The two activation domains of the CCAAT-binding factor CBF interact with the dTAF_{II}110 component of the *Drosophila* TFIID complex

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The CCAAT-binding factor CBF is a heterotrimeric transcription factor that specifically binds to CCAAT sequences in many eukaryotic genes. Previous studies have shown that CBF contains two transcription activation domains: a glutamine-rich, serine-threonine-rich domain present in the CBF-B subunit and a glutamine-rich domain in the CBF-C subunit. In this study, by using a series of deletion mutations of CBF-B and CBF-C in transcription assay *in vitro*, we further delineated smaller segments in these domains that were sufficient to support transcriptional activation by CBF. To test whether transcription activation by CBF requires co-activators, we examined the interaction between CBF and dTAF_{II}110, a component of the *Drosophila* TFIID complex. Recent work has demonstrated that

glutamine-rich domains of the Sp1 transcription factor interact with dTAF_{II}110 and that this interaction has an important role in mediating transcription activation. Here we first demonstrate in a direct interaction assay *in vitro* that CBF binds dTAF_{II}110. By using a yeast two-hybrid system we show that both of the transcription activation domains of CBF interact with dTAF_{II}110. A deletion analysis suggests that a segment of CBF-B needed for transcription activation is also involved in interaction with dTAF_{II}110. In CBF-C the C-terminal portion of the molecule seems to be needed for these two activities. Our results suggest that TAF_{II}110 might represent one of the co-activators that mediate transcriptional activation by CBF.

INTRODUCTION

The CCAAT-binding factor CBF (also named NF-Y and CP1) is a heteromeric factor composed of three subunits, CBF-A, CBF-B and CBF-C, that specifically binds to CCAAT sequences in many eukaryotic genes [1–3]. We have shown that all three subunits are needed for DNA binding and that the three polypeptides are present in the CBF–DNA complex [4,5]. The specific segments of CBF-A, CBF-B and CBF-C that are sufficient for subunit interactions and formation of the CBF–DNA complex show a high degree of sequence identity with the yeast HAP3, HAP2 and HAP5 polypeptides respectively [6–8]. The conserved segments of CBF-A and CBF-C show sequence similarity with the histone-fold motifs of histone H2B and H2A respectively [8,9]. Among the proteins containing a histone-fold motif, several are engaged in both protein–protein and protein–DNA interactions, being part of multimeric assemblies similar to those observed with the core histone. Transcriptional activation functions are specified by domains outside the DNA-binding domain. We previously identified two sequences in CBF, a glutamine-rich, serine-threonine-rich domain in CBF-B and a glutamine-rich domain in CBF-C, that activated transcription *in vivo* in a heterologous system that used fusion polypeptides [10,11]. We also demonstrated in transcription assay *in vitro* that the activation of transcription was mediated by these two domains and that they activated transcription additively [11].

CBF binds CCAAT motifs that are usually located between –60 and –100 bp 5' to the start site of transcription [12], but the mechanism by which CBF regulates expression of genes is still unknown. Protein–protein contacts between activation domains of transcriptional activators and TATA box-binding protein (TBP)-associated factors (TAFs) that are components of the general transcription machinery have been shown to have important roles in transcription activation [13,14]. The CBF ac-

tivation domains are rich in glutamine and hydrophobic residues. They show sequence similarities with each other and with the glutamine-rich activation domains of Sp1 and of the cAMP-response element-binding protein (CREB) [11]. The association of dTAF_{II}110 with Sp1 or CREB is important for mediating transcription by these activators [15–17].

In this study we used transcription system *in vitro* to delineate the activation domain of CBF to a glutamine-rich segment in both CBF-B and CBF-C. We then examined the interaction between dTAF_{II}110 and CBF. Using interaction experiments both *in vitro* and *in vivo* we show that CBF interacts with dTAF_{II}110.

