CBP/p300 double null cells reveal effect of coactivator level and diversity on CREB transactivation

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It remains uncertain how the DNA sequence of mammalian genes influences the transcriptional response to extracellular signals. Here, we show that the number of CREB-binding sites (CREs) affects whether the related histone acetyltransferases (HATs) CREB-binding protein (CBP) and p300 are required for endogenous gene transcription. Fibroblasts with both *CBP* and *p300* knocked-out had strongly attenuated histone H4 acetylation at CREB-target genes in response to cyclic-AMP, yet transcription was not uniformly inhibited. Interestingly, dependence on CBP/p300 was often different between reporter plasmids and endogenous genes. Transcription in the absence of CBP/p300 correlated with endogenous genes having more CREs, more bound CREB, and more CRTC2 (a non-HAT coactivator of CREB). Indeed, CRTC2 rescued cAMP-inducible expression for certain genes in CBP/p300 null cells and contributed to the CBP/p300-independent expression of other targets. Thus, endogenous genes with a greater local concentration and diversity of coactivators tend to have more resilient-inducible expression. This model suggests how gene expression patterns could be tuned by altering coactivator availability rather than by changing signal input or transcription factor levels.

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Introduction

One implicit view of gene regulation is that a model coactivation mechanism for a particular DNA-binding transcription factor is essential for the expression of all its target genes. Another commonly held notion is that histone hyperacetylation is required for gene expression, as it strongly correlates with active transcription. These ideas underlie reductionist

approaches to understand transcription factor function, as well as global methods to map the occurrence of transcriptional regulators and histone modifications in the genome. Complicating these notions is evidence based on synthetic plasmid reporter genes and overexpressed coactivators (Koutroubas *et al*, 2008), as well as endogenous factors and genes (Kasper *et al*, 2005; Kasper and Brindle, 2006; Xu *et al*, 2007; Ramirez-Carrozzi *et al*, 2009), that a particular signal-responsive coactivation mechanism is not required at all target genes in mammals.

The critical characteristics of individual endogenous genes that determine their coactivator requirements have been unclear for cAMP and hypoxia signalling (Kasper *et al*, 2005; Kasper and Brindle, 2006; Xu *et al*, 2007). But recent insight has been gained for Toll-like receptor (TLR)-responsive genes in macrophages, a pathway that does not involve cAMP (Hargreaves *et al*, 2009; Ramirez-Carrozzi *et al*, 2009). In one model, primary TLR-responsive genes are maintained in a permissive state by constitutive transcription factors such as SP1 that binds GC-rich motifs, and regulated by inducible factors that recruit coactivators that promote histone H4 acetylation (Hargreaves *et al*, 2009). In a related model, TLR-responsive promoters that are rich in CpG dinucleotides assemble unstable nucleosomes, which reduces their dependence on SWI/SNF nucleosome remodelling coactivators (Ramirez-Carrozzi *et al*, 2009). Other DNA characteristics besides CpG and GC richness must exist, however, as these models are insufficient to fully explain the requirements that individual genes have for different coactivators (Ramirez-Carrozzi *et al*, 2009).

The coactivators CREB-binding protein (CBP) and p300 comprise the unique KAT3 family of histone acetyltransferases (HATs) and their acetyltransferase domains share little sequence similarity with other HATs (Marmorstein, 2001), which probably contributes to why CBP and p300 are essential for normal development (Yao *et al*, 1998; Goodman and Smolik, 2000; Kung *et al*, 2000; Tanaka *et al*, 2000; Vo and Goodman, 2001). RNAi knockdown of dCBP in *Drosophila* Kc cells (Smolik and Jones, 2007), and CBP and p300 in immortal HeLa cells (Stauffer *et al*, 2007), results in death because of chromosome shredding and mitotic catastrophe. Likewise, mouse lymphocytes lacking both CBP and p300 cannot be generated *in vivo* (Kasper *et al*, 2006; Xu *et al*, 2006; Fukuyama *et al*, 2009), suggesting that most cells require some CBP or p300. Here, we describe primary mouse embryonic fibroblasts (MEFs) that are made deficient for both CBP and p300 through the use of Cre/LoxP conditional knockout alleles (Kang-Decker *et al*, 2004; Kasper *et al*, 2006), providing a first opportunity to assess transcription and histone acetylation after stably inactivating a major mammalian HAT family.

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Results

Stable inactivation of CBP and p300 in MEFs produces a non-uniform effect on cAMP-responsive endogenous gene transcription

To generate MEFs lacking CBP and p300, we treated primary $CBP^{flox/flox};p300^{flox/flox}$ MEFs with adenovirus that expresses Cre recombinase, inactivating both conditional alleles (some

MEF isolates were also engineered to carry a recombination-dependent GFP marker gene; Figure 1A). Western blot (Figure 1B; Supplementary Figure S1) indirect immunofluorescence (IF) (Figure 1C and D; Supplementary Figure S2) and semiquantitative genomic PCR and quantitative RT-PCR (qRT-PCR) (Supplementary Figure S3) showed that these double knockout (dKO) MEFs recombine all four conditional alleles efficiently and rapidly lose CBP and p300 protein.

Figure 1 *In vitro* deletion of CBP and p300 conditional alleles produces CBP/p300 double null MEFs. (A) Schematic of CBP/p300 double null (dKO) MEF derivation. LoxP sites in CBP^{flox} and $p300^{flox}$ alleles flank an exon in the KIX domain (~aa 600) and result in a frame shift mutation after recombination. eGFP expression from a recombination-dependent transgene shown. (B) Western blot of control ($CBP^{flox/flox};p300^{flox/flox}$) and FACS purified GFP⁺ dKO MEFs. Antibodies to CBP and p300 protein were raised against both N- and C-terminal peptides. A non-specific band detected by the p300 antiserum was used as a loading control. Extracts were made 4 days post-Ad-Cre infection, immediately after FACS purification. (C) Indirect immunofluorescence in dKO MEFs showing loss of CBP/p300 proteins. MEFs were stained with a cocktail of antibodies against the N- and C-termini of CBP and p300. Closed yellow arrow marks example of deleted dKO cells. Open white arrows mark examples of non-deleted cells for internal controls. Closed white arrows mark cells null for only CBP or p300 showing partial loss of signal. DAPI stained nuclei. (D) CBP and p300 protein turnover in MEFs. Indirect immunofluorescence of CBP and p300 using antibodies against the C-termini of CBP and p300 in dKO MEFs 1–4 days after Ad-Cre infection. Wild-type cells treated with Ad-Cre are provided for IF signal comparison. DAPI stained nuclei. (E) Growth curve of WT and dKO MEFs. Percent of GFP⁺ cells expressing Cre recombinase after Ad-Cre infection was assessed by flow cytometry and applied to cell count. Two independent MEF isolates of each genotype used. (F) dKO MEFs in culture 16 days after floxed gene deletion. Closed arrows indicate GFP⁺ cells that are null for CBP and p300.

