucleus and activates *hh* responsive genes (Alexandre et al. 1996; Dominguez et al. 1996; Hepker et al. 1997). The interaction of dCBP with CI suggests that dCBP is a part of the *hh* signaling pathway (Fig. 6).

hh and *wingless (wg)* (a gene that encodes another signaling morphogen) are two segment polarity genes whose main functions are to define the anterior/posterior polarity within each of the embryonic segments. Embryos that lack either *hh* or *wg* function are smaller than wild-type embryos, secrete cuticles that are covered with a lawn of denticles, and do not make naked cuticle (Nusslein-Volhard and Wieschaus 1980). This phenotype also characterizes many of the segment polarity genes involved in transducing either the *hh* or *wg* signals. One of the dCBP mutant phenotypes is a fusion of denticle belts and loss of naked cuticle, consistent with a role in modulating segment polarity.

In dCBP mutant embryos, the expression of *wg* gradually fades, as it does in *hh* mutant embryos. This suggests that dCBP is required for the *ci*-mediated expression of *wg* (Akimaru et al. 1997a). This model is supported by several other observations. First, haploinsufficiency for dCBP suppresses a subset of the wing defects seen in flies carrying a dominant mutation in *ci*, ci^D. Second, dCBP mutant cells in the wing disc do not express *patched (ptc)*, another *hh* target gene known to be activated by CI. Finally, biochemical and functional assays indicate that a discrete region in the CI activation domain interacts with dCBP and this interaction potentiates CI transcriptional activity (Chen et al. 2000). This region is highly conserved in the CBP binding domain of SREBP, a mammalian transcription factor that is activated by CBP in a phosphorylation-independent manner (Fig. 7). Moreover, CI proteins containing mutations in this conserved sequence disrupt interactions with dCBP, transactivate the CI responsive promoter only weakly in cell culture, and fail to activate the expression of *wg* in the embryo.

Mammals express three genes related to CI, Gli-1, Gli-2, and Gli-3 (for reviews, see Matise and Joyner 1999; Ruiz i Altaba 1999). Gli-1 is exclusively a transcriptional activator, but Gli-2 and Gli-3 exist in both activator and repressor forms. Like CI, the activator form of Gli-3 is regulated positively by *hh*, negatively by PKA, and interacts with CBP (Dai et al. 1999; Shin et al. 1999; Wang et al. 2000b). Gli-3 mutations have been identified in both mice and humans. In mice, these mutations result in a disorder termed extra-toes (xt^J), the key feature of which is digital abnormalities reminiscent of those seen in human RTS (Hui and Joyner 1993). Human Gli-3 mutations are somewhat more complex, as there are overlapping but distinct phenotypes (for review, see Biesecker 1997). Thus, the CI–CBP interaction is conserved from flies to humans and the mechanisms elucidated in one system should provide insights into the mechanisms and processes of the other.

Although dCBP serves as a coactivator of *ci* in the *hh* signaling cascade, it suppresses *wg* signaling in the developing gut through its interactions with dTCF, the *Drosophila* homolog of T-cell factor (Waltzer et al. 1998). Upon receiving a wg signal, LEF-1/TCF binds to Armadillo (Arm), a homolog of β-catenin, and activates *wg* target gene expression (Riese et al. 1997). The endoderm of embryos mutant for dCBP is similar to that of embryos that overexpress *wg*; that is, *wg* target genes are

