Functional Interaction of the c-Myc Transactivation Domain with the TATA Binding Protein: Evidence for an Induced Fit Model of Transactivation Domain Folding[†]

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ABSTRACT: c-Myc is a member of a family of sequence specific-DNA binding proteins that are thought to regulate the transcription of genes involved in normal cell growth, differentiation, and apoptosis. In order to understand how human c-myc functions as a transcription factor, we have studied the mechanism of action and structure of the N-terminal transactivation domain, amino acids 1−143. In a protein interaction assay, c-myc$_{1-143}$ bound selectively to two basal transcription factors, the TATA binding protein (TBP) and the RAP74 subunit of TFIIF. Furthermore, the isolated c-myc transactivation domain competed for limiting factors required for the assembly of a functional preinitiation complex. This squelching of basal transcription was reversed in a dose-dependent manner by recombinant TBP. Taken together, these results identify TBP as an important target for the c-myc transactivation domain, during transcriptional initiation. Similar to other transactivation domains, the c-myc$_{1-143}$ polypeptide showed little or no evidence of secondary structure, when measured by circular dichroism spectroscopy (CD) in aqueous solution. However, significant α-helical conformation was observed in the presence of the hydrophobic solvent trifluoroethanol. Strikingly, addition of TBP caused changes in the CD spectra consistent with induction of protein conformation in c-myc$_{1-143}$ during interaction with the target factor. This change was specific for TBP as a similar effect was not observed in the presence of TFIIB. These data support a model in which target factors induce or stabilize a structural conformation in activator proteins during transcriptional transactivation.

The product of the proto-oncogene *c-myc* is a nuclear phosphoprotein thought to be an important regulator of normal cell growth, differentiation, and programmed cell death [for review, see Marcu et al. (1992)]. In cell culture and animal models, altered expression of c-myc has been clearly demonstrated to play a role in tumorigenesis (Marcu et al., 1992). c-Myc belongs to a subfamily of transcription factors characterized by a basic helix−loop−helix (bHLH)[1] DNA binding and dimerization motif. In addition, c-myc contains a second dimerization motif, found in a number of transcription factors, the leucine zipper (LZ), which is adjacent to the HLH region at the C-terminus of the protein [see Marcu et al. (1992), Meichle et al. (1992), and Prendergast and Ziff (1992) and references therein]. Both the HLH and LZ regions are important for heterodimer formation between c-myc and a second bHLH−LZ protein, max, resulting in high-affinity DNA binding of the complex to the sequence CACGTG (Dang et al., 1989; Murre et al., 1989; Blackwell et al., 1990; Blackwood & Eisenman, 1991; Blackwood et al., 1992; Kato et al., 1992; Amati et al., 1993; Fisher et al., 1993). Further evidence that c-myc could function as a transcription factor came from domain mapping studies in which the N-terminal 143 amino acids were found to contain a potent transactivation function (Kato et al., 1990; Barrett et al., 1992).

In addition to the transactivation activity, the N-terminal region of c-myc is also required for the cotransformation of rat embryo cells, containing an activated *ras* gene (Stone et al., 1987), as well as binding of the retinoblastoma gene product, Rb (Rustgi et al., 1991), and the Rb-related protein p107 (Beijersbergen et al., 1994; Gu et al., 1994; Hoang et al., 1995). Furthermore, a number of mutations have been found in the N-terminus of c-myc, in cells derived from patients with Burkitt's Lymphoma, a disease associated with three distinct nonrandom chromosomal translocations which transpose c-myc to one of the heavy or light chain immunoglobulin gene loci (Bhatia et al., 1993; Albert et al., 1994, and references therein). A number of the mutations found in Burkitt's Lymphoma cells cluster in a region (amino acids 57−62) that is highly conserved between species and contains two known phosphorylation sites (Bhatia et al., 1993; Henriksson et al., 1993). It has been reported that mutations identified in this region predominantly increase the cotransformation activity of c-myc in rat-1A cells (Henriksson et al., 1993; Hoang et al., 1995) and result in a c-myc protein that is refractory to suppression by p107 (Gu et al., 1994; Hoang et al., 1995), both suggesting that these

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[1] Abbreviations: bHLH, basic helix−loop−helix domains; CD, circular dichroism; DBD, DNA binding domain; DDT, dithiothreitol; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; IPTG, isopropyl 1-thio-β-D-galactopyranoside; LZ, leucine zipper domain; PCR, polymerase chain reaction; PIC, preinitiation complex; PMSF, phenylmethylsulfonyl; SDS, sodium dodecyl sulfate; SRF, serum response factor; TAF, TBP-associated factor; TBP, TATA binding protein; TFE, trifluoroethanol.

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mutations lead to further deregulation of the c-myc protein in Burkitt's Lymphoma. Furthermore, several of these mutations result in a reduction of the transactivation potential of both intact c-myc (Gupta et al., 1993) and c-myc$_{1-143}$ fused to the GAL4 DBD (Albert et al., 1994). While the correlation of these mutations with the Burkitt's Lymphoma phenotype is complicated by the observation that some mutations have arisen during the propagation of Burkitt's Lymphoma cells in culture and are not found in the original tumor (Albert et al., 1994), c-myc represents one of the few examples where defects in a transactivation domain have been correlated with a pathological defect.

Initiation of transcription by RNA polymerase II requires the assembly of general or basal transcription factors, TFIIA, IIB, IID, IIE, IIF, IIH, and IIJ together with the polymerase at or near the transcription start site, to form the preinitiation complex (PIC) (Roeder, 1991; Conaway & Conaway, 1993). Activator proteins are thought to function by recruiting or modifying the activity of one or more of the general transcription factors, resulting in the formation of a more stable PIC. Two models have been proposed to explain how activators could interact with the general transcriptional apparatus. Firstly, there is good evidence that a number of viral and cellular transcription activators can contact the general transcriptional apparatus directly via contacts with basal transcription factors, most notably the TATA box binding protein (TBP), a component of the multisubunit factor TFIID [see Conaway and Conaway (1993), Nikolov and Burley (1994), and Zawel and Reinberg (1995), and references therein]. Secondly, it has been shown that a distinct class of factors, termed coactivators or adapter proteins, mediate the effects of transcription factors on the general transcriptional apparatus. The existence of these intermediate proteins was first demonstrated in squelching studies *in vivo* (Martin et al., 1990; Tasset et al., 1990) and *in vitro* (Berger et al., 1990; Kelleher et al., 1990) and in studies in which endogenous TFIID (TBP + TBP-associated factors, TAFs) but not cloned TBP could respond to sequence specific activators (Pugh & Tjian, 1992). These models are not necessarily mutually exclusive, and it is likely that multiple interactions between activators and the general transcriptional machinery are involved (Choy & Green, 1993; Goodrich et al., 1993).

