Molecular Cell, Vol. 11, 1101–1108, April, 2003, Copyright ©2003 by Cell Press

Genome-Wide Analysis of CREB Target Genes Reveals A Core Promoter Requirement for cAMP Responsiveness

Short Article

Michael D. Conkright,[3] Ernesto Guzmán,[3] Lawrence Flechner,[1] Andrew I. Su,[2] John B. Hogenesch,[2,*] and Marc Montminy*
[1]Salk Institute for Biological Studies
La Jolla, California 92037
[2]The Genomics Institute of the Novartis Research Foundation
San Diego, California 92121

Summary

We have employed a hidden Markov model (HMM) based on known cAMP responsive elements to search for putative CREB target genes. The best scoring sites were positionally conserved between mouse and human orthologs, suggesting that this parameter can be used to enrich for true CREB targets. Target validation experiments revealed a core promoter requirement for transcriptional induction via CREB; TATA-less promoters were unresponsive to cAMP compared to TATA-containing genes, despite comparable binding of CREB to both sets of genes in vivo. Indeed, insertion of a TATA box motif rescued cAMP responsiveness on a TATA-less promoter. These results illustrate a mechanism by which subsets of target genes for a transcription factor are differentially regulated depending on core promoter configuration.

Introduction

Signal-dependent transcription factors are thought to coordinate cellular responses to external stimuli by promoting the expression of specific genetic programs. The cAMP responsive factor CREB and its paralogs, ATF1 and CREM, have been shown to function in a broad array of biological processes such as glucose metabolism and cell survival and in complex neuronal functions like learning and memory (Lonze and Ginty, 2002; Mayr and Montminy, 2001). CREB family members bind to DNA via a conserved basic region/leucine zipper motif that recognizes the cAMP responsive element (CRE) (Dwarki et al., 1990; Schumacher et al., 2000).

Comparative studies on a number of cAMP responsive promoters have led to the identification of a consensus palindrome—TGACGTCA—that recognizes the CREB dimer with high affinity (Comb et al., 1986; Montminy et al., 1986; Wynshaw-Boris et al., 1986). Variant CREs containing only a half-site TGACG sequence also appear to be functional, albeit at a reduced level compared to the full palindrome (Fink et al., 1988; Yamamoto et al., 1988). The CRE is usually located proximal to the transcription start site and, in the case of the rat tyrosine hydroxylase gene, its activity is reduced when moved greater than 250 bp from the TATA box (Tinti et al., 1997). Indeed, binding of CREB to the promoter appears to stimulate cooperative interactions with TFIID that facilitate recruitment of RNA polymerase II complexes (Horikoshi et al., 1988). In this regard, CREB has been found to interact with the $hTAF_{II}130$ component of TFIID via a glutamine-rich Q2 *trans*-activation domain; and this association appears to be critical for cAMP inducibility (Felinski et al., 2001; Nakajima et al., 1997).

Despite significant progress into the mechanism of CREB activation, further insight into the biological role of this factor in different systems has been limited by the lack of comprehensive data on CREB target genes. Traditionally, determination of transcription factor target genes has required the concerted action of multiple laboratories and many years of research, usually resulting in the identification of a relatively small number of bona fide target genes. The widespread application of transcriptional profiling tools such as high-density DNA arrays has in part addressed this issue by allowing thousands of genes to be interrogated for responses to activation or inactivation of specific transcription factors. Although these tools have greatly increased the landscape of potential target genes for many transcription factors, they have concomitantly introduced a new problem: defining direct versus indirect targets of specific activators and repressors.

Recently, several groups have applied informatic techniques and comparative genomics between mouse and human to identify target genes of the circadian system (Ueda et al., 2002), P53 (Hoh et al., 2002), and myc transcriptional activators (Menssen and Hermeking, 2002; Schuldiner et al., 2002). These efforts employed consensus searching, positional weight matrices, or customized algorithms designed for the detection of target genes (Hoh et al., 2002). Here we perform a genome-wide analysis of CREB target genes in mouse and human genomic sequences using a hidden Markov model (HMM) trained on known CREB binding sites. Analysis of these data revealed a striking positional conservation of the best hits to the model. Experimental validation of these putative CREB target genes suggests that the use of positional conservation will be effective in identification of true target genes for other transcription factors as well. In addition, validation revealed an unsuspected requirement of a proximal TATA box for activation via CREB. And these results may explain, in part, how different subsets of CREB target genes are activated in response to external stimuli.

Results and Discussion

Search Strategy

The relatively short nature of response elements, and thus the likelihood of their random occurrence, prompted us to employ comparative genomic strategies. To bias our experiment for the identification of bona fide targets of CREB, we sought to (1) direct our search to regions near the start of transcription, (2) use a hidden Markov model in searching for the elements, (3) utilize comparative genomics, and (4) investigate the positional conser-

*Correspondence: montminy@salk.edu
[3]These authors contributed equally to this work.

