Interactions between p300 and Multiple NF-Y Trimers Govern Cyclin B2 Promoter Function*

Valentina Salsi‡, Giuseppina Caretti‡, Mark Wasners§, Wibke Reinhard§, Ulrike Haugwitz§, Kurt Engeland‡, and Roberto Mantovani‡¶¶

From the ‡Dipartimento di Biologia Animale, Università di Modena e Reggio, Via Campi 213/d, 41100 Modena, Italy and ¶¶Medizinische Klinik II, Max-Bürger-Forschungszentrum, Universität Leipzig, Johannisallee 30, D-04103 Leipzig, Germany

The CCAAT box is one of the most common elements in eukaryotic promoters and is activated by NF-Y, a conserved trimeric transcription factor with histone-like subunits. Usually one CCAAT element is present in promoters at positions between −60 and −100, but an emerging class of promoters harbor multiple NF-Y sites. In the triple CCAAT-containing cyclin B2 cell-cycle promoter, all CCAAT boxes, independently from their NF-Y affinities, are important for function. We investigated the relationships between NF-Y and p300. Chromatin immunoprecipitation analysis found that NF-Y and p300 are bound to the cyclin B2 promoter in vitro and that their binding is regulated during the cell cycle, positively correlating with promoter function. Cotransfection experiments determined that the coactivator acts on all CCAAT boxes and requires a precise spacing between the three elements. We established the order of in vitro binding of the three NF-Y complexes and find decreasing affinities from the most distal Y1 to the proximal Y3 site. Binding of two or three NF-Y trimers with or without p300 is not cooperative, but association with the Y1 and Y2 sites is extremely stable. p300 favors the binding of NF-Y to the weak Y3 proximal site, provided that Y1 and Y2 sites are extremely stable. NF-YB and NF-YC are tightly bound through histone-fold motifs, whose formation is required for NF-YA association and sequence-specific DNA binding. Circular permutation assays have clearly shown that NF-Y bends DNA and organizes the three-dimensional architecture of promoters; it is understood that NF-Y promotes the binding of neighboring trans-activators and makes connections with TFIID, contacting several of the TAFs (4). Moreover, NF-Y is able to interface with a well-positioned nucleosome on the major histocompatibility complex class II Ea promoter and with a chromatin-reconstituted topoisomerase IIα promoter (5, 6).

Coactivators are a large and heterogeneous family of non-DNA-binding proteins that make use of the platforms represented by DNA binding factors to access promoters. In general, coactivators are thought to serve as a bridge for transcription factors and holoenzyme interactions, having been recruited to promoters through activation domains. Many coactivators, such as CBP/p300, PCAF, GCN5, possess an enzymatic activity, histone acetyltransferase, that adds an acetyl group to lysines of the N-terminal ends of the core histones (7). The enzymatic activity is apparently essential for activation function in many, but not in all systems tested (8–10). In addition to histones, coactivators also target transcription factors, influencing different aspects of their functions, such as DNA binding affinity, nuclear localization, or retention (for review, see Ref. 11). The role of p300/CBP in control of cell growth and differentiation has been studied in many systems (12). p300/CBP exerts a profound effect on cell cycle control, as exemplified by the finding that E1A mutants that cannot bind to p300 exhibit defective cellular transformation (13). The p300/CBP-PCAF protein complex might regulate target genes that are involved in controlling the G1/S transition, such as p21\(^{\text{WAF1}}\) (14). The overexpression of E1A, whose binding to p300/CBP antagonizes PCAF association, drives cells into S phase (15). p300−/− and cbp−/− knockout mice have provided evidence that p300/CBP proteins are important for cell cycle regulation and differentiation (16).

Because NF-Y is important for the activation of many promoters, it was natural that connections between this factor and coactivators emerged. Affinity columns identified interactions

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¶ To whom correspondence should be addressed. Tel.: 39-059-2055542; Fax: 39-059-2055548; E-mail: mantor@mail.unimo.it.

The abbreviations used are: EMSA, electrophoretic mobility shift assay; CBP, cAMP-response element-binding protein (CREB)-binding protein; wt, wild type; CMV, cytomegalovirus; ChIP, chromatin immunoprecipitation.