EXPERIMENTAL

Generation of recombinant proteins

CBF-A/CBF-C, CBF-A/CBF-Cd, CBF-B and CBF-Bd cDNA species were obtained as described previously [10,11]. CBF-B1, CBF-B2 and CBF-B3 were obtained by inserting PCR fragments of CBF-B encoding amino acid residues 3–112, 68–167 and 112–224 between the *Bam*HI and *Eco*RI sites in pGEX-4T-3, in which CBF-B 225–343 had already been cloned between the *Eco*RI and *Xho*I sites. CBF-C1, CBF-C2 and CBF-C3 were obtained by cloning PCR fragments of CBF-C encoding residues 135–235, 185–285 and 235–335 in CBF-A/CBF-C cDNA [11] between the *Sac*II and *Xho*I sites, replacing the existing sequences. The recombinant proteins were expressed in *Escherichia coli* as fusion polypeptides with glutathione S-transferase and purified as described previously [10]. The amount of proteins was determined by the Bio-Rad protein assay, and the purity of the eluted proteins was examined by SDS/PAGE [10% (w/v) gel] and Coomassie Blue staining. The recombinant truncated proteins, CBF-B1, B2, B3 and CBF-AC1, AC2 AC3, had a high degree of purity, similar to that of full-length recombinant CBF subunits used previously in our experiments [10,11].

Abbreviations used: CBF, CCAAT-binding factor; CREB, cAMP response element-binding protein; TAF, TBP-associated factor; TBP, TATA box-binding protein; X-Gal, 5-bromo-4-chloroindol-3-yl β -D-galactopyranoside.

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Figure 1 Delineation of the activation domain of CBF-B by transcription experiments *in vitro*

(A) Schematic representation of CBF-B deletion mutants. CBF-B is subdivided into several domains: glutamine-rich domain (residues 3–160), serine-threonine-rich domain (residues 161–260) and conserved domain (residues 261–343). (B) Comparison of DNA binding by various combinations of truncated CBF-B with full-length CBF-A/CBF-C. Total protein (50 ng) was present in each assay. Reaction mixtures were fractionated by PAGE and the DNA–protein complexes were detected by autoradiography. (C, D) Transcriptional activation of the pFC1 DNA template by 200 ng of various deletions of CBF-B complemented with 200 ng of CBF-A/CBF-C by using CBF-depleted EL4 nuclear extracts. The newly synthesized RNA species were reverse transcribed by primer extension and the products were analysed by autoradiography after PAGE. The p120 DNA template containing an $\alpha 1(\text{III})$ collagen gene promoter was added as an internal control to all reactions; the activity of this promoter is not affected by CBF. (E) Transcriptional activities were quantified by PhosphorImager (Molecular Dynamics) analysis and are reported relative to the full-length CBF, which represents 100% activation. Activities of the $\alpha 2(\text{I})$ collagen promoter were normalized by using the activity of the $\alpha 1(\text{III})$ collagen promoter as a reference.

Nuclear extracts

Mouse lymphoma EL4 cells were grown in suspension in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) horse serum at a density of 1.5×10^6 cells/ml. Nuclear extracts were prepared as described previously [18], except that the concentration of KCl in buffer A was raised to 50 mM. Nuclear extracts were depleted of CBF by mixing with a DNA affinity resin in which the DNA sequence of the mouse MHC class II gene (from –81 to –35) [10] was linked to Sepharose. Extracts (450 μl) were incubated with 180 μl of affinity resin at 4 °C for 30 min. The supernatant was centrifuged at 1200 g; recovered, divided into small aliquots, and stored at –80 °C.

DNA binding assay

DNA binding was performed as described before [10] in a final volume of 20 μl by incubating 5 fmol of ^{32}P -end-labelled double-stranded oligonucleotide with 50 ng of the various combinations of recombinant subunits of CBF.

DNA templates for transcription *in vitro*

pFC1 contains sequences of the mouse pro $\alpha 2(\text{I})$ collagen gene promoter, including a CBF-binding site multimerized four times [10]. p120 contained a segment of the mouse $\alpha 1(\text{III})$ collagen gene [19]. Transcription assays were performed *in vitro* as described previously [10].

Expression of TAF110

An *NdeI*–*BamHI* fragment, obtained from pAS1-TAF110 [15], was cloned in a pCITE-2a expression vector between the *NdeI* and *BamHI* sites. A [^{35}S]methionine-labelled 771-residue TAF110 polypeptide was synthesized *in vitro* in a reticulocyte lysate (single tube protein system 2; Novagen).

