p300/CBP proteins: HATs for transcriptional bridges and scaffolds

Ho Man Chan and Nicholas B. La Thangue*

Division of Biochemistry and Molecular Biology, Davidson Building, University of Glasgow, Glasgow, G12 8QQ, UK
*Author for correspondence (e-mail: N.LaThangue@bio.gla.ac.uk)

Journal of Cell Science 114, 2363-2373 (2001) © The Company of Biologists Ltd

Summary

p300/CBP transcriptional co-activator proteins play a central role in co-ordinating and integrating multiple signal-dependent events with the transcription apparatus, allowing the appropriate level of gene activity to occur in response to diverse physiological cues that influence, for example, proliferation, differentiation and apoptosis. p300/CBP activity can be under aberrant control in human disease, particularly in cancer, which may inactivate a p300/CBP tumour-suppressor-like activity. The transcription regulating-properties of p300 and CBP appear to be exerted through multiple mechanisms. They act as protein bridges, thereby connecting different sequence-specific transcription factors to the transcription apparatus. Providing a protein scaffold upon which to build a multicomponent transcriptional regulatory complex is likely to be an important feature of p300/CBP control. Another key property is the presence of histone acetyltransferase (HAT) activity, which endows p300/CBP with the capacity to influence chromatin activity by modulating nucleosomal histones. Other proteins, including the p53 tumour suppressor, are targets for acetylation by p300/CBP. With the current intense level of research activity, p300/CBP will continue to be in the limelight and, we can be confident, yield new and important information on fundamental processes involved in transcriptional control.

Key words: Transcription, Signal transduction, Chromatin, p300/CBP, Acetyl transferase

Introduction

The p300/CBP proteins, which include the distinct but related proteins p300 and CBP and potentially other proteins, such as p270, are a protein family that participate in many physiological processes, including proliferation, differentiation and apoptosis (reviewed by Janknecht and Hunter, 1996; Shikama et al., 1997; Giordano and Avantaggiati, 1999; Goodman and Smolik, 2000). They function as transcriptional co-activators and are involved in multiple, signal-dependent transcription events. Viral oncoproteins, such as adenoviral E1A and SV40 large T antigen, specifically target these proteins (Arany et al., 1994; Arany et al., 1995; Eckner et al., 1994; Eckner et al., 1996a; Whyte et al., 1989; reviewed by Dyson and Harlow, 1992; Moran, 1993). The formation of viral oncoprotein complexes with p300/CBP causes a loss of cell growth control (Missero et al., 1995; Yang et al., 1996), enhances DNA synthesis (Stein et al., 1990) and blocks cellular differentiation (Eckner et al., 1996a; Eckner et al., 1996b; Puri et al., 1997a; Puri et al., 1997b, reviewed by Shikama et al., 1997; Goodman and Smolik, 2000). Recent evidence indicates that *p300/CBP* genes are altered in various human tumours (Petrij et al., 1995; Muraoka et al., 1996; Sobulo et al., 1997; Giles et al., 1998; Gayther et al., 2000), which is consistent with studies on $Cbp^{+/-}$ mice that suggest that CBP possesses tumour suppressor activity in the haematopoietic system (Kung et al., 1999). Thus, p300/CBP proteins may be regarded as having some of the hallmarks expected of a classical tumour suppressor.

Furthermore, p300/CBP proteins are endowed with histone acetyltransferase (HAT) activity (Bannister and Kouzarides, 1996; Ogryzko et al., 1996), which transfers an acetyl group to the e-amino group of a lysine residue, and the acetylation level of chromatin has been established to be a key mechanism in regulating transcription (reviewed by Brownell and Allis, 1996; Grunstein, 1997; Wade et al., 1997; Armstrong and Emerson, 1998). Although it has not been formally proven that p300/CBP HAT activity is directly involved in chromatin remodeling, a growing body of evidence suggests that other targets, including transcription factors and the transcription apparatus (Gu and Roeder, 1997b; Imhof et al., 1997; Boyes et al., 1998; Zhang and Bieker, 1998; Keirnan et al., 1999; Sartorelli et al., 1999; Bannister et al., 2000; Martinez-Balbás et al., 2000; Marzio et al., 2000, Soutogloou et al., 2000; Tomita et al., 2000, reviewed by Bayle and Crabtree, 1997), are also regulated by acetylation, thereby highlighting the potential importance of HAT activity in p300/CBP function. So far, p300/CBP proteins have been shown to interact with a multitude of signal transduction pathways, and in this respect serve as versatile transcriptional regulators (reviewed by Janknecht and Hunter, 1996).

Genes

p300 and CBP were originally identified as proteins that bind to the adenoviral E1A and the cAMP-response-element-binding protein (CREB), respectively (Chrivia et al., 1993; Eckner et al., 1994). The *p300/CBP* genes are conserved in a variety of multicellular organisms, from worms to humans. The human *CBP* locus is located in chromosomal region 16p13.3 (Chen and Korenberg, 1995; Wydner et al., 1995), which is also implicated in Rubinstein-Taybi syndrome (RTS; Petrij et al., 1995) and certain subtypes of acute myeloid leukaemia

(Borrow et al., 1996). Interestingly, the 16p13.3 region shows extensive homology to a region on chromosome 22, where p300 resides (22q13; Eckner et al., 1994). Apart from *p300/CBP*, these two regions may contain at least eight other pairs of paralogous genes (Giles et al., 1997). Giles et al. have suggested that chromosomal relationships like these could arise by tetraploidization and provide a mechanism to protect against potentially harmful mutational events during organismal evolution (Giles et al., 1997).

Proteins

p300 and CBP share several conserved regions, which constitute most of the known functional domains in the proteins (Arany et al., 1994; Fig. 1): (1) the bromodomain, which is frequently found in mammalian HATs; (2) three cysteine-histidine (CH)-rich domains (CH1, CH2 and CH3); (3) a KIX domain; and (4) an ADA2-homology domain, which shows extensive similarity to a yeast transcriptional co-activator, Ada2p. The CH1, CH3 and the KIX domains are likely to be important in mediating protein-protein interactions, and a number of cellular and viral proteins bind to these regions (Fig. 1). Crystallographic structural studies of the bromodomains of co-factor pCAF (Dhalluin et al., 1999) and the TBP-associated factor $TAF_{II}250$ (Jacobson et al., 2000) suggest that the bromodomain recognises acetylated residues. Given that an increasing number of cellular factors have been found to be acetylated by p300/CBP, bromodomains could function in recognising different acetylated motifs. Combinations of bromodomains might thus recognise distinct patterns of acetylated nucleosomes. Both the N- and the C-terminal regions of p300/CBP can activate transcription, and the HAT domain resides in the central region of the protein (Fig. 1). This modular organisation may allow p300/CBP to provide a scaffold for assembly of multicomponent transcription co-activator complexes.

The p300/CBP family is likely to include other proteins, such as p270 (Dallas et al., 1998), which shares some common antigenic determinants with p300. p270 has been reported to be part of the mammalian SWI/SNF complex, which suggests it is involved in chromatin-remodeling-related functions (Dallas et al., 1998).

Functions

Although p300 and CBP share extensive homology, genetic and molecular analyses suggest that they perform not only overlapping but also unique functions. In transfection-based assays, most sequence-specific transcription factors can be co-activated by either p300 or CBP. Similarly, adenovirus E1A binds to CBP, and CREB also can use p300 in co-activation (Arany et al., 1995; Lundbiad et al., 1995). The $p300^{-/-}$, $Cbp^{-/-}$ and $p300^{+/-}$ $Cbp^{+/-}$ mice show similar, embryonic lethal phenotypes (Yao et al., 1998), together with similar defects in growth and neural tube closure (Yao et al., 1998); this suggests that p300 and CBP have overlapping roles during embryonic development. Furthermore, some *p300* and *Cbp* heterozygous mice suffer early lethality (Yao et al., 1998), indicating that *p300/CBP* gene dosage, and therefore the level of p300/CBP proteins, is probably important during development. Consistent with this idea are the findings that the haplo-insufficient RTS syndrome is heterozygous for mutation in the *Cbp* allele (Petrij et al., 1995) and that *Cbp* heterozygous mice also show skeletal abnormalities reminiscent of RTS (Tanaka et al., 1997).

