Conditional Knockout Mice Reveal Distinct Functions for the Global Transcriptional Coactivators CBP and p300 in T-Cell Development

Lawryn H. Kasper,¹ Tomofusa Fukuyama,¹ Michelle A. Biesen,¹ Fayçal Boussouar,¹ Caili Tong,³
Antoine de Pauw,² Peter J. Murray,² Jan M. A. van Deursen,³ and Paul K. Brindle^{1*}

Department of Biochemistry¹ and Department of Infectious Diseases,² St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38105, and Department of Pediatric and Adolescent Medicine and Department of Biochemistry and Molecular Biology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905³

Received 10 July 2005/Returned for modification 8 August 2005/Accepted 9 November 2005

The global transcriptional coactivators CREB-binding protein (CBP) and the closely related p300 interact with over 312 proteins, making them among the most heavily connected hubs in the known mammalian protein-protein interactome. It is largely uncertain, however, if these interactions are important in specific cell lineages of adult animals, as homozygous null mutations in either *CBP* or *p300* result in early embryonic lethality in mice. Here we describe a Cre/LoxP conditional *p300* null allele (*p300^{lox}*) that allows for the temporal and tissue-specific inactivation of *p300*. We used mice carrying *p300^{lox}* and a *CBP* conditional knockout allele (*CBP^{lox}*) in conjunction with an *Lck-Cre* transgene to delete *CBP* and *p300* starting at the CD4⁺ CD8⁺ double-negative thymocyte stage of T-cell development. Loss of either *p300* or *CBP* led to a decrease in CD4⁺ CD8⁺ double-positive thymocytes, but an increase in the percentage of CD8⁺ single-positive thymocytes seen in *CBP* mutant mice was not observed in *p300* mutants. T cells completely lacking both *CBP* and *p300* did not develop normally and were nonexistent or very rare in the periphery, however. T cells lacking *CBP* or *p300* had reduced tumor necrosis factor alpha gene expression in response to phorbol ester and ionophore, while signal-responsive gene expression in *CBP*- or *p300*-deficient macrophages was largely intact. Thus, *CBP* and *p300* each supply a surprising degree of redundant coactivation capacity in T cells and macrophages, although each gene has also unique properties in thymocyte development.

CREB-binding protein (CBP) (*Crebbp*) and p300 (*Ep300*) are ubiquitously expressed ~2,400-residue nuclear phosphoproteins that share 61% overall sequence identity (~80 to 90% in evolutionarily conserved domains). Homologs of CBP and p300 are found in *Drosophila melanogaster* and *Caenorhabditis elegans*, but there are no other closely related family members in mammals. These two coactivators typically bind to the activation domains of transcription effectors via five unique protein-binding domains (nuclear hormone receptor binding domain, CH1, KIX, CH3, and SID/IBiD) (64, 92). CBP and p300 appear to function redundantly in many instances, but they also have unique properties, particularly in vivo (92). The lack of other closely related family members or conservation of these protein-binding domains in other mammalian proteins implies that CBP and p300 are indispensable.

Following recruitment by transcription factors, juxtaposition of the adaptor, acetyltransferase, and ubiquitin ligase functions of CBP and p300 at the promoter/enhancer is hypothesized to stimulate target gene transcription (92). Histones are believed to be the main acetylation targets of CBP and p300, thereby alleviating repressive chromatin and potentiating transcription, but the ability of CBP and p300 to regulate transcription factor activity by acetylation of specific lysine residues is also thought to be crucial (27, 176, 184). The importance of CBP and p300 for the transcription of endogenous genes, however, has not been widely investigated.

An astonishingly large fraction of the estimated 1,962 (human) transcriptional regulators (134), as well as other proteins, have been shown to interact physically and functionally with CBP and p300 (described interactions now total at least 312) (Fig. 1; more detailed and up to date information is available at <http://www.stjude.org/brindle>). This number of interactions puts CBP and p300 at the top end of known "connectivity" in the interactome, as high-throughput screens to identify *Drosophila*, *C. elegans*, and mammalian interactome networks indicate that only a very small fraction of proteins have as many as 100 to 200 interacting partners (8, 60, 118). It may even be inferred that CBP and p300 interact with the great majority of transcriptional regulators, as most have not been tested. More than half (>190) of the described CBP/p300 interactors are encoded by essential genes in mice (Fig. 1), thus the common assumption that loss of CBP or p300 will have catastrophic effects on specific cell lineages because of broad alterations in gene expression appears reasonable. Also implicit from the high level of connectivity for CBP and p300 is that cells cannot exist in the absence of both coactivators, although it has not been possible to test this rigorously because a conditional knockout allele for *p300* has not been available.

It is generally thought that CBP and p300 together are present in limiting amounts in cells, consistent with the view that they are widely involved in transcription (64, 115, 203). Mice that are homozygous for *CBP* and *p300* null alleles, or doubly heterozygous, die during embryogenesis, providing strong evidence for this notion (190, 223). Mice and humans that are haploinsufficient for *CBP* have developmental defects, also consistent with models that invoke limiting levels of CBP

* Corresponding author. Mailing address: Department of Biochemistry, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38105. Phone: (901) 495-2522. Fax: (901) 525-8025. E-mail: paul.brindle@stjude.org.

53BP1* (89)	Dach1 (81)	GR (27)	MDM2* (64)	p532 (89)	Smad7 (65)
Actin (89)	Daxx (43)	H2A (132)	MEF2C (167)	p63 (124)	SMIF (4)
AhR (193)	DBP (112)	H2B (132)	MEF-2D (180)	p68 RNA helicase (166)	SNIP1 (102)
AIRE* (159)	DDX24 (89)	H3 (132)	MEKK1 (168)	p73 (64)	Sox-2 (144)
Akt* (29)	dHAND (35)	H4 (132)	mGPBP (75)	p8 (72)	Sox9 (196)
AMF-1 (155)	E1A (64)	HCV core protein (63)	Mi (64)	Pax5 (43)	Sp1 (17)
AML1* (18)	E2 (64)	HDAC1 (179)	Mi-2 β (213)	Pax-6 (77)	Sp3* (103)
A-Myb (46)	E2F-1* (64)	HDAC6 (61)	Miz-1 (20)	Pax8 (37)	Spi-B (217)
Ap1b1 (225)	E2F-5 (140)	HEB (127)	MLL (45)	PC4 (108)	SRC1 (64)
AP-2 (21)	E47* (41)	hHR23A (232)	MN1 (200)	PCNA (67)	SRCAP (139)
APE1 (14)	E6 (64)	Hic-5 (174)	MR (56)	PDX-1 (161)	SREBP1a (64)
AR (82)	EBF (231)	HIF-1α (59)	Mre11 (89)	PEA3 (120)	SREBP2 (27)
Arnt* (105)	EBNA2 (64)	HLF (42)	MSG1 (216)	PELP1 (197)	SRF (162)
ATF-1 (64)	EBNA3C (33)	HNF1α (18)	Msh2* (89)	PGC-1α (206)	STAT1α* (59)
ATF-2* (64)	EID-1 (123)	HNF1β (7)	Msh6* (89)	PIAS1 (227)	STAT2* (59)
ATF-4 (64)	EID-2 (88)	HNF4α (64)	Msx1 (173)	PIAS3 (121)	STAT3* (142)
atrophin-1 (145)	EID3 (10)	HNF-6 (163)	Msx3 (133)	PIMT (136)	STAT4* (147)
B56 γ 3 (30)	Eif2b1 (225)	HOXA10 (172)	MyoD (59)	Pit-1 (64)	STAT5a* (157)
BCL6* (11)	EKLF (18)	HOXA11 (172)	mZac1b (76)	P-Lim (68)	STAT5b* (157)
beta-catenin (189)	Elk-1 (85)	HOXA9* (172)	NAP-1 (1)	PLZF (66)	STAT6* (58)
B-Myb (13)	Emb (138)	HOXB1 (172)	NAP-2 (175)	PML* (39)	SV40 large T (64)
BRCA1 (64)	ER1 (150)	HOXB2 (172)	NBS1 (89)	Polyoma large T (64)	SYT (27)
BRLF1 (188)	ERα (27)	HOXB3 (172)	N-Cadherin (128)	pp90 RSK (64)	TAF1 (175)
Brn-2 (181)	ESE-1 (208)	HOXB4 (172)	NEDD1 (89)	PPARα (40)	TAL1 (64)
C/EBPα (44)	Ets-1* (220)	HOXB6 (172)	NEMO (201)	PPARγ (137)	Tat (64)
C/EBPβ* (64)	Ets-2 (87)	HOXB7 (172)	Neuro D (64)	PR (27)	Tax (64)
C/EBPδ (106)	EV11 (25)	HOXB9* (172)	NFAT1* (57)	ProT α (94)	TBP (64)
C53 (226)	EWS (165)	HOXD10 (172)	NFATc1* (98)	Prox-1 (31)	TCF-4N* (99)
CARM1 (215)	Eya1* (81)	HOXD12 (172)	NFATc4 (222)	PRS1 (90)	TDG (192)
Cart1 (79)	Eya3 (81)	HOXD13 (172)	NF-E2 (64)	PU. 1* (18)	TFIIB (64)
Cdc25B (122)	FGFR1 (48)	HOXD4 (172)	NFI (2)	Rad50* (89)	TFIIE β (132)
CDP (119)	FHL2 (111)	HPV E7 (12)	NFY-B (49)	RAR* (27)	TFIIF (132)
cdx-2 (77)	FKLF2 (182)	HSF1 (73)	NF-κB p50* (55)	Rb (26)	TIF2 (204)
c-Fos (59)	FOG-2 (70)	Htt (183)	NF-κB p65* (64)	RBCK1 (191)	TR β (27)
CIITA* (64)	FoxA2 (163)	Ikka (218)	Nished (130)	Rch1 (132)	TRBP (104)
CITED4 (22)	FoxM1B (126)	importin- α 7 (132)	Notch-1* (148)	RNA helicase A (64)	TRF-132 (62)
c-Jun* (59)	FOXO1a (110)	ING2 (153)	Nrf2 (171)	RORα (113)	Trip10 (225)
CK2 (89)	FOXO3a* (125)	IRF-1* (23)	NS1 (146)	Runx2* (178)	TSG101 (187)
c-Maf* (31)	FOXO4 (199)	IRF-2* (129)	Nup98 (96)	Runx3* (151)	TTF-1 (224)
c-Myb* (59)	GABPα* (6)	IRF-3 (211)	Nur77 (210)	RXR (64)	Twist (64)
c-Myc (202)	Gak (225)	IRF-7* (221)	OLIG2 (54)	Sam68 (74)	UBF (154)
Cnot3 (225)	GATA-1 (59)	JDP2 (149)	P/CAF (64)	Sap-1a* (59)	VDR (24)
CoAA (83)	GATA-2* (18)	JMY (64)	pCIP (64)	SATB1* (53)	vIRF-1 (23)
CoCoA (101)	GATA-3* (18)	JunB (64)	p100 (198)	SF-1 (64)	VP16 (80)
COUP-TFII (5)	GATA-4 (36)	K8/K-bZIP (78)	p220(NPAT) (207)	Sh3gl1 (225)	Vpr (50)
CPAP (107)	GATA-5 (91)	KLF2* (170)	p28ING5 (177)	SIRT1 (19)	WT1 (209)
CREB* (59)	GATA-6 (205)	KLF5 (230)	p29ING4 (177)	SKIP (116)	YAP (185)
CREM (114)	GCMa (28)	MafG (18)	p30(II) (229)	Skn-1a (186)	YY1 (64)
CRX (219)	GCN5 (18)	MAM (51)	p33ING1b (195)	Smad1 (142)	ZBP-89 (3)
CtBP1 (100)	Gli3 (34)	MAML2 (214)	p34SEI-1 (71)	Smad2 (194)	ZBTB2 (89)
Cyclin D1* (52)	GMEB-1 (97)	MASH1 (127)	p35srj (15)	Smad3* (59)	ZEB-1* (160)
cyclin E-Cdk2 (156)	GMEB-2 (97)	MDC1 (89)	p53* (59)	Smad4 (86)	ZNF639 (89)

FIG. 1. The CBP and p300 interactome: 312 viral and mammalian proteins that interact physically or functionally with CBP or p300 in vitro. One hundred ninety-six proteins that are encoded by essential genes in mice (i.e., mutation results in a phenotype) are indicated in boldface type, including 56 genes that are essential for T cells (indicated by an asterisk). The source for phenotype information is Mouse Genome Informatics (<http://www.informatics.jax.org/>). For more detailed and up to date information on the interactome, see <http://www.stjude.org/brindle>. References are indicated in parentheses.

(203). The fact that *CBP*^{-/-} and *p300*^{-/-} mice survive until about embryonic day 9 (E9), however, shows that many cell types can develop and survive with only CBP or p300.

The roles of CBP and p300 in blood cells are of considerable interest because they are thought to be vital for the function of many hematopoietic transcription factors; at least 65 factors

that interact with CBP or p300 are encoded by genes that are essential for T and B cells in mice (Fig. 1). Moreover, chromosomal translocations involving *CBP* or *p300* produce gain-of-function fusion proteins in some types of human leukemia (18) (Fig. 1). Both CBP and p300 are necessary for normal definitive hematopoiesis, including lymphopoiesis, in chimeric

mice created with $CBP^{-/-}$ or $p300^{-/-}$ embryonic stem cells (164). CBP can also act as a tumor suppressor in hematopoietic cells, including T cells (93), tissue macrophages (histiocytes), and possibly the B-cell lineage (plasmacytomas developed in mice transplanted with CBP heterozygous cells) (109). There is less evidence for p300 acting as a tumor suppressor in hematopoietic cells, although histiocytic sarcoma has been reported in $p300^{-/-}$ chimeric mice (164). Still, peripheral blood T-cell numbers appear to be mostly unaffected in CBP and $p300$ heterozygous mice (109), even though CBP and p300 have been implicated by a number of studies as being important for the activity of 56 T-cell-critical transcriptional regulators (Fig. 1). In this regard, conditional knockout of CBP in multiple cell lineages that include thymocytes leads to abnormally high levels of CD8⁺ single-positive (SP) thymocytes (93). In addition, mice that are homozygous for mutations in the CREB- and c-Myb-binding domain (KIX) of p300 have thymic hypoplasia in the context of multilineage hematopoietic defects (95). It is uncertain, however, if CBP and p300 function equivalently in T-cell development. To test the hypothesis that CBP and p300 are each critically limiting in specific cell lineages in vivo, we developed mice with conditional knockout alleles for each gene. We then examined the requirement for CBP and p300 in thymocyte development and their roles in T-cell and macrophage gene expression.

