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Intracellular Association of the Protein Product of the c-*myc* Oncogene with the TATA-Binding Protein

SHYAMALA MAHESWARAN,† HAYYOUNG LEE, AND GAIL E. SONENSHEIN*

Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

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The c-*myc* proto-oncogene encodes nuclear phosphoproteins that bind DNA in a sequence-specific fashion and appear to function as transcriptional activators. Here we demonstrate that a 40-kDa nuclear protein coimmunoprecipitated with c-Myc specifically when nuclear proteins, extracted from nuclei of exponentially growing murine B-lymphoma WEHI 231 cells by using procedures for preparation of *trans*-acting factors, were reacted with anti-c-Myc antibodies made against different regions of the c-Myc protein. In contrast, preparation of nuclear lysates under denaturing conditions significantly reduced this coprecipitation. Upon incubation of WEHI 231 cells with the reversible chemical cross-linking agent dithiobis(succinimidyl propionate), the 40-kDa protein could be cross-linked to c-Myc protein intracellularly. Identification of the 40-kDa protein as the TATA-binding protein (TBP) of the TFIID transcription initiation complex was made by comigration and V-8 protease mapping, which yielded identical peptide fragments upon digestion of the 40-kDa protein and material immunoprecipitated with an anti-TBP specific antibody. Furthermore, in vitro-translated TBP bound to the amino-terminal portion of c-Myc. Column chromatography of cross-linked nuclear proteins showed TBP to be in a large-molecular-weight complex with c-Myc, consistent with a transcription initiation complex. These results indicate that intracellularly, c-Myc interacts with TBP, suggesting a mechanism of interaction of this oncoprotein with the basal transcription machinery.

The c-*myc* proto-oncogene encodes two nuclear phosphoproteins that are initiated at independent translational start sites (8, 9). Sequence analysis of the c-Myc protein shows that it contains a region rich in basic amino acids (basic region) as well as a helix-loop-helix motif and a leucine zipper domain at its carboxy terminus (15). Furthermore, the N termini of Myc protein family members contain an evolutionarily highly conserved stretch of amino acids, implicated in the transformation properties of the protein, which appears to function in transactivation (23, 26). The basic region has been implicated in DNA binding. The c-Myc protein was recently shown to bind a specific sequence of DNA: CACGTG (3, 21). Blackwood and Eisenman (4) isolated a cDNA clone that encodes an 18-kDa human protein (Max) capable of interacting with c-Myc. The helix-loop-helix and leucine zipper motifs of the Max and Myc proteins are important for their interaction. Heterodimers of c-Myc and Max bind DNA more efficiently than do homodimers of c-Myc (3, 21).

By using transfection analysis of heterologous promoter constructs driven by single or multimerized versions of the c-Myc-binding element, several groups have observed that transcription is activated by sequence-specific binding of Myc-Max heterodimers, whereas Max homodimers are inhibitory (1, 12). Although limited by the use of element-driven constructs because of the lack of identification of a normal target gene, these results suggest that binding to Max is essential for c-Myc function. This was further indicated by cotransformation assays of c-Myc and Ras (18). Very recently, however, Bello-Fernandez et al. (2) demonstrated that a c-*myc* expression vector can transactivate the ornithine decarboxylase gene promoter through its two *cis* CACGTG c-Myc binding sites. A c-*myc* expression construct mutated in the leucine zipper and incapable of interaction with Max was also able to transactivate this promoter. These results suggest that c-Myc has the ability to function as a transcriptional activator in the absence of interaction with Max.

We have recently found that a portion of the total nuclear c-Myc protein can be extracted from nuclei by using the procedure developed by Strauss and Varshavsky (24) for isolation of transcription factors (16). Western blot (immunoblot) analysis indicated that approximately 20 to 30% of the total c-Myc protein present in the nuclei of exponentially growing WEHI 231 murine B-lymphoma cells could be extracted with 0.35 M NaCl under nondenaturing conditions. The extractability of the c-Myc protein varied with the growth state of the cells (16). Furthermore, changes in extractability correlated with phosphorylation of the c-Myc protein, suggesting altered compartmentalization or protein-protein interaction. The ability to extract a portion of the total nuclear c-Myc protein under nondenaturing conditions enabled us to investigate the potential in vivo molecular interactions between c-Myc and other cellular proteins. In this study, using coimmunoprecipitation and a newly developed in vivo cross-linking procedure, we found that c-Myc protein is intracellularly associated with a 40-kDa nuclear protein (P40); V-8 protease mapping identified P40 as the TATA-binding protein (TBP) of the TFIID transcription initiation complex.