Transcriptional activation domains have been broadly classified on the basis of the abundance of particular amino acids, notably acidic residues (Glu, and Asp), glutamines (Gln), or prolines (Pro) (Mitchell & Tjian, 1989). However, the structural basis for the activity of transactivation domains remains to be determined. The c-myc transactivation domain is relatively rich in acidic residues, but there are two regions (amino acids 20−41 and 42−80) that are rich in glutamines and prolines, respectively. Secondary structural analysis of transactivation domains from VP16, Gal4, Gcn4, NF-κB (p65), and the glucocorticoid receptor by circular dichroism spectroscopy (CD; Donaldson & Capone, 1992; Van Hoy et al., 1993; Lienhard Schmitz et al., 1994; Dahlman-Wright et al., 1995) and/or nuclear magnetic resonance (Donaldson & Capone, 1992; Lienhard Schmitz et al., 1994) has shown that purified transactivation domains are largely unstructured in aqueous solution at neutral pH. In acidic conditions, the Gal4 and Gcn4 transactivation domains adopted a β-sheet conformation (Van Hoy et al., 1993), while α-helicity was induced in the domains from VP16, NF-κB (p65), and the glucocorticoid receptor in hydrophobic solvents (Donaldson & Capone, 1992; Lienhard Schmitz et al., 1994; Dahlman-Wright et al., 1995). In the case of the glucocorticoid receptor τ1core transactivation domain, the ability to activate a reporter gene *in vivo* correlates with the ability to adopt an α-helical conformation *in vitro* (Dahlman-Wright & McEwan, 1996). Thus, transactivation domains have a propensity for structure formation under specific conditions, and it has been hypothesized that such conditions might be fulfilled upon interaction with target proteins (Tjian & Maniatis, 1994).

In the present study, we have screened a panel of general transcription factors to identify target proteins for the c-myc transactivation domain (amino acids 1−143). The functional significance of these interactions was then tested in a cell-free transcription assay, where the isolated c-myc transactivation domain competed for factors required for initiation of transcription from a basal promoter. Furthermore, we show that complex formation between c-myc$_{1-143}$ and one of these target proteins, TBP, is accompanied by a change in protein conformation, consistent with a model in which target factor binding induces a more structured transactivation domain conformation.

EXPERIMENTAL PROCEDURES

Plasmid Constructions. The sequences encoding wild type and mutant forms of the human c-myc transactivation domain (amino acids 1−143) were generated from plasmid templates by PCR. The sequence of the primers was GCG ATA GAG CTC GAT GCC CCT CAA CGT TAG CTT CAC and GCG ATA GAG CTC GCC TTG GCG GCG GCC GAG AAG CC. The products were cleaved with *Sac*I, cloned into the *Sac*I site of pKV-XE (Wright et al., 1991), and confirmed by DNA sequencing the entire insert. The resulting plasmids express the wild type (pKV-c-myc-XE) or mutant c-myc sequences, containing substitutions of Thr-58 (pKV-c-mycT58A-XE) and Ser-62 (pKV-c-mycS62A-XE) with alanine, fused to the glucocorticoid receptor DNA binding domain in yeast. For bacterial expression, the sequence encoding the c-myc transactivation domain, amino acids 1−143, was excised from plasmid pKV-c-myc-XE as a *Sac*I fragment and cloned into the reciprocal site in plasmids pET-19bm and pET-LexA$_{DBD}$ to give c-myc expression plasmids pET-c-myc$_{1-143}$ and pET-c-myc-LexA$_{DBD}$, respectively. (The construction of pET-19bm and pET-LexA$_{DBD}$ will be described elsewhere.) The c-myc protein expressed from these plasmids was predicated to have the following additional amino acids, MG(H)$_{10}$SSGH(D)$_4$KHMASS and ASSGS, at the C and N terminus, respectively. c-Myc expression plasmids were transformed into *Escherichia coli* strain BL21 (plys).

Expression and Purification of Recombinant c-Myc Proteins. Bacterial cultures were grown to an OD$_{600nm}$ of 0.4−0.6 and proteins induced by adding isopropyl 1-thio-β-D-galactopyranoside (IPTG) to a final concentration of 0.5 mM. Cultures were incubated for a further 60−90 min prior to cell harvesting. After cell lysis, the His-c-myc$_{1-143}$ and His-c-myc-LexA$_{DBD}$ proteins were recovered in the 10 000 rpm (JS-13.1 rotor) pellet fraction. Proteins were solubilized in 8 M urea, 10 mM Tris (pH 8.0), and 0.1 M NaH_2PO_4 (buffer A), and the cleared supernatant was loaded directly onto a Ni^{2+}-NTA−agarose (Qiagen) column, equilibrated with

buffer A at pH 8.0. After successive washes with buffer A, at pH 8.0 and 6.3, bound proteins were eluted with buffer A at pH 5.9. Under these conditions, 1−2 mg of recombinant protein was recovered per 200 mL of culture. Proteins were renatured by dialysis for 2 h at room temperature against buffer B [25 mM HEPES (pH 7.6), 100 mM sodium acetate, 1 mM DTT, and 4 M urea] and subsequently overnight at 4 °C against buffer B without the urea.

Scintillation-Microtitration Plate-Based Protein−Protein Interaction Assay. Purified recombinant transactivation domains, c-myc$_{1-143}$, VP16$_{413-490}$, or SRF, in binding buffer [20 mM HEPES (pH 7.6), 10% glycerol, 100 mM KCl, 0.2 mM EDTA, 5 mM β-mercaptoethanol, and 0.2 mM PMSF] were adsorbed to the surface of microtiter plate wells for up to 72 h. After removal of the activator solution, the wells were blocked for 12 h with blocking buffer (binding buffer + 5 mM $MgCl_2$ and 5 mg/mL BSA) and subsequently incubated with radiolabeled human general transcription factors, synthesised in rabbit reticulocyte lysates (Promega), and diluted in binding buffer + 5 mM $MgCl_2$, and 1 mg/mL BSA. The wells were subsequently washed (binding buffer + 5 mM $MgCl_2$ and 1 mg/mL BSA), and the bound radioactivity was counted directly in a micro-beta counter (Wallac). Bound proteins were then recovered in SDS sample buffer and analyzed by SDS−PAGE.