MATERIALS AND METHODS

Generation of $p300^{lox}$ mice. The $p300^{lox}$ allele was constructed by flanking exon 9 of the mouse *Ep300* gene with LoxP sites. DNA for the targeted region was obtained from an E14 Ola mouse embryonic stem (ES) cell genomic DNA phage library. A LoxP site was inserted in the intron 3' of exon 9 using a Tn7 transposon-based system (GPS mutagenesis system, catalog no. 7101; New England Biolabs) with a modified mini-Tn7 vector (pGPS3-PM30-R¹⁻⁷⁰-Neo-CAT-TK no. 2; details available upon request) that allowed random insertion of a LoxP site and a selection cassette containing genes for neomycin resistance (Neo), chloramphenicol resistance, and thymidine kinase (TK) flanked by Frt sites (16, 135). A second LoxP site was inserted in the intron 5' of exon 9 using the Tn7 system and another modified mini-Tn7 vector (pGPS3-CAT-Tn7-LoxP no. 2; details available upon request) (16). In addition, a diphtheria toxin A cassette placed at the 3' end of the targeting construct provided negative selection to reduce the number of nonhomologous targeting events. Mouse ES cells were electroporated with linearized targeting construct and cultured with G418 to positively select for clones that had integrated the targeting construct. Clones were screened by PCR across the 3' end of the targeted region, and positive clones were confirmed by Southern blotting using a *StuI* digest with a 5' external probe as well as an *Asp718/SpeI* digest with a 3' external probe to check for homologous targeting. To remove the drug selection cassette, correctly targeted clones were subjected to transient expression of Flp recombinase and treated with 2'-fluoro-2'-deoxy-5-iodouracil- β -D-arabinofuranoside (FIAU) to enrich for clones that had lost the TK gene (169). Recombination of the Frt sites was confirmed by PCR, and positive clones were checked for loss of the selection cassette by Southern blotting using an *XbaI* digest and a probe for Neo.

Genotyping of mice. $p300$ and CBP conditional knockout mice were bred to *Lck-Cre* transgenic mice (69) or *lysM-Cre* transgenic mice (32). Recombination of the LoxP sites flanking $p300$ exon 9 in cells expressing Cre was determined by Southern blotting using an *XbaI* or *EcoRI* digest and a cDNA probe to the region encoding amino acids 615 to 681 or by semiquantitative PCR using primers p4 (CTCTACATCCTAAGTGCTAGG), p5 (TGGACTGGTTATCGGTTACCC), and p6 (CAGTAGATGCTAGAGAAAGCC), producing a 540-bp wild-type band, a 720-bp $p300$ flox band and a 1.1-kb $p300^{\Delta flox}$ band (primer locations shown in Fig. 2). This semiquantitative PCR overestimates the deletion of the $p300^{lox}$ allele in a systematic manner that was corrected for by using a standard curve of samples of known deletion as determined by quantitative Southern blotting. Generation of CBP conditional knockout mice and PCR genotyping for the deletion of the CBP^{lox} allele was reported by Kang-Decker et al. (93). Detection of the flox and Δ flox alleles of *CBP* by Southern blotting was performed using a *HindIII* digest and a 5' external probe. Thymocyte subpopu-

lations were sorted to check deletion by flow cytometry using anti-CD4 and anti-CD8 antibodies. T cells from the spleen and lymph node were purified to check deletion by positive selection using anti-Thy1.2-conjugated magnetic beads from Miltenyi Biotec.

Indirect immunofluorescent staining, nuclear extracts, and Western blots. Antibodies against CBP (A-22 and C-20) and p300 (N-15 and C-20) for immunofluorescence and Western blotting were from Santa Cruz. Indirect immunofluorescent detection of CBP and p300 was performed as previously described using single-cell suspensions of thymus and anti-p300 (1:800 dilution, C-20) and anti-CBP (1:800, C-20) antibodies (93). Nuclear extract preparations and Western blots were performed as previously described (95).

Flow cytometry and complete blood count. Antibodies for flow cytometry (fluorescence-activated cell sorting [FACS]) analysis (anti-CD3, anti-CD4, anti-CD8, anti-B220) were purchased from Becton Dickinson. FACS was performed on a FACSCalibur system using CellQuest software. Automated complete blood counts were performed using a Hemavet 3700R.

Estimation of total cell counts. Total counts for thymic subpopulations were determined by multiplying the percentage of cells in a subpopulation as measured by FACS by the total manual viable thymocyte count for that mouse. Cell counts in peripheral blood were estimated from automated complete blood count lymphocyte count per microliter multiplied by the percentages of T cells and B cells in peripheral blood as determined from anti-CD3 versus anti-B220 FACS.

Bone marrow-derived macrophages. Bone marrow-derived macrophages (BMDMs) were obtained by flushing bone cavities and isolating macrophages by differentiation in L-cell-conditioned medium as a source of macrophage colony-stimulating factor as described previously (141).

Activation of splenic T cells and qRT-PCR. Splenic T cells were sorted by FACS and cultured at 2×10^6 cells/ml in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum with 1 ng/ml phorbol myristate acetate (PMA) and 100 ng/ml ionomycin or dimethyl sulfoxide vehicle for 3 h at 37°C in round-bottom plates. cDNA was generated from 100 ng of total RNA in a 20- μ l reaction mixture using Superscript II reverse transcriptase (RT; Invitrogen). Quantitative RT-PCR (qRT-PCR) was performed on an Opticon DNA engine (MJ Research) using 1 μ l of cDNA per 25 μ l PCR with SYBR green dye. qPCR primers were designed using Primer Express software (Applied Biosystems) and confirmed to yield a single product by melting curve analysis. Samples were normalized to GAPDH mRNA.

RESULTS

Creation of mice with a $p300$ Cre/LoxP conditional knockout allele. Mouse genomic DNA harboring $p300$ exons encoding the CREB-binding domain (KIX) was used in the targeting construct. Two LoxP sites were inserted into the introns flanking exon 9, encoding residues 588 to 626 (Fig. 2a); Cre-mediated recombination results in a frameshift mutation if the flanking exons fortuitously splice and a stable mRNA is produced. The equivalent exon is targeted in the CBP^{lox} allele, so the $p300^{lox}$ allele is a comparable experimental system (93). Neomycin-resistant ES cell clones were confirmed for correct targeting of the $p300$ locus by Southern blotting using 5' and 3' probes that were external to the targeting construct (Fig. 2b) (L. H. Kasper, data not shown). The Frt site-flanked drug selection cassette was removed in the ES cells by transient expression of Flp recombinase. Chimeric mice were generated by standard methods, and germ line transmission of the $p300^{lox}$ allele was achieved. Importantly, $p300^{lox/lox}$ homozygous mice appeared normal, indicating that the conditional allele is indistinguishable from the wild type in the absence of Cre recombinase. $p300^{lox}$ recombination in the presence of Cre (yielding $p300^{\Delta flox}$) was efficient, although it showed considerable variation between mice carrying the *Lck-Cre* transgene (Fig. 2b). This is likely due to stochastic transgene expression, a relatively common phenomenon in transgenic mice (38).

$p300^{\Delta flox}$ is a null allele. The deletion efficiency in $p300^{lox/lox}; Lck-Cre$ mice could be as high as ~90 to 95% and

FIG. 2. Generation of the *p300* conditional knockout mouse. (a) Gene targeting strategy for the generation of a *p300* conditional knockout allele in mice. Exon number and relative position, selection gene cassettes, positions of external and internal Southern blot probes, PCR primer positions, LoxP sites, Frt sites, and restriction sites are indicated. (b) Southern blot using *XbaI* digest and internal and external probe showing detection of wild-type, flox, and Δ flox alleles of *p300*. #1 and #2 are separate *p300^{+/flox}*, *Lck-Cre* mice with different deletion efficiencies. (c) Western blot of control and mutant thymocyte nuclear extracts (indicated) using a *p300* N-terminal antibody (top panel) and a CBP N-terminal antibody (bottom panel).

was specific for the thymus (L. H. Kasper, data not shown) (69). Mice with high deletion frequencies were deficient for full-length p300 protein as measured by Western blots using thymic nuclear extracts and antibodies specific for the N terminus of p300 (Fig. 2c). CBP protein levels were not measurably affected in the p300 null thymuses (Fig. 2c; 3a), nor were p300 protein levels altered in thymuses deficient for CBP (Fig. 3a). There was no evidence for truncated forms of p300 appearing in $p300^{flox/flox}$; *Lck-Cre* thymic nuclear extracts as determined by Western blots using an N-terminus-specific antibody following 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, although a small amount of material that reacted with a C-terminal p300 antibody ran at the dye front (less than 37 kDa) (P. K. Brindle, data not shown). However, indirect immunofluorescence of thymocytes from $p300^{flox/flox}$; *Lck-Cre* and $CBP^{flox/flox}$; *Lck-Cre* mice using antibodies specific for the C termini of p300 or CBP confirmed that many cells lacked p300 or CBP, respectively (Fig. 3b). Together, these results indicate that $p300^{\Delta flox}$ is a null allele.

Thymic development is abnormal in both $CBP^{flox/flox}$; *Lck-Cre* and $p300^{flox/flox}$; *Lck-Cre* mice. The total number of thymocytes was significantly reduced in both $CBP^{flox/flox}$; *Lck-Cre* mice compared to $CBP^{flox/flox}$ mice ($0.61 \times 10^8 \pm 0.17 \times 10^8$ [$n = 4$] versus $2.16 \times 10^8 \pm 0.50 \times 10^8$ [$n = 4$]; t test, $P = 0.001$) and $p300^{flox/flox}$; *Lck-Cre* mice compared to $p300^{flox/flox}$ mice ($1.18 \times 10^8 \pm 0.63 \times 10^8$ [$n = 6$] versus $2.22 \times 10^8 \pm 0.28 \times 10^8$ [$n = 6$]; t test, $P = 0.004$). As *Lck-Cre* mice were found to have thymocyte numbers comparable to those of wild-type mice (T. Fukuyama, data not shown), this deficit is likely caused by inactivation of the floxed alleles.

Quantitation of individual thymocyte subtypes showed that double-negative (DN) cell numbers (the least mature major subtype) were relatively unaffected in both mutants (Fig. 4c and 5c), whereas there was a five- to threefold average decrease in double-positive (DP) cell numbers for CBP (Fig. 4d) ($n = 4$) and p300 (Fig. 5d) ($n = 5$) mutant mice, respectively, compared to flox controls ($n = 4$ and 6, respectively). This apparent conflict with the relative increase in the percentage of DN cells seen by FACS (Fig. 4a and 5a) is explained by the decrease in thymus size for both CBP and p300 mutant mice. Since *Lck-Cre* transgene expression does not initiate until the DN1 stage (the least mature stage in the thymus), DN cell numbers may be normal (Fig. 4c and 5c) because loss of CBP or p300 has no effect on DN cells or, alternatively, because CBP and p300 protein and mRNA levels are not sufficiently depleted by turnover and dilution until the next stage of development (i.e., DP thymocytes).

SP cell numbers were generally decreased about twofold in the mutants compared to controls (Fig. 4e and f and 5e and f), except that $CBP^{flox/flox}$; *Lck-Cre* mice had normal numbers of CD8⁺ cells (Fig. 4f). This indicates that the 10-fold increase in the percentage of CD8⁺ SP cells in $CBP^{flox/flox}$; *Lck-Cre* thymuses (Fig. 4a) was, in fact, due to a decrease in the absolute number of DP and CD4⁺ SP cells. Given that thymocyte development occurs in a stepwise manner from DN to DP to SP, this would indicate that the "normal" levels of CD8⁺ SP cells in $CBP^{flox/flox}$; *Lck-Cre* mice arise by an altered process, as they are derived from a much smaller pool of DP precursors than in control mice. By contrast, the ratio of CD4⁺ to CD8⁺ SP thymocytes in $p300^{flox/flox}$; *Lck-Cre* mice was normal (Fig. 5a).

We previously showed that $CBP^{flox/flox}$; *MMTV-Cre* mice had an increased percentage of CD8⁺ SP thymocytes, although it was unclear if this defect was autonomous to the thymus because CBP^{flox} inactivation occurred in multiple tissues (93). Since $CBP^{flox/flox}$; *Lck-Cre* mice also had a marked increase in the percentage of CD8⁺ SP thymocytes compared to *Lck-Cre* and $CBP^{flox/flox}$ controls (Fig. 4a), and as the *Lck-Cre* transgene is expressed exclusively in thymocytes (69), the increase in CD8⁺ SP cells is due to loss of CBP in thymocytes. Together, these results show that CBP and p300 are required for normal thymocyte development, although they have distinct roles in CD8⁺ SP development.

Modest reduction in peripheral T cells in $CBP^{flox/flox}$; *Lck-Cre* mice. Neither $CBP^{flox/flox}$; *Lck-Cre* nor $p300^{flox/flox}$; *Lck-Cre* mice had obviously abnormal proportions of CD4⁺ and CD8⁺ peripheral T cells in the spleen and blood (Fig. 4a; 5a), even when deletion in the thymus was high (~80 to 90%) (Fig. 4b; 5b), but there was a modest ~2-fold reduction in the number of peripheral blood CD4⁺ ($P = 0.028$, analysis of variance [ANOVA]) and CD8⁺ ($P = 0.020$) T cells in $CBP^{flox/flox}$; *Lck-Cre* mice that was not observed in $p300^{flox/flox}$; *Lck-Cre* mice (Fig. 6a). Peripheral blood B-cell numbers were comparable between control and mutant mice, demonstrating the lineage specificity of the mutations ($P = 0.58$, ANOVA) (Fig. 6b). Comparison of the deletion frequency in thymus and affinity-purified splenic T cells revealed that the fraction of cells harboring the nonrecombined alleles increased in peripheral T cells from $p300^{flox/flox}$; *Lck-Cre* mice (Fig. 7a) and $CBP^{flox/flox}$; *Lck-Cre* mice (Fig. 7b), indicating that late developmental stage or peripheral T cells with p300 or CBP deficiency are at a disadvantage.

Loss of p300 in thymocytes does not lead to lymphoma. We previously showed that $CBP^{flox/flox}$; *MMTV-Cre* mice develop T-cell lymphoma with high frequency starting at 3 months of age (93). Since CBP and p300 functions are often interchangeable in vitro, we expected to see T-cell lymphoma develop in $p300^{flox/flox}$; *MMTV-Cre* mice. Surprisingly, a cohort of 40 $p300^{flox/flox}$; *MMTV-Cre* mice did not develop lymphoma out to 104 weeks of age (Fig. 8a). By comparison, a cohort of 37 $CBP^{flox/flox}$; *MMTV-Cre* mice developed T-cell lymphoma with high frequency as early as 16 weeks (Fig. 8a). We observed a comparable incidence and onset of T-cell lymphoma in $CBP^{flox/flox}$; *Lck-Cre* mice (Fig. 8b) ($P = 0.03$, one-tailed log rank test, $n = 25$), although over time, the frequency of lymphoma dropped markedly as the mice were backcrossed, indicating that other genetic or environmental factors contribute to tumor development (T. Fukuyama, data not shown). We did not observe lymphoma in $p300^{flox/flox}$; *Lck-Cre* mice out past 1 year of age (Fig. 8c) ($n = 21$), in agreement with results for the $p300^{flox/flox}$; *MMTV-Cre* mice. The $CBP^{flox/flox}$; *MMTV-Cre* and $p300^{flox/flox}$; *MMTV-Cre* mice were housed in a different facility from the $CBP^{flox/flox}$; *Lck-Cre* and $p300^{flox/flox}$; *Lck-Cre* colonies, suggesting that environmental factors alone cannot account for the differences in tumor phenotype observed. Together, the data support the view that there are intrinsic differences in the tumor suppressor functions of CBP and p300, even though the contributing environmental and genetic factors required for T-cell lymphomagenesis in the absence of CBP are unclear and somewhat variable. In this regard, it has recently been shown that p300 levels can vary in different

a **b**

FIG. 3. Ablation of p300 and CBP protein in mutant thymocytes. (a) Western blot of control and mutant thymocyte whole-cell extracts (indicated) using C-terminal antibodies (α) to p300 (top panel) and CBP (middle panel) and an antibody against CREB (lower panel). (b) Single-cell suspensions of thymuses from control ($p300^{flox/flox}$) (upper panels), $p300^{flox/flox}; Lck-Cre$ (middle), and $CBP^{flox/flox}; Lck-Cre$ (lower) mice were stained with 4',6'-diaminido-2-phenylindole (DAPI) to visualize nuclei and antibodies specific for the C termini of p300 or CBP (indicated). Detection was by anti-rabbit immunoglobulin G conjugated with fluorescein isothiocyanate.