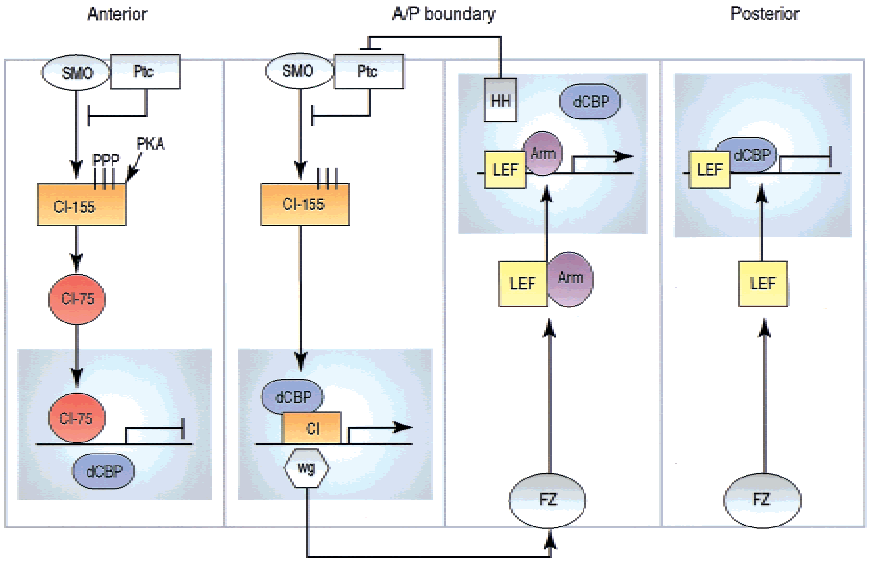

Figure 6. dCBP in *hedgehog (hh)* and *wingless (wg)* signaling. In the anterior cells that do not receive a Hh signal, Ptc suppresses Smo and the PKA phosphorylation of CI targets CI for proteolysis (Aza-Blanc et al. 1997; Chen et al. 1998,1999). The 75-kD proteolytic product migrates into the nucleus and represses *hh* target gene expression. This repressor lacks the dCBP interaction domain. In anterior cells along the anterior/posterior (A/P) boundary that receive a *hh* signal, the PKA-dependent proteolysis of CI is antagonized by the *hh* signal transduction cascade and the activator form of CI can migrate into the nucleus to activate *hh* target genes. CI requires an interaction with dCBP for its activity. Cells posterior to the A/P boundary receive a *wg* signal that allows the interaction between Armadillo (Arm) and dTCF (LEF). This heterocomplex activates *wg* target gene expression. In cells that do not receive a *wg* signal, dCBP forms a complex with LEF that represses *wg* target genes. dCBP probably exerts its inhibitory effects by acetylating LEF and rendering it inactive.

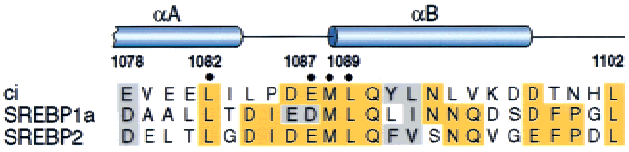

Figure 7. Similarities among the CBP interaction motifs of *Drosophila* CBP and mammalian SREBP 1 and 2. Predicted α-helical regions are designated. Dots refer to residues in CI that, when mutated, block dCBP binding. Shaded residues depict identical and similar amino acids.

misexpressed and the gut develops abnormally. This observation is most consistent with the idea that dCBP antagonizes the *wg* signaling cascade. Additional genetic analyses confirmed the repressive activity of dCBP in embryonic *wg* signaling and demonstrated that dCBP antagonizes the *wg* pathway in the wing disc as well. Mechanistically, dCBP is believed to exert its inhibitory effects by acetylating dTCF and rendering it inactive. Lys-25 is the major acetylation site in dTCF in vitro. Embryos engineered to overexpress a mutant form of dTCF that has an alanine substitution at this position show activation of a *wg* responsive midgut enhancer, whereas embryos overexpressing wild-type dTCF do not. Thus, the acetylation of dTCF by dCBP appears to delimit its activity (see Fig. 6).

The twisted germ-band phenotype associated with mutations in dCBP suggested that dCBP might mediate the expression of *twist* (*twi*), an HLH transcription factor required for the development of mesoderm in *Drosophila* (Nusslein-Volhard and Wieschaus 1980; Simpson 1983; Thisse et al. 1988). Embryos mutant for *twi* function also have a twisted germ-band phenotype. Indeed, embryos lacking dCBP activity do not express *twi* (Akimaru et al. 1997b). *twi* is a target gene for the transcription factor Dorsal (Dl), a homolog of the p65 component of NF-κB, and is activated in response to the *Toll* signal transduction pathway (for review, see Morisato and Anderson 1995; Drier and Steward 1997). The fact that dCBP mutant embryos did not express *twi* suggested that dCBP might interact with Dl to enhance *twi* expression. Binding studies demonstrated that dCBP and Dl interact in vitro and gel mobility-shift assays showed that a dCBP–Dl complex can form on the *twi* promoter. In cell culture assays, dCBP was found to enhance the transcriptional activity of Dl and genetic interactions between dCBP and Dl added further support to the idea that dCBP mediates Dl activity in vivo.