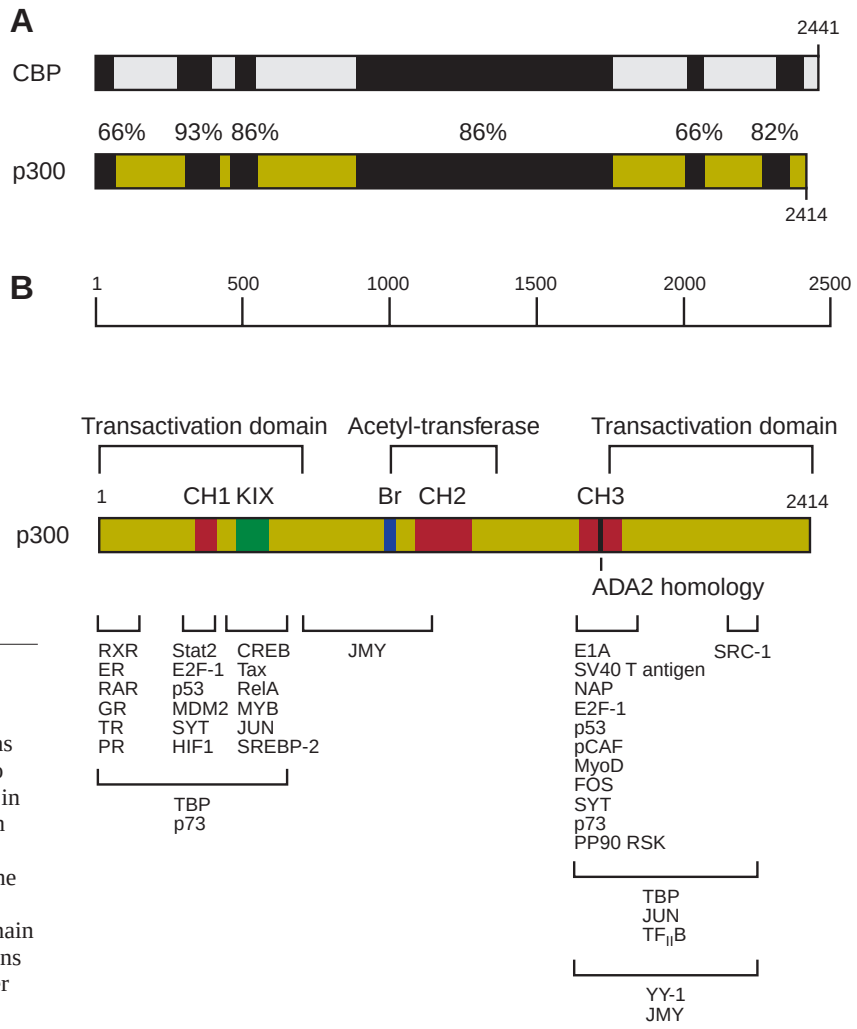

Fig. 1. Organisation of p300/CBP proteins.
(A) Comparison of p300 and CBP. The dark regions indicate the areas of highest homology, with the percentage amino acid identity between the two proteins indicated. The size of each protein, in number of amino acid residues, is indicated. (B) The functional domains in p300 are indicated, including the cysteine/histidine-rich domains CH1, CH2 and CH3, the KIX domain, the bromodomain (Br) and the ADA2 homology region. The N- and C-terminal domains of p300/CBP can act as transactivation domains, and the acetyl-transferase domain is located in the central region of the protein. The regions that have been shown to bind to target proteins, together with the identity of the interacting proteins, are shown.

An alternative but non-exclusive model argues that p300 and CBP have unique cellular functions. *p300*$^{-/-}$ fibroblasts have specific defects in retinoic-acid-dependent transcription but retain normal CREB activity (Yao et al., 1998). Furthermore, *Cbp*$^{+/-}$ mice show highly penetrant, multilineage defects in haematopoietic differentiation (Kung et al., 1999) and have an increased probability of developing haematological malignancies as they age. This pathology is specific for the *Cbp*$^{+/-}$ mice and not observed in *p300*$^{+/-}$ mice, and thus *Cbp*, but not *p300*, is required for normal haematopoietic differentiation (Kung et al., 1999). Finally, using hammerhead ribozymes to specifically target and inactivate either p300 or CBP RNA, Kawasaki et al. were able to inhibit retinoic-acid-induced differentiation of F9 embryonal carcinoma cells with a p300 mRNA ribozyme but not a CBP ribozyme (Kawasaki et al., 1998). Interestingly, ribozymes directed against either p300 or CBP can block retinoic-acid-induced apoptosis of F9 cells (Kawasaki et al., 1998). Overall, it appears that p300 and CBP have overlapping roles, but that in addition they perform unique tasks in certain physiological processes.

p300/CBP and the cell cycle

The fact that p300/CBP proteins are targets for the adenovirus E1A oncoprotein suggests that they are important in cell cycle regulation (reviewed by Moran, 1993). Studies of the interaction between E1A and p300/CBP support such a role in the control of DNA synthesis and S phase progression (Stein et al., 1990). Furthermore, E1A mutants that cannot bind to p300 exhibit defective cellular transformation (Wang et al., 1995). Studies of *p300*$^{-/-}$ and *cbp*$^{-/-}$ knockout mice have provided direct evidence that p300/CBP proteins are important for cell cycle regulation and differentiation (Yao et al., 1998). *p300*$^{-/-}$ embryos die at about E10.5d and show defects in neural tube closure and heart development; defects in cardiac myocyte differentiation are thought to contribute to this early lethality (Yao et al., 1998). Consistent with this idea is the observation that in cell-based assays p300/CBP can cooperate with members of the MyoD family of muscle-differentiation-regulating transcription factors in modulating the expression of downstream myogenic factors, including myogenin and MEF2, and promote cell cycle withdrawal in myoblasts induced to differentiate (Yuan et al., 1996; Puri et al., 1997a; Puri et al., 1997b; Sartorelli et al., 1999). The p300/CBP-interacting protein pCAF is also required for the myogenic differentiation programme, since micro-injection of either anti-p300/CBP or anti-pCAF antibodies blocks MyoD-dependent transcription in muscle cells (Puri et al., 1997a; Puri et al., 1997b). Although the p300 HAT domain is dispensable for MyoD-dependent transcription, mutation in the HAT domain of pCAF impairs MyoD-dependent transactivation (Sartorelli et al., 1999).

The p300/CBP-pCAF protein complex can arrest cell cycle progression (Yang et al., 1996) and might regulate target genes that are involved in controlling the G1/S transition, such as p21^{WAF1} (Missero et al., 1995). The overexpression of E1A, which antagonises pCAF binding to p300/CBP, drives cells into S phase (Yang et al., 1996). Given the haematological malignancies manifest in *Cbp*$^{+/-}$ mice (Kung et al., 1999), the differentiation-promoting properties of CBP may provide a tumour-suppressor-like function.

In *Caenorhabditis elegans*, inactivating the *cbp-1* gene blocks most aspects of differentiation, excluding the neuronal lineage, which in *C. elegans* is the default pathway of differentiation; mesodermal, endodermal and hypodermal cells are absent in most of the embryos (Shi and Mello, 1998). Co-inactivation of genes encoding components of the HDAC (histone deacetylase) complexes, such as *hda-1*, *rba-1* and *rba-2*, which antagonise HAT activity, rescues some of the *cbp-1* phenotype (Shi and Mello, 1998), providing evidence that p300/CBP HAT activity is required for differentiation, at least in *C. elegans*, to counteract the role of HDACs.

Cell proliferation and growth is influenced by p300/CBP activity (Stein et al., 1990; Yao et al., 1998). *p300*$^{-/-}$ embryos are significantly smaller than their wild-type littermates and show defects in cell proliferation (Yao et al., 1998). *p300*$^{-/-}$ MEFs grow more slowly compared with wild-type cells and have a phenotype reminiscent of senescence. Since binding of E1A to p300 also correlates with E1A-induced DNA synthesis (Stein et al., 1990), the E1A-p300 complex might play an active role in stimulating cellular growth and proliferation.

Analysis of the *cbp-1*-deficient *C. elegans* embryos suggested that apoptosis remains intact (Shi and Mello, 1998). However, in vitro studies in mammalian cell-based assays have provided evidence that p300/CBP is involved in apoptosis (Avantaggiati et al., 1997; Kawasaki et al., 1998; Lill et al., 1997; Lee et al., 1998; Yuan et al., 1999). Cells lacking p300 but not CBP have impaired IR (ionizing radiation) sensitivity (Yuan et al., 1999), and functional sequestration of p300/CBP activity by E1A, or a dominant negative version of p300, reduces p53-dependent apoptosis (Avantaggiati et al., 1997; Gu and Roeder, 1997a; Lill et al., 1997). The p300-interacting protein JMY (**j**unction **m**ediating and regulator**y** protein) augments p53-dependent apoptosis (Shikama et al., 1999). The p53-p300-JMY ternary complex is enriched in cells exposed to stress and upregulates a variety of p53-dependent target genes, including *bax*, which may account for its pro-apoptotic activity (Shikama et al., 1999). p300 can also interact with and co-activate the hypoxia-inducible factor HIF1a (Arany et al., 1996), which binds to and stabilises p53 during hypoxia (An et al., 1998; Carmeliet et al., 1998). The HIF1-p300/CBP-p53 pathway could play an important role in regulating apoptosis under hypoxic conditions and may prevent tumour development. Lee et al. also reported that, under certain conditions, p300 augments p53-dependent G1 arrest (Lee et al., 1998).

Another level of complexity in the p53 response became evident when p300/CBP was shown to regulate p53 stability (Grossman et al., 1998). The N-terminal region of p300 shows partial sequence homology to the E6 oncoprotein from human papilloma virus (HPV; Grossman et al., 1998), which destabilises p53. Current evidence suggests that a ternary complex containing p300, p53 and MDM2 controls p53 stability (Grossman et al., 1998). Thus, although oncoprotein MDM2 can regulate p53 stability via a ubiquitin-dependent protein degradation pathway (Honda et al., 1997; Thomas and White, 1998), MDM2 mutants that can bind to p53 but not p300 fail to promote efficient p53 degradation (Grossman et al., 1998). Similarly, a short p300 N-terminal fragment consisting of the p53-binding region in the N-terminus acts in a dominant-negative fashion to enhance p53 stability (Grossman et al., 1998), which suggests that p300 is involved with the MDM2-dependent degradation of p53. p300 therefore appears

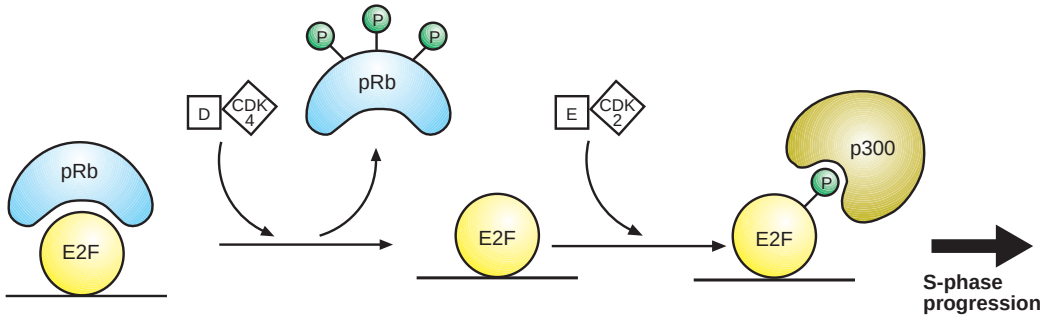

Fig. 2. Role of p300/CBP during cell cycle progression. The diagram describes the role of p300 in regulating the activity of the cell cycle transcription factor E2F. Phosphorylation of pRb by cyclin-D–CDK4 kinase releases pRb from E2F, thereafter facilitating the activation of E2F-target genes. Subsequent phosphorylation of a CDK site by cyclin-E–CDK2 in the transactivation domain of E2F augments the interaction with p300, thereafter leading to the increased transcriptional activity of E2F-target genes.

to have a dual role in controlling p53 activity: it both augments p53 transactivation function and stimulates its turnover.