* Corresponding author. Mailing address: Department of Biochemistry, Boston University Medical School, 80 East Concord St., Boston MA 02118-2394. Phone: (617) 638-4120. Fax: (617) 638-5339. Electronic mail address: gsonensh@med-biochem.bu.edu.

† Present address: Cancer Center, Massachusetts General Hospital, Charlestown, MA 02129.

MATERIALS AND METHODS

Culture conditions and radiolabeling of cells. Murine B-lymphoma WEHI 231 cells were grown in suspension as described previously (16). For radiolabeling, cells (20×10^6) were pelleted by centrifugation and washed twice in phos-

phate-buffered saline (PBS). The pellet was resuspended in 1 ml of Dulbecco's modified Eagle's medium without methionine and cysteine (Sigma), supplemented with 10% dialyzed fetal bovine serum, 4 mM L-glutamine, 5×10^{-8} M β-mercaptoethanol (BME), and 500 μCi each of [^{35}S]methionine (1,300 Ci/mmol) and [^{35}S]cysteine (1,200 Ci/mmol) (Amersham), and incubated for 40 to 60 min. Alternatively, [^{35}S]methionine (Trans-label; Amersham) was used. For chemical cross-linking, the reversible chemical cross-linking agent dithiobis (succinimidyl propionate) (DSP; 100-mg/ml freshly prepared stock in dimethyl sulfoxide; Pierce Chemical Co.) was added to the cell suspension at a final concentration of 1.5 mg/ml during the last 15 to 30 min of the labeling period. The cross-linking reaction was terminated by addition of 30 mM ammonium acetate to the labeling mixture (6).

Preparation of nuclear lysates for analysis of c-Myc. Cells were harvested by centrifugation, and subsequent manipulations were done at 4°C. Cells were washed twice in PBS, gently resuspended in 1 ml of buffer A (60 mM KCl, 15 mM NaCl, 0.25 mM $MgCl_2$, 0.5 mM NaEGTA, 0.5 mM spermine, 0.15 mM spermidine, 0.015 mM sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], 0.2 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml) containing 0.23 M sucrose and lysed with addition of 10 μl of 10% Triton X-100. The nuclei were pelleted by centrifugation at 4,000 × *g* for 5 min and washed once in buffer A containing sucrose, and proteins were extracted with either salt (10 mM HEPES, 350 mM NaCl, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin per ml) for 1 h or antibody buffer (20 mM Tris [pH 7.6], 50 mM NaCl, 0.5% sodium deoxycholate [DOC], 0.5% Nonidet P-40 [NP-40], 0.5% sodium dodecyl sulfate [SDS]) as described previously (16). Nuclear protein extracts prepared with salt (salt extracts) were either used immediately or stored at −70°C after addition of glycerol to a final concentration of 15%.