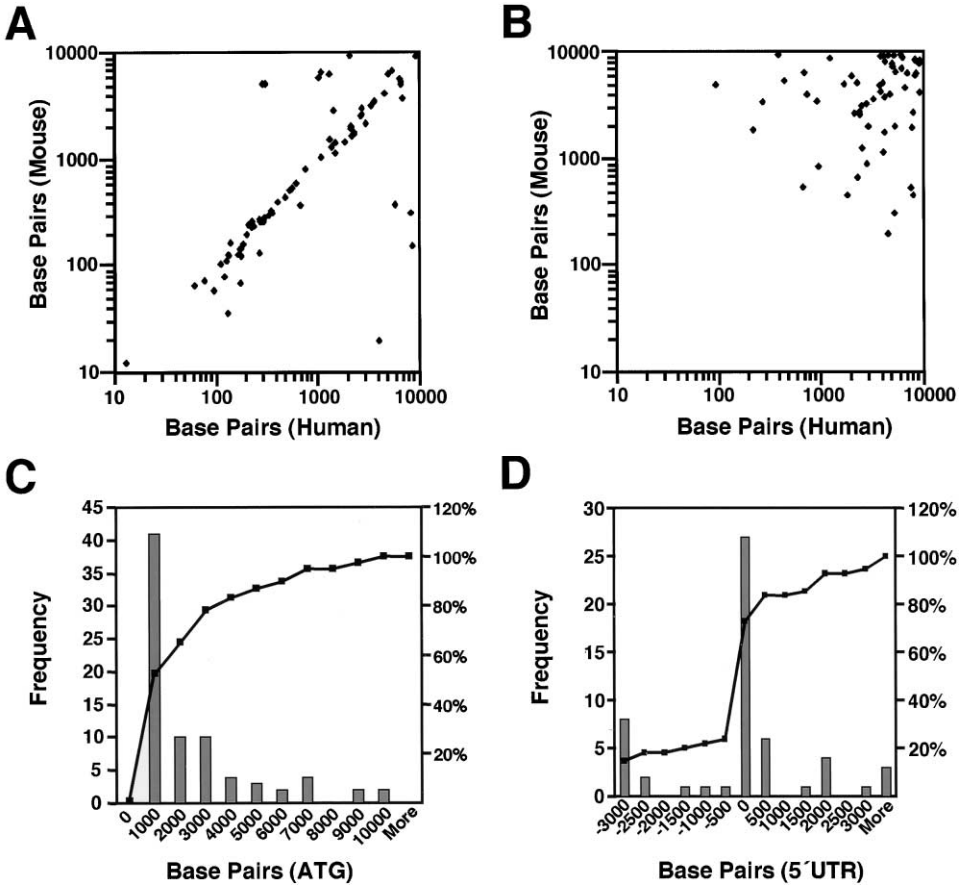

Figure 1. Positional Conservation and Distribution of Putative CREB Binding Sites

An HMM was trained on validated CREB binding sites from cAMP responsive promoters (Mayr and Montminy, 2001) and used to search mouse and human upstream regions of orthologous genes.

(A) The 78 most significant hits to the model (score $>$ 1.5, chosen based on training set) were retrieved from the human upstream regions. The positions of these motifs relative to the start methionine were plotted against the positions in the corresponding mouse ortholog (http://www.ncbi.nlm.nih.gov/HomoloGene/). Where there are multiple hits in mouse or human genes, the pair with the minimal difference in HMM score was plotted.

(B) The 60 least significant hits to the model (score $<$ –1.9) were retrieved from the human upstream regions and plotted as above.

(C) A frequency histogram and cumulative distribution (solid line) of the positions of the most significant 78 candidate CREs from human upstream regions relative to the start methionine.

(D) A frequency histogram and cumulative distribution (solid line) of the positions of the most significant 78 candidate CREs from human upstream regions relative to the position of the 5′ UTR. Negative numbers are upstream of the 5′ UTR, while positive numbers are downstream of the 5′ UTR.

vation of the enhancers between mouse and human genes. Many known transcriptional activators including CREB have been shown to function through enhancers proximal to the transcriptional start site (TSS). Analysis of a large database of putative human TSSs, Database of Human Transcriptional Start Sites (DBTSS, http://elmo.ims.u-tokyo.ac.jp/dbtss/), using BLAST revealed that the median distance from the start methionine was ~150 bp, and that 88% are located within 10 kb of the start methionine (Experimental Procedures, and H. Ueda, personal communication) (Suzuki et al., 2002). Because many more translational start sites have been mapped across species than TSSs, we chose start methionines as the fixed reference point. Therefore, in this study we chose to focus our searches to the 10 kb regions upstream of the start methionine in mouse and human genes.

Several methods have been employed to search for target genes in genomic space, including consensus searching and positional weight matrices (GuhaThakurta and Stormo, 2001). Because of their statistical properties and tolerance for insertions and deletions, we used a hidden Markov model (HMM) trained on known CREB binding sites to search mouse and human upstream regions. We also evaluated positional weight matrices (PWMs) built on the same training set and obtained qualitatively similar, but fewer, significant results. Many of the sites identified by the HMM, but not PWM, methodology, contained insertions or deletions outside of the core palindrome (TGACGTCA), although the importance of these flanking regions for transcriptional activation via CREB is unclear. Differences in parameter estimation between PWM and HMMs may also contribute to discrepancies between the two searching methods.