The E2F family of transcription factors functions in regulating cell cycle progression (reviewed by Dyson, 1998). p300 is known to control E2F activity, and an important mechanism involved relates to the role of phosphorylation. The phosphorylation of E2F by cyclin-dependent kinases (CDKs) is under cell cycle control (reviewed by Dyson, 1998). In E2F-5, a CDK-phosphorylation consensus site in the transcription activation domain is phosphorylated by cyclin-E-CDK2 as cells approach S phase (Morris et al., 2000). Morris et al. demonstrated that phosphorylation in the activation domain augments the physical interaction with p300, thereby enhancing the transcription of E2F target genes (Fig. 2). The CDK-stimulated interaction of p300 with E2F may be involved in irreversibly committing cells to cell cycle progression.

Eid et al. recently revealed an unexpected function of p300 in controlling cell adhesion (Eid et al., 2000). The nuclear proto-oncoprotein SYT (Clark et al., 1994; Brett et al., 1997) can associate with p300, and this association occurs predominantly in contact-inhibited cells. SYT is an SH2- and SH3-domain-containing protein that becomes mutated in synovial sarcomas and certain other cancers, and SYT-mutant cells are believed to exhibit deficient cell adhesion control (Eid et al., 2000). Although the mechanism by which the p300-SYT interaction affects cell adhesion remain unclear, this observation indicates that extracellular stimuli that alter cell adhesion could act through p300/CBP. In addition, the interaction between p300 and SYT could be another mechanism that contributes to the tumour suppressor activity of p300/CBP.

Transcriptional regulation by p300/CBP

It is now widely accepted that p300/CBP proteins are versatile and perhaps rather general transcriptional integrators (reviewed by Janknecht and Hunter, 1996). Current evidence suggests that they act through a variety of mechanisms (Fig. 3).

A bridge to the transcriptional machinery

The initiation of transcription by RNA polymerase II requires sequence-specific promoter/enhancer-binding transcription factors as well as the basal transcription machinery. Unless transcription factors can directly interact with the basal transcription machinery, other proteins must act as bridges that connect them to the basal machinery. p300/CBP is known to

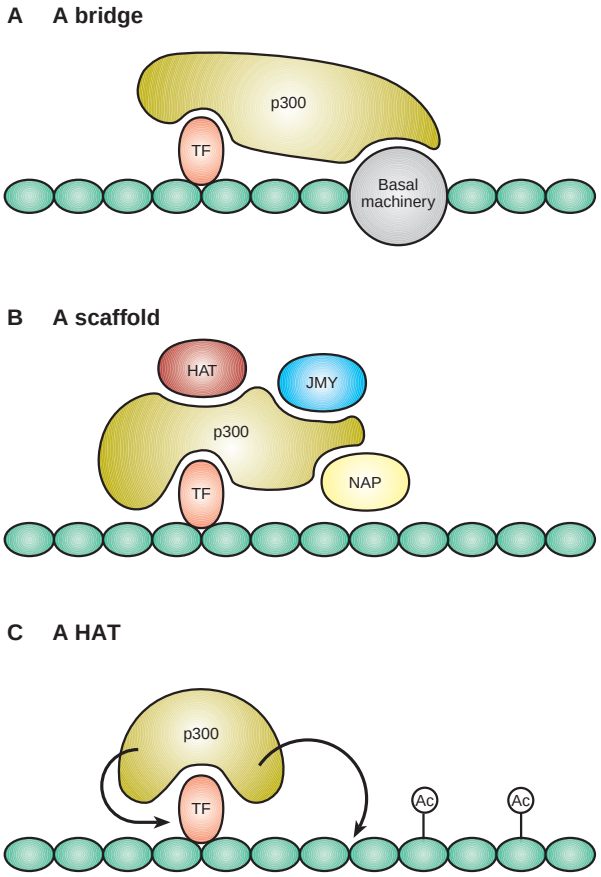

Fig. 3. Mechanisms of transcriptional activation by p300/CBP. (A) In the bridging model, p300/CBP proteins connect sequence-specific transcription factors to the transcription apparatus. (B) In the scaffold model, p300/CBP act as a protein scaffold for the assembly of multicomponent complexes that confer transcriptional activation. Examples of possible components, such as HAT (a co-factor containing acetyl-transferase activity), JMY and NAP, are indicated. (C) In the HAT model, either the intrinsic HAT activity of p300/CBP or HATs assembled in multicomponent complexes target chromatin and/or transcription factors to facilitate a transcriptional response.

interact both with a wide variety of transcription factors and with components of the basal transcriptional machinery, including TBP, TFIIB, TFIIE and TFIIF (Fig. 1; reviewed by Shikama et al., 1997; Goodman and Smolik, 2000). Therefore, in one model, p300/CBP provides such a bridge.

Since p300/CBP proteins are involved in numerous signal transduction pathways, Rosenfeld and colleagues have proposed that a coordinated re-distribution of p300/CBP activity among different classes of factor in a signal-dependent manner imparts specificity in transcriptional regulation (Kamei et al., 1996). For example, the engagement of p300/CBP by various hormone receptors inhibits AP-1 transcription (Kamei et al., 1996), and overexpression of E2F-1 can hinder p53 transactivation in a p300/CBP-dependent manner (Lee et al., 1998). Similarly, p53 represses TRE-regulated promoters, and such inhibition relies on the physical interaction between p53 and p300 (Avantaggiati et al., 1997). Agonists of Ras-dependent signal transduction can inactivate cAMP-responsive genes by interfering with CREB-dependent transcription (Nakajima et al., 1996). At a mechanistic level, activation of the Ras pathway promotes the association between p300/CBP and the mitogen-regulated S6 kinase $pp90^{Rsk}$, and the physical interaction of $pp90^{Rsk}$ with p300/CBP may be sufficient to block CREB-dependent transcription, since a catalytically inactive $pp90^{Rsk}$ can still block CREB function (Nakajima et al., 1996).

The binding of E1A to p300/CBP inactivates a number of cellular and viral promoters and enhancers (Eckner et al., 1996b; Puri et al., 1997a; Kraus et al., 1992; Lee et al., 1998). For example, E1A inhibits the expression of genes induced by muscle differentiation in a fashion that requires p300 binding to E1A (Puri et al., 1997a; Puri et al., 1997b), and E1A antagonises the SV40 and polyoma virus enhancers in a p300/CBP-dependent manner (Stein et al., 1990). Analysis of $p300^{+/-}$ and $Cbp^{+/-}$ mice suggests that the amount of p300/CBP is limiting under physiological conditions, because some embryonic lethality is observed in the heterozygotes (Yao et al., 1998). Taken together, these findings argue that different signal transduction pathways compete for a pool of p300/CBP and thereby target their effects towards specific sets of responsive genes.

A scaffold for the assembly of multiprotein complexes

p300/CBP proteins might nucleate the assembly of diverse cofactor proteins into multicomponent co-activator complexes (Yao et al., 1996; Korzus et al., 1998; Westin et al., 1998; reviewed by Xu et al., 1999). Moreover, p300/CBP has been frequently found complexed with other HATs, including pCAF (Ogryzko et al., 1996), SRC-1 (Yao et al., 1996; Spencer et al., 1997) and P/CIP/ACTR/AIB1 (Chen et al., 1997). By providing a scaffold for the assembly of transcription cofactors, it might increase the relative concentration of these factors in the local transcription environment and thereby facilitate protein-protein and protein-DNA interactions. Indeed, given a limited repertoire of activators, co-activators and cofactors available for responses to diverse regulatory cues, cells probably use cooperativity and transcriptional synergy so that a combination of a few ubiquitous, signal- and tissue-specific activators can create a potentially very large number of regulatory complexes (Carey, 1998; Kim et al., 1998).

Studies of the human interferon β (IFNβ) enhancer have shown that the surface of p300/CBP provides a scaffold for different components of the transcription apparatus (Kim et al., 1998; Munshi et al., 1998). The recruitment of p300/CBP, together with transcription factors such as ATF2/JUN, p50/p65 of NF-kB and interferon regulatory factor 1, alongside architectural proteins including high mobility group (HMG) proteins, may be important for cooperativity and transcriptional activation (Munshi et al., 1998). Both p300/CBP and pCAF are required for the activation of transcription from the *Ifnβ* enhanceosome (which is a transcription complex required for *Ifnβ* transcription) and, interestingly, p300/CBP and pCAF can acetylate the HMG protein, which leads to the destabilization and disassembly of the enhanceosome - an outcome that may be important in downregulating *Ifnβ* gene expression (Munshi et al., 1998). This suggests that HATs function at different stages during the transcription process which, in the case of *Ifnβ* allows cells to switch off *Ifnβ* gene expression. HATs might then play roles in both transcription activation and inactivation (Munshi et al., 1998).