Immunoprecipitation of c-Myc from nuclear lysates. Nuclear proteins extracted with either antibody buffer or salt were adjusted to final concentrations of 30 mM Tris (pH 7.6), 90 mM NaCl, 0.2% SDS, 0.2% NP-40, and 0.2% DOC and immunoprecipitated with either of three anti-Myc antibody preparations. A mouse monoclonal anti-Myc antibody generated against amino acids (aa) 171 to 188 of the c-Myc protein (Microbiological Associates) was used in indirect immunoprecipitation as described previously (16). Immunoprecipitation with an affinity-purified sheep polyclonal antibody, made against a c-Myc peptide composed of aa 44 to 55 (Cambridge Research Biochemicals) was done under similar conditions except that 7.5 μl of a rabbit anti-sheep immunoglobulin (Dakopatts) was used to complex 3 μg of the primary anti-Myc antibody. Competition to test the specificity of both antibodies was done by preincubation of the antibody reagents with the appropriate cognate peptides (4 and 10 μg for the monoclonal and polyclonal antibodies, respectively) for 1 h at 26°C prior to addition of the radiolabeled lysate. Immunoprecipitations of the salt extracts by using a rabbit polyclonal antibody made against the whole c-Myc protein (kindly supplied by Stephen Hann, Vanderbilt University, Nashville, Tenn.) was done with protein A-Sepharose beads. Briefly, salt extracts were adjusted as described above, precleared with 100 μl of protein A-Sepharose beads (50% bead volume; Pharmacia) at 4°C for 0.5 h, and incubated with 5 μl of the rabbit polyclonal anti-Myc antibody for 1 h at 4°C. Protein A-Sepharose beads were used to bind the Myc antibody, and the antigen complexes were then washed three times in 100 mM Tris (pH 7.6)–140 mM NaCl–1% Triton X-100–1% DOC–0.1% SDS. Immunoprecipitates were resuspended in 50 μl of 2× sample buffer (100 mM Tris [pH 6.8], 4% SDS, 20% glycerol, 20% bromophenol blue) (16), reduced with 10% BME, boiled for 3 min, and subjected to electrophoresis in either 10% or 5 to 15% gradient polyacrylamide–SDS gels as indicated. The molecular weight markers used were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa). The gels were fixed and fluorography was performed as described previously (16).

Analysis of TBP. Cells were harvested and washed, and nuclear proteins were extracted in 10S buffer (50 mM HEPES [pH 7.2], 250 mM NaCl, 0.3% NP-40, 0.1% Triton X-100, 0.005% SDS, 10 mM $NaPO_4$ [pH 7.0], 1 mM NaF, 0.1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) (13). TBP was immunoprecipitated with a rabbit anti-TBP antibody (kindly supplied by A. Berk, University of California, Los Angeles) and protein A-Sepharose as described above except that washing was performed with 10S buffer. For V-8 protease mapping, the immunoprecipitated, radiolabeled TBP and c-Myc-associated P40 bands were excised from a polyacrylamide gel, subjected to V-8 protease digestion (1 or 5 μg) (5), and analyzed on a 17.5% polyacrylamide–SDS gel.

Column chromatography. WEHI 231 cells were radiolabeled with [^{35}S]methionine and [^{35}S]cysteine and cross-linked with DSP, and nuclei were obtained as described above. The nuclei were resuspended in 400 μl of buffer A containing 0.23 M sucrose and 1 mM $CaCl_2$. Following incubation with DNase (10 μg/ml) for 30 min to digest DNA, proteins were extracted with 10 mM HEPES–0.7 M NaCl–1 mM EDTA for 1 h at 4°C. Sephacryl-300 (S-300; Pharmacia) column (50 by 0.5 cm) chromatography was performed with 10 mM HEPES (pH 7.5)–350 mM NaCl–1 mM EDTA. Calibration of the column was performed with the molecular weight markers (Sigma) blue dextran (2,000 kDa), thyroglobulin (663 kDa), and apoferritin (443 kDa). Fractions (0.5 ml) were ethanol precipitated and reconstituted in 100 μl of antibody buffer. Immunoprecipitation was performed with the mouse monoclonal antibody, and SDS-polyacrylamide gel electrophoresis (PAGE) analysis was performed as described above.

RESULTS

A 40-kDa protein specifically coprecipitates with c-Myc in immunoprecipitation reactions. To test for coprecipitation of proteins with c-Myc, radiolabeled WEHI 231 nuclear proteins were extracted under nondenaturing conditions with salt and immunoprecipitated with a monoclonal anti-c-Myc antibody generated against a synthetic peptide containing aa 171 to 188 of the c-Myc protein (Fig. 1, lane 3). The presence of c-Myc and additional proteins was noted in the immunoprecipitates. To determine whether any of these proteins were specifically coprecipitating with c-Myc, the antibody was preincubated with the cognate peptide prior to addition of the labeled lysate. In addition to c-Myc, a 40-kDa cellular protein was reproducibly competed for by addition of the peptide (lane 4). The major bands above c-Myc and at the bottom of the gel are the two membrane forms of immunoglobulin μ heavy chain and kappa light chain, respectively, of the immunoglobulin M antibody produced by the B-lymphoma WEHI 231 cells. These are recognized by the goat anti-mouse antibody used to precipitate the monoclonal anti-c-Myc antiserum and are thus seen in the resulting