Expression and Purification of Recombinant Yeast TBP, TFIIB, and TFIIA and Human TFIIB and TFIIF. Recombinant yeast and human basal transcription factors were expressed in bacteria by IPTG (1 mM) induction. Yeast TBP (Arndt et al., 1992) was partially purified by DEAE-Sephacryl and heparin sepharose chromatography (Lieberman et al., 1991). For some experiments, the eluate from the heparin sepharose column was concentrated (Centiprep-10 concentrator, Amicon) and TBP purified to near homogeneity by gel filtration on a Superose 12 column (Pharmacia). Human (Ha et al., 1991) and yeast TFIIB (Pinto et al., 1992) and the RAP74 (Finkelstein et al., 1992) subunit of human TFIIF were partially purified by differential ammonium sulfate precipitation [after Pognonec et al. (1991)]. The TOA1 and TOA2 subunits of yeast TFIIA (Ranish et al., 1992) and the RAP 30 (Sopta et al., 1989) subunit of human TFIIF were purified from inclusion bodies by urea solubilization and the proteins renatured by dialysis. The purity of recombinant proteins was judged from Coomassie blue staining of polyacrylamide gels (data not shown).

Yeast Transactivation Assays. Analysis in yeast cells and in nuclear extracts of the c-myc transactivation domain was carried out as previously described for the glucocorticoid receptor τ1 domain (Wright et al., 1990, 1991; McEwan et al., 1993, 1994).

Circular Dichroism Studies. Circular dichroism measurements were made on an AVIV 60DS CD instrument as described previously (Dahlman-Wright et al., 1995; Dahlman-Wright & McEwan, 1996). Proteins were dialyzed into 3 or 10 mM phosphate buffer and the CD spectra measured at protein concentrations of 100−140 μg/mL. For analysis of c-myc$_{1-143}$/TBP and c-myc$_{1-143}$/TFIIB mixtures, the proteins were mixed just prior to measuring the spectrum. However, there was no change in the spectra over several hours. Starting samples and the mixtures were subsequently analyzed by SDS−PAGE.

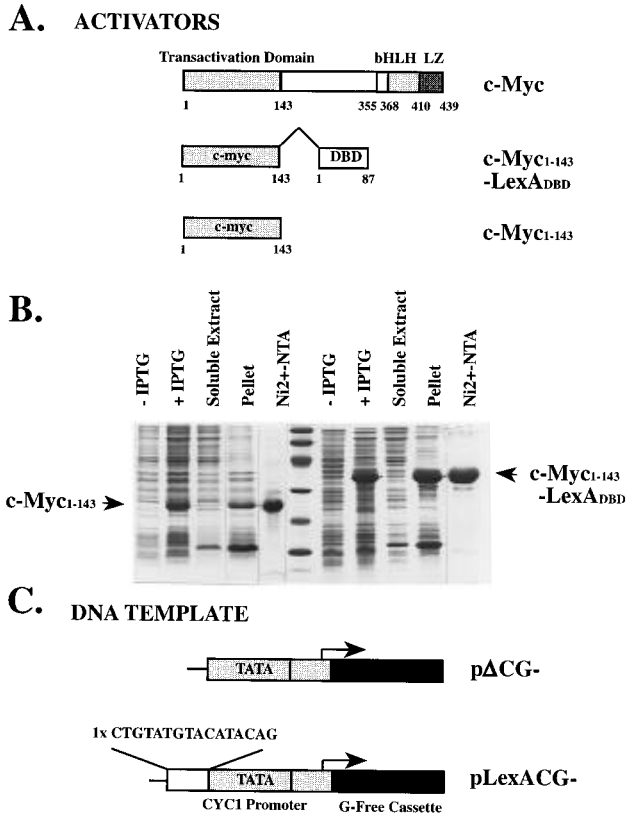

FIGURE 1: Derivatives of c-myc expressed and purified from bacteria. (A) Schematic representation of c-myc, c-myc-LexA$_{DBD}$, and c-myc$_{1-143}$ activator proteins. (B) Coomassie blue-stained SDS−polyacrylamide gel showing a typical purification of recombinant c-myc proteins after expression in *E. coli*. Shown are the total cell extracts before (−) and after (+) IPTG induction, soluble extract, urea-solubilized proteins (pellet), and the pH 5.9 elution from the Ni-NTA column. Molecular weight standards (Pharmacia) of 94K, 67K, 43K, 30K, 20.1K, and 14K are also shown (middle lane). Recombinant proteins ran slower than their expected molecular weights and were estimated to be >90% pure after Ni affinity chromatography. (C) Reporter gene constructs consisted of a 380 bp G-free cassette fused to basal promoter elements of the *CYC1* gene, pΔCG- (Lue et al., 1991). For activation studies, the DNA template, pLexCG-, containing one LexA response element cloned upstream was used.

RESULTS

The c-Myc Transactivation Domain Selectively Binds the Basal Transcription Factors TBP and TFIIF. As a first step to identifying which of the general transcription factors binds to the c-myc$_{1-143}$ transactivation domain, *in vitro* binding studies were carried out with immobilized activator proteins and radiolabeled human basal transcription factors. The c-myc$_{1-143}$ transactivation domain, containing an N-terminal histidine tag, was expressed in bacteria and purified from inclusion bodies by urea solubilization and Ni affinity chromatography (panels A and B of Figure 1). The purified protein was renatured by dialysis and estimated to be >90% pure as judged by Coomassie blue staining of SDS−polyacrylamide gels. c-Myc$_{1-143}$ protein or BSA (control) was allowed to adsorb to the surface of microtiter wells and subsequently incubated with radiolabeled human basal transcription factors TFIIB, TBP, TFIIEα, TFIIEβ, TFIIF (RAP30), and TFIIF (RAP74). After incubation, the wells were washed and the bound radioactivity was measured directly (Figure 2A). Bound protein was also recovered from the wells in SDS sample buffer and analyzed by SDS−PAGE

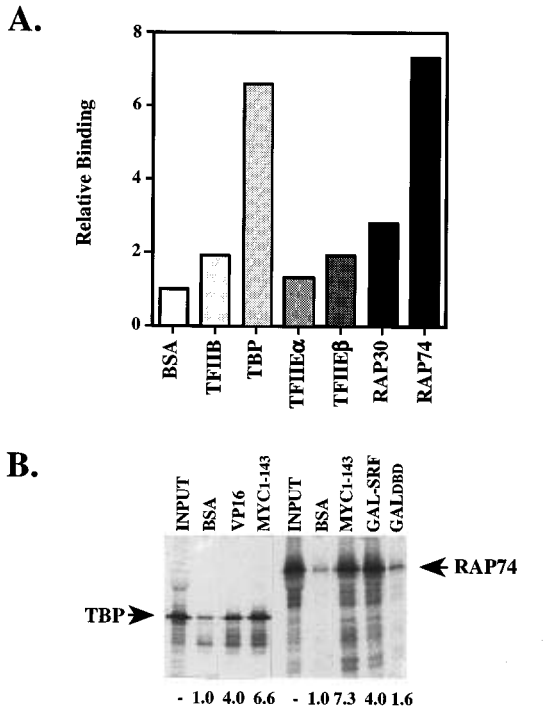

FIGURE 2: Binding of the basal factors TBP and TFIIF to the c-myc transactivation domain. (A) ^{35}S-radiolabeled human TBP and the RAP74 subunit of TFIIF (5 μL of reticulocyte lysate) bound selectively to the c-myc$_{1-143}$ polypeptide. Results are plotted relative to the BSA control (mean of duplicate samples). (B) SDS gel of radiolabeled TBP and RAP74 bound to c-myc$_{1-143}$ and the herpes simplex viral protein VP16$_{413-490}$ or serum response factor (SRF) transactivation domains, respectively. The fold binding relative to BSA is shown below each lane. Input represents 5% of the material incubated in each well.