Interaction assay *in vitro*

DNA-affinity resin (15 μg), in which the DNA sequence from –81 to –35 of the mouse MHC class II Ea gene was covalently linked to Sepharose, was equilibrated with CBTA buffer [25 mM

Figure 2 Delineation of the activation domain of CBF-C by transcription experiments *in vitro*

(A) Schematic representation of CBF-C deletion mutants. CBF-C is subdivided into a conserved domain (residues 1–139) and a glutamine-rich domain (residues 140–335). (B) Comparison of DNA binding by various forms of truncated CBF-C with full-length CBF-A/CBF-B. Total protein (50 ng) was present in each assay. Reaction mixtures were fractionated by PAGE and the DNA–protein complexes were detected by autoradiography. (C, D) Transcriptional activation of the pFC1 DNA template by 200 ng of various deletions of CBF-A/CBF-C complemented with 200 ng of CBF-B by using CBF-depleted EL4 nuclear extracts. The newly synthesized RNA species were reverse transcribed by primer extension and the products were analysed by autoradiography after PAGE. The p120 DNA template containing an α (III) collagen gene promoter was added as an internal control to all reactions; the activity of this promoter is not affected by CBF. (E) Transcriptional activities were quantified by PhosphorImager (Molecular Dynamics) analysis and are reported relative to the full-length CBF, which represents 100% activation. Activities of the α 2(I) collagen promoter were normalized by using the activity of the α 1(III) collagen promoter as a reference.

Hepes (pH 7.9)/20% (v/v) glycerol/0.05% NP40/1 mM dithiothreitol/0.2 mM EDTA/0.5 mM PMSF] containing 100 mM KCl. The resin was then incubated for 1 h at 4 °C either with CBTA buffer alone or 2 μ g of CBFA/C, 2 μ g of CBF-B or 2 μ g of CBFA/C plus CBF-B in CBTA buffer. Resin samples were washed three times, once with CBTA buffer and twice with CBTA buffer containing 50 mM KCl. Resin samples were then incubated overnight at 4 °C with CBTA buffer plus 50 mM KCl containing 35 S-labelled TAF110. The samples were then washed twice with 500 μ l of CBTA plus 50 mM KCl or 500 μ l of CBTA plus 200 mM KCl, and the proteins were eluted with 15 μ l of SDS buffer [50 mM Tris/HCl (pH 6.8)/2% (w/v) SDS/10% (v/v) glycerol/100 mM dithiothreitol]. The eluted proteins were analysed by SDS/PAGE. The gel was fixed, treated with Amplify (Amersham) and exposed for autoradiography.

Yeast two-hybrid system

The yeast strain Y190 (a gift from S. Elledge, Baylor College of Medicine, Houston, TX, U.S.A.), which contains a *Lac-Z*

reporter gene under the control of Gal4 binding sites, was co-transformed with plasmids by using the method of Gietz et al. [20]. The Gal activation-domain hybrids were constructed in pACTII (obtained from S. Elledge). Different segments of CBF-B and CBF-C, obtained by PCR, were inserted in frame with the Gal4 activation-domain coding sequence. The pAS1-TAF110 plasmid expresses the Gal4 DNA-binding domain fused to full-length TAF110. pGAD+Sp1a, which encodes the Gal4 activation domain fused to the Sp1a domain, was used as a positive control in the two-hybrid system. Both plasmids were provided by Grace Gill (Harvard Medical School, Cambridge, MA, U.S.A.) [15]. After transformation the yeast strain was selected for 3 days at 30 °C on plates lacking the appropriate amino acids. The β -galactosidase activity was monitored by colony lift assay [21]. After yeast colonies had been transferred to a nitrocellulose membrane followed by freezing and thawing, the membranes were soaked in buffer containing 5-bromo-4-chloroindol-3-yl β -D-galactopyranoside (X-Gal). The β -galactosidase activity was estimated by the appearance of staining with X-Gal. In addition, quantitative β -galactosidase assays with 2-nitrophenyl β -D-galac-

topyranoside were performed as described [22]. This activity was normalized to the number of cells assayed as estimated by attenuation.