Acetylation-dependent transcription regulation

Acetylation of multiple sites in the core histone tails is associated with transcriptional activity. Hypo-acetylation generally (but not always) correlates with transcriptional repression, and hyper-acetylation correlates with transcriptional activation (reviewed by Brownell and Allis, 1996; Grunstein, 1997; Wade et al., 1997; Armstrong and Emerson, 1998). Mechanistically, acetylation of lysine residues within the histone tails may have several outcomes: (1) it may promote transcription factors access to DNA in chromatin (Lee et al., 1993; Vetesse-Dadey et al., 1996), possibly by neutralising the positive charge associated with the lysine e-amino group (reviewed by Turner et al., 1991; Wolffe and Pruss, 1996; Wade et al., 1997); (2) it may weaken internucleosomal interactions and de-stabilise higher-order chromatin structure (Luger et al., 1997; Garcia-Ramirez et al., 1995; Tse et al., 1998a; Tse et al., 1998b); and (3) it may promote the processivity of RNA polymerase through nucleosome arrays (Ura et al., 1997; Nightingale et al., 1998). Biochemical and genetic experiments have demonstrated the importance of histone tails as key targets of acetylation, which has a significant impact in regulating transcription (Edmondson et al., 1998; Zhang et al., 1998; Schwartz et al., 1996; Durrin et al., 1991; reviewed by Gregory and Horz, 1998; Mizzen et al., 1998). Specifically, by mutating the N-terminal tail of histone H4, Durrin et al. showed that conditionally active genes, such as *Gal1* or *Pho5*, become less inducible (Durrin et al., 1991). Furthermore, mutation of certain lysine residues in the H3 and H4 tails may bypass the need for GCN5 (a key yeast HAT) for transcriptional activation (Zhang et al., 1998). Acetylation may be responsible for transcriptional repression, because deletion or lysine substitution in the histone H3 tails leads to higher basal levels of Gal1 and Pho5 transcription in budding yeast (Mann and Grunstein, 1992). Consistent with this is the finding that mutations that abolish GCN5 HAT activity reduce transcription activity (Gregory et al., 1999; Georgakopoulos and Thireos, 1992).

An important question is whether the p300/CBP HAT activity targets nucleosomal histones directly and regulates transcription by chromatin remodelling. Although p300/CBP

can acetylate all four core histone in vitro (Bannister and Kouzarides, 1996; Ogryzko et al., 1996), it is not clear whether histones are the bona fide targets of p300/CBP HAT activity in vivo and, if so, which lysine(s) in the histone tails are specifically modified by p300/CBP HAT. When tethered to the Gal4 DNA-binding domain, the p300/CBP HAT domain can stimulate transcription from some promoters, such as the adenovirus major late and E4 promoters, but not others - for example, the adenovirus E1B or the SV40 promoters (Martinez-Balbas et al., 1998). Li et al. elegantly demonstrated the requirement of p300 HAT activity in stimulating transcription from the thyroid hormone receptor βA (*trβa*) promoter and *hsp70* promoter. Importantly, these studies suggested that p300 facilitates transcription from a disrupted chromatin template but is not itself involved in disrupting chromatin structure and instead stabilises a remodelled chromatin state (Li et al., 1998; Li et al., 1999). These studies, alongside other reports, also indicated that non-chromosomal proteins may be targets of acetylation, suggesting that p300/CBP HAT acts upon transcription factors, or the basal transcriptional apparatus, to influence transcription (see below).

More recently, a functional interaction between p300/CBP and a family of proteins involved in nucleosome assembly, the nucleosome assembly proteins (NAP), has been documented (Ito et al., 2000; Shikama et al., 2000). NAP is involved in the assembly of regularly spaced nucleosomal arrays (Bulger et al., 1995). NAP and p300 cooperate in the transcriptional activation of, for example, E2F-1- and p53-target genes, and upregulate the expression of the p53-target gene p21 in a p53-dependent fashion (Shikama et al., 2000). Ito et al. demonstrated that acetylation of histones by p300 helps the transfer of H2A-H2B dimers from nucleosomes to NAP-1 (Ito et al., 2000); such a mechanism might directly couple acetylation of nucleosomes to nucleosome remodeling in transcriptional regulation. Histones H2A and H2B are known to be absent in transcriptionally active chromatin (Baer and Rhodes, 1983; Gonzalez and Palacian, 1989) and also readily exchange out of transcribed chromatin in vivo (Louters and Chalkley, 1985; Jackson, 1990). Biochemical analysis indicated that the removal of H2A/H2B from nucleosomal arrays enhances gene activity, at least in part by decreasing the level of chromatin folding (Tse et al., 1998a; Tse et al., 1998b; Hansen and Wolffe, 1994).

As mentioned previously, a growing list of transcription factors, such as p53 (Gu and Roeder, 1997b; Sakaguchi et al., 1998; Lui et al., 1999a), E2F-1, E2F-2 and E2F-3 (Martinez-Balbás et al., 2000; Marzio et al., 2000), MYB (Tomita et al., 2000), MyoD (Sartorelli et al., 1999), GATA-1 (Boyes et al., 1998), EKLF (Zhang et al., 1998), HNF-4 (Soutogloou et al., 2000) and NF-Y (Li et al., 1998), have been shown to be acetylated. In almost all cases, acetylation enhances their DNA-binding activity. Acetylation at the p53 C-terminal region may cause a conformational change that relieves the inhibitory effect of this region towards p53 DNA binding, leading to increased DNA-binding activity (Gu and Roeder, 1997b). It is also possible that acetylation creates a surface that facilitates protein-DNA recognition; this idea is consistent with the observation that many transcription factors have enhanced DNA-binding activity upon acetylation. Protein acetylation can also regulate protein-protein interaction: acetylation of *Drosophila* TCF inhibits its binding to Armadillo, thereby leading to downregulation of transcription (Waltzer and Bienz, 1998). The viral oncoprotein E1A is also acetylated, and acetylation of Lys 239 in E1A regulates its binding to CtBP (C-terminal binding protein), which is capable of interacting with various transcription repressors (Zhang et al., 2000). The retinoblastoma tumour suppressor protein (pRb) is acetylated in a fashion that influences subsequent phosphorylation of pRb, and E1A can stimulate pRb acetylation (Chan et al., 2001). Components of the basal transcription apparatus - for example, TFIIE and TFIIF, and TAF(I)68 - can also be acetylated (Imhof et al., 1997; Muth et al., 2001). Acetylation of these factors enhances DNA-binding activity and consequently stimulates gene activity (Muth et al., 2001; Wade et al., 1998).

Regulating p300/CBP activity

p300 and CBP are nuclear phosphoproteins (Yaciuk and Moran, 1991). CBP, together with various factors known to be important regulators of the cell cycle and transcription, such as p53, pRb, Daxx, PML, BLM and SUMO-1, reside in a nuclear structure called the nuclear body (reviewed by Zhong et al., 2000). The phosphorylation of p300/CBP appears to be under cell cycle control, although relatively little is known about how phosphorylation affects p300/CBP function. The cyclin-dependent kinases CDK2 and CDC2 (Banjeree et al., 1994) can phosphorylate p300 in vitro, and p300 isolated from undifferentiated F9 cells is unphosphorylated but becomes phosphorylated and competent to activate transcription upon treatment with retinoic acid, which induces F9 cell differentiation (Kitabayashi et al., 1995). The viral oncoproteins SV40 large T antigen and adenovirus E1A bind to the same region on p300/CBP but have different effects on p300/CBP phosphorylation status (Banjeree et al., 1994; Eckner et al., 1996a). Large T antigen co-immunoprecipitates with the hypophosphorylated form of p300 and might prevent p300/CBP phosphorylation (Eckner et al., 1996a). In contrast, E1A stimulates p300/CBP phosphorylation probably through cyclin-CDK complexes (Eckner et al., 1996a).

Banerjee et al., however, reported that E1A blocks p300/CBP phosphorylation by cyclin-CDC2 or CDK2 in vitro, and Perkins et al. reported that cyclin-E-CDK2 negatively regulates p300-mediated co-activation of NF-kB (Banjeree et al., 1994; Perkins et al., 1997). The *p21* gene requires p300 for transcription, which may therefore imply that a positive feedback loop relieves p300/CBP repression by cyclin-E-CDK2 (Perkins et al., 1997). The fact that p21 can increase p300-dependent transcription of NF-kB (Perkins et al., 1997) is consistent with this idea. The C-terminal region of p300/CBP interacts with cyclin-E-CDK2, as well as TFIIB and E1A. The 12S E1A protein, which is an inhibitor of p300-dependent transcription, does not affect the association between p300 and cyclin-E-CDK2 (Felzien et al., 1999). However, the 13S E1A protein, which is also a pleiotropic transcriptional activator, enhances p300-cyclin-E-CDK2 association (Felzien et al., 1999). Binding of cyclin-E-CDK2 to p300 may serve as a checkpoint to integrate extracellular signals with the cell cycle apparatus.

Protein kinase A (PKA; Chrivia et al., 1993; Xu et al., 1998), calcium/calmodulin (CaM)-dependent kinase N (CaMKIV; Chawla et al., 1998) and MAP kinase (MAPK; Janknecht and

Nordheim, 1996) can phosphorylate CBP, and this aids CBP-mediated transcriptional activation, although the critical phosphorylation sites on CBP remain to be elucidated. In PC12 cells, nerve growth factor (NGF) can stimulate CBP phosphorylation through the MAPK pathway, and inhibitors or dominant negative forms of MAPK block activation of a GAL4-CBP hybrid protein by NGF (Liu et al., 1999b). Furthermore, p300/CBP can associate with p42 MAPK in vivo (Liu et al., 1998), and p44 MAPK has been proposed to phosphorylate p300/CBP at the C-terminal region and enhance its HAT activity (Ait-Si-Ali et al., 1999). Following stimulation of the Ras pathway by insulin or NGF, $pp90^{RSK}$ associates with the CH3 domain of CBP and thereby antagonises transcriptional signalling through the cAMP pathway (see above; Nakajima et al., 1996).

