in a solution of 10 mM 3-[cyclohexylamine]-1-propane-sulfonic acid (CAPS)-NaOH (pH 11.0) and 10% methanol (the TFB1 protein does not transfer efficiently in buffers commonly used for transfer to nitrocellulose). The membrane was incubated with a 1:200 dilution of preimmune or immune serum, and bound antibodies were visualized by means of alkaline phosphatase–conjugated secondary antibodies.

28. Preimmune or immune serum was mixed with an equal volume of Affi-Gel–protein A beads (Bio-Rad), the beads were washed, and the antibodies were covalently cross-linked to the protein A with dimethyl pimelimidate (*24*). The columns were washed in elution buffer [0.1 M glycine-HCl (pH 3), glycerol (20%)] and equilibrated in binding buffer [20 mM Hepes (pH 7.5), 0.1 M potassium acetate, glycerol (20%), 1 mM DTT, 1 mM EDTA, Triton X-100 (0.1%), and protease inhibitors (*6*)]. The factor b used in Fig. 3B was a partially purified preparation obtained by fractionation of a yeast whole-cell extract on Biorex 70 (Bio-Rad) and DEAE-Sephacel (Pharmacia) columns (*25*). The preparation (1.5 ml) was applied to a preimmune or immune antibody column (0.5 ml), and the flow through was reapplied to the column five times. The column was washed with 10 volumes of binding buffer followed by 50 volumes of binding buffer containing 0.8 M potassium acetate and 50 volumes of binding buffer containing Triton X-100 (0.5%) and sodium deoxycholate (0.5%). Bound proteins were eluted with several 0.25-ml portions of elution buffer. Each eluted fraction was immediately neutralized by the addition of 15 μl of 2 M tris-acetate, pH 8.0. The eluted fractions were concentrated by trichloroacetic acid precipitation, analyzed by SDS-PAGE, and stained with Coomassie blue. The same starting material and immunoaffinity procedure were used in Fig. 4A, except that the detergent wash was omitted. In Fig. 4B, the fraction loaded on the column was the same as in Fig. 3A, and the immunoaffinity procedure was the same as in Fig. 4A.

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Cloning of the 62-Kilodalton Component of Basic Transcription Factor BTF2

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Cloning of the mammalian basic transcription factors serves as a major step in understanding the mechanism of transcription initiation. The 62-kilodalton component (p62) of one of these transcription factors, BTF2 was cloned and overexpressed. A monoclonal antibody to this polypeptide inhibited transcription in vitro. Immunoaffinity experiments demonstrated that the 62-kilodalton component is closely associated with the other polypeptides present in the BTF2 factor. Sequence similarity suggests that BTF2 may be the human counterpart of RNA polymerase II initiation factor b from yeast.

In vitro transcription of genes requires RNA polymerase B (II) and transcription factors TFIIA, TFIIB, TFIID (*1*), TFIIE, TFIIF (*2–4*), and BTF2 (*5*). The BTF2 transcription factor was purified and found to coelute or to cosediment with five polypeptides ranging from 35 to 90 kD after five chromatographic steps and one glycerol gradient (*5*). Transcription factor BTF2 is absolutely required for accurate in vitro transcription from a minimal promoter (containing the cap site and the TATA box) and sediments with a molecular size of 250 kD (*5*). Other transcription factors have also been identified and purified and include TFIIG, TFIII (*6*), TFIIH (*7*), and δ (*8*). It is unclear whether BTF2 corresponds to one or more of these factors (*5–8*). We now report the cloning and properties of the 62-kD component (p62) of BTF2.