Figure 2 cAMP-inducible genes are unequally affected in CBP/p300 null MEFs. (A) Classic model of CREB-dependent transcription. Upon cAMP or calcium signalling, constitutively DNA-bound CREB is phosphorylated on serine 133 and CRTC translocates to the nucleus allowing the binding of CBP/p300 and CRTC to CREB and transcription of target genes. TFIID is constitutively bound to CREB. (B, C) Affymetrix expression analysis of wild type and CBP/p300 dKO MEFs treated 90 min with 10 μ M forskolin + 100 μ M IBMX (FI) (mean of $N = 2$ independent MEF isolates). (B) Operationally defined cAMP-induced genes; 347 cAMP-responsive probe sets defined by all present call in wild-type MEFs and an FI/EtOH expression signal ratio ≥ 2.5 in both wild-type MEF isolates. (C) Operationally defined control genes; 204 non-cAMP-responsive probe sets defined by all present call in wild-type FI-treated samples and an FI/EtOH signal ratio of $\pm 2\%$ (non-induced) in both wild-type MEF samples. (D–H) qRT-PCR of wild type (WT) and dKO MEFs treated for 90 min with EtOH or FI. Relative gene expression values for each gene were normalized to *Pgk1* mRNA expression and the lowest value for each gene was set to one. (D–G) Mean $\pm$ s.e.m., $N = 4$. (H) Heat map colours represent the z-score normalization of the mean expression values of each gene individually and are not strictly comparable between genes. Red indicates high expression values relative to blue. $N = 2$ –4.

Although proliferation was dramatically reduced, dKO MEFs remained viable in culture for > 2 weeks, allowing them to be used for transcriptional studies (Figure 1E and F).

CBP and p300 are prototypical signal-responsive coactivators recruited to DNA-bound CREB after its phosphorylation in response to cAMP, an event that is considered essential

for the transcription of CREB-target genes (Figure 2A) (Johannessen *et al*, 2004; Carlezon *et al*, 2005; Hong *et al*, 2005; Hyman *et al*, 2006; Cohen and Greenberg, 2008; Flavell and Greenberg, 2008; Sands and Palmer, 2008). Indeed, median cAMP-inducible gene expression was lower in dKO MEFs compared with wild type (WT) as shown by Affymetrix

microarray analysis of 193 genes (347 probe sets) that were induced at least 2.5-fold in WT MEFs by 90 min treatment with cAMP agonists 10 μ M forskolin plus 100 μ M IBMX (FI) (Figure 2B). In contrast to the conventional model, however, individual cAMP-inducible genes showed a wide range of dependence on CBP/p300, including some that were unaffected, or even expressed better, in the absence of CBP/p300. As a control, microarray analysis showed that non-cAMP-responsive genes were generally less affected by loss of CBP and p300 (Figure 2C) and that many housekeeping genes, including *Pgk1* (which we used for normalization), were expressed normally in dKO MEFs (Supplementary Figure S4).

Assessment of individual cAMP-inducible genes by qRT-PCR verified that the expression of some genes, such as *Areg* and *Rgs2*, was reduced in dKO MEFs (Figure 2D and E), whereas other genes were unaffected (e.g. *Crem* Icer; Figure 2F) or displayed higher expression (e.g. *Nr4a3*; Figure 2G). Additional qRT-PCR analysis of 35 cAMP-inducible genes confirmed that their dependence on CBP/p300 for expression represented a continuum from very CBP/p300 dependent to those more highly expressed in the absence of CBP/p300 (Figure 2H).

cAMP-induced genes that are expressed well in dKO MEFs are dependent on CREB

Differences in the requirement of CBP/p300 for gene expression might be explained by reliance on non-CREB transcription factors. We next confirmed that cAMP-responsive genes in MEFs are dependent on CREB by using a retrovirus expressing the dominant-negative A-CREB, which specifically inhibits the DNA-binding activity of all three CREB family members (Ahn *et al*, 1998). We found that the cAMP-dependent expression of 34 of the 35 genes listed in Figure 2H was markedly reduced by A-CREB (*Bhlhb2* was only moderately affected by A-CREB) (Figure 3A). In addition, the increased expression seen in a subset of cAMP-inducible genes in dKO MEFs was also blocked by A-CREB, indicating that this phenomenon is CREB dependent as well.

We next tested whether the CBP/p300-independent genes *Nr4a1*, *Nr4a3*, *Fosb*, and *Junb* were also independent of the main CBP-p300 interaction surface of CREB. For this, we used another CREB dominant negative (Ser133Ala) that alters a phosphorylation site necessary for interaction with the KIX domain of CBP/p300. CREB S133A attenuates but does not block CBP/p300 recruitment (Xu *et al*, 2007). As might be expected, CREB S133A had no effect on target gene expression in dKO MEFs (Supplementary Figure S5). In contrast, it partially repressed the expression of all but *Junb* in WT MEFs. This result suggests that several of the tested CREB targets are at least partly CBP/p300 dependent in WT MEFs, but are activated through another mechanism in the absence of CBP/p300 (see Discussion). Alternatively, the CREB phospho-Ser133 interaction with KIX may potentiate transcription, whereas other domains of CBP/p300 act to repress it.

To test the latter possibility that in certain contexts either CBP or p300 acts as a repressor, we looked at gene expression in MEFs null for either CBP or p300. Here, we found modest inhibition, or little change, in the expression of *Fos*, *Fosb*, *Junb*, *Nr4a1*, *Nr4a2*, and *Nr4a3*, genes that were more highly expressed in dKO MEFs (Supplementary Figure S6). This indicates that neither CBP nor p300 individually behave as a strong repressor of these genes.

To help rule out non-transcriptional effects, primary transcript analysis of a subset of genes representing all CBP/p300 dependence categories was performed and showed that the levels of unspliced transcripts mirrored that seen for spliced mRNA (Supplementary Figures S7A–E; compare with Figure 2D–G). Thus, the great majority of cAMP-responsive genes in WT and dKO MEFs are transcriptionally regulated by CREB.

cAMP-responsive genes that recruit CBP/p300 efficiently in WT MEFs tend to be less transcriptionally inhibited in dKO MEFs

The non-uniform transcriptional response to cAMP in dKO MEFs suggested that CBP/p300-independent genes might not recruit CBP/p300 in WT MEFs, which would be inconsistent with the standard model for CREB function. Quantitative PCR chromatin immunoprecipitation (ChIP) assays showed that CBP and p300 were recruited to CREB-target genes in response to cAMP in WT MEFs, although to varying degrees, depending on the gene (Figure 3B; Supplementary Figures S7F–I). Specificity of the CBP/p300 ChIP signal was shown by its loss in dKO MEFs (Supplementary Figures S7F–I). Interestingly, the genes *Areg* and *Rgs2* that showed dependence on CBP/p300 for expression (Figure 2D and E) had weaker CBP/p300 ChIP signals (Figure 3B; Supplementary Figures S7F and G), than did *Crem* Icer and *Nr4a3* (Figure 3B; Supplementary Figures S7H and I), which were well expressed in dKO MEFs (Figure 2F and G). Paradoxically, this result implies that the amount of CBP/p300 recruited to a gene in response to cAMP cannot definitively predict its transcriptional requirement for these coactivators.