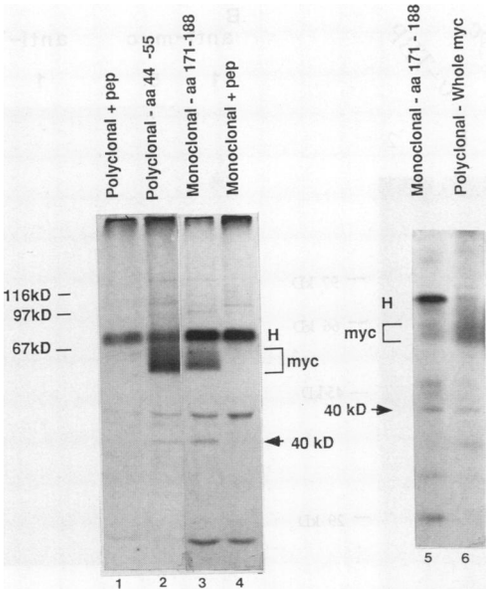

FIG. 1. A 40-kDa protein coprecipitates with c-Myc. Radiolabeled WEHI 231 cell nuclear proteins were extracted under nondenaturing conditions and immunoprecipitated with monoclonal (aa 171 to 188), sheep polyclonal (aa 44 to 55), and rabbit polyclonal (whole c-Myc) anti-c-Myc antibodies. In lanes 1 and 4, antibodies were preincubated with cognate peptides (pep) prior to addition of the lysate. Immunoprecipitates were analyzed on a 10% polyacrylamide–SDS gel. The positions of c-Myc, P40, the μ heavy (H) chain of the immunoglobulin M protein synthesized by the WEHI 231 cells, and the 116-, 97-, and 67-kDa molecular weight markers (shown on the left) are indicated.

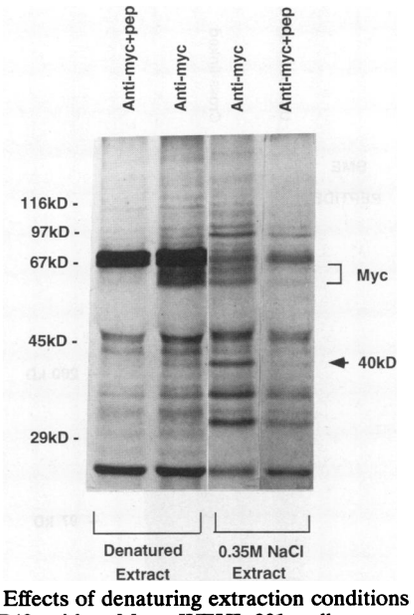

FIG. 2. Effects of denaturing extraction conditions on the interaction of P40 with c-Myc. WEHI 231 cells were labeled with [^{35}S]methionine (Trans-label), and nuclear proteins were extracted either under nondenaturing conditions as for Fig. 1 or under denaturing conditions, using 0.5% NP-40, 0.5% DOC, and 0.5% SDS as reported previously (16). Following immunoprecipitation with the mouse monoclonal (aa 171 to 188) anti-c-Myc antibody in the absence or presence of the cognate peptide (pep), proteins were separated on a 10% polyacrylamide–SDS gel. The exposure time of the resulting autoradiogram was selected to allow for adequate visualization of c-Myc in the salt-extracted lane. The positions of c-Myc, P40, and the molecular weight markers are indicated. The bands above c-Myc and at the bottom of the gel are IgM heavy and light chains, respectively, as described in the legend to Fig. 1.

fluorograph. As expected, these proteins are not competed for by the c-Myc peptide (Fig. 1, lanes 3 and 4).