We designed three degenerate oligonucleotide probes on the basis of the sequences of tryptic peptides from the purified p62 and used them for the screening of a HeLa cell cDNA library (*9*). Three independent cDNA clones were isolated and sequenced. All of them contained a predicted open reading frame (ORF) encoding a polypeptide of 548 amino acids with a calculated molecular size of 62.030 kD and an isoelectric point (pI) of 8.82 (Fig. 1). The encoded polypeptide contained the three oligopeptides that we microsequenced. We found no similarity with any of the sequences present in protein and nucleic acid databases. No known DNA binding motifs, kinase motifs, or nucleotide binding sites were found. When overexpressed in *Escherichia coli* (*10*) or in insect cells (*11*), the recombinant polypeptide (rp62) had the same electrophoretic mobility on SDS–polyacrylamide gel electrophoresis (PAGE) as the p62 from BTF2. The recombinant protein from either source did not substitute for the BTF2 activity in a HeLa cell in vitro transcription system lacking BTF2 (*12*), and no stimulation was observed when rp62 was added to an in vitro transcription system containing limiting amounts of BTF2. This result indicates that rp62 alone is not sufficient to restore BTF2 activity and that other BTF2 polypeptides may be required.

Several lines of evidence indicate that p62 is associated with BTF2 activity. First, a monoclonal antibody (M.Ab3c9) to purified rp62 (*13*), recognized a 62-kD polypeptide (p62) that cofractionated with the BTF2 activity throughout the purification (Fig. 2, A to D) (*5*, *12*, *14*). Second, inhibition of in vitro transcription indicated that p62 is a component of the BTF2 activity. Increasing amounts of either purified M.Ab3c9 or a control antibody M.AbC (*13*) were incubated for 1 hour at

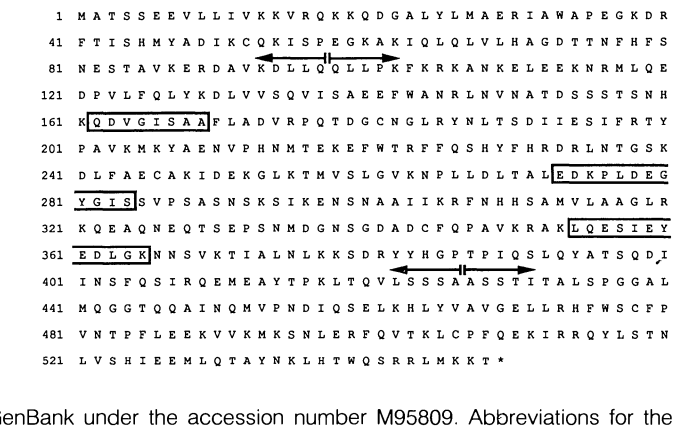

Fig. 1. Analysis of the human BTF2 p62 polypeptide sequence. Positions of the amino acids are indicated on the left. The three peptides that were microsequenced from the purified 62-kD polypeptide are boxed. Two imperfect amino acid repeats [KDLLQ/QLLPK (residues 93 to 102) and LSSSA/ASSTI (residues 422 to 431)] are shown by inverted bold arrows. The nucleotide sequence of the BTF2 p62 subunit can be found in GenBank under the accession number M95809. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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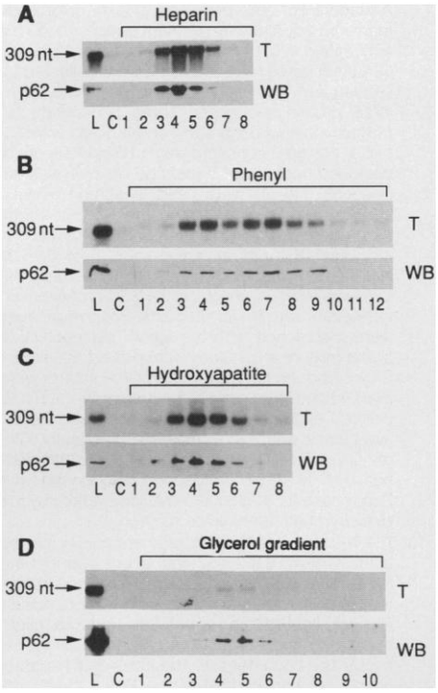