Thousands of hits were produced for the searches of

Table 1. Putative Mammalian CREB Targets Identified by Cross-Species Genome Analysis

Symbol	NM-Human	Position Human	Position Mouse	Score	CRE Sequence
BNIP3L	NM_004331	112	110	5.8	GGCCCTGACGTCAGGGGC
STXBP3	NM_007269	127	118	5.3	GCGCGTGACGTCACCGGA
SST	NM_001048	135	133	3.3	TAGCCTGACGTCAGAGAG
SULT4A1	NM_014351	141	83	3.2	CGCCATGACGTCACGCCT
SNL	NM_003088	166	113	4.7	GGCGCTGACGTCACCTCG
EPHA2	NM_004431	173	151	6	GTTGGTGACGTCACGCAG
SH3GL2	NM_003026	177	154	2.5	GCCCTTGACGTCAGAGTG
MLF1	NM_022443	186	169	3.9	CCTGGTGACGTCACAGAG
PPARGC1	NM_013261	218	219	2.1	TTGAGTGACGTCACGAGT
PPP2CA	NM_002715	237	247	6.4	GGGCGTGACGTCACCACG
PPM1D	NM_003620	269	289	4	CCGAGTGACGTCAGCGGG
SPAG6	NM_012443	269	203	6.7	GGCCGTGACGTCACGCCC
RAB25	NM_020387	288	274	1.9	CTGTCTGACGTCATCATC
EVX1	NM_001989	296	276	4.7	CCGAGTGGCGTCACCAGC
GOLPH3	NM_022130	301	295	4.7	TCCGGTGACGTCAGCCGG
CCNA2	NM_001237	321	213	3.5	TTGAATGACGTCAAGGCC
FLT1	NM_002019	351	343	5.6	CCCCTTGACGTCACCAGA
DUSP1	NM_004417	363	327	5.1	GCGGGTGACGTCACCGCC
PCK1	NM_002591	378	294	4.8	CCCCCTGACGTCAGTGGC
UCN	NM_003353	407	415	3.9	TTCGCTGACGTCAGTCGG
AUP1	NM_012103	485	464	2.7	GTTCCTGACGTCAGCAGG
WNT10A	NM_025216	536	535	5.1	CCGCGTGACGTCACGTGG
CRKL	NM_005207	566	511	1.6	CTGTGTGACGTAACGGGA
YY1	NM_003403	608	614	4	CGCGCTGACGTCACGCGC
MOAP-1	NM_022151	676	653	2.8	CCTCATGACGTCAGATCG
SEDLP	NM_015890	1321	1325	6.2	CTCTATGACGTCACCGAA
HEC	NM_006101	1440	1382	4.6	CGTAATGACGTCAGCGCC
NEUROD6	NM_022728	1507	1497	2.7	CCCTGTGACGTCACTAGC
GEM	NM_005261	1870	1524	4.3	GGCGCTGACGTCACGGAA
ADCY8	NM_001115	2134	2095	3.5	GGGCCTGACGTCATTGGC
NR4A2	NM_006186	2668	2646	6.3	GCTCGTGACGTCAGGTCG
LMO4	NM_006769	3820	3512	2.5	GTCGCTGACGTCGGAGGC
LDHA	NM_005566	2325	4173	4	CCCGCTGACGTCAGCATA
PEF	NM_012392	9322	9335	3.1	GAGTGTGACGTCAGAATC

Position of the CRE on mouse and human orthologs relative to methionine start codon are indicated. Sequence of the CRE shown for each gene. Score reflects sequence similarity to the consensus CRE palindrome and is generated by the HMMER 2 software package (http://hmmer.wustl.edu) (Eddy, 1998). Entries in Table 1 were chosen from the list of top scoring hits (78) based on the presence of a palindromic CRE sequence (TGACGTCA) and its proximity to the transcriptional start site.

both the mouse and human genomic sequences; therefore we used the match scores from known CREB binding sites as a reporting threshold. This analysis resulted in 1349 mouse sites and 1663 human sites identified. Although statistically significant, many of the hits could be spurious due to the relatively short nature of response elements, prompting us to investigate whether the cAMP response element is positionally conserved between the upstream regions of mouse and human orthologous genes (http://www.ncbi.nlm.nih.gov/HomoloGene/).

This analysis revealed a striking positional conservation of the best "hits" to the HMM, while lower scoring hits were not conserved by position in mouse and human upstream regions (Figures 1A and 1B; Supplemental Table S1 at http://www.molecule.org/cgi/content/full/11/4/1101/DC1). Several known CREB target genes including somatostatin (SST), urocortin (UCN), phosphoenolpyruvate carboxykinase 1 (PCK1), nurr1 (NR4A2), and dual specificity phosphatase 1 (DUSP1) were recovered from this analysis, as well as approximately 65 other putative CREB target genes. Several known CREB targets were also not reported by this analysis, most often because those genes did not have a defined orthologous pair between mouse and human genes. In addition, approximately 77% of significant mouse sites and 84% of significant human sites were not found as significant in their corresponding ortholog. Many of the conserved CREs across species occurred within 1000 bases of the ATG initiator codon (Figure 1C). Prompted by the earlier observation that many TSSs are likewise distributed, we mapped the 5′ UTR of Refseq genes for putative CREB target genes to the genome using BLAST. This analysis showed that many of conserved CRE-like sequences occurred within 250 bp of the 5′ UTR sequence, a rough approximation of the TSS (Figure 1D). Taken in sum, these results suggest the existence of many additional CREB target genes in the mammalian genome and show that many of these candidate sites are positionally conserved across species.