(Figure 2B). A selective interaction was observed between c-myc$_{1-143}$ transactivation domain and TBP and the RAP74 subunit of TFIIF (Figure 2A). In contrast, little or no significant binding was seen with TFIIB, TFIIEα, TFIIEβ, or the RAP30 subunit of TFIIF (Figure 2A). In order to evaluate the significance of the above interactions, we compared the binding of c-myc$_{1-143}$ with previously reported interactions between VP16 and TBP (Stringer et al., 1990; Ingles et al., 1991) and the serum response factor (SRF) and TFIIF (Joliot et al., 1995). The binding of c-myc$_{1-143}$ to TBP was as strong, if not stronger, compared to that seen for the potent viral activator VP16$_{413-490}$ transactivation domain (Figure 2B). Similarly, the binding of c-myc$_{1-143}$ to the RAP74 subunit of TFIIF was comparable to that seen with the SRF C-terminal transactivation domain (Figure 2B). Furthermore, analysis of the material recovered from the microtiter plate confirmed that the measured radioactivity predominantly reflects the binding of TBP and RAP74 proteins (Figure 2B).

c-Myc$_{1-143}$ Can Compete for a Limiting Factor(s) in the General Transcriptional Machinery. In order to determine whether interactions with general transcription factors are functionally significant during transcriptional activation by c-myc, we asked whether the interactions with c-myc$_{1-143}$ were sufficiently strong to compete for the assembly of a functional transcription complex *in vitro*. We have previously developed such a squelching assay using yeast nuclear extracts, which are particularly appropriate due to the relatively high levels of basal transcription activity (McEwan et al., 1993). Since it has been reported that random peptides can have transactivation activity in yeast (Ptashne, 1988, and references therein), we wished to correlate the activity of the N-terminal transactivation domain of c-myc in yeast with that seen in mammalian systems. Therefore, the alanine substitutions of Thr-58 and Ser-62, which reduce the transactivation activity of intact c-myc in mammalian cells to 20 and 40% of that of wild type c-myc, respectively (Seth et al., 1991; Gupta et al., 1993), were studied. Expression of wild type c-myc$_{1-143}$ fused to the DBD of the glucocorticoid receptor in yeast cells resulted in strong (almost 200-fold) induction of a lac Z reporter gene driven by a single glucocorticoid response element (Figure 3A). This level of activation was comparable to that seen with the τ1 transactivation domain of the human glucocorticoid receptor under similar conditions (Wright et al., 1991). The transcription activation activity of c-myc$_{1-143}$ was significantly reduced by 50−60% by the point mutations of Thr-58 and Ser-62 (Figure 3A). These results show localization of a strong transactivation domain to the same region mapped in mammalian cells and that its activity in yeast is reduced by point mutations that similarly reduced activity in mammalian cells.

To determine whether the c-myc transactivation domain was involved in direct interactions with one or more components of the general transcriptional machinery, we studied the effect of the isolated c-myc$_{1-143}$ transactivation domain on basal transcription. Previous studies have reported that high levels of transcriptional activators can inhibit (squelch) transcription by sequestering a limiting cellular cofactor(s) or general transcription factor(s) (Ptashne, 1988; Wright et al., 1991; McEwan et al., 1993, and references therein). Increasing concentrations of c-myc$_{1-143}$ resulted in a dose-dependent inhibition of transcription from the *CYC1* basal promoter (Figure 3B). This inhibition or squelching of transcription occurs at levels of added transactivation domain comparable to that observed previously for the τ1 transactivation domain of the human glucocorticoid receptor (McEwan et al., 1993) and VP16 (Berger et al., 1990; Kelleher et al., 1990).

In order to test whether the interactions seen in the squelching assay represent a nonspecific effect of adding the c-myc transactivation domain, we purified bacterially expressed c-myc$_{1-143}$ fused to the LexA repressor DBD (Figure 1A,B) and assayed its ability to activate a reporter gene containing a single LexA binding site (Figure 1C). Transactivation was seen over a concentration range similar to that used in the squelching experiment, indicating that the interactions occurring during squelching are likely to be functionally relevant for myc-dependent activation (Figure 3C). Therefore, taken together, these results strongly suggest that the c-myc transactivation domain interacts with one or more factors required for basal transcription.

The c-Myc Transactivation Domain Interacts Functionally with TBP. TBP is a key factor in the assembly of the preinitiation complex and has been proposed as a target factor for a number of activators (see the introductory section). Furthermore, the above physical interaction studies strongly suggested that TBP was a likely target for the c-myc transactivation domain. Therefore, to test whether TBP was being titrated out by the c-myc$_{1-143}$ transactivation domain, recombinant yeast TBP was expressed in bacteria, purified to near homogeneity, and added, together with a basal promoter template (pΔCG-), to nuclear extracts in the