FIG. 4. $CBP^{flox/flox}; Lck-Cre$ mice have abnormal T-cell subpopulations in the thymus. (a) FACS analysis of thymus, spleen, and peripheral blood from representative control ($Lck-Cre$, $CBP^{flox/flox}$) and $CBP^{flox/flox}; Lck-Cre$ mice showing ratios of $CD4^+$ and $CD8^+$ cells. Spleen and peripheral blood were pregated on $CD3^+$ cells. Percentages of cells in relevant quadrants are indicated. (b) Southern blot showing deletion in thymus of $CBP^{flox/flox}; Lck-Cre$ mouse used in panel a. (c to f) Average total counts for the $CD4^- CD8^-$ DN, $CD4^+ CD8^+$ DP, $CD4^+$ SP (CD4 SP), and $CD8^+$ SP (CD8 SP) thymocyte populations in indicated control $CBP^{flox/flox}$ (c to f) ($n = 6$) and mutant $CBP^{flox/flox}; Lck-Cre$ (c to f) ($n = 5$) 6-week-old mice (mean $\pm$ standard error of the mean).

FIG. 5. *p300^{flox/flox}; Lck-Cre* mice have reduced numbers of the more mature thymocyte subpopulations. (a) FACS analysis of thymus, spleen, and peripheral blood from representative control (*Lck-Cre*, *p300^{flox/flox}*) and *p300^{flox/flox}; Lck-Cre* mice showing ratios of CD4⁺ and CD8⁺ cells. Spleen and peripheral blood were pregated on CD3⁺ cells. Percentages of cells in relevant quadrants are indicated. (b) Southern blot of *p300^{flox/flox}; Lck-Cre* thymus used in panel a demonstrating efficient deletion. (c to f) Average total counts for the CD4⁺CD8⁻ DN, CD4⁺CD8⁺ DP, CD4⁺SP (CD4 SP), and CD8⁺SP (CD8 SP) thymocyte populations in indicated control *p300^{flox/flox}* (c to f) (*n* = 4) and mutant *p300^{flox/flox}; Lck-Cre* (c to f) (*n* = 4) 6-week-old mice (mean ± standard error of the mean).

FIG. 6. Quantitation of peripheral T-cell subpopulations in $CBP^{flox/flox}$, $Lck-Cre$ and $p300^{flox/flox}$, $Lck-Cre$ mice. Average numbers of $CD4^{+}$ and $CD8^{+}$ T cells (a) and $B220^{+}$ B cells (b) per microliter of peripheral blood for the indicated genotypes are shown (6- to 9-week-old mice, $n = 3$ to 9; mean $\pm$ standard error of the mean). WT, wild type.

mouse strains, suggesting that the combined dosage of CBP and p300 protein may also fluctuate and lead to variable phenotypic penetrance (158).

CBP and p300 combined dosage is critical for thymocyte development. The ability of T cells to develop and survive with only CBP or p300 raised the possibility that cells lacking both coactivators could be generated. We tested this by creating $CBP^{flox/flox}$; $p300^{flox/flox}$; $Lck-Cre$ mice ("quad" mutants). Recombination of the CBP^{flox} allele could be detected in quad mutant thymuses by semiquantitative PCR (Fig. 9b), albeit at a reduced level compared to $CBP^{flox/flox}$; $Lck-Cre$ mice. Similar results were obtained for the $p300^{flox}$ allele (Fig. 9b) (PCR quantitation is fairly linear for $CBP^{\Delta flox}$ but tends to overestimate the relative abundance of the $p300^{\Delta flox}$ allele; M. A. Biesen, data not shown).

FACS analysis of quad mutant mice with efficient recombination ($\sim 50\%$) of the CBP^{flox} and $p300^{flox}$ alleles in the thymus revealed about a 10-fold increase in the percentage of DN thymocytes, with a concomitant decrease in DP cells; mice with a lower deletion frequency ($\sim 30\%$) in the thymus had an

FIG. 7. Moderate selection against $p300$ and CBP null peripheral T cells in $p300^{flox/flox}$; $Lck-Cre$ and $CBP^{flox/flox}$; $Lck-Cre$ mice. (a) Representative Southern blot of genomic DNA from thymus and affinity-purified splenic T cells of a $p300^{flox/flox}$; $Lck-Cre$ mouse indicating relative abundance of the recombined allele. (b) Representative semiquantitative PCR of genomic DNA from thymus and affinity-purified splenic T cells of a $CBP^{flox/flox}$; $Lck-Cre$ mouse. Splenic T cells were purified by positive selection using Thy1.2⁺-conjugated magnetic beads.

FIG. 8. CBP but not p300 deficiency in T cells causes lymphoma with incomplete penetrance. (a to c) Percentage of lymphoma-free surviving $CBP^{flox/flox}$ and $p300^{flox/flox}$ mice with $MMTV-Cre$ (a) or $Lck-Cre$ (b and c). Ages and genotypes of mice are indicated. Mice alive at the end of the study or dying of causes other than lymphoma were censored in the analysis (tick marks). (a) $n = 37$ to 40, $P < 0.0001$, one-tailed logrank test; (b) $n = 13$ to 25, $P = 0.03$; (c) $n = 21$ to 24.

FIG. 9. *CBP^{flx/flx}, p300^{flx/flx}, Lck-Cre* mice have abnormal thymocyte subpopulations but essentially normal ratios of peripheral T cells. (a) FACS analysis of thymus, spleen, and peripheral blood from representative control and *CBP^{flx/flx}; p300^{flx/flx}, Lck-Cre* mice (quad mutant) showing ratios of CD4⁺ and CD8⁺ cells. Spleen and peripheral blood were pregated on CD3⁺ cells. Percentages of cells in relevant quadrants are indicated. (b) Semiquantitative PCR of genomic DNA showing deletion in thymus of *CBP^{flx/flx}, p300^{flx/flx}, Lck-Cre* mice used in panel a. Quad mutant no. 2 has a higher deletion frequency than no. 1. The p300 PCR tends to overestimate the relative abundance of the *p300^{Δflx}* allele.

intermediate immunophenotype (Fig. 9a and b, compare quad mutants 1 and 2). The quad mutant thymuses also lacked the increase in CD8⁺ SP cells that occurred in the *CBP^{flx/flx}; Lck-Cre* mice. The proportion of CD4⁺ and CD8⁺ T cells in the spleen and blood of the quad mutants were comparable to *Lck-Cre* and *CBP^{flx/flx}* control mice, however (Fig. 9a). We did not observe deletion frequencies greater than ~50% in quad mutant thymuses, suggesting that thymocytes lacking

FIG. 10. Very strong selection against thymocyte subpopulations but not peripheral T cells in $CBP^{flox/flox}; p300^{flox/flox}; Lck-Cre$ mice. Total counts for the $CD4^- CD8^-$ DN (a), $CD4^+ CD8^+$ DP (b), $CD4^+$ SP (CD4 SP) (c), and $CD8^+$ SP (CD8 SP) (d) thymocyte populations of $Lck-Cre$ mice ($n = 2$), $CBP^{flox/flox}; p300^{flox/flox}; Lck-Cre$ mice (quad mutant) with low deletion (<30%) in whole thymus ($n = 3$), and $CBP^{flox/flox}; p300^{flox/flox}; Lck-Cre$ mice with medium deletion (30 to 50%, $n = 2$) in whole thymus (mean $\pm$ standard error of the mean). (e) $CD3^+$ T cells per microliter of peripheral blood (mean $\pm$ standard error of the mean; $n = 4$ to 8). $Lck-Cre$ and $CBP^{flox/flox}; p300^{flox/flox}$ (quad) controls are indicated. (f) Ratio of $CD3^+$ to $B220^+$ cells in the spleen as determined by flow cytometry (mean $\pm$ standard error of the mean; $n = 2$ to 3).

both CBP and p300 are strongly selected against. We confirmed this by quantitating the number of thymocytes per subpopulation in quad mutants that showed both medium (~50%) and low (~10 to 30%) deletion frequencies. Total thymocyte counts decreased with increased deletion frequency, and as a result, DN cell numbers were unaffected in both classes of quad mutants compared to control mice (Fig. 10a), but DP cells were reduced about 10-fold in thymuses displaying medium levels of deletion (Fig. 10b). There was a deficiency of SP thymocytes in quad mutants with medium deletion, although the $CD8^+$ SP thymocytes were more variably affected (Fig. 10c and d). In further support of these data, genomic DNA isolated from FACS-purified quad mutant thymocyte subtypes showed ~30 to 40% deletion of the CBP^{flox} and $p300^{flox}$ alleles in DN cells, but there was a steady drop in the frequency of both recombined alleles in DP and SP thymocytes, with an almost complete absence (~1% deletion) in peripheral T cells purified from the spleen and lymph nodes (Fig. 11a and b). These results indicate that thymocyte development requires either CBP or p300 and that T cells need CBP or p300 to populate the peripheral lymphoid organs. The numbers of T cells in the spleen and blood of quad mutants were not significantly different from those of control mice, however, indicating that there is compensation by homeostatic proliferation of peripheral T cells that did not recombine the alleles (Fig. 10e and f) ($P > 0.05$, ANOVA, $n = 3$ to 9).

The model that concentrations of CBP and p300 are criti-

cally limiting predicts that a modest reduction (~50%) in their combined levels will have catastrophic consequences for cells. In contrast, results from this study suggest a model where CBP and p300 are not extremely limiting and that lesser amounts (between 0 and 50%) of the coactivators can still support many critical functions. We compared these models by determining whether one *CBP* or *p300* wild-type allele is sufficient for thymocyte development and for T cells to populate the periphery. Both $CBP^{+/flox}; p300^{flox/flox}; Lck-Cre$ and $CBP^{flox/flox}; p300^{+/flox}; Lck-Cre$ mice ("triple" mutants) were generated, and semi-quantitative PCR analysis of thymic and T-cell genomic DNA was performed. In this instance, we corrected the PCR data by using a standard curve made with samples of known deletion frequency as determined by quantitative Southern blotting. The corrected PCR data demonstrated that triple-mutant cells of both genotypes were selected against in the periphery, although not to the extent seen with the quad mutants (Fig. 12a and b, note that the similarity in deletion frequency for both types of alleles indicates that an individual cell either recombines all the alleles or none; the quad mutant data in Fig. 11 corroborate this). Remarkably, about 20 to 30% deletion was observed for both CBP^{flox} and $p300^{flox}$ in peripheral T cells of $CBP^{+/flox}; p300^{flox/flox}; Lck-Cre$ mice, suggesting that T cells with one wild-type *CBP* allele may be able to develop and populate the lymphoid organs to some degree (Fig. 12b). The extent of deletion in peripheral T cells does not depend strongly on the level of deletion in the thymus (compare Fig.

FIG. 11. Strong selection against thymocyte subpopulations and peripheral T cells lacking both CBP and p300 in *CBP*^{flx/flx}; *p300*^{flx/flx}; *Lck-Cre* mice. Semiquantitative PCR showing deletion of *CBP*^{flx} (a) and *p300*^{flx} (b) in FACS-purified CD4⁺ CD8⁺ DN, CD4⁺ CD8⁺ DP, CD4⁺ SP (CD4⁺ SP), and CD8⁺ SP (CD8⁺ SP) thymocyte subpopulations and affinity-purified T cells from spleen and lymph node (LN). T cells from spleen and LN were purified by positive selection using Thy1.2⁺-conjugated magnetic beads. PCR tends to overestimate the relative abundance of the *p300*^{Δflx} allele but is fairly quantitative for *CBP*^{Δflx}. Numbers indicate the approximate deletion percentages; p300 values were corrected by comparison to a standard curve of known deletion frequency derived from Southern analysis.

12a and c). All told, these results demonstrate that there is a graded effect of the combined dosage of functional *CBP* and *p300* alleles on T-cell development and their ability to populate the periphery. The inactivation of both CBP alleles (along with one p300 allele) was more detrimental to peripheral T cells than the other triple mutation combination (compare Fig. 12a and c with 12b), which is consistent with our observation that CBP is more important for T cells than p300.

TCR signal-responsive gene expression is defective in CBP and p300 null splenic T cells. To circumvent inadequate gene deletion in peripheral T cells of *CBP*^{flx/flx}; *Lck-Cre* and *p300*^{flx/flx}; *Lck-Cre* mice, we utilized a reporter transgene, *Z/EG* that expresses green fluorescent protein (GFP) after

Cre-mediated recombination, allowing us to sort for a population of splenic T cells with a high deletion frequency of *CBP* or *p300* (143). We used these cells to elucidate the role of CBP and p300 in gene expression in response to activation of signaling through the T-cell receptor (TCR). We tested two genes, interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF-α), whose expression in response to TCR signaling is critical for T-cell function and has been reported to involve p300 and CBP (47, 228). IL-2 mRNA expression was reduced less than twofold in *CBP*^{flx/flx}; *Lck-Cre* cells and was unaffected in *p300*^{flx/flx}; *Lck-Cre* splenic T cells in response to treatment with PMA and calcium ionophore. The decrease in IL-2 may be due to the increased fraction of CD8⁺ T cells relative to CD4⁺ cells in the spleen (Fig. 4a), as not all CD8⁺ T-cell subtypes express IL-2 efficiently (212). TNF-α mRNA expression, however, was decreased more than twofold in *p300*^{flx/flx}; *Lck-Cre* and more than fourfold in *CBP*^{flx/flx}; *Lck-Cre* in activated splenic T cells (Fig. 13a and b). This deficit in TNF-α expression is in agreement with a previous report by Falvo et al. demonstrating that CBP null heterozygous T cells are defective in TNF-α expression in response to TCR activation with anti-CD3 and suggests that p300 is also important (47).

Gene expression in CBP and p300 null macrophages. We used bone marrow-derived macrophages to further investigate the effects of CBP and p300 deficiency on endogenous signal-responsive gene expression. We chose several cytokine- and Toll-like receptor (TLR)-responsive target genes where either CBP or p300 has been previously implicated as playing a key role in expression or a presumed role based on chromatin immunoprecipitation assays. TNF-α, arginase (Arg1), inducible nitric oxide synthase (iNOS), and interferon regulatory factor 1 (IRF-1), are regulated by gene-specific combinations of the CBP/p300-interacting transcription factors ATF-2, c-Jun, SP1, Ets, Egr-1, NF-κB, AP-1, STAT1, PU.1, STAT6, and C/EBPβ (Fig. 1) (9, 117, 152). *CBP*^{flx/flx}; *lysMcre* mice that express Cre in macrophages yielded cells with an ~80 to 90% reduction in CBP protein when measured by Western blotting of nuclear extracts (Fig. 14a) (32). Northern blot analysis revealed that TNF-α mRNA was robustly induced in response to lipopolysaccharide (LPS) stimulation in *CBP*^{flx/flx}; *lysMcre* macrophages, although not quite as well as in control *CBP*^{flx/flx} cells (Fig. 14b) (rRNA served as a loading control). Arginase (Arg1) induction in response to the cytokine IL-4 was measured by Western blotting and was comparable between *CBP*^{flx/flx}; *lysMcre* and *CBP*^{flx/flx} macrophages (Fig. 14c) (the nonspecific band serves as a loading control).