Characterization and Validation of CREB Target Genes

Inspection of putative CREB targets revealed a number of genes that contain either full palindromes or half-site CREs (Table 1). About 30% of the top-scoring targets were transcriptional activators or coactivators, and a similar fraction was involved in cytoplasmic signaling. Consistent with the importance of CREB in the central nervous system, many CRE-containing genes were differentially expressed in neurons (Supplemental Table S1

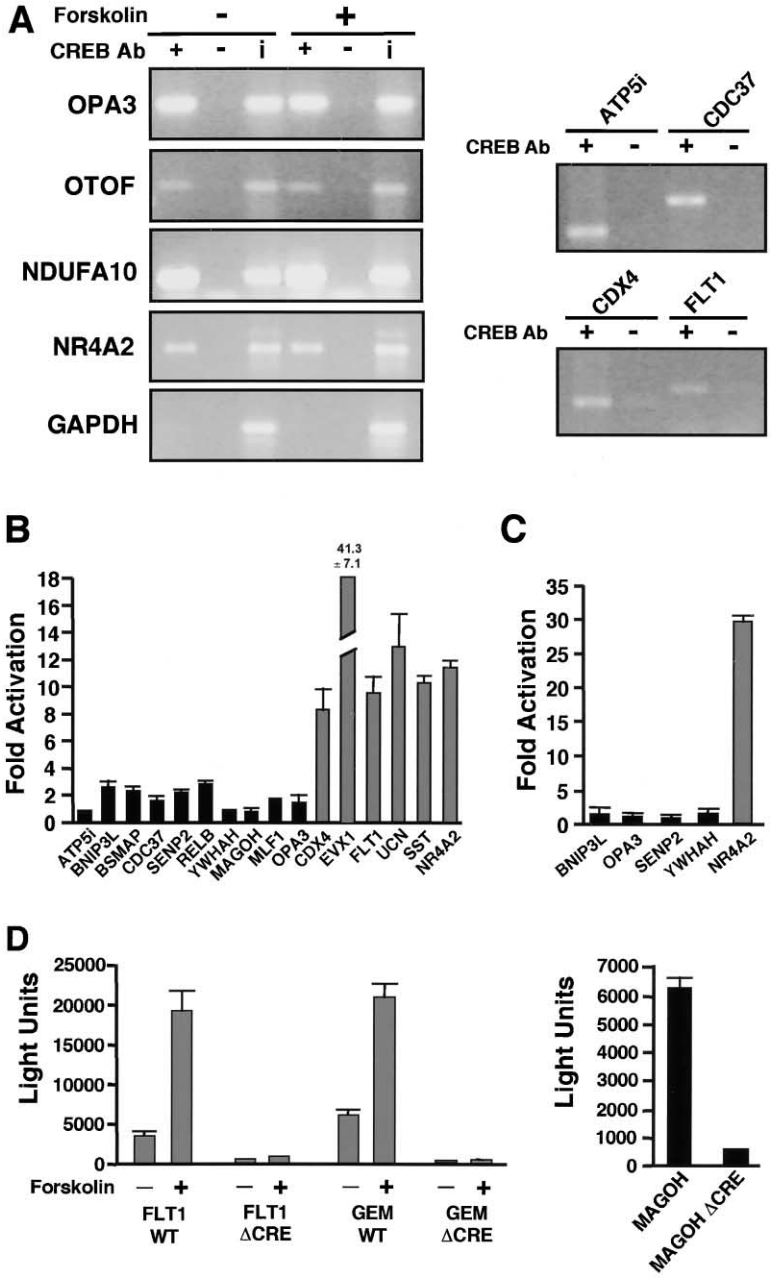

Figure 2. Functional Analysis of Putative CREB Target Genes

(A) Binding of CREB to putative target promoters in vivo. Chromatin immunoprecipitation assays of target promoters from 293T cells using anti-CREB antiserum. +, CREB-specific antiserum; -, nonspecific antiserum; i, input control DNA (1% of total). Left, immunoprecipitates prepared from control and forskolin-treated cells indicated. Right, chromatin immunoprecipitation assays of additional CREB targets from 293T cells.

(B) Transient transfection assay of luciferase reporters containing fragments of 16 CRE-containing promoters selected from the list of putative CREB target genes. Individual reporters were transfected into 293T cells, and luciferase activity was measured following treatment (4 hr) with cAMP agonist (forskolin, 10 μM). Fold-induction over control vehicle (DMSO) shown. Assays were normalized to cotransfected RSV-β galactosidase activity.

(C) Quantitative PCR analysis of YWHAH, BNIP3L, OPA3, SENP2, (black bars, TATA-less), and NR4A2 (gray bar, TATA-containing) genes in 293T cells treated with cAMP agonist or DMSO vehicle. Target genes were comparably expressed under basal conditions by gene profiling analysis (not shown).

(D) Effect of CRE mutagenesis on promoter activity from TATA-containing (FLT1, GEM) and TATA-less (MAGOH) CREB target genes. Wild-type and CRE mutant reporter plasmids were transfected into 293T cells and assayed as described above. Relative basal and camp-inducible activity indicated.

at http://www.molecule.org/cgi/content/full/11/4/1101/DC1) (http://expression.gnf.org) (Su et al., 2002). For example, a number of putative CREB targets encoded proteins involved in neuronal specification (LHX1, EVX1, LMO-4, HRY), axon guidance (UNC5c, EPHA2), synapse formation (SYN1), and neurotransmitter release (STXBP3). The majority of targets with high scores contained palindromic CREs (TGACGTCA), whereas most genes with lower scores contained half-sites (TGACG). A high proportion of putative CREB target genes were TATA-less; only about 15% of target genes contained TATA box motifs.

To determine whether the genes identified in our survey are actually bound by CREB in vivo, we performed chromatin immunoprecipitation assays on CRE-containing genes that are expressed in 293T cells by RNA profiling analysis (not shown) and that are readily amplified from genomic DNA. CREB binding was readily detected on each of eight different candidate target promoters (OPA3, OTOF, NDUFA10, NR4A2, ATP5i, CDC37, CDX4, FLT1) but not on the control CRE-less GAPDH gene (Figure 2A). CREB occupancy was comparable on TATA-containing (NR4A2, CDX4, FLT1) and TATA-less (OPA3, OTOF, NDUFA10, ATP5i, CDC37) promoters, and binding of CREB to these sites was unaffected by stimulation with cAMP agonist (Figure 2A). These results support the notion that cAMP regulates CREB *trans*-activation potential rather than its DNA binding activity.