Fig. 2. The p62 that coelutes or cosediments with BTF2 activity cross-reacts with the M.Ab3c9 antibody to the rp62. The BTF2 transcriptionally active fractions that were eluted from an SP.5PW column (*5*) were applied onto a Heparin 5PW column (**A**) equilibrated in 50 mM tris-HCl (pH 7.9) buffer containing glycerol (8.7%), 5 mM EDTA, and 50 mM KCl and eluted with an ammonium sulfate gradient (0 to 0.5 M). The transcriptionally active fractions eluted from the heparin column were then successively applied onto a phenyl 5PW column (**B**), a hydroxyapatite HPLC column (**C**), and a glycerol gradient (**D**) as described (*5*). The protein fractions were tested in a standard run-off transcription assay lacking BTF2 (*12*) (2 to 5 μl; "T") and by protein immunoblotting (*14*) (2 to 10 μl; "WB") with M.Ab3c9. L, loaded material, C, negative control. Only some of the gradient fractions are presented. (A) Lanes 1 to 8, fractions eluted between 0.35 and 0.48 M ammonium sulfate. (B) Lanes 1 to 12, fractions eluted between 0.3 and 0 M ammonium sulfate. (C) Lanes 1 to 8, fractions eluted between 0.29 and 0.50 M potassium phosphate. (D) Lanes 1 to 10, collected fractions (tubes 9 to 18).

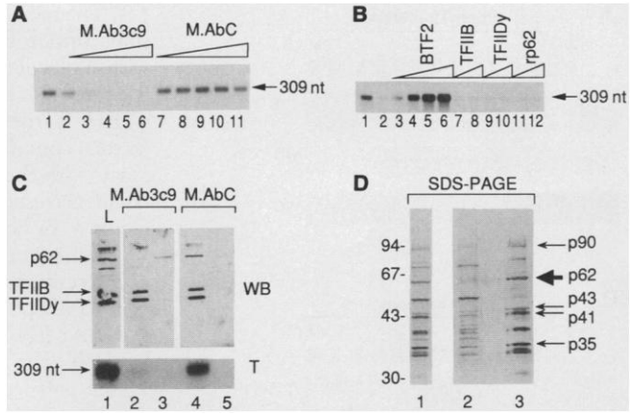

Fig. 3. Inhibition of in vitro transcription by monoclonal antibody M.Ab3c9. (**A**) The partially purified BTF2 (SP.5PW fraction) (*5*) was incubated for 1 hour at 4°C with increasing amounts of either purified M.Ab3c9 (lanes 2 to 6; 3, 12, 30, 60, 120 ng) or M.AbC (lane 7 to 11; 3, 12, 30, 60, 120 ng) and then added to a standard run-off transcription assay lacking BTF2 (*12*). Lane 1, no antibody addition. (**B**) The BTF2 fraction was first incubated with M.Ab3c9 (20 ng) for 1 hour at 4°C. We then added increasing amounts of either hydroxyapatite-purified BTF2 (lanes 3 to 6), TFIIB (lanes 7 to 8), TFIIDy (lanes 9 to 10), or partially purified rp62 overexpressed in insect cells (*11*) (lanes 11 to 12). After incubation for 5 min at 4°C, the other complementary transcription factors, RNA polymerase II(B), and the Ad2MLP template were added with the nucleotides, and incubation was continued as described (*5*). (**C**) The partially purified BTF2 (SP.5PW fraction) (*5*) was supplemented with recombinant TFIIB and TFIIDy (*15*) and loaded onto either a M.Ab3c9 Agarose column (lanes 2 to 3) or a control M.AbC Agarose column (lanes 4 to 5). After extensive washing, the proteins were eluted with a 100 mM glycine-HCl (pH 2.5) buffer (lanes 3 and 5). The protein fractions (pH 2.5) were adjusted to pH 7.9 with 2 M tris. The proteins in the various fractions were identified by protein immunoblotting with a mixture of purified M.Ab3c9 and polyclonal antibodies to TFIIB and TFIIDy (upper panel, WB) and tested for transcriptional activity in a standard run-off transcription assay lacking BTF2 (lower panel, T). Lane 1, loaded fraction; lanes 2 and 4, flow-through fractions of both columns as indicated at the top of the panel. The 50- and 70-kD polypeptides detected by protein immunoblotting correspond to polypeptides cross-reacting with the TFIIB antibody. The positions of the polypeptides corresponding to p62, TFIIB, and TFIIDy are indicated by arrows. (**D**) Purified BTF2 (500 μl) hydroxyapatite fraction (lane 1) (*5*) was loaded onto a M.Ab3c9 (200 μl) Agarose column, and the flow-through fraction (lane 2) and the fraction eluted at pH 2.5 (lane 3) were analyzed by SDS-PAGE (11%). Gels were silver-stained. The arrows on the right part of the panel indicate the previously identified polypeptides in the purified BTF2 fraction (*5*). Molecular sizes of marker proteins are indicated at the left (in kilodaltons).