We next examined gene expression in macrophages deficient for p300. Recombination of the *p300*^{flx} allele in *p300*^{flx/flx}; *lysMcre* macrophages was confirmed by semiquantitative PCR of genomic DNA (Fig. 14a). TNF-α mRNA was induced comparably by LPS in *p300*^{flx/flx} and *p300*^{flx/flx}; *lysMcre* macrophages (Fig. 14d, rRNA loading control in bottom panel). Arg1 induction after IL-4 stimulation was also comparable between *p300*^{flx/flx} and *p300*^{flx/flx}; *lysMcre* macrophages (Fig. 14e), although treatment with both gamma interferon (IFN-γ) and LPS revealed a modest deficit in the kinetics of iNOS expression but not IRF-1 (Fig. 14f, nonspecific band acts as a loading control). IRF-1 and iNOS expression in response to the same stimuli in *CBP*^{flx/flx}; *lysMcre* macrophages was in the

FIG. 12. Moderate to strong selection against peripheral T cells lacking three functional alleles of *CBP* and *p300*. Percent deletion of *CBP*^{fllox} and *p300*^{fllox} alleles in the thymus and affinity-purified T cells from the spleen and lymph node (LN) of *CBP*^{fllox/flox}; *p300*^{+/-fllox}; *Lck-Cre* (a and c) or *CBP*^{+/-fllox}; *p300*^{fllox/flox}; *Lck-Cre* (b) mice. (c) Deletion frequency in the thymus does not directly impinge on the deletion percentage in the periphery (compare panels b and c). Deletion percentages for *CBP*^{fllox} and *p300*^{fllox} were determined by semiquantitative PCR of genomic DNA corrected for nonlinearity by use of a standard curve made of samples with deletion frequencies determined by quantitative Southern blot.

FIG. 13. Examination of endogenous TCR-signaling-responsive gene expression in splenic T cells lacking p300 or CBP. (a and b) Splenocytes from mice bearing *Lck-Cre* and *Z/EG* reporter transgenes were sorted for GFP-expressing control (*Lck-Cre; Z/EG*), p300 null (*p300*^{fllox/flox}; *Lck-Cre; Z/EG*), or CBP null (*p300*^{fllox/flox}; *Lck-Cre; Z/EG*) splenic T cells. Cells were treated ex vivo with 1 ng/ml PMA and 200 ng/ml calcium ionophore (Iono) for 3 h, and qRT-PCR was performed to determine mRNA expression of IL-2 (a) and TNF-α (b). Expression was normalized to GAPDH expression, and treated control cells were arbitrarily set to 100 in each experiment. Graphs represent the average results from two independent experiments using cells from two different mice of each genotype (*n* = 2).

FIG. 14. Examination of endogenous gene expression in BMDMs deficient for either CBP or p300. (a) *lysMcre*-mediated loss of CBP in BMDMs (left panel). Nuclear extracts from BMDMs cultured ex vivo from *CBP*^{flox/flox} or two different *CBP*^{flox/flox}; *lysMcre* mice showed CBP deficiency as measured by immunoblotting with CBP-specific antisera. p300 levels were unaffected. (Right panel) p300 deletion in BMDMs was confirmed by semiquantitative PCR of genomic DNA. (b) Normal CBP levels are not required for the TLR4-mediated induction of TNF-α expression. Northern blot analysis of TNF-α mRNA induction in response to stimulation through the TLR4 pathway. BMDMs were plated at 2×10^6 cells per well and stimulated with *Escherichia coli* LPS at 100 ng/ml for the times indicated. RNA was isolated and probed with a TNF-α cDNA probe. Ethidium bromide-stained rRNA is the loading control (bottom panels). (c) CBP is not required for the IL-4-mediated induction of arginase 1 (Arg1) expression. BMDMs were stimulated with IL-4 (10 ng/ml) or left unstimulated (–) for the times shown, and lysates were analyzed for Arg1 expression by immunoblotting. The nonspecific band serves as a loading control (asterisk). (d) Normal p300 levels are not required for the TLR4-mediated induction of TNF-α expression. Total RNA was isolated and analyzed as shown in panel b. (e) Normal p300 levels are not required for the IL-4-mediated induction of Arg1 expression. BMDMs were plated and stimulated with IL-4 as shown in panel c. (f) Normal p300 levels are not required for the IFN-γ-mediated induction of IRF-1 expression but may be required for the normal kinetics of iNOS production. BMDMs were stimulated with LPS and IFN-γ to induce the expression of IRF-1 and iNOS, and lysates were analyzed by immunoblotting. The nonspecific band serves as a loading control (asterisk). Note that although IRF-1 levels are normal in *p300*^{flox/flox}; *lysMcre* BMDMs compared to control cells, the induction of iNOS expression is slightly delayed at the 6-h time point but eventually reaches robust levels.

normal range (P. J. Murray, data not shown). Together, these results indicate that CBP or p300 individually is not highly limiting for TNF-α and Arg1 expression in macrophages in response to LPS and IL-4. Interestingly, iNOS, but not IRF-1, induction in response to IFN-γ plus LPS appears to be partially sensitive to p300 deficiency, consistent with a proposed model that CBP and p300 are limiting for iNOS transcription (117). Induction of high-level iNOS expression requires IRF-1 and

NF-κB activity, so it appears that p300 levels are important for this process but expression levels can be compensated for by CBP or other factors. We did not observe a noticeable defect in TNF-α induction in macrophages even though both CBP and p300 null T cells showed a clear deficit (Fig. 13b) and B cells deficient for p300 or CBP showed a 30 to 60% decrease in TNF-α expression in response to B-cell receptor signaling (P. K. Brindle, submitted for publication). This may reflect differ-

ent transcriptional regulator usage in the three cell types. It is worth noting that the macrophages tested here had less than 100% inactivation of *CBP^{fllox}* or *p300^{fllox}*, so it is possible that contributions to gene expression by CBP or p300 were underestimated in this analysis.

DISCUSSION

Inactivation of either *CBP* or *p300* starting at the DN stage of thymocyte development reduced, but did not completely block, the ability of T cells to populate the periphery; whereas, inactivation of all four *CBP* and *p300* alleles had strong negative effects on T-cell development in the thymus. In addition, the aberrant increase in the percentage of CD8⁺ SP thymocytes seen in *CBP^{fllox/fllox}*; *Lck-Cre* mice was not observed in *p300^{fllox/fllox}*; *Lck-Cre* animals, indicating that CBP and p300 have nonredundant roles in T-cell development. Although the absolute levels of CBP and p300 protein are unknown, thymocytes express both proteins, which implies that large differences in expression do not account for the phenotypes. A provocative possibility is that differences in the biochemical properties of CBP and p300 are fundamental to the production of normal T cells and may also explain why loss of CBP, but not p300, in T cells can result in lymphoma.

Homeostatic proliferative mechanisms largely compensated for any reduction in the number of naive peripheral T cells caused by the mutations by allowing cells that did not recombine the conditional alleles (likely due to stochastic expression of the *Lck-Cre* transgene) to expand and fill the niche (84). It is somewhat counterintuitive that loss of both CBP and p300 did not cause a significant decrease in peripheral T cells, even though loss of CBP alone caused a modest ~2-fold deficit. There are several possible explanations for this observation. First, T cells lacking CBP alone may function in a non-cell-autonomous manner to suppress homeostatic proliferation of peripheral T cells. For example, it is possible that less IL-7, an essential cytokine for homeostatic proliferation, is available in *CBP^{fllox/fllox}*; *Lck-Cre* mice (84). Since T cells lacking both CBP and p300 are very rare or nonexistent outside the thymus, homeostatic proliferation would function normally in *CBP^{fllox/fllox}*; *p300^{fllox/fllox}*; *Lck-Cre* mice in this scenario. Second, homeostatic "cell counting" or "empty space detection" mechanisms may respond more efficiently to a strong deficit in early thymocyte development (as seen in *CBP^{fllox/fllox}*; *p300^{fllox/fllox}*; *Lck-Cre* mice) than if cells are less severely affected in the thymus, as with *CBP^{fllox/fllox}*; *Lck-Cre* mice. Third, recombination of the floxed alleles may be more efficient in *CBP^{fllox/fllox}*; *Lck-Cre* mice than in *CBP^{fllox/fllox}*; *p300^{fllox/fllox}*; *Lck-Cre* mice, thus homeostatic proliferation may be unable to completely fill the larger empty niche in the former.

It was surprising that loss of either CBP or p300 alone did not result in a more drastic T-cell phenotype given that the two coactivators have been shown to interact in vitro with over 190 proteins encoded by essential genes in mice, of which at least 56 are crucial for T cells (Mouse Genome Informatics) (Fig. 1). It is possible that these two coactivators are not generally limiting in cells to the extent previously believed (64, 115, 203). Indeed, *CBP^{-/-}* and *p300^{-/-}* mice are viable through the first half of development (dying at E9 to E11.5), suggestive of three possibilities with regard to the in vivo roles of CBP and p300.

(i) In vitro interactions with CBP and p300 may not be biologically relevant. Our findings, however, argue that CBP and p300 interactions based upon in vitro evidence cannot be dismissed out of hand. Although HDM2 appears to aggregate nonspecifically with the unfolded TAZ1 domain of p300 in the presence of EDTA (131), we have found that three essential transcription factors (CREB, c-Myb, and HIF-1 α) with well-characterized in vitro interactions with CBP and p300 show partial loss of activity when CBP and p300 functionality is attenuated by mutation in primary cells or mice (95; Kasper et al., in press). (ii) CBP and p300 may function redundantly in most instances and be generally nonlimiting in many cell types. Support for the second point is self-evident, since many T cells can develop and survive and mice can go through a substantial portion of development with either CBP or p300 alone. In addition, we have not observed increased expression of remaining wild-type alleles as a possible compensatory mechanism in mutant cells, so it appears that CBP and p300 levels are not highly limiting for some of their functions. Nevertheless, there are differences in the phenotypes of the various CBP and p300 mutant mice created to date, making it clear that there are either unique biochemical properties for each coactivator or that some cell types have a skewed ratio of CBP to p300 that makes one of them limiting upon mutation. (iii) Other transcriptional cofactors that are not highly related to CBP and p300 may supply redundant functions (e.g., other proteins that have histone acetyltransferase activity and bromodomains such as Gcn5, P/CAF, and TAF1). This point raises the prospect that coactivator networks are more broadly interconnected and robust than typically understood. But there are obviously limits to this proposition, as our studies show that either CBP or p300 is absolutely essential because T cells lacking both are not viable. So other cofactors may only be able to compensate under a limited number of situations. The identities of such factors are unknown, although recent genetic studies demonstrate that the combined dosage of various histone acetyltransferase proteins harboring bromodomains (p300 and Gcn5, and p300 and P/CAF) are not generally limiting for mouse development (158).

The creation of *CBP^{fllox}* and *p300^{fllox}* mice will now permit researchers to rigorously test whether either member of this small coactivator family is critical in specific cell lineages in vivo and to test their roles in endogenous gene expression ex vivo. Such analyses would be facilitated by the use of a Cre recombination-dependent GFP indicator allele to enable the FACS purification of cells with high frequencies (>90%) of floxed gene inactivation for functional and transcriptional studies ex vivo (143).

ACKNOWLEDGMENTS

We thank S. Lerach and C. Barlow for excellent technical assistance; the Transgenic core; R. Cross, J. Gatewood, J. Smith, and the Mouse Immunophenotyping core; R. Ashmun and the Flow Cytometry and Cell Sorting Shared Resource; the Animal Resource Center facilities at SJCRH; M. Biery, N. Craig, G. Martin, and S. Fiering for plasmids; and J. Opperman for helpful comments. The Hartwell Center at SJCRH provided oligonucleotides and DNA sequencing.

This work was supported by NCI grant RO1 CA076385 (to P.K.B.), the Cancer Center (CORE) support grant P30 CA021765, and by the American Lebanese Syrian Associated Charities of St. Jude Children's Research Hospital.