To investigate whether the putative CREB target genes are cAMP responsive, we generated luciferase

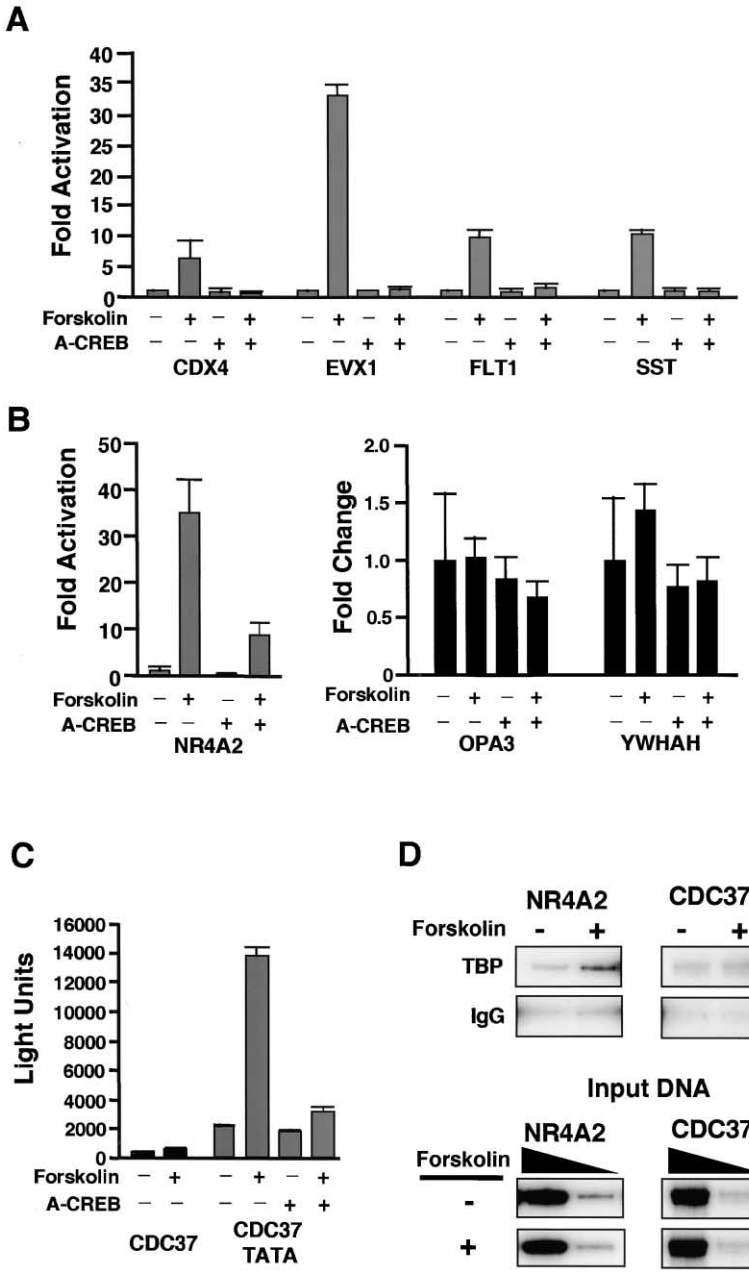

Figure 3. Transcriptional Activation via CREB Depends on Core Promoter Configuration

(A) Effect of dominant-negative CREB (A-CREB) expression on reporter activity from selected CREB target genes (CDX4, EVX1, FLT1, SST). Luciferase activity from individual reporters cotransfected with A-CREB expression or control vector as indicated. Cells were treated with forskolin or DMSO vehicle for 4 hr, and luciferase activity was determined after normalizing to β-galactosidase activity from cotransfected RSV-β gal expression vector.

(B) Effect of A-CREB inhibitor on endogenous expression of TATA-containing (NR4A2) and TATA-less (OPA3, YWHAH) genes in 293T cells by quantitative RT-PCR analysis.

(C) Insertion of a TATA box rescues cAMP inducibility on a TATA-less promoter. Wild-type or mutant CDC37 luciferase reporters were transfected into 293T cells and treated with forskolin or DMSO vehicle as shown. Effect of co-transfected A-CREB inhibitor on CDC37-TATA reporter activity in control and forskolin-treated cells indicated.

(D) Effect of cAMP agonist on recruitment of TBP to TATA-containing (NR4A2) and TATA-less (CDC37) promoters. Chromatin immunoprecipitation analysis of NR4A2 and CDC37 promoters in control and camp-treated 293T cells using anti-TBP or non-specific IgG antisera. Total input levels of DNA (1%, 0.1%) shown.

reporter constructs for 16 different promoters and analyzed these by transient transfection assay in 293T and JEG-3 cells. All TATA-containing promoters were induced an average of 15-fold by cAMP agonist in both cell types (Figure 2B; not shown). But TATA-less promoters showed only marginal responsiveness to cAMP, despite comparable basal activity in the absence of inducer (Figure 2B).

To eliminate potential regulatory artifacts associated with the use of reporter vectors, we examined the effect of cAMP agonist on endogenous gene expression using candidates that were readily expressed in 293T cells under basal conditions by gene profiling analysis (not shown). Forskolin treatment induced the TATA-containing NR4A2 gene 30-fold after 4 hr, but had minimal effects on each of the four TATA-less genes tested (BNIP3L, OPA3, SENP2, YWHAH) (Figure 2C). These results argue that activation of cellular promoters by cAMP may require a nearby TATA box.