The parallels between the Dl and NF-κB pathways are illustrated in Figure 8. The binding of NF-κB to CBP/p300 is critical for NF-κB activity and appears to depend on PKA phosphorylation of the p65 subunit (Gerristen et al. 1997; Perkins et al. 1997; Zhong et al. 1998). NF-κB-dependent gene expression also requires the recruitment of additional coactivators including p/CAF (Sheppard et al. 1999). It is of interest that the acetyltransferase function of p/CAF, but not CBP, is essential for NF-κB transcriptional activity. It is not yet known whether Dl requires PKA phosphorylation for its interaction with dCBP or whether dCBP acetyltransferase is necessary for Dl function.

TWIST, the vertebrate homolog of TWI, has been proposed to inhibit myogenesis in epithelial tissues by inhibiting the acetyltransferase activity of CBP/p300 (Hamamori et al. 1999), a coactivator of the myogenic transcription factor Myo-D (Eckner et al. 1996b; Yuan et al. 1996; Puri et al. 1997a; Sartorelli et al. 1997). This result suggests that the phenotypes of animals mutant for TWIST could, in part, result from hyperacetylation by way of CBP/p300. In humans, haploinsufficiency for TWIST leads to several developmental disorders, including the Saethre-Chotzen syndrome (El Ghouzzi et al. 1997; Howard et al. 1997; Paznekas et al. 1999; Gripp et al. 1999; Kunz et al. 1999). Patients with Saethre-Chotzen syndrome have craniosynostosis and limb abnormalities, including brachydactyly, cutaneous syndactyly, and broad big toes. Patients with RTS have craniofacial abnormalities and both broad thumbs and broad big toes. Some of these defects certainly overlap and suggest synergistic, rather than antagonistic, functions of TWIST and CBP. However, it is possible that the overlapping phenotypes reflect the misregulation of CBP acetyltransferase activity. For example, RTS patients may express CBP at a level that is insufficient to support normal levels of *TWIST* expression in specific tissues.

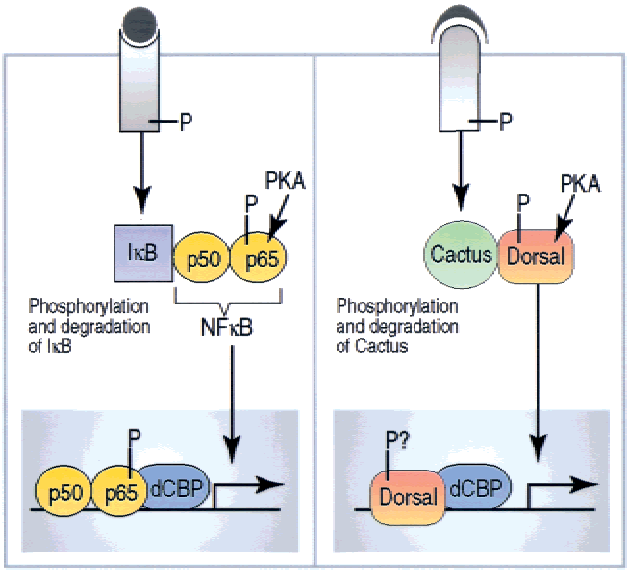

Figure 8. A comparison of the NF-κB and Dorsal pathways. In the absence of a signal, the NF-κB and Dorsal transcriptional activators are sequestered in the cytoplasm by the IκB protein and Cactus, respectively (Roth et al. 1991; Geisler et al. 1992; Kidd et al. 1992; Maniatis 1997). Stimulation of the signal transduction cascade results in the phosphorylation and degradation of the IκB and Cactus proteins that allow the NF-κB/Dorsal activators to migrate into the nucleus (for review, see Baldwin 1996; Reach et al. 1996; Ghosh et al. 1998). The activities of NF-κB and Dorsal depend on the phosphorylation of the proteins by PKA and their interaction with CBP (Norris and Manly 1992; Drier and Steward 1997; Gerristen et al. 1997; Perkins et al. 1997; Zhong et al. 1998).