2 F. Romani and R. Mantovani, unpublished information.
of the histone-fold motif subunits with human GCN5 (17); NF-Y-PCAF interactions mediate the potent activation of the multi-drug resistance-1 promoter upon treatment of human cells with trichostatin A, and binding to p300 activates the HSF70 promoter in Xenopus oocytes in the absence of heat shock or the addition of heat shock factor-1 (18, 19). Among the many genes activated by NF-Y, an emerging class is represented by those regulated differently during the cell cycle, particularly in the G$_2$/M phase. Key regulators such as CDC25A/B/C, cyclin B1/B2, Cdc2, and topoisomerase IIa contain multiple CCAAT boxes in their promoters, invariably shown to be crucial for the proper regulation of these genes (Ref. 2 and references therein; Refs. 20–26).

Cyclin B is a central regulator for progression from G$_2$ to mitosis. It associates with the Cdc2 cyclin-dependent kinase 1 and thereby regulates phosphorylation of target proteins (27). Complex formation between cyclin B and Cdc2 is essential for G$_2$/M transition. In mammalian cells cyclin B exists in two isoforms, cyclin B1 and cyclin B2 (28). We have previously analyzed expression from the cyclin B2 promoter and found NF-Y to be the major activator (21). Cell cycle-dependent transcription is mediated by a CDE-CHR repressor element (29). Three NF-Y binding CCAAT boxes in the proximal promoter spaced 33-bp apart are responsible for more than two-thirds of the total activity of the cyclin B2 promoter. A large difference in affinity to NF-Y was detected among these three CCAAT elements, with the proximal Y3 binding NF-Y with very low affinity, yet they all equally contributed to the full transcriptional activity of the promoter (21). This raised the possibility that NF-Y binding is cooperative, either directly, through interactions with intermediate factors such as coactivators, or else that another factor, for which there is no evidence at the moment, might bind and activate Y3. To solve this matter, we present data that dissect the interplay between NF-Y and p300.

**MATERIALS AND METHODS**

**Analysis of Mutants with Changed Spacing between CCAAT Boxes**—Plasmids derived from the firefly luciferase-expressing wild type cyclin B2 promoter construct B2-Luc (21, 29) were created by PCR-based targeted mutagenesis yielding constructs with insertions or deletions between different CCAAT boxes: Y$_1$–2Plus, CCAATCAGCTGCGACAAGGCTGACCTCGAGTGCTCTGGCGCCCTGC-CTGCGGTTCCTACCAAT; Y$_1$–2Del, CCAATCAACGCTGCCCACTGCATGGCTGCTCTGGCGCCCTGC-CTGCGGTTCCTACCAAT; Y$_2$–3Plus, CCAATCAACGCTGCGACAAGGCTGACCTCGAGTGCTCTGGCGCCCTGC-CTGCGGTTCCTACCAAT; Y$_2$–3Del, CCAATCAACGCTGCGACAAGGCTGACCTGCATGGCTGCTCTGGCGCCCTGC-CTGCGGTTCCTACCAAT. Fragments were incubated with NF-Y alone or in combination with p300 (purified mouse anti-p300 14991A was from Pharmingen). For footprinting assays, the wt and distance mutant cyclin B2 triple CCAAT fragments were incubated with NF-Y alone or in combination with p300 (200–400 ng) under the same conditions as in the EMSAs. After the addition of 5 μg CaCl$_2$ samples were treated with DNaI, extracted with phenol/ether, precipitated, and analyzed.