4°C with a fixed amount of partially purified BTF2 activity. The mixture was then added to an in vitro transcription system containing all the other general transcription factors, RNA polymerase B (II), and the minimal Ad2MLP template (*12*). Specific transcription was reduced as a function of the concentration of M.Ab3c9 (Fig. 3A), whereas transcription was not inhibited when equivalent amounts of the control M.AbC were added. To further establish that the inhibition resulted from the specific interaction between the monoclonal antibody M.Ab3c9 and the BTF2 factor, increasing amounts of either purified BTF2 fraction [the hydroxyapatite fraction (*5*), which contains only BTF2 activity], purified overexpressed TFIIB and TFIIDy (*15*), or the recombinant rp62 were added to an in vitro transcription reaction that had been inhibited by 50% after the addition of M.Ab3c9 (Fig. 3B). Under these conditions, the addition of increasing amounts of BTF2 overcame the inhibition of transcription produced by the antibody, whereas the addition of either TFIIB, TFIIDy, or rp62 had no effect on the rate of transcription (Fig. 3B). The addition of either recombinant TFIIB or recombinant TFIIDy did not increase the rate of transcription because both factors were present in excess in the assays. Third, to demonstrate that p62 is part of the BTF2 protein complex, a partially purified BTF2 fraction eluted from a sulfopropyl (SP.5PW) column (*5*), supplemented with recombinant TFIIB and TFIIDy [used as markers (*15*)], was loaded on either a M.Ab3c9 affinity column or on a M.AbC column, which served as the control (*13*). Both columns were equilibrated in a buffer containing 50 mM tris-HCl (pH 7.9), 500 mM KCl, and NP-40 (0.01%) to reduce nonspecific interactions. The proteins were eluted with a buffer containing 100 mM glycine-HCl (pH 2.5). The protein fractions were identified by protein immunoblotting and in vitro transcription assays (Fig. 3C). Almost no stimulation of transcription was detected when proteins from the M.Ab3c9 column flow-through fraction were tested in an in vitro transcription assay lacking BTF2, whereas the flow-through fraction of the M.AbC column displayed BTF2 activity (Fig. 3C). Thus, BTF2 activity was selectively retained on the M.Ab3c9 column. No BTF2 activity was recovered from the M.Ab3c9 column in the fraction eluted at pH 2.5 (Fig. 3C) because BTF2 is sensitive to low pH. However, protein immunoblot analysis with M.Ab3c9 revealed the presence of p62 in both fractions eluted from the M.Ab3c9 column and in the M.AbC column flow-through fraction (Fig. 3C). When similar affinity chromatography was performed with partially purified BTF2 activity, the fraction eluted from the M.Ab3c9 column contained several polypeptides in addition to p62 (Fig. 3D). Four of these polypeptides (90, 43, 41, and 35 kD) had molecular sizes corresponding to those of the polypeptides present in the most purified BTF2 fraction (*5*). In addition to the five putative BTF2 polypeptides (*5*), the M.Ab3c9 column eluate also contained three other polypep-