These low levels of TWIST may be insufficient to inhibit the CBP acetyltransferase activity. Thus, although the CBP level is low in these patients, the CBP acetyltransferase activity could be high or misregulated and capable of generating a *TWIST* haploinsufficient phenotype. Mouse knockouts of CBP, p300, and *TWIST* also have related phenotypes (Chen and Behringer 1995) that similarly cannot easily be explained by the proposed direct TWIST–CBP/p300 interaction. It is not known whether TWI affects dCBP acetyltransferase activity; however, a genetic analysis of the TWI–dCBP interaction, similar to those that have been performed to describe the role of dCBP in *hh*, *wg*, and *Toll* signaling, may shed light on the functional interactions of these factors.

In vertebrates and flies, the transcription factors that are activated in response to transforming growth factor β (TGF-β) signaling pathways, Smad-1–Smad-3 and Mad, interact with CBP/p300 through the MH2 domain (Janknecht et al. 1998; Pouponnot et al. 1998; Waltzer and Bienz 1999). In *Drosophila*, these interactions appear to have functional significance in vivo. The *decapentaplegic (dpp)* locus in *Drosophila* encodes a morphogen homologous to the TGF-β family of peptides (Padgett et al. 1987). The transcription factor Mad, activated in response to the *dpp* signal transduction pathway, drives many developmental and proliferative processes. The activity of a *dpp*-responsive enhancer in the developing *Drosophila* gut is severely reduced in embryos mutant for dCBP, as it is in embryos mutant for *mad* (Waltzer and Bienz 1999). In the developing wing disc, mutations in dCBP enhance (or worsen) the phenotype of a wing-specific *dpp* mutation, dpp^{S1}. In mammalian cells, the Smad–CBP/p300 interaction occurs in a TGF-β-dependent manner, and depends on the integrity of a putative phosphorylation site in the MH2 domain (Feng et al. 1998; Janknecht et al. 1998). The contribution of this phosphorylation site for dCBP binding in the *Drosophila* pathway has not been elucidated.

Two different genetic screens have identified mutations in dCBP that modify the function of the Hox gene *Deformed (Dfd)* (Florence and MgGinnis 1998) and affect the homeostatic regulation of synaptic function (Marek et al. 2000). *Dfd* is a homeotic gene that specifies the identity of the maxillary and mandibular head segments and is required for larval viability. To identify proteins that might interact with *Dfd* and affect its function, mutagenized X chromosomes were screened for their ability to enhance (worsen) the mutant phenotype of a weak *Dfd* allele (Florence and Mc Ginnis 1998). Five mutations of dCBP acted as dominant enhancers of the *Dfd* phenotype, whereas the dCBP null allele did not. This result suggested that the new alleles might represent dominant negative alleles of dCBP function. In support of this hypothesis, embryos from females carrying these new alleles showed more premature lethality than embryos from females carrying the null allele of dCBP. Thus, the new alleles appear to "poison" the wild-type dCBP component. In addition to enhancing the *Dfd* mutant phenotype, the dominant negative dCBP alleles enhance the mutant phenotype of another homeotic gene, *Ultrabithorax (Ubx)*. This is of interest because null mutations in dCBP do not affect mutations in *Ubx* or act as dominant modifiers of the homeotic genes *abdominal-A (abd-A)*, *Abdominal-B (Abd-B)*, *Antennapedia (Antp)*, or *Sex combs reduced (Scr)* (S. Smolik, unpubl. observations). These results suggest that the complexes including dCBP and products of the Hox genes are not sensitive to changes in the dosage of dCBP, but are sensitive to changes in dCBP activity.

Mice that carry a mutation in CBP/p300 have defects in learning and memory, somewhat reminiscent of patients with RTS (Rubinstein and Taybi 1963; Tanaka et al. 1997; Oike et al. 1999a). However, no specific role of CBP in mammalian synaptic function is known. Two hypomorphic, viable alleles of dCBP were identified in a screen for *Drosophila* genes involved in the retrograde regulation of presynaptic development (Marek et al. 2000). Larvae homozygous for these alleles have a 50% decrease in presynaptic transmitter release at the neuromuscular junction but show no change in quantal size, average muscle resting potential, or muscle input resistance when compared to controls (Marek et al. 2000). The decrease in transmitter release is not due to changes in muscle innervation or cell fate because the synapses of dCBP mutant animals are structurally wild-type. Overexpression of dCBP in the muscles of the dCBP mutant larvae can rescue the loss of transmitter release. These results (and additional genetic analyses) provide evidence that dCBP acts as both a positive and a negative regulator of the postsynaptic regulatory system that controls presynaptic function.