REFERENCES

- Asahara, H., S. Tartare-Deckert, T. Nakagawa, T. Ikehara, F. Hirose, T. Hunter, T. Ito, and M. Montminy. 2002. Dual roles of p300 in chromatin assembly and transcriptional activation in cooperation with nucleosome assembly protein 1 in vitro. *Mol. Cell. Biol.* **22**:2974–2983.
- Bachurski, C. J., G. H. Yang, T. A. Currier, R. M. Gronostajski, and D. Hong. 2003. Nuclear factor I/thyroid transcription factor 1 interactions modulate surfactant protein C transcription. *Mol. Cell. Biol.* **23**:9014–9024.
- Bai, L., and J. L. Merchant. 2000. Transcription factor ZBP-89 cooperates with histone acetyltransferase p300 during butyrate activation of p21waf1 transcription in human cells. *J. Biol. Chem.* **275**:30725–30733.
- Bai, R. Y., C. Koester, T. Ouyang, S. A. Hahn, M. Hammerschmidt, C. Peschel, and J. Duyster. 2002. SMIF, a Smad4-interacting protein that functions as a co-activator in TGF β signalling. *Nat. Cell Biol.* **4**:181–190.
- Bailey, P., V. Sartorelli, Y. Hamamori, and G. E. Muscat. 1998. The orphan nuclear receptor, COUP-TF II, inhibits myogenesis by post-transcriptional regulation of MyoD function: COUP-TF II directly interacts with p300 and myoD. *Nucleic Acids Res.* **26**:5501–5510.
- Bannert, N., A. Avots, M. Baier, E. Seifling, and R. Kurth. 1999. GA-binding protein factors, in concert with the coactivator CREB binding protein/p300, control the induction of the interleukin 16 promoter in T lymphocytes. *Proc. Natl. Acad. Sci. USA* **96**:1541–1546.
- Barbacci, E., A. Chalkiadaki, C. Masdeu, C. Haumaitre, L. Lokmane, C. Loirat, S. Cloarec, I. Talianidis, C. Bellanne-Chantelot, and S. Cereghini. 2004. HNF1 β /TCF2 mutations impair transactivation potential through altered co-regulator recruitment. *Hum. Mol. Genet.* **13**:3139–3149.
- Barrios-Rodiles, M., K. R. Brown, B. Ozdamar, R. Bose, Z. Liu, R. S. Donovan, F. Shinjo, Y. Liu, J. Dembowy, I. W. Taylor, V. Luga, N. Przulj, M. Robinson, H. Suzuki, Y. Hayashizaki, I. Jurisica, and J. L. Wrana. 2005. High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* **307**:1621–1625.
- Barthel, R., A. V. Tsytsykova, A. K. Barczak, E. Y. Tsai, C. C. Dascher, M. B. Brenner, and A. E. Goldfeld. 2003. Regulation of tumor necrosis factor alpha gene expression by mycobacteria involves the assembly of a unique enhanceosome dependent on the coactivator proteins CBP/p300. *Mol. Cell. Biol.* **23**:526–533.
- Bavner, A., J. Matthews, S. Sanyal, J. A. Gustafsson, and E. Treuter. 2005. EID3 is a novel EID family member and an inhibitor of CBP-dependent co-activation. *Nucleic Acids Res.* **33**:3561–3569.
- Bereschenko, O. R., W. Gu, and R. Dalla-Favera. 2002. Acetylation inactivates the transcriptional repressor BCL6. *Nat. Genet.* **32**:606–613.
- Bernat, A., N. Avvakumov, J. S. Mymryk, and L. Banks. 2003. Interaction between the HPV E7 oncoprotein and the transcriptional coactivator p300. *Oncogene* **22**:7871–7881.
- Bessa, M., M. K. Saville, and R. J. Watson. 2001. Inhibition of cyclin A/Cdk2 phosphorylation impairs B-Myb transactivation function without affecting interactions with DNA or the CBP coactivator. *Oncogene* **20**:3376–3386.
- Bhakat, K. K., T. Izumi, S. H. Yang, T. K. Hazra, and S. Mitra. 2003. Role of acetylated human AP-endonuclease (APE1/Ref-1) in regulation of the parathyroid hormone gene. *EMBO J.* **22**:6299–6309.
- Bhattacharya, S., C. L. Michels, M. K. Leung, Z. P. Arany, A. L. Kung, and D. M. Livingston. 1999. Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. *Genes Dev.* **13**:64–75.
- Biery, M. C., F. J. Stewart, A. E. Stellwagen, E. A. Raleigh, and N. L. Craig. 2000. A simple in vitro Tn7-based transposition system with low target site selectivity for genome and gene analysis. *Nucleic Acids Res.* **28**:1067–1077.
- Billon, N., D. Carlisi, M. B. Datto, L. A. van Grunsven, A. Watt, X. F. Wang, and B. B. Rudkin. 1999. Cooperation of Sp1 and p300 in the induction of the CDK inhibitor p21WAF1/CIP1 during NGF-mediated neuronal differentiation. *Oncogene* **18**:2872–2882.
- Blobel, G. A. 2002. CBP and p300: versatile coregulators with important roles in hematopoietic gene expression. *J. Leukoc. Biol.* **71**:545–556.
- Bouras, T., M. Fu, A. A. Sauve, F. Wang, A. A. Quong, N. D. Perkins, R. T. Hay, W. Gu, and R. G. Pestell. 2005. SIRT1 deacetylation and repression of p300 involves lysine residues 1020/1024 within the cell cycle regulatory domain 1. *J. Biol. Chem.* **280**:10264–10276.
- Bowen, H., A. Lapham, E. Phillips, I. Yeung, M. Alter-Koltunoff, B. Z. Levi, V. H. Perry, D. A. Mann, and C. H. Barton. 2003. Characterization of the murine Nramp1 promoter: requirements for transactivation by Miz-1. *J. Biol. Chem.* **278**:36017–36026.
- Braganca, J., J. J. Eloranta, S. D. Bamforth, J. C. Ibbitt, H. C. Hurst, and S. Bhattacharya. 2003. Physical and functional interactions among AP-2 transcription factors, p300/CREB-binding protein, and CITED2. *J. Biol. Chem.* **278**:16021–16029.
- Braganca, J., T. Swingle, F. I. Marques, T. Jones, J. J. Eloranta, H. C. Hurst, T. Shioda, and S. Bhattacharya. 2002. Human CREB-binding protein/p300-interacting transactivator with ED-rich tail (CITED) 4, a new member of the CITED family, functions as a co-activator for transcription factor AP-2. *J. Biol. Chem.* **277**:8559–8565.
- Burysek, L., W. S. Yeow, B. Lubyova, M. Kellum, S. L. Schafer, Y. Q. Huang, and P. M. Pitha. 1999. Functional analysis of human herpesvirus 8-encoded viral interferon regulatory factor 1 and its association with cellular interferon regulatory factors and p300. *J. Virol.* **73**:7334–7342.
- Castillo, A. I., A. M. Jimenez-Lara, R. M. Tolon, and A. Aranda. 1999. Synergistic activation of the prolactin promoter by vitamin D receptor and GHF-1: role of the coactivators, CREB-binding protein and steroid hormone receptor coactivator-1 (SRC-1). *Mol. Endocrinol.* **13**:1141–1154.
- Chakraborty, S., V. Senyuk, S. Sitailo, Y. Chi, and G. Nucifora. 2001. Interaction of EVI1 with cAMP-responsive element-binding protein-binding protein (CBP) and p300/CBP-associated factor (P/CAF) results in reversible acetylation of EVI1 and in co-localization in nuclear speckles. *J. Biol. Chem.* **276**:44936–44943.
- Chan, H. M., M. Krstic-Demonacos, L. Smith, C. Demonacos, and N. B. La Thangue. 2001. Acetylation control of the retinoblastoma tumour-suppressor protein. *Nat. Cell Biol.* **3**:667–674.
- Chan, H. M., and N. B. La Thangue. 2001. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *J. Cell Sci.* **114**:2363–2373.
- Chang, C. W., H. C. Chuang, C. Yu, T. P. Yao, and H. Chen. 2005. Stimulation of GCMA transcriptional activity by cyclic AMP/protein kinase A signaling is attributed to CBP-mediated acetylation of GCMA. *Mol. Cell. Biol.* **25**:8401–8414.
- Chen, J., S. S. Halappanavar, J. R. St-Germain, B. K. Tsang, and Q. Li. 2004. Role of Akt/protein kinase B in the activity of transcriptional coactivator p300. *Cell. Mol. Life Sci.* **61**:1675–1683.
- Chen, J., J. R. St-Germain, and Q. Li. 2005. B56 Regulatory subunit of protein phosphatase 2A mediates valproic acid-induced p300 degradation. *Mol. Cell. Biol.* **25**:525–532.
- Chen, Q., D. H. Dowhan, D. Liang, D. D. Moore, and P. A. Overbeek. 2002. CREB-binding protein/p300 co-activation of crystallin gene expression. *J. Biol. Chem.* **277**:24081–24089.
- Clausen, B. E., C. Burkhardt, W. Reith, R. Renkawitz, and I. Forster. 1999. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res.* **8**:265–277.
- Cotter, M. A., II, and E. S. Robertson. 2000. Modulation of histone acetyltransferase activity through interaction of Epstein-Barr nuclear antigen 3C with prothymosin alpha. *Mol. Cell. Biol.* **20**:5722–5735.
- Dai, P., H. Akimaru, Y. Tanaka, T. Maekawa, M. Nakafuku, and S. Ishii. 1999. Sonic Hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. *J. Biol. Chem.* **274**:8143–8152.
- Dai, Y. S., P. Cserjesi, B. E. Markham, and J. D. Molkentin. 2002. The transcription factors GATA4 and dHAND physically interact to synergistically activate cardiac gene expression through a p300-dependent mechanism. *J. Biol. Chem.* **277**:24390–24398.
- Dai, Y. S., and B. E. Markham. 2001. p300 Functions as a coactivator of transcription factor GATA-4. *J. Biol. Chem.* **276**:37178–37185.
- De Leo, R., S. Miccadei, E. Zammarchi, and D. Civitarella. 2000. Role for p300 in Pax 8 induction of thyroperoxidase gene expression. *J. Biol. Chem.* **275**:34100–34105.
- Dietrich, P., I. Dragatsis, S. Xuan, S. Zeitlin, and A. Efstratiadis. 2000. Conditional mutagenesis in mice with heat shock promoter-driven cre transgenes. *Mamm. Genome* **11**:196–205.
- Doucas, V., M. Tini, D. A. Egan, and R. M. Evans. 1999. Modulation of CREB binding protein function by the promyelocytic (PML) oncoprotein suggests a role for nuclear bodies in hormone signaling. *Proc. Natl. Acad. Sci. USA* **96**:2627–2632.
- Dowell, P., J. E. Ishmael, D. Avram, V. J. Peterson, D. J. Nevriy, and M. Leid. 1997. p300 functions as a coactivator for the peroxisome proliferator-activated receptor alpha. *J. Biol. Chem.* **272**:33435–33443.
- Eckner, R., T. P. Yao, E. Oldread, and D. M. Livingston. 1996. Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. *Genes Dev.* **10**:2478–2490.
- Ema, M., K. Hirota, J. Mimura, H. Abe, J. Yodoi, K. Sogawa, L. Poellinger, and Y. Fujii-Kuriyama. 1999. Molecular mechanisms of transcription activation by HLF and HIF1alpha in response to hypoxia: their stabilization and redox signal-induced interaction with CBP/p300. *EMBO J.* **18**:1905–1914.
- Emelyanov, A. V., C. R. Kovac, M. A. Sepulveda, and B. K. Birshtein. 2002. The interaction of Pax5 (BSAP) with Daxx can result in transcriptional activation in B cells. *J. Biol. Chem.* **277**:11156–11164.
- Erickson, R. L., N. Hemati, S. E. Ross, and O. A. MacDougald. 2001. p300 coactivates the adipogenic transcription factor CCAAT/enhancer-binding protein alpha. *J. Biol. Chem.* **276**:16348–16355.
- Ernst, P., J. Wang, M. Huang, R. H. Goodman, and S. J. Korsmeyer. 2001. MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Mol. Cell. Biol.* **21**:2249–2258.
- Facchinetti, V., L. Loffarelli, S. Schreck, M. Oelgeschlager, B. Luscher, M. Introna, and J. Golay. 1997. Regulatory domains of the A-Myb transcription factor and its interaction with the CBP/p300 adaptor molecules. *Biochem. J.* **324**:729–736.
- Falvo, J. V., B. M. Brinkman, A. V. Tsytsykova, E. Y. Tsai, T. P. Yao, A. L. Kung, and A. E. Goldfeld. 2000. A stimulus-specific role for CREB-binding

- protein (CBP) in T cell receptor-activated tumor necrosis factor alpha gene expression. *Proc. Natl. Acad. Sci. USA* **97**:3925–3929.
48. Fang, X., E. K. Stachowiak, S. M. Dunham-Ems, I. Klejbor, and M. K. Stachowiak. 2005. Control of CREB-binding protein signaling by nuclear fibroblast growth factor receptor-1: a novel mechanism of gene regulation. *J. Biol. Chem.* **280**:28451–28462.
 49. Faniello, M. C., M. A. Bevilacqua, G. Condorelli, B. de Crombrughe, S. N. Maity, V. E. Avvedimento, F. Cimino, and F. Costanzo. 1999. The B subunit of the CAAT-binding factor NFY binds the central segment of the Co-activator p300. *J. Biol. Chem.* **274**:7623–7626.
 50. Felzien, L. K., C. Woffendin, M. O. Hottiger, R. A. Subbramanian, E. A. Cohen, and G. J. Nabel. 1998. HIV transcriptional activation by the accessory protein, VPR, is mediated by the p300 co-activator. *Proc. Natl. Acad. Sci. USA* **95**:5281–5286.
 51. Fryer, C. J., E. Lamar, I. Turbachova, C. Kintner, and K. A. Jones. 2002. Mastermind mediates chromatin-specific transcription and turnover of the Notch enhancer complex. *Genes Dev.* **16**:1397–1411.
 52. Fu, M., C. Wang, M. Rao, X. Wu, T. Bouras, X. Zhang, Z. Li, X. Jiao, J. Yang, A. Li, N. D. Perkins, B. Thimmappaya, A. L. Kung, A. Munoz, A. Giordano, M. P. Lisanti, and R. G. Pestell. 2005. Cyclin D1 represses p300 transactivation through a cyclin-dependent kinase-independent mechanism. *J. Biol. Chem.* **280**:29728–29742.
 53. Fujii, Y., A. Kumatori, and M. Nakamura. 2003. SATB1 makes a complex with p300 and represses gp91(phox) promoter activity. *Microbiol. Immunol.* **47**:803–811.
 54. Fukuda, S., T. Kondo, H. Takebayashi, and T. Taga. 2004. Negative regulatory effect of an oligodendrocytic bHLH factor OLIG2 on the astrocytic differentiation pathway. *Cell Death Differ.* **11**:196–202.
 55. Furia, B., L. Deng, K. Wu, S. Baylor, K. Kehn, H. Li, R. Donnelly, T. Coleman, and F. Kashanchi. 2002. Enhancement of nuclear factor-kappa B acetylation by coactivator p300 and HIV-1 Tat proteins. *J. Biol. Chem.* **277**:4973–4980.
 56. Fuse, H., H. Kitagawa, and S. Kato. 2000. Characterization of transactivational property and coactivator mediation of rat mineralocorticoid receptor activation function-1 (AF-1). *Mol. Endocrinol.* **14**:889–899.
 57. Garcia-Rodriguez, C., and A. Rao. 1998. Nuclear factor of activated T cells (NFAT)-dependent transactivation regulated by the coactivators p300/CREB-binding protein (CBP). *J. Exp. Med.* **187**:2031–2036.
 58. Gingras, S., J. Simard, B. Groner, and E. Pfizner. 1999. p300/CBP is required for transcriptional induction by interleukin-4 and interacts with Stat6. *Nucleic Acids Res.* **27**:2722–2729.
 59. Giordano, A., and M. L. Avantaggiati. 1999. p300 and CBP: partners for life and death. *J. Cell. Physiol.* **181**:218–230.
 60. Giot, L., J. S. Bader, C. Brouwer, A. Chaudhuri, B. Kuang, Y. Li, Y. L. Hao, C. E. Ooi, B. Godwin, E. Vitols, G. Vijayadamodar, P. Pochart, H. Machineni, M. Welsh, Y. Kong, B. Zerhusen, R. Malcolm, Z. Varrone, A. Collis, M. Minto, S. Burgess, L. McDaniel, E. Stimpson, F. Spriggs, J. Williams, K. Neurath, N. Joime, M. Agee, V. Voss, K. Furtak, R. Renzulli, N. Aanensen, S. Carrola, E. Bickelhaupt, Y. Lazovatsky, A. DaSilva, J. Zhong, C. A. Stanyon, R. L. Finley, Jr., K. P. White, M. Braverman, T. Jarvie, S. Gold, M. Leach, J. Knight, R. A. Shimkets, M. P. McKenna, J. Chant, and J. M. Rothberg. 2003. A protein interaction map of *Drosophila* melanogaster. *Science* **302**:1727–1736.
 61. Girdwood, D., D. Bumpass, O. A. Vaughan, A. Thain, L. A. Anderson, A. W. Snowden, E. Garcia-Wilson, N. D. Perkins, and R. T. Hay. 2003. P300 transcriptional repression is mediated by SUMO modification. *Mol. Cell* **11**:1043–1054.
 62. Gizard, F., B. Lavalley, F. DeWitte, and D. W. Hum. 2001. A novel zinc finger protein TRP-132 interacts with CBP/p300 to regulate human CYP11A1 gene expression. *J. Biol. Chem.* **276**:33881–33892.
 63. Gomez-Gonzalo, M., I. Benedicto, M. Carretero, E. Lara-Pezzi, A. Maldonado-Rodriguez, R. Moreno-Otero, M. M. Lai, and M. Lopez-Cabrera. 2004. Hepatitis C virus core protein regulates p300/CBP co-activation function. Possible role in the regulation of NF-AT1 transcriptional activity. *Virology* **328**:120–130.
 64. Goodman, R. H., and S. Smolik. 2000. CBP/p300 in cell growth, transformation, and development. *Genes Dev.* **14**:1553–1577.
 65. Gronroos, E., U. Hellman, C. H. Heldin, and J. Ericsson. 2002. Control of Smad7 stability by competition between acetylation and ubiquitination. *Mol. Cell* **10**:483–493.
 66. Guidez, F., L. Howell, M. Isalan, M. Cebat, R. M. Alani, S. Ivins, I. Hormaeche, M. J. McConnell, S. Pierce, P. A. Cole, J. Licht, and A. Zelen. 2005. Histone acetyltransferase activity of p300 is required for transcriptional repression by the promyelocytic leukemia zinc finger protein. *Mol. Cell. Biol.* **25**:5552–5566.
 67. Hasan, S., P. O. Hassa, R. Imhof, and M. O. Hottiger. 2001. Transcription coactivator p300 binds PCNA and may have a role in DNA repair synthesis. *Nature* **410**:387–391.
 68. Hashimoto, K., K. Zanger, A. N. Hollenberg, L. E. Cohen, S. Radovick, and F. E. Wondisford. 2000. cAMP response element-binding protein-binding protein mediates thyrotropin-releasing hormone signaling on thyrotropin subunit genes. *J. Biol. Chem.* **275**:33365–33372.
 69. Hennet, T., F. K. Hagen, L. A. Tabak, and J. D. Marth. 1995. T-cell-specific deletion of a polypeptide N-acetylgalactosaminyl-transferase gene by site-directed recombination. *Proc. Natl. Acad. Sci. USA* **92**:12070–12074.
 70. Hirai, M., K. Ono, T. Morimoto, T. Kawamura, H. Wada, T. Kita, and K. Hasegawa. 2004. FOG-2 competes with GATA-4 for a transcriptional co-activator p300 and represses hypertrophic responses in cardiac myocytes. *J. Biol. Chem.* **279**:37640–37650.
 71. Hirose, T., R. Fujii, H. Nakamura, S. Aratani, H. Fujita, M. Nakazawa, K. Nakamura, K. Nishioka, and T. Nakajima. 2003. Regulation of CREB-mediated transcription by association of CDK4 binding protein p34SEI-1 with CBP. *Int. J. Mol. Med.* **11**:705–712.
 72. Hoffmeister, A., A. Ropolo, S. Vasseur, G. V. Mallo, H. Bodeker, B. Ritz-Laser, G. R. Dressler, M. I. Vaccaro, J. C. Dagorn, S. Moreno, and J. L. Iovanna. 2002. The HMG-I/Y-related protein p8 binds to p300 and Pax2 trans-activation domain-interacting protein to regulate the trans-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter. *J. Biol. Chem.* **277**:22314–22319.
 73. Hong, S., S. H. Kim, M. A. Heo, Y. H. Choi, M. J. Park, M. A. Yoo, H. D. Kim, H. S. Kang, and J. Cheong. 2004. Coactivator ASC-2 mediates heat shock factor 1-mediated transactivation dependent on heat shock. *FEBS Lett.* **559**:165–170.
 74. Hong, W., R. J. Resnick, C. Rakowski, D. Shalloway, S. J. Taylor, and G. A. Blobel. 2002. Physical and functional interaction between the transcriptional cofactor CBP and the KH domain protein Sam68. *Mol. Cancer Res.* **1**:48–55.
 75. Hsu, L. C., S. Liu, F. Abedinpour, R. D. Beech, J. M. Lahti, V. J. Kidd, J. A. Greenspan, and C. Y. Yeung. 2003. The murine G+C-rich promoter binding protein mGPBP is required for promoter-specific transcription. *Mol. Cell. Biol.* **23**:8773–8785.
 76. Huang, S. M., and M. R. Stallcup. 2000. Mouse Zacl, a transcriptional coactivator and repressor for nuclear receptors. *Mol. Cell. Biol.* **20**:1855–1867.
 77. Hussain, M. A., and J. F. Habener. 1999. Glucagon gene transcription activation mediated by synergistic interactions of pax-6 and cdx-2 with the p300 co-activator. *J. Biol. Chem.* **274**:28950–28957.
 78. Hwang, S., Y. Gwack, H. Byun, C. Lim, and J. Choe. 2001. The Kaposi's sarcoma-associated herpesvirus K8 protein interacts with CREB-binding protein (CBP) and represses CBP-mediated transcription. *J. Virol.* **75**:9509–9516.
 79. Iio, T., K. Furukawa, A. Yamaguchi, H. Shindo, S. Yamashita, and T. Tsukazaki. 2003. P300/CBP acts as a coactivator to cartilage homeoprotein-1 (Cart1), paired-like homeoprotein, through acetylation of the conserved lysine residue adjacent to the homeodomain. *J. Bone Miner. Res.* **18**:1419–1429.
 80. Ikeda, K., T. Stuehler, and M. Meisterernst. 2002. The H1 and H2 regions of the activation domain of herpes simplex virion protein 16 stimulate transcription through distinct molecular mechanisms. *Genes Cells* **7**:49–58.
 81. Ikeda, K., Y. Watanabe, H. Ohto, and K. Kawakami. 2002. Molecular interaction and synergistic activation of a promoter by Six, Eya, and Dach proteins mediated through CREB binding protein. *Mol. Cell. Biol.* **22**:6759–6766.
 82. Ikonen, T., J. J. Palvimo, and O. A. Janne. 1997. Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. *J. Biol. Chem.* **272**:29821–29828.
 83. Iwasaki, T., W. W. Chin, and L. Ko. 2001. Identification and characterization of RRM-containing coactivator activator (CoAA) as TRBP-interacting protein, and its splice variant as a coactivator modulator (CoAM). *J. Biol. Chem.* **276**:33375–33383.
 84. Jameson, S. C. 2002. Maintaining the norm: T-cell homeostasis. *Nat. Rev. Immunol.* **2**:547–556.
 85. Janknecht, R., and A. Nordheim. 1996. MAP kinase-dependent transcriptional coactivation by Elk-1 and its cofactor CBP. *Biochem. Biophys. Res. Commun.* **228**:831–837.
 86. Janknecht, R., N. J. Wells, and T. Hunter. 1998. TGF-beta-stimulated cooperation of smad proteins with the coactivators CBP/p300. *Genes Dev.* **12**:2114–2119.
 87. Jayaraman, G., R. Srinivas, C. Duggan, E. Ferreira, S. Swaminathan, K. Somasundaram, J. Williams, C. Hauser, M. Kurkinen, R. Dhar, S. Weitzman, G. Buttice, and B. Thimmappaya. 1999. p300/cAMP-responsive element-binding protein interactions with ets-1 and ets-2 in the transcriptional activation of the human stromelysin promoter. *J. Biol. Chem.* **274**:17342–17352.
 88. Ji, A., D. Dao, J. Chen, and W. R. MacLellan. 2003. EID-2, a novel member of the EID family of p300-binding proteins inhibits transactivation by MyoD. *Gene* **318**:35–43.
 89. Jung, S. Y., A. Malovannaya, J. Wei, B. W. O'Malley, and J. Qin. 2005. Proteomic analysis of steady-state nuclear hormone receptor coactivator complexes. *Mol. Endocrinol.* **19**:2451–2465.
 90. Kaida, A., Y. Ariumi, K. Baba, M. Matsubae, T. Takao, and K. Shimotohno. 2005. Identification of a novel p300-specific associating protein, phospho-