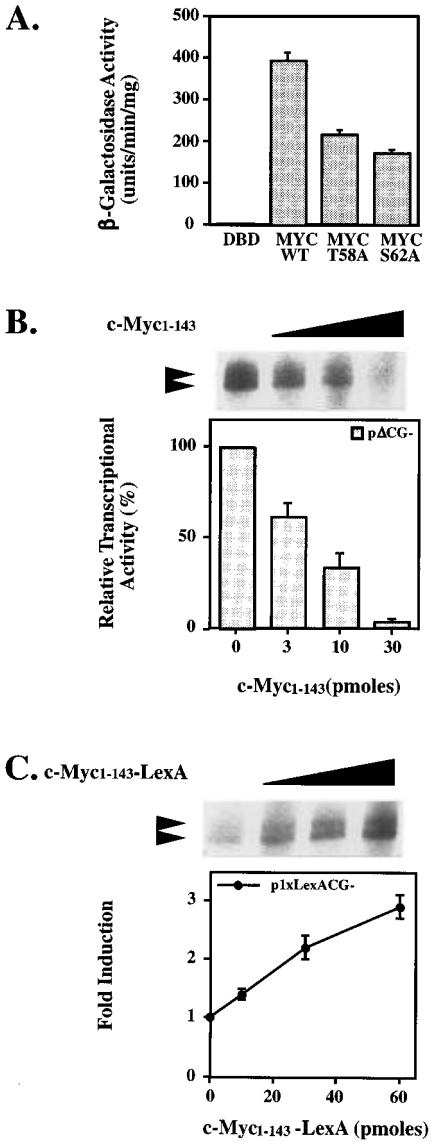

FIGURE 3: c-Myc transactivation domain interacts with a limiting factor(s) required for basal transcription. (A) Transactivation of gene expression by the c-myc$_{1-143}$ transactivation domain in yeast cells. Transactivation of β-galactosidase expression from a reporter gene, consisting of a basal *CYC1* promoter with glucocorticoid responsive elements cloned upstream, in plasmid pLGZ-TAT (Wright et al., 1990). Bars represent β-galactosidase expression after induction of c-myc$_{1-143}$ derivatives from pKV-c-myc-XE (MYC WT), pKV-c-mycT58A-XE (MYC T58A), and pKV-c-mycS62A-XE (MYCS-62A) or the DBD of the human glucocorticoid receptor alone (DBD). (B) Basal transcription from pΔCG- (100 ng) reporter construct in the presence of increasing amounts of c-myc$_{1-143}$. Specific RNase T_1-resistant transcripts were resolved by urea−PAGE and quantitated using a BAS2000 bioimaging analyzer (Fuji Film). A representative gel and the quantitated data, the mean ± the standard deviation, from three independent experiments, are shown; arrowheads indicate correctly initiated transcripts. (C) Transcription the pLexACG- (100 ng) reporter construct by increasing amounts of c-myc-LexA. A representative gel and the quantitated data, the mean ± the standard deviation, from three independent experiments, are shown; arrowheads indicate correctly initiated transcripts.

absence or presence of a squelching concentration of c-myc$_{1-143}$. Increasing amounts of TBP resulted in higher levels of transcription in both the absence and presence of c-myc$_{1-143}$ (Figure 4B). Significantly, the ratio of transcription in the presence of c-myc$_{1-143}$ as a proportion of transcription in the absence increased from 0.3 up to 0.72, indicating a dose-dependent reversal of squelching by TBP (Figure 4A). At the highest concentration used, TBP was itself inhibitory, presumably as a result of squelching of other components of the general transcription machinery (Figure 4B). Interestingly, this inhibitory effect of TBP is reduced in the presence of c-myc$_{1-143}$, consistent with these two factors interacting (Figure 4B). In contrast, similar studies with recombinant yeast TFIIB, another general transcription factor, showed no significant ability to reverse squelching (Figure 4A), which is in agreement with its observed low binding to c-myc$_{1-143}$ in the interaction assay (Figure 2A). Another basal factor, TFIIA, similarly failed to reverse c-myc-dependent squelching, although again the overall levels of transcription were increased (Figure 4A). Attempts to reverse the squelching of basal transcription with recombinant TFIIF are hampered by the lack of functional recombinant yeast TFIIF (Henry et al., 1994), and although bacterially produced human TFIIF (RAP30 + RAP74) did not appear to reverse squelching (data not shown), the significance of the interaction observed between c-myc$_{1-143}$ and RAP74 (Figure 2A,B) remains to be determined. Therefore, we conclude that the c-myc$_{1-143}$ transactivation domain probably interacts functionally with TBP during transcriptional activation by c-myc, although interactions with other factors are probably also involved.

Structural Analysis of the c-Myc$_{1-143}$ Polypeptide. We have used circular dichroism (CD) spectroscopy to analyze the secondary structural characteristics of the c-myc transactivation domain. In aqueous solution and over a range of temperatures, the c-myc$_{1-143}$ polypeptide showed little or no secondary structure (Figure 5A). The spectra showed the characteristics of a random conformation with a minimum at around 200 nm and progressively nearing zero at wavelengths above 210 nm. However, α-helical structures were detected in the presence of increasing amounts of the α-helix-stabilizing, hydrophobic solvent TFE (Figure 5B). This is seen as an increased negative CD signal at 222 nm (Figure 5B, inset). Thus, the c-myc$_{1-143}$ transactivation domain has the potential to form secondary structure under certain conditions.

Structural analysis of several transactivation domains has revealed a general lack of secondary structure in aqueous solution, and this has led to the suggestion of an induced fit model according to which these domains remain flexible in solution and only become structured upon interaction with appropriate target protein(s). In order to test this model experimentally, we were interested to determine the CD spectrum of c-myc$_{1-143}$ in aqueous solution in the presence of TBP (Figure 6A). While the spectrum of c-myc$_{1-143}$ alone was again characteristic of a random coil conformation, that of TBP alone showed a clear secondary structure conformation, with a minimum at 222 nm characteristic of α-helical structures (Figure 6A), in agreement with the known crystal structure (Nikolov & Burley, 1994, and references therein). If there is no conformational change when c-myc$_{1-143}$ binds TBP, the spectrum for the mixture would be expected to represent the sum of the spectra for the individual proteins. However, the observed spectrum for the mixture is clearly distinct from the calculated additive spectra (Figure 6B). The most striking difference is the shift to the right and reduction in the minimum at around 200 nm derived from the c-myc$_{1-143}$ polypeptide. Furthermore, the change in the 200 to 222 nm ratio to near unity is consistent with conversion