CBP/p300 in Xenopus *development*

Studies of CBP/p300 in other model systems have confirmed that these proteins are important for development and have suggested a particular function in neural determination. Kato et al. (1999) used 12S E1A and a dominant negative fragment of p300 to demonstrate that the block of CBP/p300 function in *Xenopus* embryos activates a variety of neurogenic transcription factors. In contrast, non-neural gene expression was blocked by the inhibition of CBP/p300 function. These findings led Kato et al. to conclude that all embryonic cells possess intrinsic neuralizing capability and that CBP/p300 plays an essential role in suppressing neural fate.

One limitation of this type of analysis is that it depends on the specificity of the agents used to block CBP/p300 function. As described above, E1A affects several key components of the transcriptional process in addition to CBP/p300, and it has not been determined whether the purportedly specific E1A mutants (such as RG2, del3-36) interfere with these other functions as well. Because of the multiplicity of CBP/p300 actions and interactions, it is also difficult to assess the specificity of the various dominant negative isoforms that have been developed. In this regard, Fujii et al. (1998) reported that microinjection of a carboxy-terminal truncated form of *Xenopus* p300 mRNA at the two-cell stage

disrupted many aspects of embryonic development, including neural formation.

By using the ras recruitment protein interaction system, Takemaru and Moon (2000) recently determined that the activation domain of *Xenopus* β-catenin binds to the CREB-binding domain of *Xenopus* CBP (xCBP). Association of these two factors was confirmed biochemically and xCBP was shown to augment xβ-catenin function in transfection assays. Moreover, injection of E1A RNA into *Xenopus* embryos was found to block the expression of Wnt target genes in vivo. These findings appear to contradict the earlier report by Waltzer and Bienz (1998; see above), which proposed that dCBP antagonizes Wnt signaling. It is possible that this discrepancy relates to differences between the *Xenopus* and *Drosophila* systems.

CBP/p300 has also been linked to the actions of *Xenopus* NGN-1, a member of the neurogenin family of bHLH proteins (Koyano-Nakagawa et al. 1999). E1A was shown to block xNGN-1 function, presumably at the level of CBP/p300. The result of this inhibition would be expected to be complex, however, as xNGN-1 also induces processes that result in the lateral inhibition of neurogenesis.

CBP in Caenorhabditis elegans

In vivo evidence for the importance of the CBP HAT function in developmental processes has come from studies in *C. elegans*. Three sequences related to mammalian CBP/p300 have been identified in the *C. elegans* genome, two of which might be pseudogenes. Examination of the function of the remaining gene, *cbp-1*, has been accomplished through the use of RNA interference (RNAi) technology (Shi and Mello 1998). Embryos treated with *cbp-1* RNAi show arrested development with an increased total number of cells. On further analysis, these embryos demonstrate defects in muscle, intestinal, and hypodermal differentiation and an increased number of neurons, suggesting that *cbp-1* is required for early mesoderm and endoderm development. This finding is reminiscent of the effects of blocking CBP/p300 function in *Xenopus*. The inhibition of endoderm differentiation can be overcome by preventing expression of *hda-1*, a homolog of the human histone deacetylases (HDACs) or *rba-1* and *rba-2*, homologs of the mammalian RbAp-46 and RbAp-48. RbAp46 and RbAp-48 have been proposed to be required for certain aspects of HDAC function, such as targeting the HDAC complexes to their histone substrates (Hassig et al. 1997; Verreault et al. 1998). The most straightforward conclusion from these studies is that the functions of *cbp-1* are normally antagonized by HDACs. Although these observations could be interpreted as suggesting that the HAT function of cbp-1 is essential, other explanations are also tenable. For example, cbp-1 might serve to organize the recruitment of other HATs that provide the acetyltransferase function. Unfortunately, the RNAi approach does not allow an assessment of the importance of the cbp-1 HAT function specifically. Presumably, the lack of cbp-1 in the RNAi animals would perturb multiple transcriptional pathways that are important for development; therefore, it may be difficult to confirm these studies further without alternative genetic approaches.