To confirm the association of P40 with c-Myc, two additional anti-c-Myc antibody preparations were used: an affinity-purified sheep polyclonal antibody made against the amino-terminal peptide 44-55 of the c-Myc protein and a rabbit polyclonal antibody prepared against the whole c-Myc protein. These antibodies immunoprecipitate c-Myc and P40 (Fig. 1). Furthermore, preincubation of the sheep polyclonal antibody with its cognate peptide, prior to addition of labeled lysate, reduced immunoprecipitation of both c-Myc and P40, as described above for the monoclonal antibody. Thus, coimmunoprecipitation of the P40 appears to be based on its intracellular association with the c-Myc oncoprotein, not shared antigenicity. Apparent differences in stoichiometry are probably not significant since different anti-Myc antibodies would behave slightly differently in terms of the ratio of free to bound polypeptide that they would precipitate, and the labeling, extraction, and storage conditions varied slightly between experiments.

To verify that P40 is noncovalently associated with c-Myc protein, we similarly analyzed WEHI 231 nuclear proteins isolated under denaturing conditions with detergents for coimmunoprecipitation of P40. Equal volumes of a labeled WEHI 231 cell preparation was extracted either with antibody buffer, containing 0.5% each SDS, DOC, and NP-40 (Materials and Methods), or salt buffer as described above. The intensity of the c-Myc protein band is greater in the immunoprecipitate from denatured extracts, consistent with our previous results indicating that the salt buffer does not extract all of the c-Myc protein (16). In contrast, the intensity of P40 was highly reduced in the immunoprecipitate of the denatured lysates compared with that from salt extracts (Fig. 2). Again, competition with the cognate peptide reduced coimmunoprecipitation of P40, consistent with an association of P40 with c-Myc. Furthermore, P40 was not detected in Western blot analysis of salt-extracted proteins with use of the sheep polyclonal anti-c-Myc antibody and was not radiolabeled with $^{32}P_i$, unlike c-Myc (data not shown). These results indicate P40 coprecipitates with c-Myc and does not itself either contain an epitope that is recognized directly by the anti-c-Myc antibodies or represent an internally initiated form of c-Myc protein.

Cross-linking of P40 to c-Myc in vivo. To confirm the intracellular association of c-Myc with P40, a procedure was developed for in vivo cross-linking of proteins. The reversible, membrane-permeable chemical cross-linking agent DSP was used. DSP covalently links lysine residues of proteins that are appropriately oriented to interact. WEHI 231 cells were treated with DSP during the final 15 min of a 45-min period of radiolabeling with ^{35}S-labeled methionine and cysteine, and free cross-linker was inactivated by the addition of ammonium acetate (Fig. 3). Immunoprecipitation of c-Myc protein from DSP-cross-linked nuclear proteins extracted with salt yielded a broad band of larger complexes migrating at the upper region of a 5 to 15% gradient gel following SDS-PAGE analysis under nonreducing conditions (Fig. 3). Broad migration patterns are frequently observed with cross-linked material (6). These high-molecular-weight complexes could be abolished upon preincubation of the