**RESULTS**

**p300 Is Bound to the Cyclin B2 Promoter in Vivo**—The chromatin immunoprecipitation (ChIP) technique is a valuable system to detect the binding of transcription factors and coactivators to promoter sequences. We immunoprecipitated chromatin derived from exponentially growing NIH3T3 cells with anti-NF-YB, anti-NF-YC, and anti-p300, and PCR amplifications with cyclin B2-specific oligonucleotides detected the target promoter in the NF-Y immunoprecipitations (not shown); this is expected from the previously detected in vitro binding of NF-Y to the CCAAT boxes and activity of the NF-YA dominant negative vector (21). Next we wished to determine the association of NF-Y and p300 to the cyclin B2 promoter during the cell cycle. NIH3T3 cells were serum-starved and restimulated. Chromatin was prepared at different time points from these cells, and ChIPs were performed. Fig. 1 shows that G$_0$ cells have no NF-Y or p300 on the promoter. The two proteins do not associate with the cyclin B2 promoter until after 18 h of restimulation, when most cells are completing S phase and cyclin B2 expression starts. At 24 h, where most cells are in G$_2$/M, NF-Y and p300 are still largely bound. As a control for earlier time points, we checked a promoter, JunB, which is rapidly

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**FIG. 1. Binding of p300 and NF-Y to the cyclin B2 promoter in vivo.** Chromatin immunoprecipitations were performed with NIH3T3 mouse fibroblasts using the indicated antibodies. NIH3T3 serum-starved cells (G$_0$) were used as well as cells at 6, 12, 18, and 24 h post-restimulation by the addition of 10% fetal calf serum.Ctl, control.
induced by serum, and found NF-Y and p300 bound in G0 cells (Data not shown). We conclude that NF-Y and p300 association to the cyclin B2 promoter is regulated during the cell cycle, positively correlating with transcriptional activation of the gene.

Transcriptional Activation of the Cyclin B2 Promoter by p300—Because of the p300 interactions with the cyclin B2 promoter observed in ChIP assays in vivo, we decided to investigate their role in the transcriptional regulation of this gene. We cotransfected a p300 expression vector and the cyclin B2-luciferase reporter (21) in NIH3T3 cells. In reporter assays we observed a reproducible, although not spectacular, activation of transcription (Fig. 2A) that is well in line with numerous reports of similar experiments performed in other promoter systems (8–10). To verify the CCAAT dependence of the p300 activation, we used several mutant constructs with alterations in the Y1, Y2, and/or Y3 pentanucleotides described previously (21): Fig. 2A shows that compared with the wt cyclin B2, all mutant constructs are variously crippled in transcription and, most importantly, in p300 activation. In particular, mutation of the Y1/Y2 high affinity NF-Y sites and change of the all three Y1–3 boxes are essentially refractory to the activity of p300 overexpression. Additionally, mutation of Y3, which is a poor NF-Y binding site, is also severely down in p300-stimulated transcription.

It is well known that correct spacing between different transcription factors is a crucial aspect in promoter proficiency. The three CCAAT boxes of the cyclin B2 promoter are aligned, with a conserved spacing of 33 base pairs between them. Because the presence of all three CCAAT boxes is important for optimal transcription and for p300 activation, we figured that cyclin B2 transcriptional regulation represents a good system to study the role of activator alignment for p300 function. The distance between the three CCAAT sites is 33 base pairs, which represents an almost perfect alignment of the three binding elements on the same side of the double helix. We derived a set of mutants in which the spacing between Y1/Y2 and Y2/Y3 was altered, either by eliminating 10 base pairs, 1 turn of the double helix, or adding 5 base pairs. In the first case one expects to be at the limit of simultaneous NF-Y/CCAAT binding, based on experiments performed on the double CCAAT boxes of the human γ-globin promoter (32). In the second, the two NF-Y sites would be positioned on opposite sides of the DNA helix (see the scheme in Fig. 2B). These mutants were tested in the cotransfection assays used above with and without p300 overexpression. Fig. 2B shows that all distance mutants exhibited decreased activity, about 3/4-fold lower than wt, even without p300 cotransfection. More importantly, p300-dependent activation was reduced by about 2/3-fold but not completely abolished. Interestingly, the most affected mutant was Y2/Y3Plus, which is expected to affect the interactions between the two lowest affinity NF-Y sites. Overall, these data indicate that p300 activates cyclin B2 transcription by acting on intact and correctly spaced CCAAT boxes. Despite their differences in relative NF-Y affinities, all three CCAAT elements are important for p300-dependent activation.