Contribution of CBP/p300 to differentiation of specialized cell lineages

Hematopoiesis

The hematopoietic system has provided a good model for examining the development of specialized cell types from multipotential progenitors. In particular, this system has been useful in elucidating the interplay between specific transcription factors and the protein complexes that mediate chromatin remodeling. Not surprisingly, CBP/p300 have been found to contribute importantly to these mechanisms. A more extensive discussion of the role of CBP and p300 in blood formation can be found in Blobel et al. (2000).

One of the most extensively studied hematopoietic transcription factors is GATA-1, which is required for differentiation of erythroid and megakaryocytic lineages (Pevny et al. 1991; Shivdasani et al. 1997). GATA-1 has been found to interact biochemically with CBP and the expression of GATA-1 dependent genes is blocked by E1A (Blobel et al. 1998), implicating CBP in GATA-1 function. Precisely how CBP stimulates GATA-1 remains somewhat obscure. CBP has been shown to acetylate residues near the GATA-1 DNA-binding domain, but how this affects transcription factor function is controversial. In one study, acetylation of GATA-1 was shown to increase DNA-binding (Boyes et al. 1998). In another, acetylation increased GATA-1 mediated transcriptional activity but did not affect DNA binding (Hung et al. 1999). Further studies will be required to resolve this discrepancy. Interestingly, the transcription factor c-*myb*, which also binds to CBP/p300, has been shown to oppose GATA-1 functionally and block erythroid differentiation. It has been proposed that this block might occur through the mutually inhibitory interactions of c-*myb* and GATA-1 with CBP (Takahashi et al. 2000).

EKLF is also involved in erythroid differentiation. Like GATA-1, EKLF activates β-globin gene expression through a mechanism that depends on CBP/p300 (Zhang and Bieker 1998). Although EKLF has been shown to be acetylated by CBP/p300, the consequences of this modification are currently unknown.

GATA-1 and EKLF both bind to DNA elements within the locus control region (LCR), a domain that is required in some experimental models for transcriptional activity of the β-globin gene cluster. Another transcription factor that interacts with this region is NF-E2. NF-E2 is a heterodimeric complex consisting of a tissue-restricted p45 subunit and a more widely expressed member of the maf gene family. Although loss of NF-E2 primarily affects platelet formation, this transcription factor may also

contribute to expression of globin genes. The lack of a predominant erythroid phenotype in NF-E2 knockouts may be due to the redundancy of factors that interact with the LCR. Studies have shown that the p45 subunit of NF-E2 binds to CBP and that this interaction may contribute to the synergistic signaling of hematopoietic factors with thyroid hormone (Cheng et al. 1997).

Several other transcription factors involved in hematopoiesis have also been shown to interact with CBP/p300, including c-*myb*, Ets-1, PU.1, AML-1, E47, Tal1, and C/EBPα. The functional consequences of these interactions are discussed by Blobel et al. (2000) and elsewhere in this review.

Myogenesis

The processes involved in skeletal muscle differentiation provide another useful model for elucidating the developmental functions of CBP/p300. Skeletal muscle development is controlled by four bHLH proteins—myf-5, myo-D, myogenin, and MRF-4. In this process, myf-5 and myo-D are believed to be responsible for the specification and maintenance of myoblast identity, whereas myogenin is thought to regulate the differentiation of myoblasts into myotubes. Other more general factors also appear to be essential for muscle differentiation, such as the bHLH proteins E12 and E47 and the myocyte enhancer factor MEF-2.

The first clues to the involvement of CBP/p300 in muscle development were provided by Webster et al. (1988), who showed that adenovirus E1A suppressed myogenic differentiation. The ability of E1A to block the transactivation function of myf-5 was subsequently reported by Braun et al. (1992). Caruso et al. (1993) showed that the amino-terminal (CBP/p300 binding) portion of E1A inhibited myo-D transcription, but suggested that the Rb-interacting portion (CR2) blocked myo-D-mediated transactivation. Mymryk et al. (1992) and Taylor et al. (1993) suggested that the amino-terminal portion of E1A blocks myo-D-mediated transactivation as well.