An important question regarding p300/CBP function is whether the HAT activity is controlled during the cell cycle, and if so why? Ait-Si-Ali et al. showed that HAT activity peaks at the G1/S transition (Ait-Si-Ali et al., 1998). Furthermore, phosphorylation of p300/CBP by cyclin-E-CDK2 in the C-terminal region of the protein appears to stimulate its HAT activity, and E1A also activates the HAT (Ait-Si-Ali et al., 1998). These observations suggest that the HAT is involved in cell cycle progression and that viral oncoproteins mimic physiological signals that activate p300/CBP HAT. Similarly, E1A was shown to immunoprecipitate enzymatically active HAT fractions of p300/CBP and pCAF (Banjeree et al., 1994). However, this model has been challenged by several reports suggesting that E1A inhibits p300/CBP and pCAF HAT activity in vitro (Chakravarti et al., 1999; Hamamori et al., 1999). Furthermore, p53, TFIIE and TFIIF acetylation is compromised in the presence of E1A (Chakravarti et al., 1999; Li et al., 1999), whereas MyoD acetylation is enhanced (Li et al., 1999). It is possible that these results on the effect of E1A are influenced by the level of E1A protein.

p300/CBP genes and disease

Mutation in *p300/CBP* genes has been observed in a number of human tumours (Petrij et al., 1995; Muraoka et al., 1996; Sobulo et al., 1997; Gayther et al., 2000, reviewed by Giles et al., 1998), which, together with the properties discussed earlier, suggests that p300/CBP proteins possess classical tumour-suppressor-like activity. This idea relates to the possibility that viral oncoproteins, such as E1A and SV40 LT, target p300/CBP to overcome negative effects on cell proliferation (reviewed by Dyson and Harlow, 1992; Moran, 1993). Mutations in *CBP* were first described in RTS, an autosomal-dominant disease characterised by mental retardation, skeletal abnormalities and a high incidence of neoplasia (Petrij et al., 1995). Most RTS patients are heterozygous for mutation in *CBP*, which suggests that a full complement of *CBP* gene dosage is crucial for normal development. $Cbp^{+/-}$ mice exhibit skeletal abnormalities reminiscent of RTS patients (Tanaka et al., 1997; Yao et al., 1998). As discussed above, they have defects in haematopoietic differentiation, which leads to haematological malignancies with advancing age; again this suggests that CBP functions as a tumour suppressor (Kung et al., 1999). Furthermore, bi-allelic somatic mutations in the *p300* gene have been observed in gastric, colon and breast cancers, and mutations in *p300* that result in truncated proteins were detected in primary tumours and tumour cell lines (Muraoka et al., 1996; Gayther et al., 2000). In many cases, the somatic mutations are associated with loss of heterozygosity of the second allele (Gayther et al., 2000). These observations are consistent with Knudson's hypothesis and suggest that in some circumstances *p300* behaves as a classical tumour suppressor gene.

The *p300/CBP* genes are involved in various chromosomal translocation events during haematological malignancy and might contribute to aberrant growth control possibly through a gain of function mutation. For example, an MOZ-CBP fusion resulting from a translocation between *MOZ* (for monocytic leukaemia zinc finger protein) and *CBP* was reported in AML (acute myeloid leukemia; Borrow et al., 1996). The MOZ protein is predicted to be a HAT on the basis of its homology to the yeast HAT SAS2. The hybrid protein results from a 5′-*moz-cbp*-3′ organisation and retains the HAT domains from both proteins. Similarly, a second translocation of the *CBP* gene to the *MLL* (mixed lineage leukaemia) gene occurs in chronic myeloid leukaemia and myelodysplastic syndrome (Sobulo et al., 1997), which arise as a consequence of cancer therapies. In another case, a patient suffering from therapy-related AML was identified as having an in-frame fusion of *MLL* with *p300* (Ida et al., 1997). Since both MOZ and MLL have been implicated in chromatin remodeling, these fused proteins could deregulate gene expression, thereby preventing proper differentiation.

Concluding comments

It has become increasingly clear that p300/CBP proteins are versatile transcriptional co-activators that can influence different physiological processes, including cell growth, proliferation and differentiation. They are likely to participate in DNA replication, tissue differentiation (such as skeletal myogenesis, adipogenesis and B cell differentiation), cell cycle checkpoints, cAMP nuclear signalling, the response to hypoxia, cell adhesion control and the interplay between distinct signal transduction pathways. Indeed, p300/CBP proteins are biologically multifunctional.

However, many key questions remain. For example, the HAT activity of p300/CBP undoubtedly plays a crucial role in its physiological function, and many cellular proteins are known to be acetylated. In cells, protein acetylation may well be analogous to protein phosphorylation and play an equally important role in transducing signals along pathways. It has yet to be confirmed that the HAT activity of p300/CBP is directly involved in nucleosome remodeling, although this possibility seems likely. However, if this is the case, how is p300/CBP HAT activity integrated with chromatin remodeling and other HAT activities? Acetylated nucleosomes may act as a signal to recruit multicomponent transcription complexes to facilitate transcription. In this model, acetylation plays more of a role in signalling instead of directly in chromatin remodeling.

There is much to be learnt about how p300/CBP proteins are regulated. p300 and CBP are nuclear phosphoproteins and, although many kinases have been implicated in the phosphorylation control of p300/CBP, the physiological cues and roles of phosphorylation are not understood. The role of acetylation in the control of p300/CBP also awaits further elucidation.

The recent reports establishing a function for the p300-MDM2 complex in regulating p53 stability through ubiquitination are tantalising. MDM2 is known to possess E3 ubiquitin ligase activity, and p300 might possess a similar enzymatic activity that assists the ubiquitination of p53. It is of considerable interest that acetylation and ubiquitination are both modifications that occur on lysine residues that, in all probability, result in very different consequences for a particular protein target. How are these enzymatic modifications integrated and coordinated during physiological processes?

The importance of p300/CBP in malignancy remains to be elucidated. Increasing evidence supports the view that *p300/CBP* can be under aberrant control in tumour cells. For example, the chromosomal translocation events that affect *CBP* give rise to tumour-specific hybrid proteins. At a mechanistic level, it is important to gain insights into the significance of these translocation events for tumorigenesis. It is certainly possible that such information could have therapeutic benefit in facilitating the design of new approaches towards controlling haematological malignancy and perhaps other types of cancer.

We are left with a feeling of expectation. It is clear that p300/CBP proteins are placed at the centre of the stage, and we can confidently predict that new complexities and hidden mechanisms will emerge from this flourishing area of biomedical research.

We thank Marie Caldwell for help in preparation of this manuscript. Research in our laboratory is supported by the Medical Research Council, Cancer Research Campaign, Leukaemia Research Fund, Wellcome Trust and European Union.

References

Ait-Si-Ali, S., Ramirez, S., Barre, F.-X., Dkhissi, F., Magnaghi-Jaulin, L., Girault, J. A., Robin, P., Knibiehler, M., Pritchard, l. L., Ducommun, B. et al. (1998). Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature* **396**, 184-186.

Ait-Si-Ali, S., Carlisi, D., Ramirez, S., Upegui-Gonzalez, L. C., Duquet, A., Robin, P., Rudkin, B., Harel-Bellan, A. and Trouche, D. (1999). Phosphorylation by p44 MAP kinase/ERK1 stimulates CBP histone acetyl transferase activity in vitro. *Biochem. Biophys. Res. Commun.* **262**, 157-162.

An, W. G., Kanekal, M., Simon, M., Maltepe, E., Blagosklonny, M. V. and Neckers, L. M. (1998). Stabilisation of wild-type p53 by hypoxia-inducible factor-1a. *Nature* **392**, 405-408.

Arany, Z., Sellers, W. R., Livingston, D. M. and Eckner, R. (1994). E1A-associated p300 and CREB-associated CBP belong to a conserved family of co-activators. *Cell* **77**, 799-800.

Arany, Z., Newsome, D., Oldread, E., Livingston, D. M. and Eckner, R. (1995). A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. *Nature* **374**, 81-84.

Arany, Z., Huang, L. E., Eckner, R., Bhattacharya, S., Jiang, C., Goldberg, M. A., Bunn, H. F. and Livingston, D. M. (1996). An essential role for p300/CBP in the cellular response to hypoxia. *Proc. Natl. Acad. Sci. USA* **93**, 12969-12973.

Armstrong, J. A. and Emerson, B. M. (1998). Transcription of chromatin: these are complex times. *Curr. Opin. Genet. Dev.* **8**, 165-172.

Avantaggiati, M. L., Ogrykko, V., Garner, K., Giordano, A., Levine, A. S. and Kelly, K. (1997). Recruitment of p300/CBP in p53-dependent signal pathways. *Cell* **89**, 1175-1184.

Baer, B. W. and Rhodes, D. (1983). Eukaryotic RNA polymerase II binds to nucleosome cores from transcribed genes. *Nature* **301**, 482-488.

Banerjee, A. C., Recupero, A. J., Mal, A., Piotrkowski, A. M., Wang, D. M. and Harter, M. L. (1994). The adenovirus E1A 289R and 243R proteins inhibit the phosphorylation of p300. *Oncogene* **9**, 1733-1737.