Histone acetylation in response to cAMP is highly dependent on CBP/p300

Acetylation of histone N-terminal tail lysines correlates strongly with active transcription (Li *et al*, 2007; Rando and Chang, 2009), and acetylated H4 in particular is important for signal-responsive gene expression by binding bromodomain protein-4, which recruits transcriptional elongation factors (Hargreaves *et al*, 2009). We therefore expected that non-CBP/p300 HATs would acetylate H4 in response to cAMP at genes that were well expressed in the absence of CBP/p300. We performed ChIP assays of 13 cAMP-inducible genes that span the continuum of CBP/p300-transcriptional dependence, using WT and dKO MEFs and antibodies against acetylated H4 lysines K5, K8, K12, and K16. We found that inducible acetylation of these residues was measurable at the CRE-containing regions of most but not all promoters and was CBP/p300 dependent (Figure 3C–F). Querying the region located ~500 bases downstream of the transcriptional start site (TSS) of cAMP-inducible genes gave similar results (data not shown). Surprisingly, the genes with the highest levels of cAMP-inducible H4 acetylation were among those that least required CBP/p300 for their expression (Figure 3C–F). In contrast, most CBP/p300-dependent genes showed little or no cAMP-responsive acetylation of the H4 lysines tested (Figure 3C–F).

The inducible acetylation of histone H3 K9, K14, and K18 was also examined by ChIP at cAMP-responsive promoters and was found to correlate with H4 acetylation in WT cells (compare Figure 3C–F with Supplementary Figure S8). Unlike histone H4, cAMP-inducible acetylation of histone H3 K9 and K14 was only partially dependent on CBP/p300

(Supplementary Figure S8A). In contrast, inducible acetylation of H3 K18 was extremely dependent on CBP and p300 at all promoters tested by ChIP; the same was true for basal K18 acetylation in bulk H3 (Horwitz *et al*, 2008) (Supplementary Figures S8B and C).

As with CBP/p300 recruitment, these results show that there is generally an inverse correlation between the amount of cAMP-inducible H3 and H4 acetylation and transcriptional dependence on CBP/p300. These findings also indicate that it is unlikely that there is a redundant HAT that inducibly acetylates H4 and H3K18 in the absence of CBP/p300. This suggests that inducible H4 and H3K18 acetylation is incidental and is not strictly required for the cAMP-responsive transcription of at least some genes.

CBP with HAT domain mutations can rescue certain CREB-target genes

The poor inducible histone acetylation seen at some CBP/p300-dependent genes suggested that they require a coactivator function of CBP/p300 other than HAT activity for their cAMP-dependent expression. To test this, we used retroviruses to express either WT or HAT mutant HA epitope-tagged mouse CBP (CBP-HA) in dKO MEFs and looked for rescue of CBP/p300-dependent gene expression. We used two HAT mutants, W1503A/Y1504S (WY/AS) that strongly affect binding to acetyl-coenzyme A and catalysis, and F1390A/P1391A/Y1392A (FPY/AAA) that inhibits histone acetylation, but not autoacetylation (Bordoli *et al*, 2001; Liu *et al*, 2008a; Bowers *et al*, 2010). These CBP-HA proteins were expressed at levels comparable with endogenous CBP (Supplementary Figure S9). WT CBP-HA rescued cAMP-responsive transcription in dKO MEFs, and both HAT mutants were able to partially rescue expression of *Areg* (Figure 3G). The FPY/AAA, but not WY/AS, mutant was able to rescue *Rgs2* expression (Figure 3H), suggesting that this gene may have particular acetylation requirements for proteins besides histones. Neither CBP HAT mutant could rescue the CBP/p300-dependent genes *Kcne4* and *Nppc*, however (Figure 3I and J). These results suggest that a subset of CREB-target genes use non-HAT coactivator functions of CBP/p300 for their cAMP-inducible expression, possibly explaining why inducible H4 acetylation correlates imperfectly with transcription.

The quantity of CREB-binding sites (CREs), bound CREB, and recruited coactivators at endogenous genes correlates with increased transcriptional resilience in response to cAMP in dKO cells

The limited correlation between the requirement of CBP/p300 for transcription and the level of CBP/p300 recruitment

(and CBP/p300-dependent histone acetylation) led us to search for additional mechanisms to explain CBP/p300 dependence for cAMP-inducible genes. We noticed that several CBP/p300-independent genes had more than one CRE close to the TSS (not shown), implying that multiple CREs confer resistance to the loss of CBP/p300. We tested this hypothesis using synthetic luciferase reporters driven by two, four, or six copies of a canonical CRE, as well as reporters driven by –500 to +60 bp promoter fragments from four different CREB-target genes. We found that there was no correlation between the number of CREs and CBP/p300 dependence; however, the activities of the promoter fragments poorly matched that of the cognate endogenous genes (Supplementary Figure S10). This suggested that the non-uniform dependence of CREB-target promoters on CBP/p300 is dictated by DNA sequences that are missing in the plasmids or requires an endogenous gene context.

We, therefore, focused on endogenous loci by determining the number of CREs (TGACG) in the 2 kb region centered on the TSS of 172 genes having clearly annotated promoters that are induced at least 2.5-fold by cAMP in WT MEFs based on Affymetrix microarrays. We found that the number of CREs correlated significantly with CBP/p300 independence as measured by the $\log_2$ ratio of dKO to WT cAMP-induced expression signals, in which negative numbers denote CBP/p300 dependence (Figure 4A; Pearson's correlation, $P = 8.8 \times 10^{-10}$, $N = 172$, $r = 0.44$, 95% CI of $r = 0.32$ to 0.56 , where $r = 1$ denotes a perfect positive correlation, $r = 0$ is no correlation and $r = -1$ is a perfect inverse correlation). The number of CpG dinucleotides (corrected for TGACG CREs; the bold, underlined CG in the TGACG sequence is to indicate that every canonical CRE contains a CG dinucleotide, hence the need to correct for the number of CREs) in the region 200 bp upstream of the TSS (–200 to –1) was also determined for comparison with a described CpG-dependent mechanism for TLR-responsive transcription (Ramirez-Carrozzi *et al*, 2009), but it correlated less well with CBP/p300 independence (Supplementary Figure S11A; $P = 0.0015$, $r = 0.24$, 95% CI = 0.09 to 0.38 , $N = 172$). This suggests that CpG-associated mechanisms contribute less to CREB-target gene regulation than those involving CREs. Indeed, the total variation in gene expression explained by CRE motifs was about 20%, whereas for CG motifs it was only 2%. In addition, as the effect of CG motifs becomes almost negligible after adjusting for the presence of CREs, it suggests that there is no significant synergistic effect between the two motifs (regression analysis for multiple variables). Another described TLR-responsive mechanism involving GC-rich SP1 sites (Hargreaves *et al*, 2009) did not appear to be important