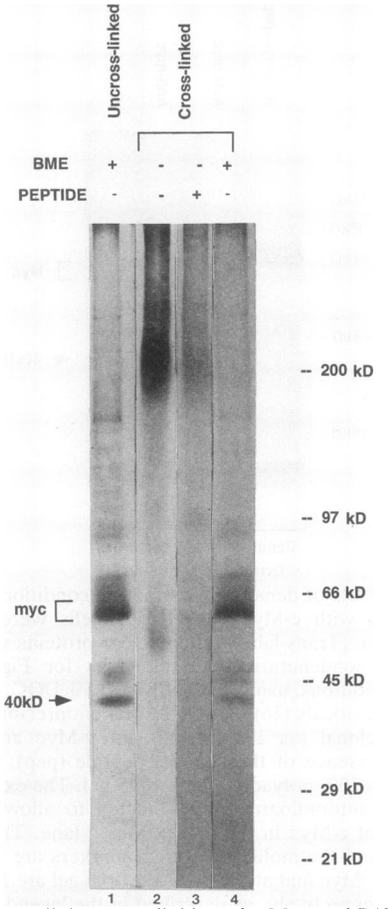

FIG. 3. Intracellular cross-linking of c-Myc and P40. WEHI 231 cells were radiolabeled for 40 min with [^{35}S]methionine and [^{35}S]cysteine. The cross-linking agent DSP was added to a portion of culture during the last 20 min of the labeling period. Nuclear proteins were extracted with salt buffer and immunoprecipitated with the mouse monoclonal (aa 171 to 188) antibody in the absence or presence of the cognate peptide. Immunoprecipitates were either reduced or not with 10% BME, as indicated, and separated on a 5 to 15% gradient polyacrylamide–SDS gel.

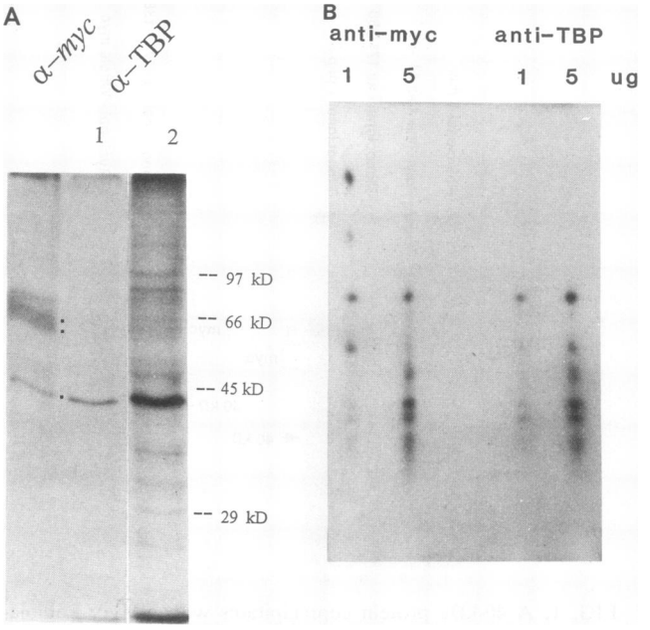

FIG. 4. c-Myc-associated P40 is antigenically related to TBP. (A) Comigration with TBP. WEHI 231 cells were radiolabeled, and cross-linked nuclear proteins were either extracted with antibody buffer and immunoprecipitated with rabbit polyclonal anti-whole c-Myc antibody or extracted with 10S buffer (13) and immunoprecipitated with a rabbit anti-TBP antibody, using protein A-Sepharose. Immunoprecipitated proteins were reduced with BME and analyzed on a 10% polyacrylamide–SDS gel. The positions of the 97-, 66-, 45-, and 29-kDa molecular weight markers and of Myc (:) and TBP (·) are indicated. For the TBP immunoprecipitate, a longer exposure of the autoradiogram is shown in lane 2 to facilitate visualization of the coprecipitating bands. (B) V-8 protease mapping. The immunoprecipitated, radiolabeled TBP and c-Myc-associated P40 bands, prepared as described above, were excised from a polyacrylamide gel, subjected to V-8 protease digestion (1 or 5 μg), and analyzed on a 17.5% polyacrylamide–SDS gel.

antibody with the cognate peptide (Fig. 3, lanes 2 and 3). Reduction of the c-Myc immunoprecipitate with 10% BME prior to electrophoresis resulted in the release of c-Myc and P40 (Fig. 3, lane 4). These results strongly suggest that P40 associates with c-Myc in vivo.

c-Myc-associated P40 is TBP. The viral oncoprotein E1A and c-Myc share homologous protein domains and have been demonstrated to be functionally analogous in transformation assays. In adenovirus-infected cells, E1A interacts with TBP of the transcription initiation complex TFIID (13). Since TBP has a molecular mass of approximately 40 kDa, we compared the migrations of c-Myc-associated P40 and TBP by SDS-PAGE. Radiolabeled nuclear proteins were extracted from cross-linked WEHI 231 cells for immunoprecipitation with an antiserum against c-Myc or TBP in antibody buffer or 10S buffer, respectively, as used previously by Lee et al. (13). Figure 4A shows that the P40 present in immunoprecipitates of c-Myc comigrates with the TBP present in WEHI 231 cells. Since TBP is believed to associate with many transactivating factors (TAF) in the nucleus, c-Myc is not likely to represent a major band in the TBP immunoprecipitate. The darker exposure does indicate the presence of material that comigrates with c-Myc among the polypeptides in the anti-TBP immunoprecipitate.