Bannister, A. J. and Kouzarides, T. (1996). The CBP co-activator is a histone acetyltransferase. *Nature* **384**, 641-643.

Bannister, A. J., Miska, E. A., Gorlich, D. and Kouzarides, T. (2000). Acetylation of importin-a nuclear import factor by CBP/p300. *Curr. Biol.* **10**, 467-470.

Bayle, J. H. and Crabtree, G. R. (1997). Protein acetylation: more than chromatin modification to regulate transcription. *Chem. Biol.* **4**, 885-888.

Borrow, J., Stanton, V. P., Andresen, J. M., Becher, R. Behm, F. G., Chaganti, R. S., Civin, C. I., Discteche, C., Dube, I., Frichauf, A. M. et al. (1996). The translocation t(8;16)(p11,p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat. Genet.* **14**, 33-41.

Boyes, J., Byfield, P., Nakatani, Y. and Ogryzko, V. (1998). Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* **396**, 594-598.

Brett, D., Whitehouse, S., Antonson, P., Shipley, J., Cooper, C. and Goodwin, G. (1997). The SYT protein involved in the t(x;18) synovial sarcoma translocation is a transcriptional activator localised in nuclear bodies. *Hum. Mol. Genet.* **6**, 1559-1564.

Brownell, J. E. and Allis, C. D. (1996). Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr. Opin. Genet. Dev*. **6**, 176-184.

Bulger, M., Ito, T., Kamakaka, R. T. and Kadonaga, J. T. (1995). Assembly of regularly spaced nucleosome arrays by *Drosophila* chromatin assembly factor 1 and a 56-kDa histone-binding protein. *Proc. Natl. Acad. Sci. USA* **92**, 11726-11730.

Carmeliet, P., Dor, Y., Herbert, J. M., Fukumura, D., Brusselmans, K., Dewerchin, M., Neeman, M., Bono, F., Abramovitch, R., Maxwell, P. et al. (1998). Role of HIF-1a in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature* **394**, 485-490.

Carey, M. (1998). The enhanceosome and transcriptional synergy. *Cell* **92**, 5-8.

Chakravarti, D., Ogryzko, V., Kao, H.-Y., Nash, A., Chen, H., Nakatani, Y. and Evans, R. M. (1999). A viral mechanism for inhibition of p300 and pCAF acetyltransferase activity. *Cell* **96**, 393-403.

Chan, H.-M., Krstic-Demonacos, M., Smith, L., Demonacos, C. and La Thangue, N. B. (2001). Acetylation control of the retinoblastoma tumour suppressor protein. *Nat. Cell Biol.* (in press).

Chawla, S., Hardingham, G. E., Quinn, D. R. and Bading, H. (1998). A signal-regulated transcriptional co-activator controlled by nuclear calcium and CaM kinase IV. *Science* **281**, 1505-1509.

Chen, X. and Korenberg, J. R. (1995). Localisation of human CREBBP to 16p13.3 by fluorescence in situ hybridisation. *Cytogenet. Cell Genet.* **71**, 56-57.

Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y. and Evans, R. M. (1997). Nuclear receptor co-activator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**, 569-580.

Chrivia, J. C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R. and Goodman, R. H. (1993). Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature* **365**, 855-859.

Clark, J., Rocques, P. J., Crew, A. J., Gill, S., Shipley, J., Chan, A. M., Gusterson, B. A. and Cooper, C. S. (1994). Identification of novel genes, SYT and SSX, involved in the t(x;18)9p11.2;q11.2) translocation found in human synovial sarcoma. *Nat. Genet*. **7**, 502-508.

Dallas, P. B., Cheney, I. W., Liao, D., Bowrin, V., Byam, W., Pacchione, S., Kobayashi, R., Yaciuk, P. and Moran, E. (1998). p300/CREB binding protein-related protein p270 is a component of mammalian SWI-SNF complexes. *Mol. Cell. Biol.* **18**, 3596-3603.

Dhalluin, C., Carlson, J. E., Zeng, L., He, C., Aggarwal, A. K. and Zhou, M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491-496.

Durrin, L. K., Mann, R. K., Kayne, P. S. and Grunstein, M. (1991). Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. *Cell* **65**, 1023-1031.

Dyson, N. (1998). The regulation of E2F by pRB-family proteins. *Genes Dev.* **12**, 2245-2262.

Dyson, N. and Harlow, E. (1992). Adenovirus E1A targets key regulators of cell proliferation. *Cancer Surv.* **12**, 161-195.

Durrin, L. K., Mann, R. K., Kayne, P. S. and Grunstein, M. (1991). Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. *Cell* **65**, 1023-1031.

Eckner, R., Ewen, M. E., Newsome, D., Gerdes, M., DeCaprio, J. A., Lawrence, J. B. and Livingston, D. M. (1994). Molecular cloning and functional analysis of the adenovirus E1A-associated 300-KD protein

(p300) reveals a protein with properties of a transcriptional adaptor. *Genes Dev.* **8**, 869-884.

Eckner, R., Ludlow, J. W., Lill, N. L., Oldread, E., Arany, Z., Modjtahedi, N., DeCaprio, J. A., Livingston, D. M. and Morgan, J. A. (1996a) Association of p300 and CBP with simian virus 40 large T antigen. *Mol. Cell. Biol*. **16**, 3454-3464.

Eckner, R., Yao, T. P., Oldread, E., Arany, Z., Modjtahedi, N., DeCaprio, J. A. and Livingston, D. M. (1996b). Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. *Genes Dev.* **10**, 2478-2490.

Edmondson, D. G., Zhang, W., Watson, A., Xu, W., Bone, J. R., Yu, Y., Stillman, D. and Roth, S. Y. (1998). In vivo functions of histone acetylation/deacetylation in Tup1p repression and Gcn5p activation. *Cold Spring Harbor Symp. Quant. Biol.* **63**, 459-468.

Eid, J. E., Kung, A. L., Scully, R. and Livingston, D. M. (2000). p300 interacts with the nuclear proto-oncoprotein SYT as part of the active control of cell adhesion. *Cell* **102**, 839-848.

Felzien, L. K., Farrell, S., Betts, J. C., Mosavin, R. and Nabel, G. (1999). Specificity of Cyclin E-Cdk2, TFIIB, and E1A interactions with a common domain of the p300 co-activator. *Mol. Cell. Biol.* **19**, 4241-4246.

Garcia-Rameriez, M., Rocchini, C. and Ausio, J. (1995). Modulation of chromatin folding by histone acetylation. *J. Biol. Chem*. **270**, 17923-17928.

Gayther, S. A., Batley, S. J., Linger, L., Bannister, A., Thorpe, K., Chin, S., Daigo, Y., Russell, P., Wilson, A., SowJoy, H. M. et al. (2000). Mutations truncating the EP300 acetylase in human cancers. *Nat. Genet*. **24**, 300-304.

Georgakopoulos, T. and Thireos, G. (1992). Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.* **11**, 4145-4151.

Giles, R. H., Dauwerse, H. G., Van Ommen, G. B. and Breuning, M. H. (1997). Do human chromosomal bands 16p13 and 22q11-13 share ancestral origins? *Am. J. Hum. Genet.* **63**, 1240-1242.

Giles, R. H., Peters, D. J. M. and Breuning, M. H. (1998). Conjunction dysfunction: CBP/p300 in human disease. *Trends Genet.* **14**, 178-183.

Giordano, A. and Avantaggiati, M. L. (1999). p300 and CBP: partners for life and death. *J. Cell. Physiol*. **181**, 218-230.

Gonzalez, P. J. and Palacian, E. (1989). Interaction of RNA polymerase II with structurally altered nucleosomal particles. Transcription is facilitated by loss of one H2A.H2B dimer. *J. Biol. Chem.* **264**, 18457-18462.

Goodman, R. H. and Smolik, S. (2000). CBP/p300 in cell growth, transformation, and development. *Genes Dev.* **14**, 1553-1577.

Gregory, P. D. and Horz, W. (1998). Life with nucleosomes: chromatin remodelling in gene regulation. *Curr. Opin. Cell Biol.* **10**, 339-345.

Gregory, P. D., Schmid, A., Zavari, M., Lui, L., Berger, S. L. and Horz, W. (1999). Absence of Gcn5 HAT activity defines a novel state in the opening of chromatin at the PHO5 promoter in yeast. *Mol. Cell* **1**, 495-505.

Grossman, S. R., Perez, M., Kung, A. L., Joseph, M., Mansur, C., Xiao, Z. X., Kumar, S., Howley, P. M. and Livingston, D. M. (1998). p300/MDM2 complexes participate in MDM2-mediated p53 degradation. *Mol. Cell* **2**, 405-415.

Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature* **389**, 349-352.

Gu, W. and Roeder, R. G. (1997a). Synergistic activation of transription by CBP and p53. *Nature* **387**, 819-822.

Gu, W. and Roeder, R. G. (1997b). Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**, 595-606.

Hamamori, Y., Sartorelli, V., Ogryzko, V., Puri, P. L., Wu, H.-Y., Wang, J. Y. J., Nakatani, Y. and Kedes, L. (1999). Regulation of histone acetyltransferases p300 and pCAF by the bHLH protein twist and adenoviral oncoprotein E1A. *Cell* **96**, 405-413.

Hansen, J. C. and Wolffe, A. P. (1994). A role for histones H2A/H2B in chromatin folding and transcriptional repression. *Proc. Natl. Acad. Sci. USA* **91**, 2339-2343.

Honda, R., Tanaka, H. and Yasuda, H. (1997). Oncoprotein MDM2 is a ubiquitin ligase E3 for tumour suppressor p53. *FEBS Lett.* **420**, 25-27.