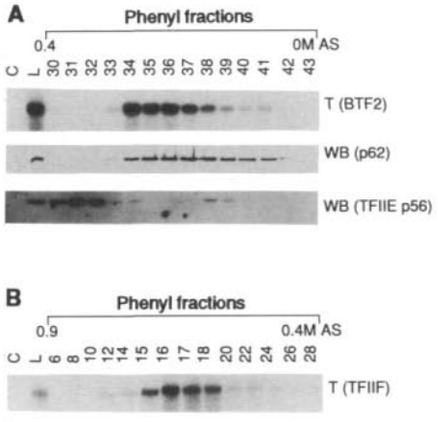

Fig. 4. BTF2 has different chromatographic properties than TFIIE and TFIIF. The SP.5PW fraction containing BTF2 was loaded onto a phenyl 5PW column, and elution was performed with an ammonium sulfate (AS) gradient (0.9 to 0 M). Eluted fractions 6 to 28 and 30 to 43 were tested for BTF2 transcription activity (**A**), TFIIF transcription activity (**B**), and the presence of either the p62 or the TFIIE p56 polypeptide (A) with the corresponding antibodies (*14*). L, loaded SP.5PW fraction. C, control.

tides with molecular sizes of 80, 38, and 32 kD. These polypeptides, which were not detected in the eluate of the M.AbC column, could have resulted from partial proteolysis (Fig. 3D). These results demonstrate that p62 is part of a multiprotein complex that exhibits BTF2 transcriptional activity.

The BTF2 factor appears to be different from the TFIIE and TFIIF factors. First, the primary sequence of rp62 is different from the large subunits of the previously reported sequences of TFIIE and TFIIF (*2*). Second, the TFIIE and TFIIF polypeptides were not detected by immunoblotting with either RAP30 or TFIIE p56 antibodies (*16*). Third, the BTF2 transcription activity was eluted from a phenyl column in fractions 33 to 41 (Fig. 4A), whereas the TFIIF transcription activity was mainly present in fractions 15 to 18 (Fig. 4B). Furthermore, protein immunoblots (*16*) revealed the presence of RAP30 and TFIIE 56-kD polypeptides in fractions 15 to 18 and 30 to 33, respectively (Fig. 4A). Trace amounts of RNA polymerase B (II) and TFIIE p56 were detected in fractions 38 and 39 (Fig. 4A), which indicates that TFIIE may interact with the polymerase (*17*). Increasing amounts of the fraction containing TFIIE p56 (fraction 31) did not substitute for BTF2 activity. This suggests that BTF2 and TFIIE are different proteins. Recent studies revealed that a partially purified TFIIH preparation contains polypeptides with molecular sizes of about 60 and 40 kD, in addition to a 33- and a 95-kD polypeptide (*7*). The most highly purified preparation of transcription factor δ contains polypeptides with molecular sizes of 35, 38, 43, 46, 68, 85, and 94 kD (*8*). The purified HeLa TFIIH or the purified rat δ transcription factors contain a polypeptide that cross-reacts with the M.Ab3c9 (*12*). Thus, BTF2 could correspond to TFIIH, transcription factor δ, or both.

The amino acid sequence of the human p62 described in this report is similar to that of a 75-kD polypeptide that is a subunit of the yeast RNA polymerase II transcription factor b. The amino acid sequences of the 75-kD polypeptide (*19*) from residues 233 to 302 and of p62 from residues 171 to 236 show an overall similarity of 59% (41% identity and 18% similarity). On the basis of this sequence similarity, it seems likely that BTF2 may be the human counterpart of yeast factor b, which has CTD kinase activity (*19*). No CTD kinase activity has been found to be associated with either the yeast 75-kD subunit or the human rp62. Whether such kinase activity is indeed associated with BTF2 is presently under investigation.