To more definitively identify the c-Myc-associated P40, V-8 protease peptide mapping was performed. Labeled WEHI 231 cells were cross-linked, and nuclear proteins were extracted and immunoprecipitated for TBP and c-Myc as described above. Following reduction, the proteins were separated by gel electrophoresis, and the P40 and TBP bands in the gel, identified from the resulting autoradiogram, were excised and subjected to V-8 digestion and reelectrophoreses. The patterns of V-8 fragments yielded by digestion of the c-Myc-associated P40 are essentially identical to that of immunoprecipitated TBP (Fig. 4B). These results suggest that the c-Myc-associated P40 is TBP of the TFIID initiation complex. Bernards and coworkers have very recently shown that TBP can interact with c-Myc protein in vitro (2a, 10). Using the amino-terminal 204 aa of c-Myc linked to glutathione *S*-transferase protein, we have confirmed the direct association of in vitro-transcribed and -translated TBP with c-Myc (data not shown); these results, therefore, indicate the interaction of these two proteins.

Gel filtration column chromatography of cross-linked WEHI 231 nuclear lysates. A TFIID complex, which is composed of multiple TAF proteins as well as TBP, is very

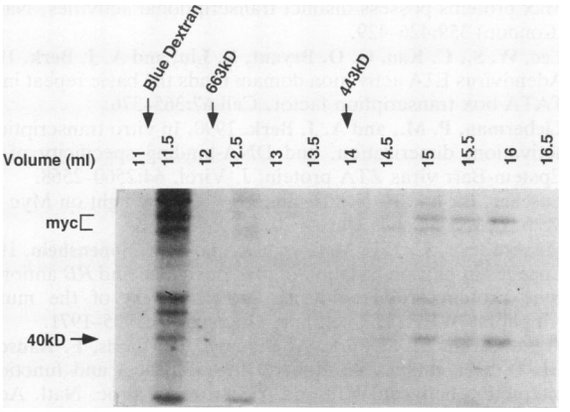

FIG. 5. S-300 column chromatography analysis of c-Myc in cross-linked, salt-extracted WEHI 231 nuclear proteins. Fractions (0.5 ml) were ethanol precipitated, reconstituted in 100 μl of antibody buffer, and immunoprecipitated with the mouse monoclonal (aa 171 to 188) antibody. Samples were reduced and subjected to 10% polyacrylamide–SDS gel analysis as for Fig. 4. Positions of c-Myc and P40 are shown.

large and thus elutes slightly behind the void volume upon chromatography in an S-300 gel filtration column (25). To determine whether c-Myc could be detected in such a large complex, S-300 column chromatography was performed. WEHI 231 cells were cross-linked with DSP during the final 30 min of a 60-min radiolabeling with ^{35}S-labeled methionine and cysteine. Nuclear proteins were extracted with 700 mM NaCl to maximize c-Myc protein extraction and fractionated on an S-300 column. Fractions (0.5 ml) were immunoprecipitated with the monoclonal anti-c-Myc antibody. SDS-PAGE analysis of the immune complexes following reduction with 10% BME shows c-Myc protein to be present in multiple fractions: fractions close to the void volume of the column and fractions of samples retarded in the column (Fig. 5). This analysis shows a 40-kDa protein to be present in the same fractions as c-Myc. Immunoprecipitation analysis of uncross-linked nuclear salt extracts fractionated on the same column also indicated that the two proteins were present in a large complex with multiple other proteins (data not shown). These results suggest that the c-Myc protein is present with the 40-kDa TBP in a large complex, similar in size to those seen for transcriptional initiation complexes (25).