Association of p300 to Multiple NF-Y/CCAAT Complexes—The results obtained with in vivo assays prompted us to set up an in vitro system with recombinant proteins to dissect the interplay between p300 and the NF-Y binding to the three CCAAT boxes in the cyclin B2 promoter. Recombinant NF-Y proteins were purified from E. coli, and p300 was purified from baculovirus. These reagents were employed in EMSAs. It should be noted that NF-Y-p300 direct interactions in solution were described in Xenopus (18), but we found no evidence of such interactions under our immunoprecipitation conditions.3

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Initially, we wished to test the possibility that p300 directly binds to DNA-bound NF-Y. A cyclin B2 fragment of 200 base pairs containing the three Y boxes was labeled and used with increasing amounts of NF-Y in the absence or presence of 20 ng of p300 (Fig. 3A, lanes 1–4 and 5–8, respectively). As expected, multiple bands are generated with NF-Y, corresponding to the formation of complexes with one, two, or three trimers bound to the Y boxes (see below). Two types of information were manifest from this experiment. (i) At high NF-Y concentrations a slow migrating complex is observed only in the presence of p300 (compare lanes 4 and 8); (ii) at low NF-Y concentrations the addition of p300 induces an increase of double CCAAT binding (compare lanes 2 and 3 with lanes 6 and 7). In parallel, the same amount of proteins was incubated with probes of identical lengths containing mutations in either Y1 and Y2 (Fig. 3A, lanes 9–17) or all three CCAAT boxes (Fig. 3A, lanes 17–24). With the Y1/Y2m probe a single band was generated only at high NF-Y concentrations, representing a single NF-Y bound to the low affinity Y3. This complex is not affected by the addition of p300. With the Y1/Y2/Y3m probe, no interaction was observed in the presence or absence of p300 even at high NF-Y concentrations. To verify the effect of p300 addition, we performed supershift EMSAs; as shown in Fig. 3B, formation of the upper, slow migrating complex was partially inhibited by the addition of anti-p300 antibodies, with the appearance of the non-p300 complexed NF-Y single and double complexes (compare lane 4 with lane 2). A the same time, the addition of anti-p300 had no effect on the NF-Y complexes, much like an irrelevant anti-Gata antibody on the NF-Y-p300 complexes (Fig. 3B, lanes 3 and 5). Having detected a complex of discrete mobility between NF-Y and p300 on the wt cyclin B2 probe, we used a recombinant NF-Y trimer deleted of domains outside of the homology domains of NF-YA/B/C: Fig. 3C shows that unlike wt NF-Y, which can be bound by p300, the YA9-YB4-YC5 mutant (33, 34) is incapable of doing so.

Taken together these results indicate that a complex can be formed in vitro between NF-Y and p300 provided that (i) multiple CCAAT boxes are present and bind the bridging NF-Y, and (ii) domains outside the histone-fold motifs of NF-YB/NF-YC and the conserved part of NF-YA are present. Binding of p300 apparently favors NF-Y-DNA interactions.

Lack of Cooperativity of the NF-Y/p300 Complex—Our analysis on the double CCAAT box of the γ-globin promoter suggested that if a certain spacing is respected (32 bp), then cooperative binding of NF-Y molecules is possible, mainly thanks to the presence of Q-rich regions of NF-YA and NF-YC. In other systems, NF-Y is capable of improving the DNA binding affinity of neighboring factors, forming extremely stable DNA-protein complexes (3). Because of the presence of a 33-bp spacing between all three Y boxes, we considered the possibility that three NF-Ys could bind DNA cooperatively; alternatively, cooperativity could be mediated by the association of the coactivator. To investigate this point, we performed off-rate EMSA experiments with the cyclin B2 probe used above. We incubated two different quantities of NF-Y in two sets of assays for 30 min, until the binding equilibrium was reached, then added a large ~200-fold excess of an unlabeled oligonucleotide containing the high affinity CCAAT box of the major histocompatibility complex class II Ea promoter. After the indicated periods of time, an aliquot of the sample was loaded on a running polyacrylamide gel. At low doses (Fig. 4A, lanes 1–5), under conditions where only single and double CCAAT binding is observed, the upper band, corresponding to Y1-Y2 occupation (see below), was rapidly competed (compare lanes 1–3), whereas the single interaction only slowly decreased. This behavior is not indicative of a cooperative effect, because in the latter case, we would have observed a slower decrease of the upper complex compared with the lower, single CCAAT binding activity. At higher NF-Y amounts (Fig. 4A, lanes 6–10), the triple interaction appeared. In this case, double and single CCAAT binding was still observed at late time points (lanes 9 and 10), but triple binding was rapidly competed, being minimal after 15 min (compare lanes 6–8). Because of the persistence of NF-Y binding at relatively late points (90 min in lanes 5 and 10), we extended the off-rates to 4, 8, 16, and 24 h, starting with NF-Y concentrations that generate triple binding. Fig. 4B shows that double and single, but not triple NF-Y binding, persisted after 24 h of incubation with the competing oligonucleotide. By comparison, we performed a parallel experiment in the absence of unlabeled competitor, which showed a slower off-rate for the triple complex (Fig. 4B, compare lanes 1–3 with 6–8). These latter experiments reinforce the notion that CCAAT binding by NF-Y to high, that medium affinity sites are stunningly sta...
ble, and that no cooperativity is observed in vitro with either Y1-Y2 or the low affinity Y3.