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9. The purified BTF2 fraction (*5*) (200 pmol) was concentrated by ammonium sulfate (60%) precipitation. Proteins separated by SDS-PAGE (15% gel) were transferred onto a polyvinylidene difluoride membrane (Immobilon, Millipore) and stained with amido black. The region containing p62 was excised and hydrolyzed with trypsin (Promega). The digested polypeptides were released with formic acid (70%) and resolved by reversed-phase chromatography (Brownlee Aquapore RP-300; pore size, 7 μm; column dimension, 1 × 250 mm; gradient elution, 10 to 70% acetonitrile in 0.1% trifluoroacetic acid; flow rate, 50 μl/min for 70 min). Fractions were collected for subsequent analysis on an Applied Biosystems 477A protein sequencer. Three degenerate oligodeoxynucleotide probes end-labeled with ^{32}P (~5 × 10^8 cpm per microgram of oligonucleotide) designed from microsequenced oligopeptide sequences (Fig. 1) were used to screen a HeLa cDNA library constructed in the λZAP-II vector (Stratagene). Hybridization was performed at 42°C for 18 hours in a 6× solution of 0.15 M NaCl–0.015 M sodium citrate, pH 7.0 (SSC). Filters were washed at 46°C in 6× SSC and exposed to Kodak x-ray film for 36 hours. Positive plaques were rescreened with the same oligonucleotide probe mixture and clones were picked. Bluescript SK(−) phagemids containing cDNA inserts were excised and rescued with the helper phage R408. Dot blot analysis was performed on double positive clones with each probe independently. Single-stranded DNA was prepared by superinfection with M13K07 phage and sequenced with Sequenase (U.S. Biochemical Corporation) by the dideoxy chain termination method.
10. A 1.7-kb Bsp MI–Bsp MI fragment containing the entire ORF of BTF2 p62 was subcloned into the Sma I site of a Bluescript SK(−) phagemid and the Bam HI–Sac I fragment of the latter construction was ligated to an Xba I–Sac I adaptor oligonucleotide and the pET11a plasmid (Novagene) cut by Xba I and Bam HI. The Xba I–Sac I adaptor oligonucleotide contained the Xba I–Nde I sequence of pET11a followed by the first 68 nucleotides of the BTF2 p62 cDNA. *Escherichia coli* strain BL21(DE3) (Novagene) containing pET11a-BTF2 p62 was grown in NZY medium supplemented with ampicillin (100 μg/ml) at 37°C. Cells were induced with isopropylthiogalactoside (0.4 mM) at an optical density at 600 nm (OD_{600}) of 0.8. After 3 hours at 37°C, cells were collected by centrifugation, and the pellet was resuspended in 50 mM tris-HCl buffer (pH 7.8), 2 mM EDTA, and 100 mM KCl and stored at −80°C. The frozen cells were thawed and lysed by sonication on ice. Cell debris and inclusion bodies were removed by centrifugation at 6000*g* for 20 min at 4°C.
11. The rp62 cDNA and the AcMNPV genome were transfected into Sf9 cells as described [M. D. Summers and G. E. Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures* (Bulletin 1555, Texas Agricultural Experiment Station, 1987)]. The rp62 was extracted in 50 mM tris-HCl pH (7.8), glycerol (20%), 1 mM EDTA, and 500 mM KCl and purified on a DEAE Spherodex column (IBF, France) equilibrated in the same buffer. The flow-through fraction was subsequently diluted to 50 mM KCl and further purified on a heparin Ultrogel column. The rp62 polypeptide was eluted with 400 mM KCl.
12. The in vitro transcription assays were performed in a reaction volume of 20 to 40 μl containing 50 mM tris-HCl (pH 7.9), 6.5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM dithiothreitol, 50 mM KCl, glycerol (8.7% v/v), Ad2MLP Eco RI–Sal I template (60 ng), TFIIDy (16 ng) (*15*), TFIIB (15 ng) (*15*), TFIIA (4 μg of DE0.35), TFIIF (0.9 μg of phenyl-5PW 0.65), RNA-polymerase B(II) containing TFIIE [(0.002 unit of eluted fraction from the phenyl column with 0.12M KCl (*5*)], and aliquots of the various BTF2 fractions as described in (*5*).
13. The rp62 inclusion body, purified from *E. coli*, was emulsified in phosphate-buffered saline (PBS) with complete Freund's adjuvant buffer, and aliquots (100 μg) were injected intraperitoneally into BALB/c mice. The spleen cells were fused with Sp2.0 Ag14 myeloma cells (*20*). Culture supernatants were first tested by enzyme-linked immunosorbent assay with the solubilized rp62 and by protein immunoblotting on rp62. For production of ascites fluid, cells (2 × 10^6) were injected into pristane-primed BALB/c mice. The monoclonal antibody M.Ab3c9 was purified from ascite by caprilic acid and ammonium sulfate precipitations (*21*) and further purified by chromatography on a