RESULTS

Delineation of the activation domains of CBF by transcription experiments *in vitro*

To delineate further which segments of the activation domains in CBF-B and CBF-C are directly involved in the activation of transcription, we generated several truncated forms of CBF-B and CBF-C. CBF-B represents the full-length B subunit, whereas CBF-Bd is a minimal form of the B subunit containing only the conserved sequence necessary for CBF-DNA complex formation. The other constructs, CBF-B1, CBF-B2 and CBF-B3, contain the conserved sequence and a different segment of the glutamine-rich, serine-threonine-rich activation domain of CBF-B, residues 3–112, 68–167 and 112–224 respectively (Figure 1A). Each CBF-B deletion mutant bound with a similar efficiency to that of full-length CBF-B to an oligonucleotide containing a CBF-binding site, after complementation with full-length CBF-A/CBF-C (Figure 1B). We then tested the activity of the deletion mutants in transcription assay *in vitro*. The DNA template pFC1, which contains four copies of a CBF-binding site, was transcribed with nuclear extracts of the mouse cell line EL4, which had been previously depleted of endogenous CBF by incubation of the extracts with an affinity resin containing a CBF-binding site. The different deleted forms of CBF-B were added to the depleted nuclear extracts complemented with CBF-A and CBF-C. CBF-B1 and CBF-B2, complemented with full-length CBF-A/CBF-C, activated the transcription of pFC1 to the same level as that activated by full-length CBF-B (Figure 1C, lanes 1, 2 and 3; Figure 1E). In contrast, in the presence of CBF-B3 and full-length CBF-A/CBF-C, the level of transcriptional activity was decreased by half (Figure 1C, lane 4; Figure 1E). This decrease is similar to that seen with truncated CBF-Bd together with full-length CBF-A/CBF-C (Figure 1D, lane 2). The results indicate that the amino acid sequence between residues 68 and 112 of CBF-B is needed for CBF-B-mediated transcription activation.

As shown in Figure 2(A), CBF-C represents the full-length CBF-C, whereas CBF-Cd is the minimal form of CBF-C containing only the conserved sequence of CBF-C necessary for the formation of the CBF-DNA complex. CBF-C1, CBF-C2 and CBF-C3 contain the conserved sequence and various segments of the glutamine-rich activation domain of CBF-C, residues 237–335, 186–286 and 140–237 respectively. Each of these mutants bound DNA with equal efficiency (Figure 2B). CBF-C1 and CBF-C2, when complemented with full-length CBF-B and CBF-A, were able to activate transcription of pFC1 to the same level as that activated by full-length CBF-C (Figure 2C, lanes 1, 3 and 4; Figure 2E). In the reaction containing CBF-C3, the transcriptional activity was decreased to 40% compared with that obtained with full-length CBF-C (Figure 2C, lane 2; Figure 2E) and was similar to the activity of the minimal form CBF-Cd (Figure 2D). Thus the results indicate that the amino acid sequence between residues 237 and 286 of CBF-C is required for CBF-C-mediated transcription activation.

Interaction between heterotrimeric CBF and dTAF110 in an assay *in vitro*

One possible way in which the two CBF activation domains activate transcription is by interacting with co-activators. Co-activators function as intermediary factors between DNA-bound

Figure 3 CBF interacts with dTAF110 *in vitro*

Upper panel: DNA-affinity resin samples were incubated with the CBTA buffer (control) or recombinant CBF before incubation with ^{35}S -labelled dTAF110. Two separate wash conditions were used, either 50 mM KCl (lanes 1 and 2) or 200 mM KCl (lanes 3 and 4). The bound proteins were resuspended with SDS buffer, resolved by SDS/PAGE and analysed by autoradiography. The position of dTAF110 is indicated by the arrow. The numbers at the left correspond to the molecular masses (in kDa) of prestained protein markers. Lower panel: DNA-affinity resin samples were incubated with the CBTA buffer, CBF-A/CBF-C, CBF-B or CBF (CBF-A/CBF-C plus CBF-B) before incubation with ^{35}S -labelled dTAF110. After being washed with 200 mM KCl CBTA buffer, the bound proteins were resuspended with SDS buffer, resolved by SDS/PAGE and analysed by autoradiography. The position of dTAF110 is indicated by the arrow. The numbers at the left correspond to the molecular masses (in kDa) of prestained protein markers.

transcription factors and the basal transcription machinery. Recent studies have shown that the glutamine-rich domain of the Sp1 transcription factor interacts with dTAF110, a component of the *Drosophila* TFIID complex, and transcription studies *in vitro* have demonstrated that dTAF110 functions as a co-activator to mediate the Sp1-dependent activation of transcription. Because the two activation domains of CBF showed similarity with the activation domain of Sp1, we tested for interaction between CBF and dTAF110 by using an assay *in vitro*.