Ida, K., Kitabayashi, I., Taki, T., Taniwaki, M., Noro, K., Yamamoto, M., Ohki, M. and Hayashi, Y. (1997). Adenoviral E1A-associated protein p300 is involved in acute myeloid leukemia with t(11;22)(q23;q13). *Blood* **90**, 4699-4704.

Imhof, A., Yang, X. J., Ogryzko, V. V., Nakatani, Y., Wolffe, A. P. and Ge, H. (1997). Acetylation of general transcription factors by histone acetyltransferases. *Curr. Biol*. **7**, 689-692.

Ito, T., Ikehara, T., Nakagawa, T., Lee Kraus, W. and Muramatsu, M. (2000). p300-mediated acetylation facilitates the transfer of histone H2A-H2B dimers from nucleosomes to a histone chaperone. *Genes Dev.* **14**, 1899-1907.

Jackson, V. (1990). In vivo studies on the dynamics of histone-DNA interaction: evidence for nucleosome dissolution during replication and transcription and a low level of dissolution independent of both. *Biochemistry* **29**, 719-731.

Jacobson, R. H., Ladurner, A. G., King, D. S. and Tjian, R. (2000). Structure and function of a human TAFII250 double bromodomain module. *Science* **288**, 1422-1425.

Janknecht, R. and Hunter, T. (1996). Transcription control: versatile molecular glue. *Curr. Biol.* **6**, 22-23.

Janknecht, R. and Nordheim, A. (1996). MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. *Biochem. Biophys. Res. Commun.* **228**, 831-837.

Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass C. K. et al. (1996). A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85**, 403-414.

Kawasaki, H., Eckner, R., Yao, T. P., Taira, K., Chiu, R., Livingston, D. M. and Yokoyama, K. K. (1998). Distinct role of the co-activators p300 and CBP in retinoic-acid-induced F9-cell differentiation. *Nature* **393**, 284-289.

Keirnan, R., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K.-T. et al. (1999). HIV-1 Tat transcriptional activity is regulated by acetylation. *EMBO J*. **18**, 6106-6118.

Kim, T. K., Kim, T. H. and Maniatis, T. (1998). Efficient recruitment of TFIIB and CBP-RNA polymerase II holoenzyme by an interferon-beta enhanceosome in vitro. *Proc. Natl. Acad. Sci. USA* **95**, 12191-12196.

Kitabayashi, I., Eckner, R., Arany, Z., Chiu, R., Gachelin, G., Livingston, D. M. and Yokoyama, K. K. (1995). Phosphorylation of the adenovirus E1A-associated 300KDa protein in response to retinoic acid and E1A during the differentiation of F9 cells. *EMBO J.* **14**, 33496-33509.

Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K. and Rosenfeld, M. G. (1998). Transcription factor-specific recruitment of co-activators and their acetyltransferase functions. *Science* **279**, 703-707.

Kraus, V. B., Moran, E. and Nevins, J. R. (1992). Promoter-specific *trans*-activation by the adenovirus $E1A_{12S}$ product involves separate E1A domains. *Mol. Cell. Biol.* **12**, 4391-4399.

Kung, A. L., Rebel, V. I., Bronson, R. T., Ch'ng, L., Sieff, C. A., Livingston, D. M. and Yao, T. (1999). Gene dosage-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev.* **14**, 272-277.

Lee, D. Y., Hayes, J. J., Pruss, D. and Wolffe, A. P. (1993). A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* **72**, 73-84.

Lee, C. W., Sørensen, T. S., Shikama, N. and La Thangue, N. B. (1998). Functional interplay between p53 and E2F through co-activator p300. *Oncogene* **16**, 2695-2710.

Li, Q., Herrler, M., Landsberger, N., Kaludov, N., Ogryzko, V. V., Nakatani, Y. and Wolffe, A. P. (1998). *Xenopus* NF-Y pre-sets chromatin to potentiate p300 and acetylation-responsive transcription from the *Xenopus hsp70* promoter in vivo. *EMBO J.* **17**, 6300-6315.

Li, Q., Imhof, A., Collingwood, T. N., Urnov, F. D. and Wolffe, A. P. (1999). p300 stimulates transcription instigated by ligand-bound thyroid hormone receptor at a step subsequent to chromatin disruption. *EMBO J.* **18**, 5634-5652.

Lill, N. L., Grossman, S. R., Ginsberg, D., DeCaprio, J. and Livingston, D. M. (1997). Binding and modulation of p53 by p300/CBP co-activators. *Nature* **387**, 823-827.

Liu, Y. Z., Chirivia, J. C. and Latchman, D. S. (1998). Nerve growth factor up-regulates the transcriptional activity of CBP through activation of the p42/p44 (MAPK) cascade. *J. Biol. Chem.* **273**, 32400-32407.

Liu, L., Scolnick, D. M., Trievel, C., Zhang, H. B., Marmorstein, R., Halazonetis, T. D. and Berger, S. L. (1999a). p53 sites acetylated in vitro by pCAF and p300 are acetylated in vivo in response to DNA damage. *Mol. Cell. Biol.* **19**, 1202-1209.

Liu, Y. Z., Thomas, N. S. B. and Latchman, D. S. (1999b). CBP associates with the p42/p44 MAPK enzymes and is phosphorylated following NGF treatment. *NeuroReport* **10**, 1239-1243.

Louters, L. and Chalkley, R. (1985). Exchange of histones H1, H2A, and H2B in vivo. *Biochemistry* **24**, 3080-3085.

Lundbiad, J. R., Kwok, R. P. S., Laurance, M. E., Harter, M. L. and Goodman, R. H. (1995). Adenoviral E1A-associated protein p300 as a

functional homologue of the transcriptional co-activator CBP. *Nature* **374**, 85-88.

Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. and Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8A resolution. *Nature* **389**, 251-260.

Mann, R. K. and Grunstein, M. (1992). Histone H3 N-terminal mutations allow hyperactivation of yeast GAL1 gene in vivo. *EMBO J.* **11**, 3297-3306.

Martinez-Balbas M. A., Bannister A. J., Martin K., Haus-Seuffert P., Meisterernst, M. and Kouzarides, T. (1998). The acetyltransferase activity of CBP stimulates transcription. *EMBO J.* **17**, 2886-2893.

Martinez-Balbás, M. A., Bauer, U.-M., Nielsen, S. J., Brehm, A. and Kouzarides, T. (2000). Regulation of E2F-1 activity by acetylation. *EMBO J.* **19**, 662-271.

Marzio, G., Wagnerer, C., Gutierez, M. I., Cartwright, P., Helin, K. and Giacca, M. (2000). E2F family members are differentially regulated by reversible acetylation. *J. Biol. Chem.* **275**, 10887-10892.

Missero, C., Calautti, E., Eckner, R., Chin, J., Tsai, L. H., Livingston, D. M. and Dotto, G. P. (1995). Involvement of the cell-cycle inhibitor Cip1/WAF1 and the E1A-associated p300 protein in terminal differentiation. *Proc. Natl. Acad. Sci. USA* **92**, 5451-5455.

Mizzen, C., Kuo, M. H., Smith, E., Brownell, J., Zhou, J., Ohba, R., Wei, Y., Monaco, L., Sassone-Corsi, P. and Allis, C. D. (1998). Signaling to chromatin through histone modifications: how clear is the signal? *Cold Spring Harbor Symp. Quant. Biol.* **63**, 469-481.

Moran, E. (1993). DNA tumour virus transforming proteins and the cell cycle. *Curr. Opin. Genet. Dev.* **3**, 63-70.

Morris, L., Allen, K. E. and La Thangue, N. B. (2000). Regulation of E2F transcription by cyclin E/cdk2 kinase mediated through p300/CBP co-activators. *Nat. Cell Biol.* **12**, 232-239.

Munshi, N., Merika, M., Yie, J., Senger, K., Chen, G. and Thanos, D. (1998). Acetylation of HMG I(Y) by CBP turns off IFNβ expression by disrupting the enhanceosome. *Mol. Cell* **2**, 457-467.

Muraoka, M., Konishi, M., Kikuchi, R., Tanaka, K., Shitara, N., Chong, J. M., Iwama, T. and Miyaki, M. (1996). p300 gene alterations in colorectal and gastric carcinomas. *Oncogene* **12**, 1565-1569.

Muth, V., Nadaud, S., Grummt, I. and Voit, R. (2001). Acetylation of TAF(I)68, a subunit of TIF-IB/SL1, activtes RNA polymerase I transcription. *EMBO J.* **20**, 1353-1362.

Nakajima, T., Fukamizy, A., Takahashi, J., Gage, F. H., Fisher, T., Blenis, J. and Montminy, M. R. (1996). The signal-dependent co-activator CBP is a nuclear target for pp90RSK. *Cell* **86**, 465-474.

Nightingale, K. P., Wellinger, R. E., Sogo, J. M. and Becker, P. B. (1998). Histone acetylation facilitates RNA polymerase II transcription of the *Drosophila hsp26* gene in chromatin. *EMBO J.* **17**, 2865-2875.

Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. and Nakatani, T. (1996). The transcriptional co-activators p300 and CBP are histone acetyltransferases. *Cell* **87**, 1107-1112.

Perkins, N. D., Felzien, L. K., Betts, J. C., Leung, K., Beach, D. H. and Nabel, G. L. (1997). Regulation of NF-kB by cyclin-dependent kinases associated with the p300 co-activator. *Science* **275**, 523-527.

Petrij, F., Giles, R. H., Dauwerse, H. G., Saris, J. J., Hennekam, R. C., Masuno, M., Tommerup, N., van Ommen, G. J., Goodman, R. H. and Peters D. J. (1995). Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* **376**, 348-351.