DISCUSSION

We have demonstrated an intracellular association of c-Myc with a 40-kDa nuclear protein that is antigenically related to TBP of the TFIID complex in murine WEHI 231 B-lymphoma cells. Coimmunoprecipitation of a 40-kDa protein was noted with use of three different antibody preparations generated against different regions of c-Myc when nuclear proteins were extracted under nondenaturing conditions. Furthermore, this protein could be cross-linked in vivo with c-Myc. A 40-kDa protein was similarly coimmunoprecipitable with c-Myc in salt extracts of nuclei from human HL-60 promyelocytic leukemia cells, 3T3 fibroblasts, and Ba/F3 pre-B cells (data not shown), suggesting that the association is a general phenomenon. Identification of this protein as TBP was made using an antiserum specific for TBP and V-8 protease mapping. Consistent with this identification, we found that P40, when associated with c-Myc, is unphosphorylated (data not shown) and present within a large complex. Furthermore, the ability of the amino terminus of c-Myc, which contains the transactivation domain (11, 23), to interact with TBP was demonstrated directly in vitro, confirming the results of Haterboer et al. (10). The intracellular association of c-Myc with TBP suggests a mechanism for the interaction of this transactivating oncoprotein with the RNA synthetic machinery within the cell.

In addition to P40, there were several other proteins that appeared to coprecipitate with c-Myc in antibody-peptide competition studies, consistent with our finding that P40 and c-Myc were present within large-molecular-weight complexes (data not shown). For transcription by RNA polymerase II, recent evidence has demonstrated that binding of TBP to the TATA box allows for binding of the other general transcription factors, thus mediating basal, i.e., nonactivated, transcription. Transactivation is then believed to be mediated by the direct interaction of the activator protein with TBP. Interaction of TBP with several activator proteins of viral origin, including adenovirus E1A, herpes simplex virus VP16, and Epstein-Barr virus Zta (13, 14, 25), has been demonstrated. The c-Myc and P53 (22) proteins represent some of the first cellular transactivators to be demonstrated to interact with TBP.

Progress in the identification of specific cellular proteins which complex with c-Myc has been slow because of the protein's unusual properties of extractability, i.e., a requirement for high concentrations of either ionic detergents such as SDS or salt for full extraction (7, 16). These conditions weaken hydrophobic, noncovalent interactions commonly involved in protein-protein interactions. In fact, we observed decreased association of P40 with c-Myc when nuclear extracts were prepared with higher salt concentrations (data not shown). The cross-linking procedure that we have developed enabled us to characterize the molecular interactions of c-Myc protein within the cell. This procedure has recently been used to demonstrate the interaction of p53 with the Wilms' tumor protein as well as to confirm its known interaction with the adenovirus E1B protein (17). Furthermore, a similar method, developed independently, has been used to study collagen interaction with the heat shock protein HSP47 (19). Thus, the in vivo use of the cross-linking agent DSP, which is resistant to detergents but reversible with reducing agents, should be widely applicable for the study of intracellular protein-protein interactions.

We were unable to detect a coprecipitating protein with mobility corresponding to that of Max in immunoprecipitates of c-Myc from extracts of WEHI 231 cells. While the detergent DOC used in the immunoprecipitations might have resulted in some dissociation of such complexes in salt extracts (4), this would not be a problem in cross-linked samples. In fact, a band migrating at the appropriate molecular mass for Max (18 to 22 kDa) was noted in c-Myc immunoprecipitates of cross-linked protein extracts obtained from human HL-60 cells (data not shown). However, we do not know whether the lysine residues are in the correct configuration to be cross-linked in the murine proteins, and thus normal interaction of these partners escaped detection in immunoprecipitation assays. While Max is expressed in a wide variety of cells, PC12 cells have undetectable levels of Max protein (28). These results and the findings of Bello-Fernandez et al. (2) raise the possibility that other c-Myc-binding partners exist or that c-Myc can function without a partner. It is likely, however, that interaction of c-Myc with TBP plays an important role in mediating the transactivation ability of this oncoprotein.

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