Figure 3 cAMP-inducible histone H4 lysine acetylation at CREB-dependent promoters is CBP/p300 dependent, but not strictly required for gene expression. (A) Wild-type (WT) and dKO MEFs infected with GFP (control) or A-CREB (dominant negative that inhibits DNA binding for CREB family members) expressing retrovirus treated for 90 min with ethanol vehicle (EtOH) or 10 μ M forskolin $\pm$ 100 μ M IBMX (FI). Relative gene expression values for each gene were normalized to *Pgk1* mRNA expression and the lowest value for each gene was set to one. Heat map colours represent the z-score normalization of the mean expression values of each gene individually and are not strictly comparable between genes. $N = 2$. (B–F) ChIP at the CREs of 13 CREB-target gene promoters in WT MEFs treated for 30 min with EtOH or FI. Genes are arranged from most to least CBP/p300 dependent for expression based on data in Figure 1K. (B) ChIP signal for normal rabbit serum (NRS) or CBP/p300 was normalized to chromatin input signal. Heat map shows the mean $\log_2$ normalized ChIP signals. $N = 2$. (C–F) Histone H4 acetylated K5 (C), K8 (D), K12 (E) and K16 (F) ChIP was normalized to chromatin input signal, then to histone H4 ChIP signal from a non-discriminating H4 antibody. Heat maps show the mean normalized ChIP signals; $N = 2$. In all heat maps (A–F), red indicates high values relative to blue. (G–J) WT and dKO MEFs, as well as dKO MEFs infected with CBP-HA retroviruses (WT, HAT mutant WY/AS or HAT mutant FPY/AAA) treated for 90 min with EtOH or FI. Relative expression values for each gene were normalized to *Pgk1* mRNA expression and the lowest value for each gene was set to one. Mean $\pm$ s.e.m., $N = 2$.

Figure 4 Increased transcription factor and coactivator recruitment correlates broadly with CBP/p300-independent gene expression. (A) Analysis of the promoters (−1 to +1 kb from TSS) of 172 genes with clearly annotated TSS induced at least 2.5-fold by 90 min 10 μ M forskolin + 100 μ M IBMX (FI) treatment in wild-type (WT) MEFs, comparing relative dependence on CBP/p300 ($\log_2$ of ratio of WT FI signal to CBP/p300 dKO FI signal) to number of CREs (TGACG). Expression data for unambiguously annotated genes in the Affymetrix experiment described in Figure 1 were used. (B) Pearson's correlation of the CREB ChIP signal in WT MEFs (treated 30 min with FI) at the CREs in 24 CREB-target gene promoters against the number of CREs in the region 600 base pairs centered on the primers used for ChIP analysis. (C–E) Pearson's correlation of CREB versus CBP/p300 (C), CRT2 (D), and CBP/p300 versus CRT2 (E) ChIP signals in WT MEFs treated 30 min with FI at the CREs in 24 CREB-target gene promoters. (F) Model showing that increased recruitment of multiple coactivator types at promoters with more bound transcription factor enhances transcriptional resilience. (G–I) Comparison of CREB (G), CBP/p300 (H), and CRT2 (I) ChIP signals in WT MEFs treated 30 min with FI at the CREs in 24 CREB-target gene promoters with the relative dependence of these genes on CBP/p300 for their expression (expressed as the $\log_2$ of ratio of WT FI signal to CBP/p300 dKO FI signal). All ChIP signals normalized to chromatin input signal (B–E and G–I). Pearson's correlation coefficients (r) and P -values indicated (where $r=0$ indicates no correlation and $r=1$ is a perfect positive correlation) (A–E and G–I).

for cAMP-responsive genes as there was neither a significant correlation between the number of SP1 sites (−1 kb to +1 kb from TSS) and CBP/p300 independence (Supplementary Figure S11B; $P = 0.14$, $N = 172$), nor did GC nucleotide content in the −200 to −1 region correlate significantly (Supplementary Figure S11C; $P = 0.054$, $N = 172$). Together, these findings indicate that the cAMP-induced expression of genes with more CREs tends to be more resistant to the loss of CBP/p300, whereas CpG-associated mechanisms may contribute, but less so, to CBP/p300 independence.

‘CREB-rich’ promoters are ‘coactivator-rich’ and are apt to be more resistant to the loss of CBP/p300

The highly significant (albeit broad) correlation between the number of CREs and CBP/p300 independence could reflect differences in the way CRE-containing promoters recruit transcriptional co-factors or in how such co-factors function. We tested the first of these hypotheses that promoters with more CREs would recruit CREB (and thereby coactivators) more efficiently than promoters with fewer CREs. We performed ChIP assays using antibodies against CREB, CBP/p300, and the non-HAT protein CRT2 (TORC2, a cAMP-responsive CREB coactivator unrelated to CBP/p300; Konkright *et al*, 2003; Iourgenko *et al*, 2003; Bittinger *et al*, 2004; Sreaton *et al*, 2004; Koo *et al*, 2005; Dentin *et al*, 2007, 2008; Liu *et al*, 2008b; Wang *et al*, 2009) in FI-treated WT MEFs ($N = 3$) and analysed the levels of these proteins using primers located near the CREs of 24 cAMP-inducible gene promoters. We found that the number of CREs in the 600 bp promoter region centered on each primer set correlated well with the CREB ChIP signal (Figure 4B; Pearson’s $r = 0.73$, $P < 0.0001$, $N = 24$). In addition, as we anticipated, the CREB ChIP signal at the CREs in these 24 promoters correlated very strongly with both CBP/p300 (Figure 4C; Pearson’s $r = 0.84$, $P < 0.0001$) and CRT2 ChIP signals (Figure 4D; Pearson’s $r = 0.92$, $P < 0.0001$). These results indicate that CRE-rich promoters have more bound CREB, which recruits CBP/p300 and CRT2 more efficiently. Moreover, the strong correlations suggest that both CBP/p300 and CRT2 are recruited by CREB, rather than by some other transcription factor.