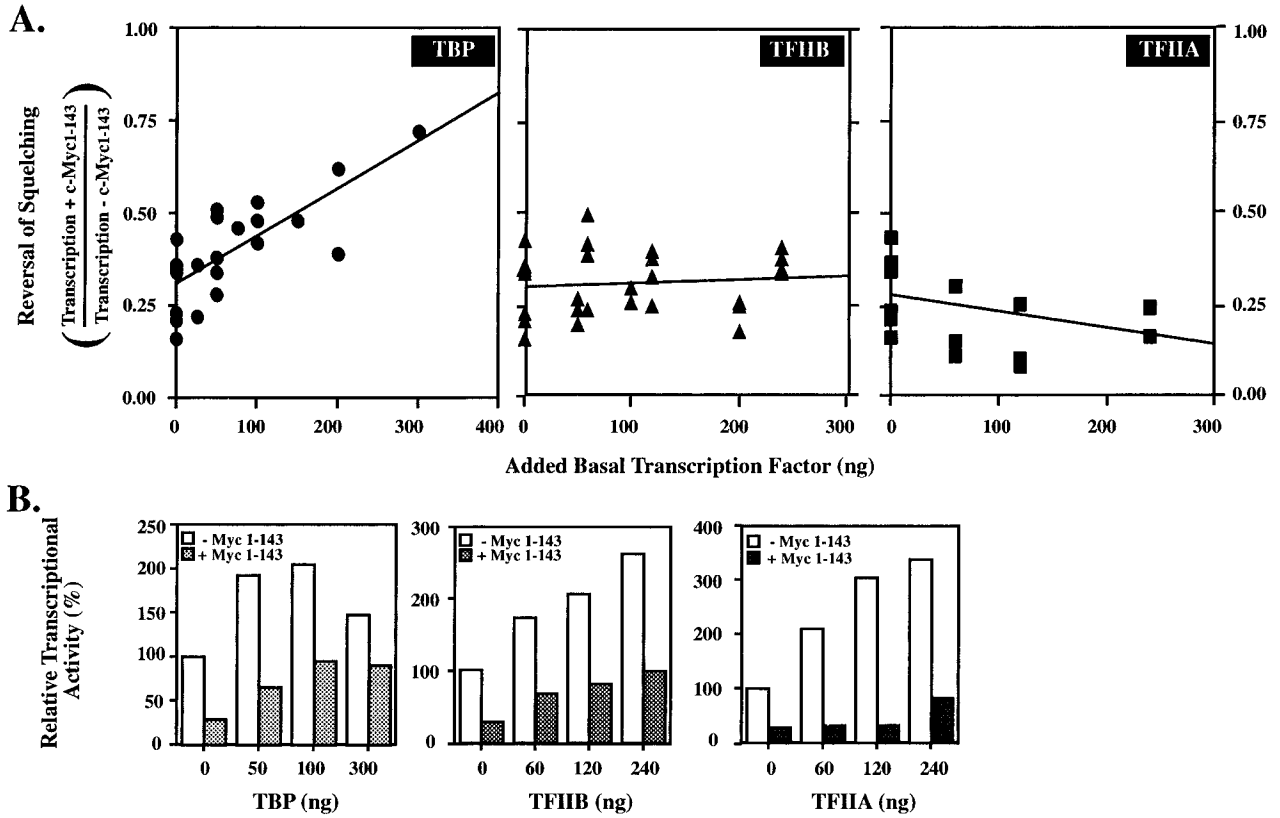

FIGURE 4: Recombinant yeast TBP selectively reverses squelching of basal transcription by c-myc$_{1-143}$. (A) Reversal of squelching was calculated as the ratio of transcription in the presence of c-myc$_{1-143}$ versus transcription in the absence of c-myc$_{1-143}$ and plotted against the amount of recombinant basal factor added to transcription reactions (data for up to seven experiments are shown). In the absence of TBP, TFIIB, or TFIIA the mean of this ratio was 0.3 ± 0.1 and an increase in this ratio is indicative of reversal of c-myc$_{1-143}$-dependent squelching. The linear regression coefficient, *r*, for TBP, TFIIB, and TFIIA data was 0.76, 0.1, and 0.37, respectively, with *p* values ≤0.001. (B) Representative experiments showing relative transcription levels after addition of recombinant yeast TBP, TFIIB, and TFIIA to nuclear extracts in the absence (open bars) or presence (filled bars) of c-myc$_{1-143}$. Transcripts were quantitated as described in the legend to Figure 3.

of a random coil to an α-helical conformation. A trivial explanation for the reduced signal at around 200 nm for the mixture would be a preferential loss of the c-myc$_{1-143}$ polypeptide during the CD measurements. However, analysis of the proteins after CD analysis clearly shows that the relative amounts of each protein are not significantly altered (Figure 6E). Furthermore, these changes in the c-myc$_{1-143}$ spectra were specific for TBP as they were not seen with another basal transcription factor, TFIIB (Figure 6C,D), which interacts poorly, if at all, with c-myc$_{1-143}$. The CD spectrum for TFIIB, indicating a predominantly α-helical conformation, was in good agreement with the recently published crystal and solution structures of an N-terminal truncated TFIIB core (Figure 6C) (Bagby et al., 1995; Nikolov et al., 1995). However, in contrast to the result with TBP, the observed CD spectrum for the mixture and the calculated spectrum were essentially superimposable, strongly indicating that TFIIB had no significant structural effect on the c-myc polypeptide (Figure 6D). As with the TBP samples, the relative amounts of the proteins in the c-myc$_{1-143}$/TFIIB mixture do not significantly change during the CD measurements (Figure 6E). Taken together with the above binding and functional studies, these data indicate that the c-myc transactivation domain can interact specifically with TBP and that this interaction is accompanied by changes in protein conformation that are consistent with the induction or stabilization of secondary structure conformation in the c-myc polypeptide.

DISCUSSION

In this study, we have shown that the c-myc transactivation domain, amino acids 1−143, interacts functionally with components of the general transcription machinery. *In vitro* binding studies identified a relatively specific interaction between the c-myc$_{1-143}$ transactivation domain and the basal transcription factors TBP and TFIIF (RAP74). The ability of recombinant TBP to reverse c-myc$_{1-143}$-dependent squelching of basal transcription demonstrates the functional relevance of the c-myc$_{1-143}$−TBP interaction in the context of preinitiation complex formation. These results confirm and extend a previous report describing direct binding of c-myc to TBP *in vitro* (Hateboer et al., 1993) and are consistent with the results of Maheswran et al. showing that c-myc and TBP exist as a complex intracellularly (Maheswaran et al., 1994). TBP plays a central role in the assembly of the preinitiation complex and has been shown to bind to a number of viral and cellular transcriptional activators (see the introductory section). The interaction of the c-myc$_{1-143}$ transactivation domain with TBP suggests that the c-myc protein functions at an early stage in the initiation of transcription and that its mechanism of transcriptional activation possibly involves recruitment of TBP to the TATA element and/or stabilization of the TBP−TATA complex.