- ribosylpyrophosphate synthetase subunit 1 (PRS 1). *Biochem. J.* **391**:239–247.
91. Kakita, T., K. Hasegawa, T. Morimoto, S. Kaburagi, H. Wada, and S. Sasayama. 1999. p300 protein as a coactivator of GATA-5 in the transcription of cardiac-restricted atrial natriuretic factor gene. *J. Biol. Chem.* **274**: 34096–34102.
 92. Kalkhoven, E. 2004. CBP and p300: HATs for different occasions. *Biochem. Pharmacol.* **68**:1145–1155.
 93. Kang-Decker, N., C. Tong, F. Boussouar, D. J. Baker, W. Xu, A. A. Leontovich, W. R. Taylor, P. K. Brindle, and J. M. Van Deursen. 2004. Loss of CBP causes T cell lymphomagenesis in synergy with p27(Kip1) insufficiency. *Cancer Cell* **5**:177–189.
 94. Karetsov, Z., A. Kretsovali, C. Murphy, O. Tsolas, and T. Papamarcaki. 2002. Prothymosin alpha interacts with the CREB-binding protein and potentiates transcription. *EMBO Rep.* **3**:361–366.
 - 94a. Kasper, L. H., F. Boussouar, K. Boyd, W. Xu, M. Biesen, J. Reh, T. A. Baudino, J. L. Cleveland, and P. K. Brindle. 2005. Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. *EMBO J.* **24**:3846–3858.
 95. Kasper, L. H., F. Boussouar, P. A. Ney, C. W. Jackson, J. Reh, J. M. van Deursen, and P. K. Brindle. 2002. A transcription-factor-binding surface of coactivator p300 is required for haematopoiesis. *Nature* **419**:738–743.
 96. Kasper, L. H., P. K. Brindle, C. A. Schnabel, C. E. J. Pritchard, M. L. Cleary, and J. M. A. van Deursen. 1999. CREB binding protein interacts with nucleoporin-specific FG repeats that activate transcription and mediate NUP98-HOXA9 oncogenicity. *Mol. Cell. Biol.* **19**:764–776.
 97. Kaul, S., J. A. Blackford, Jr., J. Chen, V. V. Ogryzko, and S. S. Simons, Jr. 2000. Properties of the glucocorticoid modulatory element binding proteins GMEB-1 and -2: potential new modifiers of glucocorticoid receptor transactivation and members of the family of KDWK proteins. *Mol. Endocrinol.* **14**:1010–1027.
 98. Kawamura, T., K. Ono, T. Morimoto, M. Akao, E. Iwai-Kanai, H. Wada, N. Sowa, T. Kita, and K. Hasegawa. 2004. Endothelin-1-dependent nuclear factor of activated T lymphocyte signaling associates with transcriptional coactivator p300 in the activation of the B cell leukemia-2 promoter in cardiac myocytes. *Circ. Res.* **94**:1492–1499.
 99. Kennell, J. A., E. E. O'Leary, B. M. Gummow, G. D. Hammer, and O. A. MacDougald. 2003. T-cell factor 4N (TCF-4N), a novel isoform of mouse TCF-4, synergizes with beta-catenin to coactivate C/EBPalpha and steroidogenic factor 1 transcription factors. *Mol. Cell. Biol.* **23**:5366–5375.
 100. Kim, J. H., E. J. Cho, S. T. Kim, and H. D. Youn. 2005. CtBP represses p300-mediated transcriptional activation by direct association with its bromodomain. *Nat. Struct. Mol. Biol.* **12**:423–428.
 101. Kim, J. H., H. Li, and M. R. Stallcup. 2003. CoCoA, a nuclear receptor coactivator which acts through an N-terminal activation domain of p160 coactivators. *Mol. Cell* **12**:1537–1549.
 102. Kim, R. H., K. C. Flanders, S. Birkey Refeffy, L. A. Anderson, C. S. Duckett, N. D. Perkins, and A. B. Roberts. 2001. SNIP1 inhibits NF-kappa B signaling by competing for its binding to the C/H1 domain of CBP/p300 transcriptional co-activators. *J. Biol. Chem.* **276**:46297–46304.
 103. Kishikawa, S., T. Murata, H. Kimura, K. Shiota, and K. K. Yokoyama. 2002. Regulation of transcription of the Dnmt1 gene by Sp1 and Sp3 zinc finger proteins. *Eur. J. Biochem.* **269**:2961–2970.
 104. Ko, L., G. R. Cardona, and W. W. Chin. 2000. Thyroid hormone receptor-binding protein, an LXXLL motif-containing protein, functions as a general coactivator. *Proc. Natl. Acad. Sci. USA* **97**:6212–6217.
 105. Kobayashi, A., K. Numayama-Tsuruta, K. Sogawa, and Y. Fujii-Kuriyama. 1997. CBP/p300 functions as a possible transcriptional coactivator of Ah receptor nuclear translocator (Arnt). *J. Biochem. (Tokyo)* **122**:703–710.
 106. Kovacs, K. A., M. Steinmann, P. J. Magistretti, O. Halfon, and J. R. Cardinaux. 2003. CCAAT/enhancer-binding protein family members recruit the coactivator CREB-binding protein and trigger its phosphorylation. *J. Biol. Chem.* **278**:36959–36965.
 107. Koyanagi, M., M. Hijikata, K. Watashi, O. Masui, and K. Shimotohno. 2005. Centrosomal P4.1-associated protein is a new member of transcriptional coactivators for nuclear factor-kappaB. *J. Biol. Chem.* **280**:12430–12437.
 108. Kumar, B. R., V. Swaminathan, S. Banerjee, and T. K. Kundu. 2001. p300-mediated acetylation of human transcriptional coactivator PC4 is inhibited by phosphorylation. *J. Biol. Chem.* **276**:16804–16809.
 109. Kung, A. L., V. I. Rebel, R. T. Bronson, L. E. Ch'ng, C. A. Sieff, D. M. Livingston, and T. P. Yao. 2000. Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev.* **14**:272–277.
 110. Kwon, H. S., B. Huang, T. G. Untermyer, and R. A. Harris. 2004. Protein kinase B-alpha inhibits human pyruvate dehydrogenase kinase-4 gene induction by dexamethasone through inactivation of FOXO transcription factors. *Diabetes* **53**:899–910.
 111. Labalette, C., C. A. Renard, C. Neuveut, M. A. Buendia, and Y. Wei. 2004. Interaction and functional cooperation between the LIM protein FHL2, CBP/p300, and beta-catenin. *Mol. Cell. Biol.* **24**:10689–10702.
 112. Lamprecht, C., and C. R. Mueller. 1999. D-site binding protein transactivation requires the proline- and acid-rich domain and involves the coactivator p300. *J. Biol. Chem.* **274**:17643–17648.
 113. Lau, P., P. Bailey, D. H. Dowhan, and G. E. Muscat. 1999. Exogenous expression of a dominant negative RORalpha1 vector in muscle cells impairs differentiation: RORalpha1 directly interacts with p300 and myoD. *Nucleic Acids Res.* **27**:411–420.
 114. Laurance, M. E., R. P. Kwok, M. S. Huang, J. P. Richards, J. R. Lundblad, and R. H. Goodman. 1997. Differential activation of viral and cellular promoters by human T-cell lymphotropic virus-1 tax and cAMP-responsive element modulator isoforms. *J. Biol. Chem.* **272**:2646–2651.
 115. Legube, G., and D. Trouche. 2003. Regulating histone acetyltransferases and deacetylases. *EMBO Rep.* **4**:944–947.
 116. Leong, G. M., N. Subramaniam, L. L. Issa, J. B. Barry, T. Kino, P. H. Driggers, M. J. Hayman, J. A. Eisman, and E. M. Gardiner. 2004. Ski-interacting protein, a bifunctional nuclear receptor coregulator that interacts with N-CoR/SMRT and p300. *Biochem. Biophys. Res. Commun.* **315**: 1070–1076.
 117. Li, M., G. Pascual, and C. K. Glass. 2000. Peroxisome proliferator-activated receptor gamma-dependent repression of the inducible nitric oxide synthase gene. *Mol. Cell. Biol.* **20**:4699–4707.
 118. Li, S., C. M. Armstrong, N. Bertin, H. Ge, S. Milstein, M. Boxem, P. O. Vidalain, J. D. Han, A. Chesneau, T. Hao, D. S. Goldberg, N. Li, M. Martinez, J. F. Rual, P. Lamesch, L. Xu, M. Tewari, S. L. Wong, L. V. Zhang, G. F. Berriz, L. Jacotot, P. Vaglio, J. Reboul, T. Hirozane-Kishikawa, Q. Li, H. W. Gabel, A. Elewa, B. Baumgartner, D. J. Rose, H. Yu, S. Bosak, R. Sequerra, A. Fraser, S. E. Mango, W. M. Saxton, S. Strome, S. Van Den Heuvel, F. Piano, J. Vandenhaute, C. Sardet, M. Gerstein, L. Doucette-Stamm, K. C. Gunsalus, J. W. Harper, M. E. Cusick, F. P. Roth, D. E. Hill, and M. Vidal. 2004. A map of the interactome network of the metazoan *C. elegans*. *Science* **303**:540–543.
 119. Li, S., B. Aufiero, R. L. Schiltz, and M. J. Walsh. 2000. Regulation of the homeodomain CCAAT displacement/cut protein function by histone acetyltransferases p300/CREB-binding protein (CBP)-associated factor and CBP. *Proc. Natl. Acad. Sci. USA* **97**:7166–7171.
 120. Liu, Y., G. L. Borchert, and J. M. Phang. 2004. Polyoma enhancer activator 3, an ets transcription factor, mediates the induction of cyclooxygenase-2 by nitric oxide in colorectal cancer cells. *J. Biol. Chem.* **279**:18694–18700.
 121. Long, J., G. Wang, I. Matsuura, D. He, and F. Liu. 2004. Activation of Smad transcriptional activity by protein inhibitor of activated STAT3 (PIAS3). *Proc. Natl. Acad. Sci. USA* **101**:99–104.
 122. Ma, Z. Q., Z. Liu, E. S. Ngan, and S. Y. Tsai. 2001. Cdc25B functions as a novel coactivator for the steroid receptors. *Mol. Cell. Biol.* **21**:8056–8067.
 123. MacLellan, W. R., G. Xiao, M. Abdellatif, and M. D. Schneider. 2000. A novel Rb- and p300-binding protein inhibits transactivation by MyoD. *Mol. Cell. Biol.* **20**:8903–8915.
 124. Macpartlin, M., S. X. Zeng, H. Lee, D. Stauffer, Y. Jin, M. Thayer, and H. Lu. 2005. p300 regulates p63 transcriptional activity. *J. Biol. Chem.* **280**: 30604–30610.
 125. Mahmud, D. L., M. G. Amlak, D. K. Deb, L. C. Platanias, S. Uddin, and A. Wickrema. 2002. Phosphorylation of forkhead transcription factors by erythropoietin and stem cell factor prevents acetylation and their interaction with coactivator p300 in erythroid progenitor cells. *Oncogene* **21**:1556–1562.
 126. Major, M. L., R. Lepe, and R. H. Costa. 2004. Forkhead box M1B transcriptional activity requires binding of Cdk-cyclin complexes for phosphorylation-dependent recruitment of p300/CBP coactivators. *Mol. Cell. Biol.* **24**:2649–2661.
 127. Mandolesi, G., S. Gargano, M. Pennuto, B. Illi, R. Molfetta, L. Soucek, L. Mosca, A. Levi, R. Jucker, and S. Nasi. 2002. NGF-dependent and tissue-specific transcription of vgf is regulated by a CREB-p300 and bHLH factor interaction. *FEBS Lett.* **510**:50–56.
 128. Marambaud, P., P. H. Wen, A. Dutt, J. Shioi, A. Takashima, R. Siman, and N. K. Robakis. 2003. A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. *Cell* **114**:635–645.
 129. Masumi, A., and K. Ozato. 2001. Coactivator p300 acetylates the interferon regulatory factor-2 in U937 cells following phorbol ester treatment. *J. Biol. Chem.* **276**:20973–20980.
 130. Mathew, S., E. Mascareno, and M. A. Siddiqui. 2004. A ternary complex of transcription factors, Nishid and NFATc4, and co-activator p300 bound to an intronic sequence, intronic regulatory element, is pivotal for the up-regulation of myosin light chain-2v gene in cardiac hypertrophy. *J. Biol. Chem.* **279**:41018–41027.
 131. Matt, T., M. A. Martinez-Yamout, H. J. Dyson, and P. E. Wright. 2004. The CBP/p300 TAZ1 domain in its native state is not a binding partner of MDM2. *Biochem. J.* **381**:685–691.
 132. McManus, K. J., and M. J. Hendzel. 2001. CBP, a transcriptional coactivator and acetyltransferase. *Biochem. Cell Biol.* **79**:253–266.
 133. Mehra-Chaudhary, R., H. Matsui, and R. Raghow. 2001. Mx3 protein recruits histone deacetylase to down-regulate the Mx1 promoter. *Biochem. J.* **353**:13–22.
 134. Messina, D. N., J. Glasscock, W. Gish, and M. Lovett. 2004. An ORFeome-