A DNA-affinity resin containing a CBF-binding site was incubated with recombinant CBF subunits. After incubation, the CBF-bound affinity resins were washed several times, then incubated with ^{35}S -labelled dTAF110. After three column-volume washes with either CBTA buffer plus 50 mM KCl (Figure 3, upper panel, lanes 1 and 2) or CBTA buffer plus 200 mM KCl (Figure 3, upper panel, lanes 3 and 4), the bound proteins were resuspended in SDS buffer and subjected to SDS/PAGE, after which the labelled dTAF110 was detected by autoradiography.

Figure 4 CBF-B and dTAF110 interact in yeast

Yeast bearing an integrated *GAL1-LacZ* reporter gene were transformed with plasmids encoding two different fusion polypeptides: various deletions of CBF-B activation domains fused to the acidic activation domain of Gal4, and dTAF110 fused to the DNA-binding domain of Gal4. Interactions between the two fusion proteins recruit the acidic activation domain to the promoter bearing Gal4-binding sites, resulting in expression of the β -galactosidase gene. The level of β -galactosidase activity was estimated both by staining with X-Gal in a colony lift assay and by a liquid assay. Staining with X-gal obtained after interaction between Sp1 and dTAF110 was considered as a positive control (blue staining appeared within 3 h). (+, blue staining appeared after 3 h; —, no colour). In the liquid assay, β -galactosidase activity was measured using 2-nitrophenyl β -D-galactopyranoside as substrate, normalized to the number of cells and reported relative to the positive control SP1A considered as 100. Abbreviation: nd, not determined.

For control experiments the labelled dTAF110 was also incubated with the unbound affinity resin (Figure 3, upper panel, lanes 1 and 3; Figure 3, lower panel, lane 1) or with affinity resin that had been preincubated with either CBF-B (Figure 3, lower panel, lane 3) or the CBFA/CBF-C heterodimer (Figure 3, lower panel, lane 2). In each of these experiments, the CBF-containing resin retained significantly more labelled dTAF110 (Figure 3, upper panel, lanes 2 and 4; Figure 3, lower panel, lane 4) than the control resins (Figure 3, upper panel, lanes 1 and 3; Figure 3, lower panel, lanes 1 to 3).

Interaction between the CBF activation domains and dTAF110 in a yeast two-hybrid system

We used a yeast two-hybrid method to determine whether each of the CBF activation domains interacts with dTAF110. Expression plasmids encoding various polypeptide sequences of either CBF-B or CBF-C fused to the activation domain of Gal4 were co-transformed with a second plasmid expressing a dTAF110-Gal4 DNA-binding domain fusion in yeast bearing an

integrated *GAL1-LacZ* reporter gene. When there is interaction between dTAF110 and CBF, the activation domain of Gal4 is recruited to the promoter containing the Gal4-binding site, resulting in the induction of β -galactosidase gene expression (Figures 4A and 5A). CBF-B1-184, which contains the entire glutamine-rich domain of CBF-B fused with Gal4, induces β -galactosidase expression when co-transformed with the dTAF110 plasmid. A similar level of β -galactosidase expression was also observed when the dTAF110 plasmid was co-transformed with SP1A, which contains the glutamine-rich domain of Sp1 fused with Gal4 (Figure 4B). The results indicate that the glutamine-rich domain of CBF-B interacts with dTAF110 *in vitro* similarly to the interaction between the glutamine-rich domain of Sp1 and dTAF110. Several deletion mutants in the glutamine-rich region of CBF-B were generated and were tested in the yeast two-hybrid system for interaction with dTAF110. This experiment showed that CBF-Bdel 112–184, containing a deletion of the C-terminal portion of the glutamine-rich domain, interacts with dTAF110; instead CBF-Bdel 1–112, containing a deletion of the N-terminal part of the domain, did not interact with dTAF110, indicating

Figure 5 CBF-C and dTAF110 interact in yeast

Yeast bearing an integrated *GAL1–LacZ* reporter gene were transformed with similar plasmids to those described in the legend to Figure 4: various deletions of CBF-C activation domains fused to the acidic activation domain of Gal4, and dTAF110 fused to the DNA-binding domain of Gal4. β -Galactosidase activities were measured as described in the legend to Figure 4 (+, X-gal staining appeared after 3 h; —, no staining). Abbreviation: nd, not determined.

that the N-terminal part of CBF-B between residues 1 and 112 is essential for interaction with dTAF110. Thus we generated several smaller deletion mutants between residues 1 and 112 of CBF-B and tested them for their ability to interact with dTAF110. Deletion of segment 1–25 in CBF-Bdel 1–25, and segment 25–54 in CBF-Bdel 25–54, did not affect interaction with dTAF110. In contrast, deletion of segment 54–84 in CBF-Bdel 54–84, and segment 84–112 in CBF-Bdel 84–112, abolished the interaction between CBF and dTAF110. These results indicate that the sequence between residues 54 and 112 of CBF-B is involved in the interaction with dTAF110.