One possibility to explain the transfection data of Fig. 2 could be that cooperativity is mediated by p300. We therefore performed the same type of off-rate EMSAs in the presence of p300 using as a competitor a long oligonucleotide comprising the Y1-Y2 boxes (Fig. 4C, lanes 1–8) or a Y1 oligonucleotide (Fig. 4C, lanes 9–16). Results indicate that the addition of the coactivator had no effect on the stability of the NF-Y/p300 or NF-Y triple, double, or single complexes using the Y1 and Y2 (21) was used as a cold competitor; lanes 9–16 used the Y1 oligonucleotide.

EMSA and Footprinting Analysis of Distance Mutants between Y Boxes—The functional data on the distance mutants shown in Fig. 2 lead us to analyze the role of spacing on NF-Y interactions with and without p300. Initially, we verified the NF-Y binding capacity in EMSA experiments with probes containing the alterations described in Fig. 2. Results in Fig 5 show that, with the exception of Y1-Y2Del, all other mutants showed normal interactions with NF-Y. The apparent difficulty in forming double CCAAT box-NF-Y complexes observed with the latter mutant (Fig. 5, compare lane 3 with 1) was not unexpected, because the distance between Y1 and Y2 becomes 23 base pairs, which is the lower limit for simultaneous NF-Y binding (30).

Next we switched to footprinting analysis of these five probes after incubation with NF-Y and p300 (Fig. 6). Panel A shows that increasing concentrations of NF-Y progressively protects Y1 and Y2, but hardly Y3 (Fig. 6A, lanes 1–4). However, in the presence of p300, protections over Y2 and Y3 are observed already at lower NF-Y concentrations (Fig. 6A, compare lanes 2–4 with lanes 5–7). Furthermore, protection of Y3 is only observed with p300 (Fig. 6A, compare lanes 4 and 7). In addition to CCAAT protections, NF-Y and NF-Y/p300 also generated hypersensitive sites at the 5’ of Y1,
between Y1 and Y2 and between Y2 and Y3. Note that p300 alone was incapable of DNA binding (Fig. 6A, compare lanes 1 and 8 with lane 7). The same set of experiments was performed on the four mutant promoters; Fig. 6B shows the Y1-Y2Del probe, with weaker protections over Y2, no protection on Y3, and absence of hypersensitive sites between Y1-Y2 and Y2-Y3. On the other hand, the binding on Y1 is apparently normal (Fig. 6B, compare lanes 1–3 and 4–6 with lane 8). Fig. 6C shows the 1–2Plus probe, which has essentially wt-like patterns of NF-Y protections and hypersensitive sites, with a decrease of the hypersensitive site between Y2-Y3 (compare lanes 1–4 in Fig. 6A with lanes 1–3 in Fig. 6C); the addition of p300 has negligible effects on Y1 or Y2 binding but clearly decreases Y3 interactions (Fig. 6C, lanes 5–7 and 9). With the Y2–3Del probe, binding of Y3 and to a lesser extent Y2, but not Y1, is decreased; the Y2–3 hypersensitive site is also abolished (Fig. 6D). Finally, with the Y2–3Plus, binding of Y1 and Y2 is normal as well as the Y1–2 hypersensitive site, but Y3 binding is negligible; the addition of p300 has essentially no effect on NF-Y binding on Y3 but has a modest effect on Y2 (Fig. 6E, compare lanes 2–4 with lanes 5–7). Altogether, these results indicate p300 acts on NF-Y bound to Y1 and Y2 to increase the otherwise very low affinity for the Y3 CCAAT; alterations of the correct spacing between the Y1-Y2 or Y2-Y3 CCAAT boxes abolish the p300-facilitation effect.