To determine the importance of the CRE site for promoter activation, we performed mutagenesis studies in which the central 4 nucleotides of the CRE palindrome were deleted in several putative targets. Compared to wild-type constructs, the CRE mutant reporters for both TATA-containing (GEM, FLT1) and TATA-less (MAGOH) promoters showed markedly lower basal activity, and, in the case of TATA-containing genes, cAMP-inducible promoter activity (Figure 2D). These results indicate that the CRE contributes to both basal transcriptional activity and cAMP responsiveness in these promoter contexts.

In order to investigate the role of CREB in regulating the potential targets, we employed a dominant-negative CREB expression vector referred to as acidic CREB (A-CREB). A-CREB contains the CREB leucine zipper dimerization domain fused to an acidic peptide that extends the dimerization interface and disrupts binding of CREB family members but not other bZIP proteins to DNA (Ahn et al., 1998). Expression of A-CREB in 293T cells blocked induction of target reporter constructs for TATA-containing genes (CDX4, EVX1, FLT1, SST) by cAMP, but had no effect on basal promoter activity (Figure 3A). A-CREB also blocked endogenous expression from the TATA-containing NR4A2 gene in forskolin-treated 293T cells (Figure 3B, left) but had no effect on basal activity from TATA-less promoters (OPA3, YWHAH; Figure 3B, right). These results suggest that other CRE binding proteins besides CREB stimulate target gene expression in the absence of cAMP agonist.

The apparent requirement of both CRE and TATA sequences for cAMP inducibility prompted us to test this notion directly by inserting a TATA motif into the TATA-less CDC37 promoter. In transient transfection assays of 293T cells, a wild-type CDC37-luciferase reporter was only marginally induced by cAMP agonist (1.3-fold). By contrast, a CDC37-TATA construct containing a consensus TATA box motif showed robust activation (6-fold) in response to forskolin (Figure 3C). Moreover, cotransfection of an A-CREB expression vector blocked camp-inducible transcription from the mutant CDC37-TATA construct, demonstrating the importance of CREB in this context (Figure 3C).

CREB has been found to stimulate transcription, in part, by recruiting TFIID via a direct interaction with $TAF_{II}135$ (Felinski and Quinn, 1999; Ferreri et al., 1994; Nakajima et al., 1997; Saluja et al., 1998). The requirement of a proximal TATA box for target gene activation via CREB led us to examine whether recruitment of TFIID complexes by CREB might be impaired on TATA-less promoters. In chromatin immunoprecipitation assays of 293T cells, treatment with cAMP agonist stimulated recruitment of TBP to the TATA-containing NR4A2 promoter but had no effect on TBP occupancy over the TATA-less CDC37 gene (Figure 3D). Taken together, these experiments indicate that core promoter configuration imposes important constraints on coactivator recruitment and transcriptional activation via CREB in response to cAMP stimulus.

In addition to CREB, core promoter selectivity has been described for a number of activators. The Ets family protein Elf-1, for example, displays a striking preference for TATA-less promoters whereas c-*fos* appears to function selectively on TATA-containing genes (Smale, 2001). The biochemical basis for this specificity is unclear, but the ability of CREB to discriminate between different core promoters provides a mechanism for selective activation of target gene subsets depending on cellular context. Notably, many of the TATA-less genes identified by our analysis appear to be involved in cell cycle progression, DNA repair, and apoptosis-cellular functions that are not typically regulated by cAMP. Thus CREB is likely to activate such genes in a selective fashion, depending on cell context and costimulatory signals.

In summary, we describe a method in which known sequence-specific DNA binding sites for a transcriptional activator are used to determine additional likely target genes. Our strategy employs a model-based approach with excellent statistical properties, comparative genomics, and the positional conservation of putative target genes across mouse and human upstream regions. Validation of this strategy using chromatin immunoprecipitation confirmed CREB binding to all of the targets tested. Activation studies, however, indicated that relatively few of the candidate genes responded to CREB activation by forskolin. Those that did harbored TATA sequences proximal to the CREB sites, supporting the notion that CREB requires TATA sequences for trans-activation. The informatic and experimental strategies discussed here are especially timely as the proliferation of genomics tools gains wider acceptance in the study of transcription. Preliminary studies on a number of additional sequence specific trans-activators including steroid receptors and bHLH-factors reveal similar positional conservation of enhancers across mouse and human upstream regions. Characterization of these putative new target genes should propel understanding of the biology mediated by signal dependent activators including CREB.

Experimental Procedures

Retrieval of Upstream Regions

The coding region was identified for all human and mouse genes in Refseq. These sequences were mapped to the genome assemblies for human (Celera R26h) and mouse (Celera R13) using BLAST. Up to 10 kb sequence was retrieved upstream of the translational start ATG.

Mapping of TSS and 5′ UTR Sequences

The database of TSS for humans was retrieved from, and used in a BLAST search against, the upstream regions for human genes above. Results were stored in a relational database, and hits initiating in the first 20 bp of the query sequence were tabulated.