A structural analysis of c-myc$_{1-143}$ using CD spectroscopy revealed that this polypeptide was relatively unstructured in aqueous solution but acquires significant α-helical structure in the presence of TFE, a hydrophobic solvent that favors

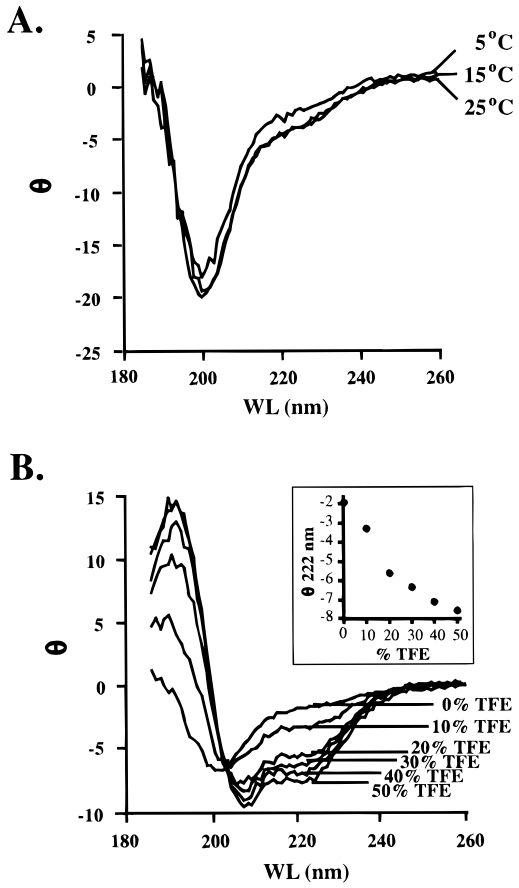

FIGURE 5: c-Myc transactivation domain assumes an α-helical conformation in the presence of the hydrophobic solvent TFE. (A) CD spectra of c-myc$_{1-143}$ protein recorded at different temperatures as indicated. The CD signal is given as the molar ellipticity, θ, calculated as $\theta = \Delta\epsilon \times 3300$ in units of deg cm^2 dmol^{-1} and where $\Delta\epsilon$ is the differential molar coefficient. The values plotted are times 10^{-3}. The minimum at around 200 nm is characteristic of a random coil conformation (Johnson, 1990). (B) CD spectra of c-myc$_{1-143}$ recorded at different concentrations of TFE (percent as indicated). The maximum at 190 nm and the minima at 220 and 208 nm are characteristic of an α-helical conformation (Johnson, 1990). The inset shows the θ values at a wavelength of 222 nm plotted relative to the concentration of TFE.

secondary structure formation in regions which have a propensity to form an α-helix. Thus, the c-myc$_{1-143}$ polypeptide conforms to the current picture of transactivation domains being relatively flexible and unstructured, until they interact with a specific target protein within the general transcriptional machinery. It is of considerable significance therefore that, in the presence of a target factor TBP, there was a change in the structural conformation of c-myc$_{1-143}$ as seen by a reduction in random coil signal at around 200 nm and a reduction in the 200 to 222 nm ratio, indicative of an increase in α-helix conformation. Together with a recent fluorescent spectroscopy study of the VP16 transactivation domain (Shen et al., 1996), the present study provides the first experimental evidence for an induced conformational change in a transactivation domain upon binding to a target protein and thus provides support for the above model. A precedent for the functional relevance of such conformational changes in nature is eloquently illustrated by the recent crystallographic analysis of the influenza hemagglutinin protein (Bullough et al., 1994). A critical event during viral infection of a host cell is thought to be the pH-induced conformational change in the hemagglutinin molecule, which has been suggested to be important for subsequent fusion of the viral and host cell membranes. One of the striking features of this conformational change was found to be the folding of a region of random coil (loop) into an α-helix to form part of an extended helical rod or spike (Bullough et al., 1994).

The interaction of c-myc$_{1-143}$ transactivation domain with the RAP74 subunit of TFIIF is of particular note. TFIIF interacts with both RNA polymerase II, another basal factor, TFIIB, and TAF$_{II}$250, a component of multisubunit factor TFIID (Ha et al., 1993; Ruppert & Tjian, 1995, and references therein). The role of TFIIF in initiation seems to involve specific recruitment of the polymerase to promoter sequences, preventing nonspecific DNA binding by the polymerase enzyme (Conaway & Conaway, 1993, and references therein). In addition, TFIIF has been reported to be associated with RNA polymerase II in the elongation complex (Zawel et al., 1995), and it is therefore tempting to speculate that, in addition to functioning during transcriptional initiation, c-myc may play a role during elongation of the nascent RNA. To date, only one other cellular activator, SRF, has been reported to bind to this basal transcription factor. The C-terminal transactivation domain of SRF specifically bound to the RAP74 subunit of TFIIF, and the interaction with TFIIF was important for SRF-dependent transcriptional activation (Johansen & Prywes, 1993; Joliot et al., 1995).

The binding of c-myc$_{1-143}$ to known components of the general transcriptional machinery is consistent with the ability of this protein to squelch basal transcription. Although recombinant TBP, at least partially, relieves this squelching, attempts to overcome the squelching of basal transcription by adding recombinant TFIIF have so far proved unsuccessful. These results suggest that both factors together or additional factors may be important for c-myc-dependent transactivation and squelching. On the basis of the described sequence homology between the Rb pocket domain and the basal factors TBP and TFIIB (Hagemeier et al., 1993), c-myc would be predicted to bind to TFIIB. Indeed, the region of Rb (amino acids 697−731) critical for c-myc binding (Rustgi et al., 1991) shares extensive homology with TFIIB (Hagemeier et al., 1993). However, as yet there is no experimental evidence for either a physical interaction [this study and Hateboer et al. (1993)], or a functional interaction (this study) between c-myc and TFIIB. Previously, c-myc was found to interact with TFII-I, a factor involved in initiation of transcription from the initiator element [see Roeder (1991) and references therein]. This interaction appears distinct from the interactions observed in the present study since the binding of c-myc to TFII-I requires sequences in the C terminus of c-myc, not the N-terminal transactivation domain, and because this interaction is involved in c-myc-dependent repression of transcription (Roy et al., 1993). Taken together, these findings suggest that c-myc can make multiple interactions with the general transcription machinery, the specificity of which results in either positive or negative regulation of transcription.

Interestingly, another member of the c-myc family of proteins, B-myc, that lacks the b-HLH−LZ region but shows considerable homology to the N-terminal transactivation domain of c-myc has been identified (Ingvarsson et al., 1988). This protein can activate transcription when fused to the GAL4 DBD and compete (squelch) for a factor or