The near perfect correlation between the CBP/p300 and CRT2 recruitment levels (Figure 4E; Pearson’s $r = 0.95$, $P < 0.0001$) led us to model the existence of ‘coactivator-rich’ and ‘coactivator-poor’ promoters (Figure 4F). In this model, a given promoter recruits a transcription factor poorly or strongly based on the quality and number of binding sites; coactivators of different types are recruited correspondingly (i.e. more transcription factor corresponds to more coactivator recruitment). Our model predicts that promoters with low levels of CREB and coactivator recruitment will be more dependent on each individual type of coactivator (and their particular biochemical function that leads to transactivation, e.g. histone acetylation) for gene expression. On the other hand, promoters that recruit coactivators strongly will benefit from buffering between coactivator transactivating mechanisms and be less dependent on any particular coactivator class. Indeed, comparison of the ChIP signals for CREB, CBP/p300, and CRT2 with the CBP/p300 dependence of expression for these 24 genes all gave significant correlations (Figure 4G–I; Pearson’s correlation, $N = 24$, CREB ChIP $r = 0.52$, $P = 0.0087$; CBP/p300 ChIP $r = 0.54$, $P = 0.0065$; CRT2 ChIP $r = 0.48$, $P = 0.0171$). This model helps explain

the paradox of why the genes that recruit the most CBP and p300 in response to cAMP tend to need them the least for their inducible expression; in these cases, abundant CBP/p300 recruitment is a proxy for abundant coactivator recruitment in general (in this instance, CRT2 and perhaps others).

CRTC2 overexpression increases its recruitment to CREB targets and rescues the expression of certain genes in the absence of CBP/p300

Implicit in our coactivator recruitment model are two hypotheses: (1) different classes of coactivators can provide redundancy for each other in certain promoter contexts and (2) rescue of gene expression in dKO cells could occur if the local concentration of a different coactivator could be increased. To test these hypotheses, we overexpressed CRT2 in WT and dKO MEFs and examined CBP/p300-dependent genes for expression and CRT2 recruitment. ChIP showed that CRT2 overexpression in both WT and dKO MEFs increased the recruitment of CRT2 to CBP/p300-dependent promoters in a cAMP-dependent manner (Figure 5A–D; $N = 2$). Interestingly, increased CRT2 recruitment occurred at CBP/p300-dependent genes that were rescued by CRT2 (*Areg*, *Has1*, Figure 5A, B, E and F; $N = 2–3$), as well as at genes that were not rescued by CRT2 overexpression (*1810011O10Rik*, *Dio2*; Figure 5C, D, G and H; $N = 2–3$). Another six CBP/p300-dependent genes also showed partial to complete rescue by CRT2 (Supplementary Figure S12), although most genes examined did not (data not shown). This data indicates that distinct types of coactivators (e.g. HATs versus non-HATs) can provide redundancy in certain gene contexts if there is a sufficiently high local coactivator concentration. Consistent with our model (Figure 4F), coactivator recruitment could be driven both by a greater local concentration of DNA-bound transcription factor (i.e. CREB), and by increasing the total amount of coactivator in the cell.

This result suggested that CBP/p300-independent gene expression might in part be due to increased expression of endogenous *Crtc1* and *Crtc2* in dKO MEFs. Indeed, qRT-PCR showed about a three-fold increase of *Crtc1* and *Crtc2* mRNA in dKO MEFs compared with WT (Supplementary Figure S13). CRT2 protein level was similarly increased in dKO MEFs (Figure 6J). Endogenous CRT2 recruitment as measured by ChIP also tended to increase somewhat at CBP/p300-independent genes (e.g. *Fosb*, *Nr4a3*) in dKO MEFs, an effect that was not observed for genes such as *Areg* and *1810011O10Rik* that are highly dependent on CBP/p300 (Figure 5; Supplementary Figure S14). These results agree with the notion that promoters for CBP/p300-independent genes such as *Nr4a3* compete more effectively for limiting amounts of coactivators, which helps buffer their expression.

CRTC knockdown reduces the CBP/p300-independent expression of some CREB-target genes in dKO MEFs

The data and model (Figure 4F) predicts that the CBP/p300-independent expression of some cAMP-inducible genes in dKO MEFs is due to the presence of another CREB-recruited coactivator such as CRT2 at these promoters. To test this, we knocked down CRT2 by transient transfection with *Crtc1* and *Crtc2* siRNAs and observed the effect on the expression of eight CBP/p300-independent genes (Figure 6A–H). Knockdown of CRT2 protein and/or mRNA was verified (Figures 6J–L) and was found to reduce

Figure 5 CRTC2 overexpression rescues CBP/p300-dependent expression of some CREB-target genes. (A–D) ChIP of WT and dKO MEFs infected with control (GFP) or CRTC2 retrovirus using normal rabbit serum control (NRS), a mix of CBP and p300 antibodies or CRTC2 antibody, and primers at the CRE in CREB-target gene promoters. MEFs for ChIP were treated 30 min with 10 μ M forskolin + 100 μ M IBMX (FI) or ethanol vehicle (EtOH). ChIP signal is normalized to chromatin input signal. Mean $\pm$ s.e.m., $N=2$. (E–H) Gene expression measured by qRT-PCR of WT and dKO MEFs infected with control (GFP) or CRTC2 retrovirus and treated 90 min with EtOH or FI. Relative gene expression values for each gene were normalized to *Pgk1* mRNA expression and the lowest value for each gene was set to one. Mean $\pm$ s.e.m., $N=2-3$.

the average expression of the CBP/p300-independent genes tested in WT MEFs to 87% of control siRNA-treated cells (95% CI=98 to 75%, $N=8$ genes), whereas in dKO MEFs, it was reduced to 53% (95% CI=62 to 45%) (Figure 6I; $P<0.0001$). These results were confirmed using a retrovirus expressing an shRNA targeting a different part of the *Crtc2* mRNA (Supplementary Figure S15). The *Crtc2* shRNA reduced

CREB-target gene expression in dKO MEFs to 58% of the control shRNA (95% CI=71 to 45%, $N=8$ genes), whereas the *Crtc2* shRNA had little effect in WT MEFs, as gene expression was 97% of that seen with the control shRNA (95% CI=107 to 88%, $N=8$) (Supplementary Figure S15; $P<0.0001$). These findings agree with our model, which predicts that the presence of CBP/p300 in WT cells should

Figure 6 CRTC knockdown reduces CBP/p300-independent CREB-target gene expression in dKO MEFs. (**A–H**) Gene expression measured by qRT–PCR of WT and dKO MEFs 72 h after transfection with non-specific scramble siRNA or a mix of siRNAs targeting CRTC1 and CRTC2. In the presence of serum (which tends to dampen the fold response to cAMP), MEFs were treated for 90 min with 10 μ M forskolin + 100 μ M IBMX (FI) or ethanol vehicle (EtOH) before RNA harvest. Mean $\pm$ s.e.m., $N = 7$. (**I**) Ratio of the average FI-induced gene expression of the eight genes in (**A–H**) in WT or dKO MEFs treated with Crtc1 plus Crtc2 siRNA relative to scramble siRNA. Mean $\pm$ 95% confidence interval. (**J**) IP western of CRTC2 in WT and dKO MEF whole cell extracts 3 days after transient transfection with scramble siRNA or siRNAs targeting CRTC1 and CRTC2. Western blot for actin in same extracts used for IP western in upper panel. Note increased CRTC2 protein in dKO MEFs compared with WT MEFs. (**K**, **L**) CRTC1 and CRTC2 gene expression measured by qRT–PCR of mRNA from WT and dKO MEFs transfected with a scramble siRNA or a mixture of siRNAs against CRTC1 and CRTC2 (CRTC siRNA) and treated 90 min with FI or ethanol vehicle (EtOH) ($N = 7$, mean $\pm$ s.e.m.).

buffer certain genes from lower amounts of CRTC. Together, this suggests that CRTC coactivators are at least partly responsible for the CBP/p300-independent CREB-target gene expression seen in dKO MEFs.