Construction, Search, and Analysis of Putative CREs

Nucleotide sequences for known CREB binding sites flanking 10 bp from the CGA, CHGA, FN1, DUSP1, NR4A2, PCK1, PCSK1, SST, DIO2, and VIP genes were identified and retrieved from GenBank. These sites were aligned using the Clustal algorithm using the following parameters: Multiple Alignment Parameters, Gap Penalty 10, Gap Length Penalty 10; Pairwise Alignment Parameters, Ktuple 2, Gap Penalty 5, Window 4, Diagonals Saved 4. (Megalign, DNAstar, Madison, WI). The alignment was saved as a multiple sequence format file, and used to build a HMM using hmmbuild in the HMMER 2 software package (http://hmmer.wustl.edu) (Eddy, 1998). Following construction, the model was calibrated using hmmcalibrate, and used to search upstream regions of mouse and human genes using hmmsearch. Results of the search were parsed and stored in relational database. The model and alignments are available upon request.

Plasmid Constructs

CRE-containing promoters were amplified from 293T cell human genomic DNA and cloned into pXP2, a luciferase reporter vector (Nordeen, 1988). CRE deletions were created by AatII digestion followed by blunting the 3′ overhangs with T4 DNA polymerase. CDC37 TATA insertion mutant promoter was generated by site-directed mutagenesis (Wang and Wilkinson, 2000).

Chromatin Immunoprecipitation Assays

Human embryonal kidney cells (293T) were plated in 15 cm dishes and grown to approximately 80%–90% confluence. To test for forskolin induction, cells were treated with 10 μM forskolin dissolved

in DMSO for 1 hr. The cells were treated with 1% formaldehyde as previously described (Asahara et al., 2001). For CREB immunoprecipitations, rabbit polyclonal antibody raised against CREB (244) was used along with nonspecific antiserum for negative controls. CREB target promoters were PCR amplified using appropriate oligos. Reference number (NM) and position of CRE relative to initiator methionine (in italics) for putative human CREB target promoters are OPA3 (NM_025136: *156*), OTOF (NM_004802: *396*), NDUFA10 (NM_004544: *95*), NR4A2 (NM_006186: *2668*), ATP5I (NM_007100: *193*), CDC37 (NM_007065: *135*), CDX4 (NM_005193: *223*), FLT1 (NM_002019: *372*). Anti-TBP immunoprecipitations were performed with monoclonal TBP antibody SL30-3-563 (generous gift of Nouria Hernandez).

Transfections and Luciferase Assays

293T or human placental choriocarcinoma cells (JEG-3) were plated in 12-well tissue culture dishes at a density of 1×10^5 cells/ well. The following day, cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Where indicated, 100 ng reporter vector, 100 ng RSV-β-galactosidase expression plasmid, and 250 ng SV40-A-CREB expression plasmid per well were cotransfected, and total DNA was kept constant by addition of empty vector. Twenty hours posttransfection, cells were treated for 4 hr with 10 μM forskolin or vehicle (DMSO). Cell extracts were prepared after stimulation and luciferase assays were performed as described previously (Brasier et al., 1989), normalizing to activity from cotransfected Rous sarcoma virus-β-galactosidase expression plasmid. Additional information for reporter constructs used to evaluate CREB target genes (Figure 1B) is available upon request.

Quantitative PCR

Total RNA was extracted from 293T cells using RNeasy (Qiagen) kit according to manufacturer's instructions. cDNA was prepared by reverse transcription of 500 ng total RNA using Superscript II enzyme and oligo dT primer. The resulting cDNAs were amplified using the SYBR green PCR kit and an ABIPRISM 7700 sequence detector (Perkin Elmer). All mRNA expression data was normalized to GAPDH expression in the corresponding sample.

Acknowledgments

We thank Nouria Hernandez for the gift of TBP antiserum. This work was supported by NIH grant RO1 GM37828. M.D.C. is a fellow of the George Hewitt Foundation.

Received: October 15, 2002
Revised: January 21, 2003
Accepted: January 23. 2003
Published: April 24, 2003

References

Ahn, S., Olive, M., Aggarwal, S., Krylov, D., Ginty, D., and Vinson, C. (1998). A dominant negative inhibitor of CREB reveals that it is a general mediator stimulus-dependent transcription of c-fos. Mol. Cell. Biol. *18*, 967–977.

Asahara, H., Santoso, B., Du, K., Cole, P., and Montminy, M. (2001). Chromatin dependent cooperativity between constitutive and inducible activation domains in CREB. Mol. Cell. Biol. *21*, 7892–7900.

Brasier, A., Tate, J., and Habener, J. (1989). Optimized use of the firefly luciferase assay as a reporter gene in mammalian cell lines. Biotechniques *7*, 1116–1122.

Comb, M., Birnberg, N.C., Seasholtz, A., Herbert, E., and Goodman, H.M. (1986). A cyclic AMP- and phorbol ester-inducible DNA element. Nature *323*, 353–356.

Dwarki, V.J., Montminy, M., and Verma, I.M. (1990). Both the basic region and the "leucine zipper" domain of cyclic AMP response element binding (CREB) protein are essential for transcriptional activation. EMBO J. *9*, 225–232.

Eddy, S. (1998). Profile hidden Markov models. Bioniformatics *14*, 755–763.

Felinski, E.A., and Quinn, P.G. (1999). The CREB constitutive activation domain interacts with TATA-binding protein-associated factor 110 (TAF110) through specific hydrophobic residues in one of the three subdomains required for both activation and TAF110 binding. J. Biol. Chem. *274*, 11672–11678.

Felinski, E.A., Kim, J., Lu, J., and Quinn, P.G. (2001). Recruitment of an RNA polymerase II complex is mediated by the constitutive activation domain in CREB, independently of CREB phosphorylation. Mol. Cell. Biol. *21*, 1001–1010.