The CBF-C 135–335 plasmid, which contains the entire glutamine-rich domain of CBF-C fused with Gal4, when co-transformed with the dTAF110 plasmid, induces β -galactosidase expression, indicating that the glutamine-rich domain of CBF-C interacts with dTAF110 (Figure 5B). To analyse this interaction further, we generated several deletion mutants in the glutamine-rich region of CBF-C. CBF-C 135–235, containing a deletion of a large part of the C-terminal domain, did not interact with dTAF110. For both CBF-C 185–285 and CBF-C 235–335, containing a deletion of the N-terminus of the glutamine-rich domain, staining with X-Gal was observed in the assay *in situ*, but very low values of β -galactosidase were seen after liquid growth of the colonies. This discrepancy might be caused by the instability of either the mutants CBF-C 185–285 and CBF-C 235–335 themselves or by the instability of their interactions with dTAF110 under the conditions of liquid growth. These results indicate that residues 135–335 of CBF-C interact with dTAF110, whereas the N-terminal segment 135–235 is unable to interact

with dTAF110. Our results are further consistent with the hypothesis that the interaction of CBF-C segments 185–285 and 235–335 with dTAF110 are likely to be weaker than for the larger segment 135–335.

DISCUSSION

Previously we showed that the heterotrimeric CBF consists of at least two transcriptional activation domains, a 211-residue sequence in the N-terminal portion of CBF-B and a 195-residue sequence in the C-terminal portion of CBF-C [10,11]. In the present study we have further dissected the two CBF activation domains and determined that a 45-residue sequence in CBF-B and a 50-residue sequence in CBF-C are involved in CBF-dependent transcription activation. The amino acid sequences of the two CBF activation domains are rich in glutamine and hydrophobic residues, and show sequence similarities with the activation domains of the Sp1 and CREB transcription factors [11]. Recent studies showed that the activation domain of Sp1 interacts with dTAF110, a component of *Drosophila* TFIID transcription factor, which functions as a co-activator to mediate Sp1-dependent transcription activation [16]. Several hypotheses have been proposed to explain how interaction between an activator and a TAF increase transcription. The protein–protein interaction between the activator and a TAF might stabilize the binding of TFIID to the TATA box [23], or it might induce a conformational change in the TFIID complex that promotes the assembly of the initiation factors into an active complex [24]. TAF110 does not bind TBP directly but does bind at least two

other TAFs, 250 and 30 α , which bind TBP [25–27]. dTAF110 has also been shown to bind the large subunit of TFIIA [28]. Thus we tested the interaction between dTAF110 and CBF by using protein–protein interaction assays both *in vitro* and *in vivo*. The interaction assay *in vitro* demonstrated that the heterotrimeric CBF specifically associates with dTAF 110. The interaction assay *in vivo* showed that each of the CBF activation domains interacts with dTAF110. Our deletion analysis suggests that, in CBF-B, a 59-residue sequence in the activation domain is involved in the interaction with dTAF110. Because a similar segment also seems to be needed for CBF-dependent transcription activation, we hypothesize that dTAF110 might function as a co-activator in CBF-dependent transcription. In CBF-C the C-terminal portion of the molecule is involved in both transcription activation and interaction with dTAF110. It is possible that the presence of two activation domains in two different subunits of CBF and their potential equivalent interactions with dTAF110 allow CBF to activate transcription irrespective of the orientation of the CBF-binding site in individual promoters.

Both Sp1 and CBF are ubiquitous transcription factors, and they interact with promoter DNAs of many higher eukaryotic genes. The CBF-binding site in many promoters is often found adjacent to an Sp1-binding site [29,30]. The binding of Sp1 and CBF in the same promoter region could generate a surface that contains multiple binding sites for dTAF110. Thus Sp1 and CBF together could interact more efficiently with dTAF110, which then could lead to a synergistic increase in transcription activation.

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