Direct evidence for the involvement of p300 in myo-D function was provided by Yuan et al. (1996), who showed that E1A blocked the ability of myo-D to activate an E-box-containing reporter gene and that this block could be overcome by overexpression of p300. These workers also showed that myo-D interacted with the third zinc finger domain of p300 and that the addition of p300 augmented myo-D-directed transcriptional activity. Similar findings were reported by Eckner et al. (1996), who noted that the interaction involved the bHLH domain of myo-D and suggested that p300 could bind to MEF-2 as well. This latter interaction was subsequently shown to involve the MEF-2 MADS domain (Sartorelli et al. 1997). Eckner et al. also demonstrated that microinjected antibodies directed against CBP/p300 blocked the expression of myo-D responsive genes and differentiation of C2C12 cells. They were able to confirm the association of myo-D and p300 in vitro, but only in the presence of a DNA fragment containing two adjacent E-boxes. This result suggested that p300 might promote myogenesis by participating in cooperative protein–protein interactions at myogenic gene promoters. Of note, they found that the transactivation domain at the amino terminus of myo-D was dispensable for p300 binding.

Similar findings were reported by Puri et al. (1997a), who showed that the microinjected p300 antibodies additionally blocked the cell cycle arrest that precedes differentiation. This cell cycle effect was attributed to the inability of the microinjected cells to induce p21 in response to differentiating conditions. Of note, p300, but not CBP, was detected in complexes containing E-box sequences and myo-D. This finding was one of the first indications that CBP and p300 might have different functions during developmental processes. A similar dependence on p300, but not CBP, for differentiation of F9 cells has also been reported (Kawasaki et al. 1998; Ugai et al. 1999).

These initial studies describing the binding of myo-D to CBP/p300 were perplexing, however, because the region of CBP/p300 that interacted with myo-D appeared to overlap with the binding sites for TFIIB, P/CAF, and the RNA polymerase II holoenzyme. Thus, it was unclear how CBP/p300 could associate with myo-D and the various effectors of CBP/p300 action simultaneously. It was subsequently determined that myo-D and P/CAF actually bound to slightly different CBP/p300 sequences (Puri et al. 1997b). Despite the fact that myo-D and P/CAF bind to distinct CBP/p300 sites, both interactions are disrupted by E1A.

Antibody microinjection studies indicate that both p300 and P/CAF activities are required for muscle differentiation. In the model proposed by Puri et al. (1997b), p300 is required primarily for recruiting P/CAF to the myo-D-binding sites. Deletion of sequences responsible for the P/CAF HAT activity blocked differentiation in transfection assays, whereas deletion of p300 HAT sequences had no effect. These observations led the authors to suggest that the HAT function of p300 was not required for differentiation. Whether p300 simply serves as a scaffold for P/CAF or whether it provides other activation functions has not been determined. Subsequent studies from this group suggested that one of the key targets for the P/CAF acetyltransferase activity was myo-D (Sartorelli et al. 1999). Consistent with this hypothesis, replacement of the potentially acetylated lysine residues in myo-D with arginines impaired its binding to DNA and its ability to activate transcription. These experiments focused on the binding properties of myo-D homodimers, however, which are known to interact with E-box sequences relatively poorly.

A different model for the E1A-mediated inhibition of myogenesis was proposed by Taylor et al. (1993). These workers found that E1A bound directly to the bHLH domains of the myogenic factors and blocked their transcriptional activities. In support of this model, one E1A mutation (E1A del 38-67) was identified that retained the ability to interact with bHLH factors and blocked expression of muscle-specific promoters, but did not interact with p300. This result led Taylor et al. to suggest that

the capacity of E1A to interact with bHLH domains, rather than with p300, correlated with the transcriptional repression. Confirmation of this model will require the development of point mutants that specifically disrupt the bHLH–E1A interaction. In contrast, Sandmoller et al. (1996) determined that this same region of E1A (amino acids 38–62) could block myo-D and myf-5 activation of myogenin and muscle creatine kinase promoters. The inhibition in this second study also appeared to be independent of p300. However, these workers were not able to confirm the direct interaction of E1A and bHLH proteins.