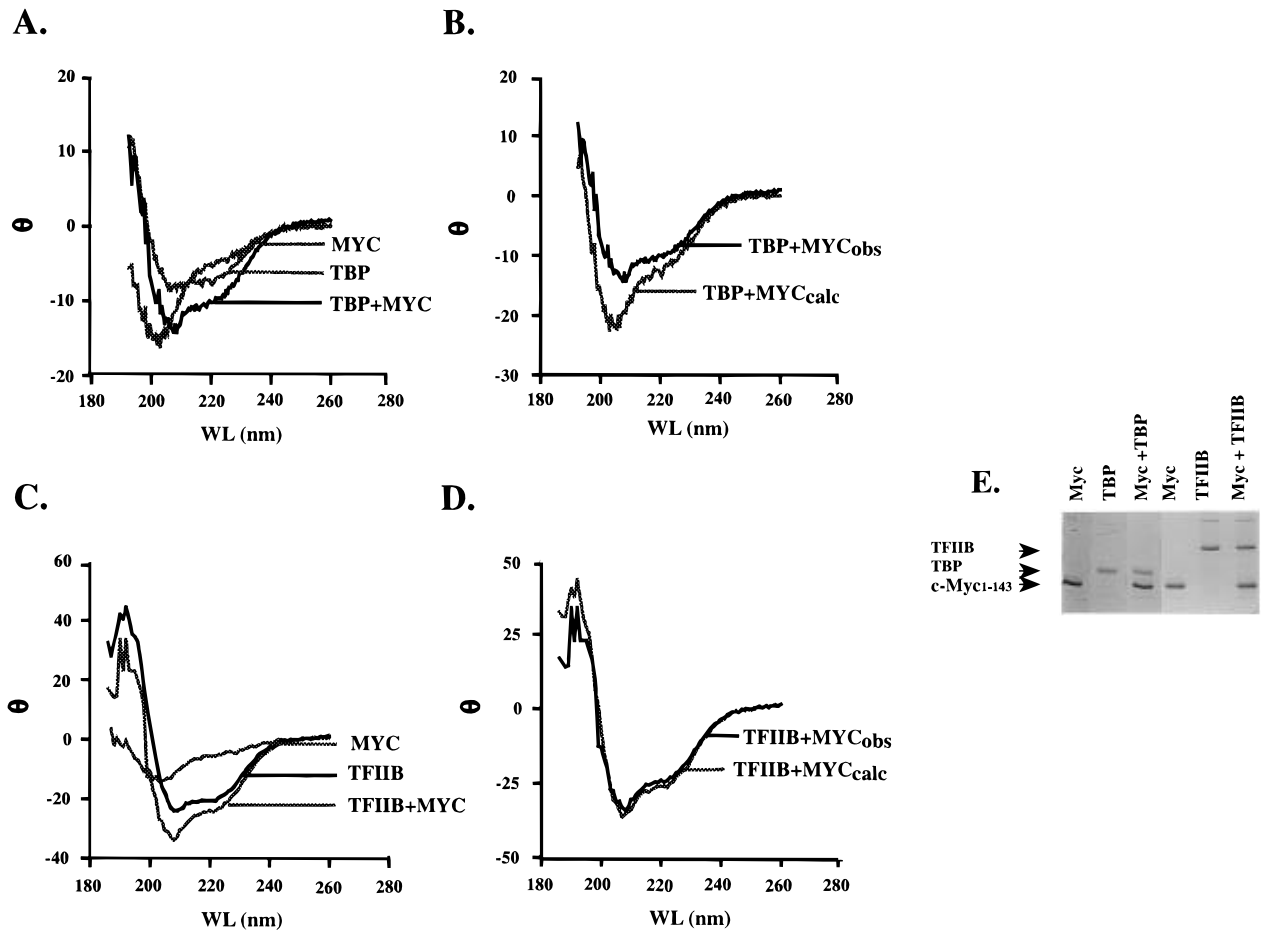

FIGURE 6: Interaction of c-myc$_{1-143}$ with TBP causes a conformational change. (A) The CD spectrum was measured for c-myc$_{1-143}$ and TBP alone and for a mixture of the two proteins together. (B) In order to determine the effect of TBP on the c-myc$_{1-143}$ conformation, the observed CD spectrum for the mixture was compared with the additive spectrum calculated from the individual protein spectra. (C) The CD spectra for c-myc$_{1-143}$ and TFIIB alone and for a mixture of both proteins together. (D) A comparison of the observed and calculated spectra for the c-myc$_{1-143}$ + TFIIB mixture. (E) SDS gel of the proteins used in parts A and C after CD measurements and centrifugation to remove possible particulate material.

factors required for c-myc-dependent transactivation (Resar et al., 1993). Although the factor(s) involved was not identified, it seems likely that it is distinct from the basal transcription factors, as the activity of VP16 was unaffected by B-myc. Thus a model for c-myc-dependent transactivation would involve a conformational change in the transactivation domain concomitant with the binding to basal transcription factors and possible coactivator or adapter proteins. Significantly, both TBP and TFIIF exist in cells, from yeast to humans, as part of the multiprotein complexes TFIID and holo-RNA polymerase, respectively (Pugh & Tjian, 1992; Reese et al., 1994; Koleske & Young, 1995; Ossipow et al., 1995; Poon et al., 1995). Although these complexes appear to be biochemically distinct, they share the common property of being necessary for activated levels of transcription (Chen et al., 1994; Kim et al., 1994; Koleske & Young, 1994; Chiang & Roeder, 1995; Hengartner et al., 1995). An attractive possibility is that the interactions with TBP and RAP74 are important for the recruitment of these key complexes to the promoter during c-myc-dependent gene activation. It will therefore be of importance to determine which, if any, other components of these complexes play a role in the c-myc-dependent transcriptional activation.

The N-terminal 143 amino acids of c-myc can efficiently transactivate a reporter gene in yeast cells, which is in good agreement with a study that demonstrated that full length c-myc could function in yeast, when coexpressed with the dimerization partner max (Amati et al., 1992; Crouch et al., 1993; Fisher et al., 1993). In contrast, when almost the intact protein, amino acids 39−440, was fused to the LexA DBD, only modest levels of activity were observed (Lech et al., 1988). An intriguing possibility is that sequences C-terminal of amino acid 143 have a negative or repressive function and that this accounts for the poor activity of the LexA constructs. Alternatively, the lack of the first 39 amino acids may have a deleterious effect on transactivation by c-myc, as the first 41 amino acids have been shown to have a strong positive effect on transactivation in the context of GAL fusion proteins tested in mammalian cells (Kato et al., 1990). However, most importantly, the results of the present study show localization of a strong transactivation domain to the same region mapped in mammalian cells and show that its activity in yeast is reduced by point mutations that similarly reduced activity in mammalian cells. Thus, yeast offers an attractive model system for further studies on the mechanism of transcriptional activation by c-myc.

In order to better understand the role of c-myc in normal cell proliferation and tumorigenesis, it is important not only to identify target genes for the c-myc−max transcription complex but also to study how these proteins function as transcription factors. In this study, we have demonstrated a functional interaction between the c-myc transactivation

domain and the general transcription factor TBP. Furthermore, the binding of TBP to c-myc$_{1-143}$ resulted in a change in the CD spectrum compatible with an induced conformational change in the c-myc transactivation domain. It will now be important to identify more clearly the nature of the conformational changes taking place and to use mutations affecting transactivation to probe the structure−function relationships involved in c-myc−TBP binding.

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