- based analysis of human transcription factor genes and the construction of a microarray to interrogate their expression. *Genome Res.* **14**:2041–2047.
135. Meyers, E. N., M. Lewandoski, and G. R. Martin. 1998. An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination. *Nat. Genet.* **18**:136–141.
 136. Misra, P., C. Qi, S. Yu, S. H. Shah, W. Q. Cao, M. S. Rao, B. Thimmapaya, Y. Zhu, and J. K. Reddy. 2002. Interaction of PIMT with transcriptional coactivators CBP, p300, and PBP differential role in transcriptional regulation. *J. Biol. Chem.* **277**:20011–20019.
 137. Mizukami, J., and T. Taniguchi. 1997. The antidiabetic agent thiazolidinedione stimulates the interaction between PPAR gamma and CBP. *Biochem. Biophys. Res. Commun.* **240**:61–64.
 138. Molinari, S., F. Relaix, M. Lemonnier, B. Kirschbaum, B. Schafer, and M. Buckingham. 2004. A novel complex regulates cardiac actin gene expression through interaction of Emb, a class VI POU domain protein, MEF2D, and the histone transacetylase p300. *Mol. Cell. Biol.* **24**:2944–2957.
 139. Monroy, M. A., D. D. Ruhl, X. Xu, D. K. Granner, P. Yaciuk, and J. C. Chrivia. 2001. Regulation of cAMP-responsive element-binding protein-mediated transcription by the SNF2/SWI-related protein, SRCAP. *J. Biol. Chem.* **276**:40721–40726.
 140. Morris, L., K. E. Allen, and N. B. La Thangue. 2000. Regulation of E2F transcription by cyclin E-Cdk2 kinase mediated through p300/CBP co-activators. *Nat. Cell. Biol.* **2**:232–239.
 141. Murray, P. J., L. Wang, C. Onufryk, R. I. Tepper, and R. A. Young. 1997. T cell-derived IL-10 antagonizes macrophage function in mycobacterial infection. *J. Immunol.* **158**:315–321.
 142. Nakashima, K., M. Yanagisawa, H. Arakawa, N. Kimura, T. Hisatsune, M. Kawabata, K. Miyazono, and T. Taga. 1999. Synergistic signaling in fetal brain by STAT3-Smad1 complex bridged by p300. *Science* **284**:479–482.
 143. Novak, A., C. Guo, W. Yang, A. Nagy, and C. G. Lobe. 2000. Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis* **28**:147–155.
 144. Nowling, T. K., L. R. Johnson, M. S. Wiebe, and A. Rizzino. 2000. Identification of the transactivation domain of the transcription factor Sox-2 and an associated co-activator. *J. Biol. Chem.* **275**:3810–3818.
 145. Nucifora, F. C., Jr., M. Sasaki, M. F. Peters, H. Huang, J. K. Cooper, M. Yamada, H. Takahashi, S. Tsuji, J. Troncoso, V. L. Dawson, T. M. Dawson, and C. A. Ross. 2001. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **291**:2423–2428.
 146. Ohshima, T., E. Yoshida, T. Nakajima, K. I. Yagami, and A. Fukamizu. 2001. Effects of interaction between parvovirus minute virus of mice NS1 and coactivator CBP on NS1- and p53-transactivation. *Int. J. Mol. Med.* **7**:49–54.
 147. O'Sullivan, A., H. C. Chang, Q. Yu, and M. H. Kaplan. 2004. STAT4 is required for interleukin-12-induced chromatin remodeling of the CD25 locus. *J. Biol. Chem.* **279**:7339–7345.
 148. Oswald, F., B. Tauber, T. Dobner, S. Bourtelee, U. Kostezka, G. Adler, S. Liptay, and R. M. Schmid. 2001. p300 acts as a transcriptional coactivator for mammalian Notch-1. *Mol. Cell. Biol.* **21**:7761–7774.
 149. Pan, J., C. Jin, T. Murata, and K. K. Yokoyama. 2003. Sequence specific transcription factor, JDP2 interacts with histone and inhibits p300-mediated histone acetylation. *Nucleic Acids Res. Suppl.* **2003**:305–306.
 150. Papoutsopoulou, S., and R. Janknecht. 2000. Phosphorylation of ETS transcription factor ER81 in a complex with its coactivators CREB-binding protein and p300. *Mol. Cell. Biol.* **20**:7300–7310.
 151. Park, S. R., E. K. Lee, B. C. Kim, and P. H. Kim. 2003. p300 cooperates with Smad3/4 and Runx3 in TGFbeta1-induced IgA isotype expression. *Eur. J. Immunol.* **33**:3386–3392.
 152. Pauleau, A. L., R. Rutschman, R. Lang, A. Pernis, S. S. Watowich, and P. J. Murray. 2004. Enhancer-mediated control of macrophage-specific arginase I expression. *J. Immunol.* **172**:7565–7573.
 153. Pedoux, R., S. Sengupta, J. C. Shen, O. N. Demidov, S. Saito, H. Onogi, K. Kumamoto, S. Wincovitch, S. H. Garfield, M. McMenamin, M. Nagashima, S. R. Grossman, E. Appella, and C. C. Harris. 2005. ING2 regulates the onset of replicative senescence by induction of p300-dependent p53 acetylation. *Mol. Cell. Biol.* **25**:6639–6648.
 154. Pelletier, G., V. Y. Stefanovsky, M. Faubladiet, I. Hirschler-Laszkiewicz, J. Savard, L. I. Rothblum, J. Cote, and T. Moss. 2000. Competitive recruitment of CBP and Rb-HDAC regulates UBF acetylation and ribosomal transcription. *Mol. Cell. Biol.* **20**:1059–1066.
 155. Peng, Y. C., D. E. Breiding, F. Sverdrup, J. Richard, and E. J. Androphy. 2000. AMF-1/Gps2 binds p300 and enhances its interaction with papillomavirus E2 proteins. *J. Virol.* **74**:5872–5879.
 156. Perkins, N. D., L. K. Felzien, J. C. Betts, K. Leung, D. H. Beach, and G. J. Nabel. 1997. Regulation of NF-kappaB by cyclin-dependent kinases associated with the p300 coactivator. *Science* **275**:523–527.
 157. Pfützner, E., R. Jahne, M. Wissler, E. Stoecklin, and B. Groner. 1998. p300/CREB-binding protein enhances the prolactin-mediated transcriptional induction through direct interaction with the transactivation domain of Stat5, but does not participate in the Stat5-mediated suppression of the glucocorticoid response. *Mol. Endocrinol.* **12**:1582–1593.
 158. Phan, H. M., A. W. Xu, C. Coco, G. Srajer, S. Wyszomierski, Y. A. Evrard, R. Eckner, and S. Y. Dent. 2005. GCN5 and p300 share essential functions during early embryogenesis. *Dev. Dyn.* **233**:1337–1347.
 159. Pitkanen, J., V. Doucas, T. Sternsdorf, T. Nakajima, S. Aratani, K. Jensen, H. Will, P. Vahamurto, J. Ollila, M. Vihinen, H. S. Scott, S. E. Antonarakis, J. Kudoh, N. Shimizu, K. Krohn, and P. Peterson. 2000. The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common coactivator CREB-binding protein. *J. Biol. Chem.* **275**:16802–16809.
 160. Postigo, A. A., J. L. Depp, J. J. Taylor, and K. L. Kroll. 2003. Regulation of Smad signaling through a differential recruitment of coactivators and corepressors by ZEB proteins. *EMBO J.* **22**:2453–2462.
 161. Qiu, Y., M. Guo, S. Huang, and R. Stein. 2002. Insulin gene transcription is mediated by interactions between the p300 coactivator and PDX-1, BETA2, and E47. *Mol. Cell. Biol.* **22**:412–420.
 162. Ramirez, S., S. Ait-Si-Ali, P. Robin, D. Trouche, A. Harel-Bellan, and S. Ait Si Ali. 1997. The CREB-binding protein (CBP) cooperates with the serum response factor for transactivation of the c-fos serum response element. *J. Biol. Chem.* **272**:31016–31021.
 163. Rausa, F. M., Y. Tan, and R. H. Costa. 2003. Association between hepatocyte nuclear factor 6 (HNF-6) and FoxA2 DNA binding domains stimulates FoxA2 transcriptional activity but inhibits HNF-6 DNA binding. *Mol. Cell. Biol.* **23**:437–449.
 164. Rebel, V. I., A. L. Kung, E. A. Tanner, H. Yang, R. T. Bronson, and D. M. Livingston. 2002. Distinct roles for CREB-binding protein and p300 in hematopoietic stem cell self-renewal. *Proc. Natl. Acad. Sci. USA* **99**:14789–14794.
 165. Rossow, K. L., and R. Janknecht. 2001. The Ewing's sarcoma gene product functions as a transcriptional activator. *Cancer Res.* **61**:2690–2695.
 166. Rossow, K. L., and R. Janknecht. 2003. Synergism between p68 RNA helicase and the transcriptional coactivators CBP and p300. *Oncogene* **22**:151–156.
 167. Sartorelli, V., J. Huang, Y. Hamamori, and L. Kedes. 1997. Molecular mechanisms of myogenic coactivation by p300: direct interaction with the activation domain of MyoD and with the MADS box of MEF2C. *Mol. Cell. Biol.* **17**:1010–1026.
 168. See, R. H., D. Calvo, Y. Shi, H. Kawa, M. P. Luke, and Z. Yuan. 2001. Stimulation of p300-mediated transcription by the kinase MEK1. *J. Biol. Chem.* **276**:16310–16317.
 169. Seibler, J., D. Schubeler, S. Fiering, M. Groudine, and J. Bode. 1998. DNA cassette exchange in ES cells mediated by Flp recombinase: an efficient strategy for repeated modification of tagged loci by marker-free constructs. *Biochemistry* **37**:6229–6234.
 170. SenBanerjee, S., Z. Lin, G. B. Atkins, D. M. Greif, R. M. Rao, A. Kumar, M. W. Feinberg, Z. Chen, D. I. Simon, F. W. Lusinskas, T. M. Michel, M. A. Gimbrone, Jr., G. Garcia-Cardena, and M. K. Jain. 2004. KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. *J. Exp. Med.* **199**:1305–1315.
 171. Shen, G., V. Hebbur, S. Nair, C. Xu, W. Li, W. Lin, Y. S. Keum, J. Han, M. A. Gallo, and A. N. Kong. 2004. Regulation of Nrf2 transactivation domain activity. The differential effects of mitogen-activated protein kinase cascades and synergistic stimulatory effect of Raf and CREB-binding protein. *J. Biol. Chem.* **279**:23052–23060.
 172. Shen, W. F., K. Krishnan, H. J. Lawrence, and C. Largman. 2001. The HOX homeodomain proteins block CBP histone acetyltransferase activity. *Mol. Cell. Biol.* **21**:7509–7522.
 173. Shetty, S., T. Takahashi, H. Matsui, R. Ayengar, and R. Raghov. 1999. Transcriptional autorepression of Msx1 gene is mediated by interactions of Msx1 protein with a multi-protein transcriptional complex containing TATA-binding protein, Sp1 and cAMP-response-element-binding protein-binding protein (CBP/p300). *Biochem. J.* **339**:751–758.
 174. Shibanuma, M., J. R. Kim-Kaneyama, S. Sato, and K. Nose. 2004. A LIM protein, Hic-5, functions as a potential coactivator for Sp1. *J. Cell. Biochem.* **91**:633–645.
 175. Shikama, N., H. M. Chan, M. Krstic-Demonacos, L. Smith, C. W. Lee, W. Cairns, and N. B. La Thangue. 2000. Functional interaction between nucleosome assembly proteins and p300/CREB-binding protein family coactivators. *Mol. Cell. Biol.* **20**:8933–8943.
 176. Shikama, N., W. Lutz, R. Kretzschmar, N. Sauter, J. F. Roth, S. Marino, J. Wittwer, A. Scheidweiler, and R. Eckner. 2003. Essential function of p300 acetyltransferase activity in heart, lung and small intestine formation. *EMBO J.* **22**:5175–5185.
 177. Shiseki, M., M. Nagashima, R. M. Pedoux, M. Kitahama-Shiseki, K. Miura, S. Okamura, H. Onogi, Y. Higashimoto, E. Appella, J. Yokota, and C. C. Harris. 2003. p29ING4 and p28ING5 bind to p53 and p300, and enhance p53 activity. *Cancer Res.* **63**:2373–2378.
 178. Sierra, J., A. Villagra, R. Paredes, F. Cruzat, S. Gutierrez, A. Javed, G. Arriagada, J. Olate, M. Imshenetzky, A. J. Van Wijnen, J. B. Lian, G. S. Stein, J. L. Stein, and M. Montecino. 2003. Regulation of the bone-specific osteocalcin gene by p300 requires Runx2/Cbfa1 and the vitamin D3 receptor but not p300 intrinsic histone acetyltransferase activity. *Mol. Cell. Biol.* **23**:3339–3351.
 179. Simone, C., P. Stiegler, S. V. Forcales, L. Bagella, A. De Luca, V. Sartorelli,