Despite general acceptance of the concept that p300 (and possibly CBP) are involved in myo-D signaling, precisely how this occurs is unclear. As discussed above, Eckner et al. (1996b) found that p300 bound to the bHLH motif of myo-D. In contrast, Sartorelli et al. (1997) determined that p300 interacted primarily with the amino-terminal myo-D activation domain. These workers suggested that, in addition to the previously reported third zinc finger binding site, myo-D also interacted with the first zinc finger domain of p300. In related factors such as NeuroD1/BETA2, the bHLH domain has been reported to be dispensible (Sharma et al. 1999) or required (Mutoh et al. 1998) for CBP/p300 binding.

p300 binding to myo-D may occur through indirect mechanisms as well. For example, Lau et al. (1999) showed that the amino-terminal activation domain of myo-D interacted with the retinoid-related orphan receptor RORα. In turn, RORα bound to an amino-terminal region of p300 and all three proteins could be identified in a single complex. The importance of the ROR interaction was demonstrated by showing that a dominant negative isoform blocked the induction of myo-D, myogenin, and p21 expression after serum withdrawal. Another nuclear orphan receptor, COUP-TFII, has been shown to associate with the amino-terminal activation domain of myo-D and block its function by competing for p300 binding (Bailey et al. 1998).

Adenovirus E1A blocks cardiac myocyte-specific gene expression as well, but the mechanisms underlying these effects remain even more obscure. Kirshenbaum and Schneider (1995) showed that 12S E1A inhibited transcription of the cardiac-restricted α actin promoter if expressed in the presence of E1B. Their studies indicated that both the amino terminus and CR2 regions of E1A were required for this effect, implicating both p300 and p107, the predominant "pocket protein" expressed in these cells. Hasegawa et al. (1997) reported similar findings, but argued that the inhibition was due entirely to the block in p300 function. The capacity to bind p300 was not required for E1A-mediated inhibition of cardiac-specific promoters in the studies of Bishopric et al. (1997), but overexpression of p300 was found to partially overcome the E1A effect. The heart defects detected in CBP- and p300-deficient knockout mice (Yao et al. 1998; Kung et al. 2000) support the idea that these coactivators are indeed required for normal cardiac development but do not provide much insight into precisely how they mediate their effects.

Conclusions

The complex functions of CBP and p300 are beginning to come into focus, but much remains unknown. To date, most studies of CBP/p300 have been carried out under the assumption that these proteins serve as transcriptional activators. Several clear examples of CBP/p300 participating in transcriptional repression pathways have been described, however, and it is likely that genetic approaches in model organisms, such as *Drosophila*, will yield even more. The complex phenotypes associated with a loss of CBP/p300 function in humans and mice reflect the diverse roles of CBP/p300 in multiple developmental processes. Potentially, the contributions of CBP/p300 activity to each of these processes can be dissected pathway by pathway in model systems such as *Drosophila*. As illustrated above, the signaling pathways that rely on dCBP for their transduction into the nucleus are highly conserved in vertebrates and, so far, the interactions of dCBP with the various signal-responsive transactivators are conserved as well. Therefore, the work in *Drosophila* development can provide the groundwork for similar studies in mammals. For example, it will be interesting to learn whether CBP/p300 acts as a negative regulator of TCF in mammalian cells and whether these coactivators similarly modulate retrograde signaling systems at the vertebrate neuromuscular junction. Likewise, it will be important to determine whether the CBP interactions described in cell culture have functional consequences for *Drosophila* development.

Most of the interactions between viral gene products and CBP/p300 result in transcriptional repression. This is not surprising because a major function of these viral proteins is to inhibit cellular gene expression. Nonetheless, several viral proteins have been identified that clearly activate specific promoters by recruiting CBP/p300 and more examples undoubtedly will be uncovered as functional genomic technology develops. Many other aspects of CBP/p300 function also remain unknown. For example, understanding of the regulation of CBP/p300 by phosphorylation is, at best, rudimentary. This mechanism is likely to be critically important for allowing the integration of information from different signal transduction pathways. Evidence suggests that CBP/p300 levels are limiting in cells, but how (or whether) this situation contributes to inhibitory crosstalk between transcriptional pathways remains unresolved. Finally, the ability of CBP/p300 to serve as mediators of both cell proliferation and growth arrest pathways remains a paradox. Nonetheless, the absolute requirement for these coactivators in the actions of many (or even most) transcription factors indicates that they will continue to provide a fruitful target for studies aimed at understanding the complex interactions that underlie the control of cell growth and differentiation.

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CBP/p300 in cell growth, transformation, and development

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