Discussion

This study provides a first glimpse at endogenous gene expression and histone acetylation in primary cells that are

stably deficient for one of the major mammalian HAT families (CBP and p300 having at least 400 described interacting protein partners; Bedford *et al*, 2010). It was unexpected that many CBP/p300-independent genes would robustly recruit CBP/p300 in WT MEFs, but not retain inducible histone acetylation through redundant HATs in dKO MEFs. This suggests that at least some CBP/p300-dependent histone acetylation marks are bystander events that are not critical for transcription.

In a previous study using MEFs bearing point mutations in the KIX domain of CBP and p300 that disrupt the CREB interaction surface (Xu *et al*, 2007), we attributed the non-uniform dependence on the KIX domain for cAMP-inducible gene expression to the residual recruitment of mutant CBP/p300 through CRTC. We were, therefore, surprised in this study to find that not all CREB-target genes were profoundly inhibited by the absence of CBP/p300. In some but not all cases, there is an interesting lack of concordance in the expression of CREB targets in KIX mutant MEFs compared with dKO MEFs. For example, *Nr4a3*, which is partially sensitive to the KIX and CREB Ser133Ala mutations (Xu *et al*, 2007), is actually expressed better in dKO MEFs than in WT cells. The reason for this distinction between different types of CBP/p300 mutant cells is not entirely clear, but the simplest hypothesis invokes a compensatory mechanism that is more active in dKO cells. *A priori*, a non-CBP/p300 HAT would seem to be a probable candidate, but our data suggests that this is not the case, as acetylation of certain histone residues was not compensated in the dKO MEFs. Instead, non-HAT coactivators may be involved, and evidence presented here and in our previous study of KIX mutant MEFs (Xu *et al*, 2007) implicates CRTC, at least in part. For example, *Nr4a3* expression in KIX mutant MEFs could be rescued by CRTC2 overexpression (Xu *et al*, 2007) and the increased expression of *Nr4a3* in dKO MEFs was inhibited by CRTC knockdown. Consistent with the idea that endogenous CRTC upregulation is providing some compensation in dKO MEFs, the expression of *Crtc1* and *Crtc2* was increased, as was CRTC2 recruitment to 'coactivator-rich' genes such as *Nr4a3*. CRTC2 recruitment did not increase at genes such as *Areg* in dKO MEFs unless CRTC2 was highly overexpressed, which agrees with the 'coactivator-poor' and 'coactivator-rich' model.

The DNA sequences or other gene characteristics (e.g. location in the nucleus) that determine the extent to which a CREB target requires CBP/p300 are not entirely apparent, but our data indicates that the number of CREs, and to a lesser degree CpG motifs, appears to contribute. The amount of CREB bound at a promoter correlated strongly with more efficient recruitment of coactivators, which we propose buffers the mechanisms that can activate a particular gene. For example, CREB has two signal-responsive activation domains (that bind CBP/p300 and CRTC, respectively), and constitutive glutamine-rich domains similar to SP1 that interact with TFIID (Figure 2A) (Ferreri *et al*, 1994), thus the amount of CREB at a promoter may singly influence coactivator dependence much as SP1 and NF- κ B do together at TLR-responsive genes (Hargreaves *et al*, 2009). Accordingly, 'CREB-rich, coactivator-rich' promoters tend to be more resistant to the loss of CBP/p300. Whether CRE number influences the number of CREB and coactivator molecules recruited to individual promoter loci, or affects the stochastic probability that a promoter will recruit CREB and coactivators is an

interesting question, but one that cannot be distinguished using bulk cell culture methods.

Our results also refine the popular view that the local occurrence of a coactivator or histone modification equates with their functional importance at a locus. Instead, our findings suggest that CBP/p300, and concomitant histone acetylation, tends to be more abundant at loci in which they are least required for gene expression. Broadly, this implies that functional importance correlates better with the low to moderate local abundance of a particular coactivator or histone modification. This would have implications for interpreting genome-wide mapping studies (e.g. ChIP-seq). Finally, it is interesting to speculate that the different requirements genes have for coactivators may provide a physiological mechanism for tuning the pattern of signal-responsive gene expression by modulating the availability of coactivators.

Materials and methods

Cell culture, plasmids and antibodies

MEFs were generated from E14.5 embryos; experiments were performed in primary MEFs between passages 3 and 6 and growth rates and morphology were comparable for WT and floxed allele cells before recombination. To recombine floxed alleles, mutant and control MEFs were treated with adenovirus expressing Cre recombinase at an MOI of 100. All experiments were performed 4 days after recombination (see Figure 1D) and deletion efficiency was monitored by PCR. Cre reporter transgenes that express eGFP (Novak *et al*, 2000) or eYFP (Srinivas *et al*, 2001) after Cre-mediated recombination were bred into the mice and used for MEFs to allow for FACS purification of dKO cells, although this was found to be unnecessary for most experiments; unsorted MEFs were, therefore, used except where noted. MEFs were serum starved overnight before treatment with cAMP agonists, except for siRNA experiments (note that serum tended to blunt the fold response to cAMP). Typically, multiple independent primary MEF isolates were used for each assay to account for biological variation. The cDNA for HA epitope-tagged mouse CBP was cloned out of the vector pRC/RSV-mCBP-HA-RK (from R Goodman) into the MSCV retroviral expression vector and mutations were generated by QuickChange Mutagenesis (Stratagene). The A-CREB construct was cloned into MSCV-IRES-GFP. MSCV-CRTC2-IRES-GFP was generated from a cDNA obtained from the mammalian gene collection. The CREB and CREB-S133A MSCV retroviral constructs were described previously (Xu *et al*, 2007). Antibodies against bulk histone H3 (ab1791) and H3K18Ac (ab1191) (Abcam) were used for ChIP and western antibodies against bulk histone H4 (05–858), H3K9,14Ac (06–599), acetyl histone H4 antibody set (17–211; including antibodies against acetylated H4K5, K8, K12, and K16) (Upstate/Millipore) were used for ChIP. HA-11 monoclonal antibody against the HA epitope (Boehringer Mannheim) and CBP (A-22 and C-20) and p300 (N-20 and C-20) antibodies (Santa Cruz) were used for IF. CBP and p300 antisera used for western and ChIP were generated against peptides from the N- and C-termini of CBP and p300, respectively. CBP/p300 antiserum (2574) used for IF was generated against GST-p300 1–328, but detects both CBP and p300 similarly by immunoprecipitation and IF. CRTC2 antiserum was described previously (Xu *et al*, 2007). CREB antiserum (244) used for western was a gift from Marc Montminy. Normal rabbit serum used for ChIP was from Sigma.