- A. Giordano, and P. L. Puri. 2004. Deacetylase recruitment by the C/H3 domain of the acetyltransferase p300. *Oncogene* **23**:2177–2187.
180. Slepak, T. I., K. A. Webster, J. Zang, H. Prentice, A. O'Dowd, M. N. Hicks, and N. H. Bishopric. 2001. Control of cardiac-specific transcription by p300 through myocyte enhancer factor-2D. *J. Biol. Chem.* **276**:7575–7585.
181. Smit, D. J., A. G. Smith, P. G. Parsons, G. E. Muscat, and R. A. Sturm. 2000. Domains of Brn-2 that mediate homodimerization and interaction with general and melanocytic transcription factors. *Eur. J. Biochem.* **267**: 6413–6422.
182. Song, C. Z., K. Keller, K. Murata, H. Asano, and G. Stamatoyannopoulos. 2002. Functional interaction between coactivators CBP/p300, PCAF, and transcription factor FKL2. *J. Biol. Chem.* **277**:7029–7036.
183. Steffan, J. S., A. Kazantsev, O. Spasic-Boskovic, M. Greenwald, Y. Z. Zhu, H. Gohler, E. E. Wanker, G. P. Bates, D. E. Housman, and L. M. Thompson. 2000. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl. Acad. Sci. USA* **97**:6763–6768.
184. Sterner, D. E., and S. L. Berger. 2000. Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.* **64**:435–459.
185. Strano, S., O. Monti, N. Pediconi, A. Baccarini, G. Fontemaggi, E. Lapi, F. Mantovani, A. Damalas, G. Citro, A. Sacchi, G. Del Sal, M. Levero, and G. Blandino. 2005. The transcriptional coactivator Yes-associated protein drives p73 gene-target specificity in response to DNA damage. *Mol. Cell* **18**:447–459.
186. Sugihara, T. M., E. I. Kudryavtseva, V. Kumar, J. J. Horridge, and B. Andersen. 2001. The POU domain factor Skin-1a represses the keratin 14 promoter independent of DNA binding. A possible role for interactions between Skn-1a and CREB-binding protein/p300. *J. Biol. Chem.* **276**:33036–33044.
187. Sun, Z., J. Pan, W. X. Hope, S. N. Cohen, and S. P. Balk. 1999. Tumor susceptibility gene 101 protein represses androgen receptor transactivation and interacts with p300. *Cancer* **86**:689–696.
188. Swenson, J. J., E. Holley-Guthrie, and S. C. Kenney. 2001. Epstein-Barr virus immediate-early protein BRLF1 interacts with CBP, promoting enhanced BRLF1 transactivation. *J. Virol.* **75**:6228–6234.
189. Takamaru, K. I., and R. T. Moon. 2000. The transcriptional coactivator CBP interacts with beta-catenin to activate gene expression. *J. Cell Biol.* **149**:249–254.
190. Tanaka, Y., I. Naruse, T. Maekawa, H. Masuya, T. Shiroishi, and S. Ishii. 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.
191. Tatematsu, K., N. Yoshimoto, T. Koyanagi, C. Tokunaga, T. Tachibana, Y. Yoneda, M. Yoshida, T. Okajima, K. Tanizawa, and S. Kuroda. 2005. Nuclear-cytoplasmic shuttling of a RING-IBR protein RBCK1 and its functional interaction with nuclear body proteins. *J. Biol. Chem.* **280**:22937–22944.
192. Tini, M., A. Benecke, S. J. Um, J. Torchia, R. M. Evans, and P. Chambon. 2002. Association of CBP/p300 acetylase and thymine DNA glycosylase links DNA repair and transcription. *Mol. Cell* **9**:265–277.
193. Tohkin, M., M. Fukuhara, G. Elizondo, S. Tomita, and F. J. Gonzalez. 2000. Aryl hydrocarbon receptor is required for p300-mediated induction of DNA synthesis by adenovirus E1A. *Mol. Pharmacol.* **58**:845–851.
194. Topper, J. N., M. R. DiChiara, J. D. Brown, A. J. Williams, D. Falb, T. Collins, and M. A. Gimbrone, Jr. 1998. CREB binding protein is a required coactivator for Smad-dependent, transforming growth factor beta transcriptional responses in endothelial cells. *Proc. Natl. Acad. Sci. USA* **95**:9506–9511.
195. Toyama, T., and H. Iwase. 2004. p33ING1b and estrogen receptor (ER) alpha. *Breast Cancer* **11**:33–37.
196. Tsuda, M., S. Takahashi, Y. Takahashi, and H. Asahara. 2003. Transcriptional co-activators CREB-binding protein and p300 regulate chondrocyte-specific gene expression via association with Sox9. *J. Biol. Chem.* **278**: 27224–27229.
197. Vadlamudi, R. K., R. A. Wang, A. Mazumdar, Y. Kim, J. Shin, A. Sahin, and R. Kumar. 2001. Molecular cloning and characterization of PELP1, a novel human coregulator of estrogen receptor alpha. *J. Biol. Chem.* **276**: 38272–38279.
198. Valineva, T., J. Yang, R. Palovuori, and O. Silvennoinen. 2005. The transcriptional co-activator protein p100 recruits histone acetyltransferase activity to STAT6 and mediates interaction between the CREB-binding protein and STAT6. *J. Biol. Chem.* **280**:14989–14996.
199. van der Horst, A., L. G. Tertoolen, L. M. de Vries-Smits, R. A. Frye, R. H. Medema, and B. M. Burgering. 2004. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1). *J. Biol. Chem.* **279**:28873–28879.
200. van Wely, K. H., A. C. Molijn, A. Buijs, M. A. Meester-Smoor, A. J. Aarnoudse, A. Hellemons, P. den Besten, G. C. Grosveld, and E. C. Zwartthoff. 2003. The MN1 oncoprotein synergizes with coactivators RAC3 and p300 in RAR-RXR-mediated transcription. *Oncogene* **22**:699–709.
201. Verma, U. N., Y. Yamamoto, S. Prajapati, and R. B. Gaynor. 2004. Nuclear role of I kappa B Kinase-gamma/NF-kappa B essential modulator (IKK gamma/NEMO) in NF-kappa B-dependent gene expression. *J. Biol. Chem.* **279**:3509–3515.
202. Vervoorts, J., J. M. Luscher-Firzlaff, S. Rottmann, R. Lilischkis, G. Walsemann, K. Dohmann, M. Austen, and B. Luscher. 2003. Stimulation of c-MYC transcriptional activity and acetylation by recruitment of the cofactor CBP. *EMBO Rep.* **4**:484–490.
203. Vo, N. K., and R. H. Goodman. 2001. CBP and p300 in transcriptional regulation. *J. Biol. Chem.* **276**:13505–13508.
204. Voegel, J. J., M. J. Heine, M. Tini, V. Vivat, P. Chambon, and H. Grone-meyer. 1998. The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. *EMBO J.* **17**:507–519.
205. Wada, H., K. Hasegawa, T. Morimoto, T. Kakita, T. Yanazume, and S. Sasayama. 2000. A p300 protein as a coactivator of GATA-6 in the transcription of the smooth muscle-myosin heavy chain gene. *J. Biol. Chem.* **275**:25330–25335.
206. Wallberg, A. E., S. Yamamura, S. Malik, B. M. Spiegelman, and R. G. Roeder. 2003. Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1alpha. *Mol. Cell* **12**: 1137–1149.
207. Wang, A., T. Ikura, K. Eto, and M. S. Ota. 2004. Dynamic interaction of p220(NPAT) and CBP/p300 promotes S-phase entry. *Biochem. Biophys. Res. Commun.* **325**:1509–1516.
208. Wang, H., R. Fang, J. Y. Cho, T. A. Libermann, and P. Oettgen. 2004. Positive and negative modulation of the transcriptional activity of the ETS factor ESE-1 through interaction with p300, CREB-binding protein, and Ku 70/86. *J. Biol. Chem.* **279**:25241–25250.
209. Wang, W., S. B. Lee, R. Palmer, L. W. Ellisen, and D. A. Haber. 2001. A functional interaction with CBP contributes to transcriptional activation by the Wilms tumor suppressor WT1. *J. Biol. Chem.* **276**:16810–16816.
210. Wansa, K. D., J. M. Harris, and G. E. Muscat. 2002. The activation function-1 domain of Nur77/NR4A1 mediates trans-activation, cell specificity, and coactivator recruitment. *J. Biol. Chem.* **277**:33001–33011.
211. Weaver, B. K., K. P. Kumar, and N. C. Reich. 1998. Interferon regulatory factor 3 and CREB-binding protein/p300 are subunits of double-stranded RNA-activated transcription factor DRAF1. *Mol. Cell. Biol.* **18**:1359–1368.
212. Wherry, E. J., and R. Ahmed. 2004. Memory CD8 T-cell differentiation during viral infection. *J. Virol.* **78**:5535–5545.
213. Williams, C. J., T. Naito, P. G. Arco, J. R. Seavitt, S. M. Cashman, B. De Souza, X. Qi, P. Keables, U. H. Von Andrian, and K. Georgopoulos. 2004. The chromatin remodeler Mi-2beta is required for CD4 expression and T cell development. *Immunity* **20**:719–733.
214. Wu, L., J. Liu, P. Gao, M. Nakamura, Y. Cao, H. Shen, and J. D. Griffin. 2005. Transforming activity of MECT1-MAML2 fusion oncoprotein is mediated by constitutive CREB activation. *EMBO J.* **24**:2391–2402.
215. Xu, W., H. Chen, K. Du, H. Asahara, M. Tini, B. M. Emerson, M. Montminy, and R. M. Evans. 2001. A transcriptional switch mediated by cofactor methylation. *Science* **294**:2507–2511.
216. Yahata, T., M. P. de Caestecker, R. J. Lechleider, S. Andriole, A. B. Roberts, K. J. Isselbacher, and T. Shioda. 2000. The MSG1 non-DNA-binding transactivator binds to the p300/CBP coactivators, enhancing their functional link to the Smad transcription factors. *J. Biol. Chem.* **275**:8825–8834.
217. Yamamoto, H., F. Kihara-Negishi, T. Yamada, M. Suzuki, T. Nakano, and T. Oikawa. 2002. Interaction between the hematopoietic Ets transcription factor Spi-B and the coactivator CREB-binding protein associated with negative cross-talk with c-Myb. *Cell Growth Differ.* **13**:69–75.
218. Yamamoto, Y., U. N. Verma, S. Prajapati, Y. T. Kwak, and R. B. Gaynor. 2003. Histone H3 phosphorylation by IKK-alpha is critical for cytokine-induced gene expression. *Nature* **423**:655–659.
219. Yanagi, Y., Y. Masuhiro, M. Mori, J. Yanagisawa, and S. Kato. 2000. p300/CBP acts as a coactivator of the cone-rod homeobox transcription factor. *Biochem. Biophys. Res. Commun.* **269**:410–414.
220. Yang, C., L. H. Shapiro, M. Rivera, A. Kumar, and P. K. Brindle. 1998. A role for CREB binding protein and p300 transcriptional coactivators in Ets-1 transactivation functions. *Mol. Cell. Biol.* **18**:2218–2229.
221. Yang, H., C. H. Lin, G. Ma, M. O. Baffi, and M. G. Wathlet. 2003. Interferon regulatory factor-7 synergizes with other transcription factors through multiple interactions with p300/CBP coactivators. *J. Biol. Chem.* **278**:15495–15504.
222. Yang, T., R. J. Davis, and C. W. Chow. 2001. Requirement of two NFATc4 transactivation domains for CBP potentiation. *J. Biol. Chem.* **276**:39569–39576.
223. Yao, T. P., S. P. Oh, M. Fuchs, N. D. Zhou, L. E. Ch'ng, D. Newsome, R. T. Bronson, E. Li, D. M. Livingston, and R. Eckner. 1998. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* **93**:361–372.
224. Yi, M., G. X. Tong, B. Murry, and C. R. Mendelson. 2002. Role of CBP/p300 and SRC-1 in transcriptional regulation of the pulmonary surfactant protein-A (SP-A) gene by thyroid transcription factor-1 (TTF-1). *J. Biol. Chem.* **277**:2997–3005.
225. Yin, X., D. R. Warner, E. A. Roberts, M. M. Pisano, and R. M. Greene.

2005. Identification of novel CBP interacting proteins in embryonic orofacial tissue. *Biochem. Biophys. Res. Commun.* **329**:1010–1017.
226. **Yin, X., D. R. Warner, E. A. Roberts, M. M. Pisano, and R. M. Greene.** 2005. Novel interaction between nuclear co-activator CBP and the CDK5 activator binding protein-C53. *Int. J. Mol. Med.* **16**:251–256.
227. **Yin, X., D. R. Warner, E. A. Roberts, M. M. Pisano, and R. M. Greene.** 2005. Novel interaction between nuclear coactivator CBP and the protein inhibitor of activated Stat1 (PIAS1). *J. Interferon Cytokine Res.* **25**:321–327.
228. **Yu, C. T., M. H. Feng, H. M. Shih, and M. Z. Lai.** 2002. Increased p300 expression inhibits glucocorticoid receptor-T-cell receptor antagonism but does not affect thymocyte positive selection. *Mol. Cell. Biol.* **22**:4556–4566.
229. **Zhang, W., J. W. Nisbet, B. Albrecht, W. Ding, F. Kashanchi, J. T. Bartoe, and M. D. Lairmore.** 2001. Hum. T-lymphotropic virus type 1 p30(II) regulates gene transcription by binding CREB binding protein/p300. *J. Virol.* **75**:9885–9895.
230. **Zhang, Z., and C. T. Teng.** 2003. Phosphorylation of Kruppel-like factor 5 (KLF5/IKLF) at the CBP interaction region enhances its transactivation function. *Nucleic Acids Res.* **31**:2196–2208.
231. **Zhao, F., R. McCarrick-Walmsley, P. Akerblad, M. Sigvardsson, and T. Kadesch.** 2003. Inhibition of p300/CBP by early B-cell factor. *Mol. Cell. Biol.* **23**:3837–3846.
232. **Zhu, Q., G. Wani, M. A. Wani, and A. A. Wani.** 2001. Human homologue of yeast Rad23 protein A interacts with p300/cyclic AMP-responsive element binding (CREB)-binding protein to down-regulate transcriptional activity of p53. *Cancer Res.* **61**:64–70.