**DISCUSSION**

In this study we investigated the relationship between NF-Y and p300 on the cyclin B2 promoter that is active in the G2M phase of the cell cycle. We found that p300 activates in a manner that requires a precise spacing between the three CCAAT boxes. Indeed, NF-Y and the coactivator are bound to the promoter in vivo in a cell cycle-dependent way. In vitro the order of binding of three NF-Y protein complexes is Y1 > Y2 >> Y3, with decreasing affinities from the most distal to the proximal site. The binding of two or three NF-Y molecules with or without p300 is not cooperative. However, p300 favors the association of NF-Y to the proximal site, and the distance between the three CCAAT boxes is crucial for this activity. We conclude that the precise alignment of multiple CCAAT boxes is crucial for coactivator function.

**NF-Y Binding to Multiple CCAAT Boxes**—A recent analysis of 1031 human promoters indicated that the CCAAT box is present in 65% of them (1). We have catalogued >500 CCAAT promoters regulated by NF-Y and found that the majority contain only one CCAAT box, either in the forward or reverse orientation. In general, NF-Y cooperates with neighboring factors to regulate gene expression. In inducible systems, such as those of the heat shock, endoplasmic reticulum stress response, and genes involved in cholesterol metabolism, NF-Y teams up with heat shock factor, ATF6, and sterol regulatory element binding protein. In the major histocompatibility complex class II genes setting, NF-Y cooperates with the regulatory factor X box trimer (Ref. 3 and references therein; Refs. 18 and 34). The mechanism of the cooperative effect has been studied and, baring the NF-Y-C/EBP connections on the albumin promoter, in all other cases NF-Y mediates a profound increase in DNA-binding affinity of the neighboring factor.

Alterations in the spacing between the CCAAT boxes and nearby sites provoke a decrease and sometimes abolition of the activation potential. In cell cycle-regulated promoters, NF-Y is essential for the timing of activation (35, 36) and repression (37, 38). In the case of G2/S promoters, NF-Y cooperates with E2Fs; in G0/M promoters NF-Y binding CCAAT boxes are found near the CDE-CHR element (29, 37–39). Biochemically, it is unclear at what level the cooperation is exerted. Many cell cycle promoters are peculiar in that they contain at least two and sometimes more NF-Y binding sites, in particular in promoters of key G2/M cell cycle regulators Cdc2, topoisomerase IIα, CDC25C, and cyclin B1/B2 (Table I). The distance between these elements is (i) highly conserved among species and (ii) relatively constant; in the case of CDC25C, three CCAAT boxes are spaced 32 bp apart, in cyclin B2 the distances are 33 bp, in
Cdc2 two CCAAT elements are spaced by 32 bp. Promoters for which both human and mouse sequences are available show that the sequences of NF-Y binding sites as well as their distance is strictly conserved. Especially in CDC25C and cyclin B2 mouse and human promoters nucleotides in between CCAAT boxes are not conserved, contrasting the sequence identity of and distance between NF-Y binding elements (32). In all the promoters mentioned above, the affinities of NF-Y for the individual CCAAT sites differ; in CDC25C one of the CCAAT boxes is not even a perfect pentanucleotide. In cyclin B2 it is apparent that between Y1 and Y3 there is a 50-fold difference in binding affinity, yet the integrity of Y3 is as important as the high affinity Y1 binding in terms of function. The cyclin B2 systems is also conspicuous for another reason; that is, the NF-Y sites are apparently sufficient on their own to activate this promoter essentially without the need of additional factors. This is unlike the other systems tested so far, in which NF-Y is unable to activate alone. In a previous study on the γ-globin promoter, we found that NF-Y binding to double CCAAT boxes spaced by 27 nucleotides was not cooperative unless an extra 5 nucleotides were added. In such a case, a complex formed by two NF-Y trimers bound to the two sites was much more stable in off-rate experiments thanks to the extra nucleotide present between the cyclin B2 CCAAT boxes or to the intervening sequences. Nevertheless, it is clear to the extra nucleotide present between the cyclin B2 CCAAT boxes that that the sequences of NF-Y binding sites as well as their distance is strictly conserved. Especially in CDC25C and cyclin B2 mouse and human promoters nucleotides in between CCAAT boxes are not conserved, contrasting the sequence identity of and distance between NF-Y binding elements (32).