Puri, P. L., Avantaggiati, M. L., Balsano, C., Sang, N., Graessmann, A., Giordano, A. and Levrero, M. (1997a). p300 is required for MyoD-dependent cell cycle arrest and muscle-specific gene transcription. *EMBO J.* **16**, 369-383.

Puri, P. L., Sartorelli, V., Yang, X., Hamamori, Y., Ogryzko, V. V., Howard, B. H., Kedes, L., Wang, Y. J., Graessmann, A., Nakatani, Y. et al. (1997b). Differential roles of p300 and pCAF acetyltransferase in muscle differentiation. *Mol. Cell* **1**, 35-45.

Sakaguchi, K., Herrera, J. E., Saito, S.-I., Miki, T., Bustin, M., Vassilev, A., Anderson, C. W. and Appella, E. (1998). DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* **12**, 2831-2841.

Sartorelli, V., Puri, P. L., Hamamori, Y., Ogryzko, V., Chung, G., Nakatani, Y., Wang, J. Y. J. and Kedes, L. (1999). Acetylation of MyoD directed by pCAF is necessary for the execution of the muscle program. *Mol. Cell* **4**, 725-734.

Schwarz, P. M., Felthauser, A., Fletcher, T. M. and Hansen, J. C. (1996). Reversible oligonucleotide self-association: dependence on divalent cations and core histone tail domains. *Biochemistry* **35**, 4009-4015.

Shi, Y. and Mello, C. (1998). A CBP/p300 homolog specifies multiple differentiation pathways in *Caenorhabditis elegans*. *Genes Dev.* **12**, 943-955.

Shikama, N., Lyon, J. and La Thangue, N. B. (1997). The p300/CBP family: integrating signals with transcription factors and chromatin. *Trends Cell Biol.* **7**, 230-236.

Shikama, N., Lee, C. W., France, S., Delavaine, L., Lyon, J., Krstic-Demonacos, M. and La Thangue, N. B. (1999). A novel cofactor for p300 that regulates the p53 response. *Mol. Cell* **4**, 365-376.

Shikama, N., Chan, H., Smith, L., Krstic-Demonacos, M., Lee, C., Cairns, W. and La Thangue, N. (2000). Functional interaction between nucleosome assembly proteins and p300/CBP co-activators. *Mol. Cell. Biol.* **20**, 8933-8943.

Sobulo, O. M., Borrow, J., Tomek, R., Reshmi, S., Harden, A., Schlegelberger, B., Housman, D., Doggett, N. A., Rowley, J. D. and Zeleznik-Le, N. J. (1997). MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;q13.3). *Proc. Natl. Acad. Sci. USA* **94**, 8732-8737.

Soutogloou, E., Katrakili, N. and Talianidis, I. (2000). Acetylation regulates transcription factor activity at multiple levels. *Mol. Cell* **5**, 745-751.

Spencer, T. E., Jenster, G., Burcin, M. M., Assis, C. D., Zhou, J., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai, S. Y., Tsai, M. J. et al. (1997). Steroid receptor co-activator-1 is a histone acetyltransferase. *Nature* **289**, 194-198.

Stein, R. W., Corrigan, M., Yaciul, P., Whelan, J. and Moran, E. (1990). Analysis of E1A-mediated growth regulation functions: binding of the p300-kilodalton cellular product correlates with E1A enhancer repression function and DNA synthesis-inducing activity. *J. Virol.* **64**, 4421-4427.

Tanaka, Y., Naruse, I., Maekawa, T., Masuya, H., Shiroshi, T. and Ishii, S. (1997). Abnormal skeletal patterning in embryos lacking a single Cbp allele: a partial similarity with Rubinstein-Taybi syndrome. *Proc. Natl. Acad. Sci. USA* **94**, 10215-10220.

Thomas, A. and White, E. (1998). Suppression of the p300-dependent mdm2 negative-feedback loop induces the p53 apoptotic function. *Genes Dev.* **12**, 1975-1985.

Tomita, A., Towatari, M., Tsuzuki, S., Hayakawa, F., Kosugi, H., Tamai, K., Miyazaki, T., Kinoshita, T. and Saito, H. (2000). c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300. *Oncogene* **19**, 444-451.

Tse, C., Fletcher, T. M. and Hansen, J. C. (1998a). Enhanced transcription factor access to arrays of histone H3/H4 tetramer. DNA complexes in vitro: implications for replication and transcription. *Proc. Natl. Acad. Sci. USA.* **21**, 12169-121673.

Tse, C., Sera, T., Wolffe, A. P. and Hansen, J. C. (1998b). Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. *Mol. Cell Biol.* **18**, 4629-4638.

Turner, B. M. (1991). Histone acetylation and control of gene expression. *J. Cell Sci.* **99**, 13-20.

Ura, K., Kurumizaka, H., Dimitrov, S., Almouzni, G. and Wolffe, A. P. (1997). Histone acetylation: influence on transcription, nucleosome mobility and positioning, and linker histone-dependent transcriptional repression. *EMBO J.* **16**, 2096-2107.

Vettese-Dadey, M., Grant, P. A., Hebbes, T. R., Crane-Robinson, C., Allis, C. D. and Workman, J. L. (1996). Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. *EMBO J.* **15**, 2508-2518.

Wade, P. A., Pruss, D. and Wolffe, A. P. (1997). Histone acetylation: chromatin in action. *Trends Biochem. Sci.* **22**, 128-32.

Wade, P. A., Jones, P. L., Vermaak, D., Veenstra, G. J., Imhof, A., Sera, T., Tse, C., Ge, H., Shi, Y. B., Hansen, J. C. et al. (1998). Histone deacetylase directs the dominant silencing of transcription in chromatin: association with MeCP2 and the Mi-2 chromodomain SW1/SNF ATPase. *Cold Spring Harbor Symp. Quant. Biol.* **63**, 435-446.

Waltzer, L. and Bienz, M. (1998). Drosophila CBP represses the transcription factor TCF to antagonize wingless signalling. *Nature* **395**, 521-525.

Wang, H.-G. H., Moran, E. and Yacuik, P. (1995). E1A promotes association between p300 and pRb in multimeric complexes required for normal biological activity. *J. Virol.* **69**, 7917-7924.

Westin, S., Kurokowa, R., Nolte, R. T., Wisely, G. B., McInerney, E. M., Rose, D. W., Milburn, M. V., Rosenfeld, M. G. and Glass, C. K. (1998). Interactions controlling the assembly of nuclear-receptor heterodimers and co-activators. *Nature* **305**, 199-202.

Whyte, P., Williamson, N. M. and Harlow, E. (1989). Cellular targets for transformation by the adenovirus E1A proteins. *Cell* **56**, 67-75.

Wolffe, A. P. and Pruss, D. (1996). Targeting chromatin disruption: transcription regulators that acetylate histones. *Cell* **84**, 817-819.

Wydner, K., Bhattacharya, S., Eckner, R., Lawrence, J. B. and Livingston, D. M. (1995). Localisation of human CREB binding protein gene to 16p13.2-13.3 by fluorescent in situ hybridisation. *Genomics* **30**, 395-396.

Xu, L., Lavinsky, R. M., Dasen, J. S., Flynn, S. E., McInerney, E. M., Mullen, T. M., Heinzel, T., Szeto, D., Korzus, E. and Kurokawa, R. (1998). Signal-specific co-activator domain requirement for Pit-1 activation. *Nature* **395**, 301-306.

Xu, L., Glass, C. K. and Rosenfeld, M. G. (1999). Co-activator and co-repressor complexes in nuclear receptor function. *Curr. Opin. Genet. Dev.* **9**, 140-147.

Yaciuk, P. and Moran, E. (1991). Analysis with specific polyclonal antiserum indicates that the E1A-associated 300-KDa product is a stable nuclear phosphoprotein that undergoes cell cycle phase-specific modification. *Mol. Cell. Biol.* **11**, 5389-5397.

Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H. and Nakatani, Y. (1996). A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* **382**, 319-324.

Yao, T., Ku, G., Zhou, N., Scully, R. and Livingston, D. M. (1996). The nuclear hormone receptor co-activator SRC-1 is a specific target of p300. *Proc. Natl. Acad. Sci. USA* **93**, 10626-10631.

Yao, T., Oh, S. P., Fuchs, M., Zhou, N., Ch'ng, L., Newsome, D., Bronson, R. T., Li, E., Livingston, D. M. and Eckner, R. (1998). Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* **93**, 361-372.

Yuan, W., Condorelli, G., Caruso, M., Felsani, A. and Giordano, A. (1996). Human p300 protein is a co-activator for the transcription factor MyoD. *J. Biol. Chem.* **271**, 9009-9013.

Yuan, Z., Huang, Y., Ishiko, T., Nakada, S., Utsugisawa, T., Shioya, H., Utsugisawa, Y., Shi, Y., Weichselbaum, R. and Kufe, D. (1999). Function for p300 and not CBP in the apoptotic response to DNA damage. *Oncogene* **18**, 5714-5717.

Zhang, W. and Bieker, J. J. (1998). Acetylation and modulation of erythroid Krüppel-like factor (EKLF) activity by interaction with histone acetyltransferases. *Proc. Natl. Acad. Sci. USA* **95**, 9855-9860.

Zhang, W., Bone, J. R., Edmondson, D. G., Turner, B. M. and Roth, S. Y. (1998). Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. *EMBO J.* **17**, 3155-3167.

Zhang, Q., Yao, H., Vo, N. and Goodman, R. H. (2000). Acetylation of adenovirus E1A regulates binding of the transcriptional corepressor CtBP. *Proc. Natl. Acad. Sci. USA.* **26**, 14323-14328.

Zhong, S., Salomoni, P. and Pandolfi, P. P. (2000). The transcriptional role of PML and the nuclear body. *Nat. Cell Biol.* **2**, 85-90.