Nuclear extracts, histone extraction, western blots, and ChIP assays

Nuclear extract preparation and western blots were performed as described (Kasper *et al*, 2002) except CBP/p300 were transferred with 0.1% SDS for 90 min at 400 mA and histones were transferred overnight at 30 V without SDS. Histones were acid extracted as described in Shechter *et al* (2007) and run on a 15% SDS-PAGE gel for western blotting. ChIP assays followed a modified Upstate Biotechnology Inc. protocol as described previously (Xu *et al*, 2007). ChIP primer sets were checked for linear amplification and

qPCR ChIP signals were normalized to chromatin input allowing comparison between different samples and primer sets.

Indirect IF

MEFs were seeded on 12-well microscope slides for at least 12 h, fixed in 3% formaldehyde for 10 min at room temperature (RT), washed three times with phosphate-buffered saline (PBS), permeabilized in PBS–0.2% Triton X-100 for 15 min, washed five times in PBS–2% non-fat milk, and incubated overnight with the primary antibodies diluted 1:1000 in PBS–2% non-fat milk. Primary antibodies were visualized with Cy3-conjugated donkey anti-mouse or anti-rabbit antibodies (Jackson ImmunoResearch) diluted 1:500 in PBS–2% non-fat milk. Slides were examined by fluorescence microscopy.

qRT–PCR, microarrays, and primary transcript analysis

qRT–PCR and microarray analysis was performed as described previously (Xu *et al*, 2007) except that gene expression for qRT–PCR was normalized to *Pgk1* RNA. Primary transcript analysis was performed as described previously (Kasper *et al*, 2005).

CBP-HA, A-CREB, CREB, CREB-S133A, CRTC2, and shRNA retroviral experiments

WT and *CBP^{fllox/fllox}*; *p300^{fllox/fllox}* MEFs were infected with retrovirus expressing WT CBP-HA, mutant CBP-HA, GFP (control), A-CREB, CREB, CREB-S133A, CRTC2, or shRNA (shRNA scramble ‘complement’ sequence: GCACTACCAGAGCTAACTCAGATAGTACT; shRNA CRTC2 complement sequence: GACTGCCGTCTGCACTTAACAGGAC AAGC; shRNA loop sequence; and reverse complement sequence not shown). Percentage of cells infected was determined for CBP-HA by indirect IF and for A-CREB, CREB, CREB-S133A, CRTC2, and shRNA by flow cytometry for GFP (GFP is coexpressed by the retrovirus). The CBP-HA experiments were performed using MEFs in which the infection rate was >50% and comparable between WT and mutant CBP-HA. Cells for A-CREB, CREB, CREB-S133A, CRTC2, and shRNA experiments were sorted for GFP-positive cells if the infection was <90%. After 2 days, cells were treated with adenovirus expressing Cre recombinase at an MOI of 100 and gene expression was tested 4 days later.

siRNA knockdown of CRTC1 and CRTC2

WT and dKO MEFs were transfected as follows: 1 ml of DMEM + L-glutamine was placed in a 6 cm dish; to it was added 20 µl of

20 µM siRNA (either a non-specific scramble siRNA or a mixture of siRNAs targeting CRTC1 and CRTC2) and 10 µl of RNAiMax Lipfectamine (Invitrogen). Scramble siRNA: CTGTATTGGCGTGACCA GTTT; CRTC1 siRNA: GTCACGGGGCCACCTGCACATT; CRTC2 siRNAs: AGACTGCCGTCTGCACTTAACCT, CTGAAACCTTGGGATGCTAAGTT, and CCTGCAGTCTCCCTAAGCAATT (last two bases are dT). This mixture was incubated at RT for 20 min, then 300 000 MEF cells in 5 ml DMEM + 10% FBS + L-glutamine were added. After 72 h in a 37°C incubator, the cells were treated with 10 µM forskolin/100 µM IBMX for 90 min and RNA was harvested. For protein extracts, the transfection was scaled up 10-fold in a 15 cm dish.

Statistical analysis and software

Graphs and basic statistical analyses (including Pearson’s correlation) were generated using GraphPad Prism. Regression for multiple variables was performed using SAS version 9.1.3 Service Pack 4 (SAS Institute, Inc., Cary, NC). Affymetrix microarray analyses and heat maps were generated using Spotfire. Pearson’s correlation *P*-values <0.0001 were calculated using Free Statistics Software, Wessa, P (2009), Office for Research Development and Education, version 1.1.23-r4 (URL: <http://www.wessa.net/>).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

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Conflict of interest

The authors declare that they have no conflict of interest.

References

- Ahn S, Olive M, Aggarwal S, Krylov D, Ginty DD, Vinson C (1998) A dominant-negative inhibitor of CREB reveals that it is a general mediator of stimulus-dependent transcription of *c-fos*. *Mol Cell Biol* **18**: 967–977
- Bedford DC, Kasper LH, Fukuyama T, Brindle PK (2010) Target gene context influences the transcriptional requirement for the KAT3 family of CBP and p300 histone acetyltransferases. *Epigenetics* **5**: 9–15
- Bittinger MA, McWhinnie E, Meltzer J, Iourgenko V, Latario B, Liu X, Chen CH, Song C, Garza D, Labow M (2004) Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. *Curr Biol* **14**: 2156–2161
- Bordoli L, Husser S, Luthi U, Netsch M, Osmani H, Eckner R (2001) Functional analysis of the p300 acetyltransferase domain: the PHD finger of p300 but not of CBP is dispensable for enzymatic activity. *Nucleic Acids Res* **29**: 4462–4471
- Bowers EM, Yan G, Mukherjee C, Orry A, Wang L, Holbert MA, Crump NT, Hazzalin CA, Liszczak G, Yuan H, Larocca C, Saldanha SA, Abagyan R, Sun Y, Meyers DJ, Marmorstein R, Mahadevan LC, Alani RM, Cole PA (2010) Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. *Chem Biol* **17**: 471–482
- Carlezon Jr WA, Duman RS, Nestler EJ (2005) The many faces of CREB. *Trends Neurosci* **28**: 436–445
- Cohen S, Greenberg ME (2008) Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. *Annu Rev Cell Dev Biol* **24**: 183–209
- Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, Hogenesch JB, Montminy M (2003) TORCs: transducers of regulated CREB activity. *Mol Cell* **12**: 413–423
- Dentin R, Hedrick S, Xie J, Yates III J, Montminy M (2008) Hepatic glucose sensing via the CREB coactivator CRTC2. *Science* **319**: 1402–1405
- Dentin R, Liu Y, Koo SH, Hedrick S, Vargas T, Heredia J, Yates III J, Montminy M (2007) Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. *Nature* **449**: 366–369
- Ferreri K, Gill G, Montminy M (1994) The cAMP-regulated transcription factor CREB interacts with a component of the TFIID complex. *Proc Natl Acad Sci USA* **91**: 1210–1213
- Flavell SW, Greenberg ME (2008) Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. *Annu Rev Neurosci* **31**: 563–590
- Fukuyama T, Kasper LH, Boussouar F, Jeevan T, van Deursen J, Brindle PK (2009) Histone acetyltransferase CBP is vital to demarcate conventional and innate CD8+ T-cell development. *Mol Cell Biol* **29**: 3894–3904
- Goodman RH, Smolik S (2000) CBP/p300 in cell growth, transformation, and development. *Genes Dev* **14**: 1553–1577
- Hargreaves DC, Horng T, Medzhitov R (2009) Control of inducible gene expression by signal-dependent transcriptional elongation. *Cell* **138**: 129–145
- Hong EJ, West AE, Greenberg ME (2005) Transcriptional control of cognitive development. *Curr Opin Neurobiol* **15**: 21–28
- Horwitz GA, Zhang K, McBrien MA, Grunstein M, Kurdiani SK, Berk AJ (2008) Adenovirus small e1a alters global patterns of histone modification. *Science* **321**: 1084–1085

- Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* **29**: 565–598
- Iourgenko V, Zhang W, Mickanin C, Daly I, Jiang C, Hexham JM, Orth AP, Miraglia L, Meltzer J, Garza D, Chirn GW, McWhinnie E, Cohen D, Skelton J, Terry R, Yu Y, Bodian D, Buxton FP, Zhu J, Song C *et al* (2003) Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. *Proc Natl Acad Sci USA* **100**: 12147–12152
- Johannessen M, Delghandi MP, Moens U (2004) What turns CREB on? *Cell Signal* **16**: 1211–1227
- Kang-Decker N, Tong C, Boussouar F, Baker DJ, Xu W, Leontovich AA, Taylor WR, Brindle PK, Van Deursen JM (2004) Loss of CBP causes T cell lymphomagenesis in synergy with p27(Kip1) insufficiency. *Cancer Cell* **5**: 177–189
- Kasper LH, Boussouar F, Boyd K, Xu W, Biesen M, Reh J, Baudino TA, Cleveland JL, Brindle PK (2005) Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. *EMBO J* **24**: 3846–3858
- Kasper LH, Boussouar F, Ney PA, Jackson CW, Reh J, van Deursen JM, Brindle PK (2002) A transcription-factor-binding surface of coactivator p300 is required for haematopoiesis. *Nature* **419**: 738–743
- Kasper LH, Brindle PK (2006) Mammalian gene expression program resiliency: the roles of multiple coactivator mechanisms in hypoxia-responsive transcription. *Cell Cycle* **5**: 142–146
- Kasper LH, Fukuyama T, Biesen MA, Boussouar F, Tong C, de Pauw A, Murray PJ, van Deursen JM, Brindle PK (2006) Conditional knockout mice reveal distinct functions for the global transcriptional coactivators CBP and p300 in T-cell development. *Mol Cell Biol* **26**: 789–809
- Koo SH, Flechner L, Qi L, Zhang X, Screaton RA, Jeffries S, Hedrick S, Xu W, Boussouar F, Brindle P, Takemori H, Montminy M (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. *Nature* **437**: 1109–1114
- Koutroubas G, Merika M, Thanos D (2008) Bypassing the requirements for epigenetic modifications in gene transcription by increasing enhancer strength. *Mol Cell Biol* **28**: 926–938
- Kung AL, Rebel VI, Bronson RT, Ch'ng LE, Sieff CA, Livingston DM, Yao TP (2000) Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes Dev* **14**: 272–277
- Li B, Carey M, Workman JL (2007) The role of chromatin during transcription. *Cell* **128**: 707–719
- Liu X, Wang L, Zhao K, Thompson PR, Hwang Y, Marmorstein R, Cole PA (2008a) The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. *Nature* **451**: 846–850
- Liu Y, Dentin R, Chen D, Hedrick S, Ravnskjaer K, Schenk S, Milne J, Meyers DJ, Cole P, Yates III J, Olefsky J, Guarente L, Montminy M (2008b) A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. *Nature* **456**: 269–273
- Marmorstein R (2001) Structure of histone acetyltransferases. *J Mol Biol* **311**: 433–444
- Novak A, Guo C, Yang W, Nagy A, Lobe CG (2000) Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis* **28**: 147–155
- Ramirez-Carrozzi VR, Braas D, Bhatt DM, Cheng CS, Hong C, Doty KR, Black JC, Hoffmann A, Carey M, Smale ST (2009) A unifying model for the selective regulation of inducible transcription by CpG islands and nucleosome remodeling. *Cell* **138**: 114–128
- Rando OJ, Chang HY (2009) Genome-wide views of chromatin structure. *Annu Rev Biochem* **78**: 245–271
- Sands WA, Palmer TM (2008) Regulating gene transcription in response to cyclic AMP elevation. *Cell Signal* **20**: 460–466
- Screaton RA, Conkright MD, Katoh Y, Best JL, Canettieri G, Jeffries S, Guzman E, Niessen S, Yates III JR, Takemori H, Okamoto M, Montminy M (2004) The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. *Cell* **119**: 61–74
- Shechter D, Dormann HL, Allis CD, Hake SB (2007) Extraction, purification and analysis of histones. *Nat Protoc* **2**: 1445–1457
- Smolik S, Jones K (2007) Drosophila dCBP is involved in establishing the DNA replication checkpoint. *Mol Cell Biol* **27**: 135–146
- Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* **1**: 4
- Stauffer D, Chang B, Huang J, Dunn A, Thayer M (2007) p300/CREB-binding protein interacts with ATR and is required for the DNA replication checkpoint. *J Biol Chem* **282**: 9678–9687
- Tanaka Y, Naruse I, Hongo T, Xu M, Nakahata T, Maekawa T, Ishii S (2000) Extensive brain hemorrhage and embryonic lethality in a mouse null mutant of CREB-binding protein. *Mech Dev* **95**: 133–145
- Vo NK, Goodman RH (2001) CBP and p300 in transcriptional regulation. *J Biol Chem* **276**: 13505–13508
- Wang Y, Vera L, Fischer WH, Montminy M (2009) The CREB coactivator CRTC2 links hepatic ER stress and fasting gluconeogenesis. *Nature* **460**: 534–537
- Xu W, Fukuyama T, Ney PA, Wang D, Reh J, Boyd K, van Deursen JM, Brindle PK (2006) Global transcriptional coactivators CREB-binding protein and p300 are highly essential collectively but not individually in peripheral B cells. *Blood* **107**: 4407–4416
- Xu W, Kasper LH, Lerach S, Jeevan T, Brindle PK (2007) Individual CREB-target genes dictate usage of distinct cAMP-responsive coactivation mechanisms. *EMBO J* **26**: 2890–2903
- Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM, Eckner R (1998) Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* **93**: 361–372