DNA in the nucleosomal structure (30, 39, 40). The stunningly slow off-rates observed in this study are a premiere for sequence-specific transcription factors and can only be compared with the highly stable nucleosomal structures. Indeed, two of the NF-Y subunits, NF-YB and NF-YC, have histone-like features, as predicted from amino acid alignments, with the highly stable nucleosomal structures. Indeed, two of the NF-Y subunits, NF-YB and NF-YC, have histone-like features, as predicted from amino acid alignments, with the highly stable nucleosomal structures.
acetyltransferase enzymatic activity. We find that the positive role of p300 on NF-Y function is exerted through an increase in DNA affinity, mostly observed on the weak Y3 binding site. To the best of our knowledge, this is the first such demonstration for p300 binding on multiple sites for the same factor. Essentially three types of action are supposed to be exerted by p300/CBP: (i) the protein serves as a platform, a bridge, through which the direct interactions with multiple DNA binding activators are supported; recruitment of p300/CBP stabilizes the otherwise weak binding of these factors or even makes them possible. In keeping with this, interactions of p300/CBP with many transcription factors have been mapped in one and sometimes multiple subdomains of the coactivators. Evidence for this mechanism is still largely circumstantial, and the biochemical dissection of this mechanism on DNA was obtained only on the β-interferon promoter (43). (ii) Once on a promoter, p300/CBP proteins modify the chromatin structures nearby the sites by virtue of their histone acetylation activity, rendering nucleosomes more “accessible” to the general transcription apparatus. (iii) The same histone acetyltransferase activity would be used to increase the affinity of the DNA binding factor for the targeted sequence. This latter property is less well understood. In many cases, in fact, the opposite happens; acetylation of high mobility group(I) inhibits formation of the enhanceosome on the β-interferon promoter (43). In the case of p53, whose function is positively affected by p300, acetylation apparently affects recruitment of p300/CBP (44). Interestingly, in keeping with these latter results, we find that p300 acylates NF-YB and that this modification increases NF-Y-p300 interactions. In many promoters, the histone acetyltransferase activity of p300/CBP is apparently dispensable (8–10); p300/CBP and PCAF cooperate with members of the MyoD family of muscle transcription factors in modulating the expression of downstream myogenic factors, including myogenin and MEF2, leading to terminal withdrawal from the cell cycle of myotubes (45, 46). The p300 histone acetyltransferase domain is dispensable for MyoD-dependent transcription, suggesting that the “bridging” mechanism is predominant in this case.

One important result that stems from the ChIP analysis is that the cyclin B2 promoter is devoid of NF-Y and p300 in cells arrested in G0, and the two activators become bound only in that the cyclin B2 promoter is devoid of NF-Y and p300 in cells of cycling cells as compared with Y3 (21). The presence of two or three molecules of NF-Y tends to the general transcriptional machinery. It is also possible that the low affinity for NF-Y at Y3 has evolved by fluctuation of the sequences flanking CCAAT to create the possibility of an on-off system, less likely to happen on high affinity sites such as Y1. This type of mechanisms might not be restricted to the cyclin B2 promoter but rather a constant for cell cycle and, in general, growth-regulated promoters.

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