Ferreri, K., Gill, G., and 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.

Fink, J.S., Verhave, M., Kasper, S., Tsukada, T., Mandel, G., and Goodman, R.H. (1988). The CGTCA sequence motif is essential for biological activity of the vasoactive intestinal peptide gene cAMP-regulated enhancer. Proc. Natl. Acad. Sci. USA *85*, 6662–6666.

GuhaThakurta, D., and Stormo, G.D. (2001). Identifying target sites for cooperatively binding factors. Bioinformatics *17*, 608–621.

Hoh, J., Jin, S., Parrado, T., Edington, J., Levine, A., and Ott, J. (2002). The p53MH algorithm and its application in detecting p53-responsive genes. Proc. Natl. Acad. Sci. USA *99*, 8467–8472.

Horikoshi, M., Hai, T., Lin, Y., Gree, M.R., and Roeder, R.G. (1988). Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. Cell *54*, 1033–1042.

Lonze, B., and Ginty, D. (2002). Function and regulation of CREB family transcription factors in the nervous system. Neuron *35*, 605.

Mayr, B., and Montminy, M. (2001). Tanscriptional regulation by the phosphorylation dependent factor CREB. Nat. Rev. Mol. Cell. Biol. *2*, 599–609.

Menssen, A., and Hermeking, H. (2002). Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes. Proc. Natl. Acad. Sci. USA *99*, 6274–6279.

Montminy, M.R., Sevarino, K.A., Wagner, J.A., Mandel, G., and Goodman, R.H. (1986). Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. Proc. Natl. Acad. Sci. USA *83*, 6682–6686.

Nakajima, T., Uchida, C., Anderson, S.F., Parvin, J.D., and Montminy, M. (1997). Analysis of a cAMP-responsive activator reveals a two-component mechanism for transcriptional induction via signal-dependent factors. Genes Dev. *11*, 738–747.

Nordeen, S. (1988). Luciferase reporter gene vectors for analysis of promoters and enhancers. Biotechniques *6*, 454–458.

Saluja, D., Vassallo, M., and Tanese, N. (1998). Distinct subdomains of human TAFII130 are required for interactions with glutamine-rich transcriptional activators. Mol. Cell. Biol. *18*, 5734–5743.

Schuldiner, O., Shor, S., and Benvenisty, N. (2002). A computerized database-scan to identify c-MYC targets. Gene *292*, 91–99.

Schumacher, M.A., Goodman, R.H., and Brennan, R.G. (2000). The structure of a CREB bZIP.somatostatin CRE complex reveals the basis for selective dimerization and divalent cation-enhanced DNA binding. J. Biol. Chem. *275*, 35242–35247.

Smale, S. (2001). Core promoters: active contributors to combinatorial gene regulation. Genes Dev. *15*, 2503–2508.

Su, A.I., Cooke, M.P., Ching, K.A., Hakak, Y., Walker, J.R., Wiltshire, T., Orth, A.P., Vega, R.G., Sapinoso, L.M., Moqrich, A., et al. (2002). Large-scale analysis of the human and mouse transcriptomes. Proc. Natl. Acad. Sci. USA *99*, 4465–4470.

Suzuki, Y., Yamashita, R., Nakai, K., and Sugano, S. (2002). DBTSS: dataBase of human transcriptional start sites and full-length cDNAs. Nucleic Acids Res. *30*, 328–331.

Tinti, C., Yang, C., Seo, H., Conti, B., Kim, C., Joh, T.H., and Kim, K.S. (1997). Structure/function relationship of the cAMP response element in tyrosine hydroxylase gene transcription. J. Biol. Chem. *272*, 19158–19164.

Ueda, H.R., Chen, W., Adachi, A., Wakamatsu, H., Hayashi, S., Takasugi, T., Nagano, M., Nakahama, K., Suzuki, Y., Sugano, S., et al. (2002). A transcription factor response element for gene expression during circadian night. Nature *418*, 534–539.

Wang, J., and Wilkinson, M. (2000). Site-directed mutagenesis of large (13-kb) plasmids in a single PCR procedure. Biotechniques *29*, 976–978.

Wynshaw-Boris, A., Short, J.M., Loose, D.S., and Hanson, R.W. (1986). Characterization of the phosphoenolpyruvate carboxykinase (GTP) promoter-regulatory region. I. Multiple hormone regulatory elements and the effects of enhancers. J. Biol. Chem. *261*, 9714–9720.

Yamamoto, K.K., Gonzalez, G.A., Biggs, W.H., III, and Montminy, M.R. (1988). Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. Nature *334*, 494–498.

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Molecular Cell
Volume 11, Issue 5, May 2003, Page 1417

DOI: https://doi.org/10.1016/S1097-2765(03)00199-0

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Erratum

Genome-Wide Analysis of CREB Target Genes Reveals A Core Promoter Requirement for cAMP Responsiveness

In this article (Molecular Cell *11*, 1101–1108, April 2003), several of the authors' affiliations were not attributed correctly, and John Hogenesch's correspondence information was omitted. The corrected list of authors and affiliations appears below.

Michael D. Conkright,[1,3] Ernesto Guzmán,[1,3]
Lawrence Flechner,[1] Andrew I. Su,[2]
John B. Hogenesch,[2,*] and Marc Montminy[1,*]
[1]Salk Institute for Biological Studies
La Jolla, California 92037
[2]The Genomics Institute of the Novartis Research Foundation
San Diego, California 92121

*Correspondence: jhogenesch@gnf.org; montminy@salk.edu
[3]These authors contributed equally to this work.