DEAE-Spherodex column with the use of a phosphate gradient. The purified M.Ab3c9 and the M.AbC (M.IgG, Nordic Immunology) were conjugated to Affi-Gel10 (Bio-Rad) columns (2.5 mg antibody per milliliter of swollen gel). The columns were then equilibrated in 50 mM tris-HCl (pH 7.8), glycerol (20%), 1 mM EDTA, 500 mM KCl, and NP-40 (0.01%).
14. Protein immunoblotting was performed as described (*15*), except that the proteins were detected with the Amersham chemiluminescence protein immunoblotting detection reagents.
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Modulation of the Dimerization of a Transcriptional Antiterminator Protein by Phosphorylation

Orna Amster-Choder and Andrew Wright

The transcriptional antiterminator protein BglG inhibits transcription termination of the *bgl* operon in *Escherichia coli* when it is in the nonphosphorylated state. The BglG protein is now shown to exist in two configurations, an active, dimeric nonphosphorylated form and an inactive, monomeric phosphorylated form. The migration of BglG on native polyacrylamide gels was consistent with it existing as a dimer when nonphosphorylated and as a monomer when phosphorylated. Only the nonphosphorylated dimer was found to bind to the target RNA. When the dimerization domain of the λ repressor was replaced with BglG, the resulting chimera behaved like an intact λ repressor in its ability to repress λ gene expression, which suggests that BglG dimerizes in vivo. Repression by the λ-BglG hybrid was significantly reduced by BglF, the BglG kinase, an effect that was relieved by conditions that stimulate dephosphorylation of BglG by BglF. These results suggest that the phosphorylation and the dephosphorylation of BglG regulate its activity by controlling its dimeric state.

Control of transcription involves the interaction between specific DNA or RNA sequence elements and protein factors (*1*, *2*), many of which have been shown to bind DNA as dimers (*3–5*). Changes in the oligomeric state of transcription factors, which can change their sequence specificity or activation function, increase the diversity of regulation by a limited number of regulatory molecules in the cell. The regulation of dimerization has not yet been shown to participate in the regulation of transcription by RNA binding proteins.

The processes that control the switch between dimers and monomers in the cell are poorly understood. These processes may involve interaction with other cellular accessory proteins or covalent modification of the proteins being dimerized. In the case of hepatocyte nuclear factor–1α, a mammalian homeodomain protein, a protein dimerization cofactor has been identified (6). In no case has a covalent modification been shown to be involved in the regulation of the dimerization process.

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Transcription of the *bgl* operon in *E. coli* is regulated by reversible protein phosphorylation (*7*, *8*). BglG is a sequence-specific RNA binding protein whose ability to inhibit termination of transcription of the *bgl* operon is modulated by BglF, a membrane-bound protein kinase–phosphatase that senses the presence of β glucosides (*7–10*).

To increase our understanding of how BglG functions as a transcriptional antiterminator, we constructed a series of hybrid proteins that contained various portions of BglG and complementary portions of Sac Y, a similar antiterminator protein from *Bacillus subtilis* (*11*). The hybrids (*12*) were tested for their ability to prevent termination of transcription of a *bgl-lacZ* fusion gene in *E. coli*. One of the hybrids failed to antiterminate, giving only 2 to 3 units of β-galactosidase activity compared to 60 units obtained with wild-type BglG. Moreover, this hybrid exerted a dominant negative effect on antitermination by wild-type BglG co-expressed in the same cell, reducing β galactosidase production from 60 units to 2 to 3 units. One possible explanation for this result is that the dominance resulted from the formation of an inactive hetero-oligomer between the hybrid protein and BglG, which implies that an oligomeric form of BglG is the active species in antitermination.

To test this hypothesis, we determined the molecular size of native BglG on a set of nondenaturing polyacrylamide gels (Fig. 1A). The molecular size of nonphosphorylated BglG, estimated by extrapolation of its coefficient of retardation (Fig. 1), was ~60,000 daltons. The molecular size of BglG, calculated from its amino acid sequence and confirmed by denaturing gels, is 32,067 daltons. On the basis of this result, we conclude that BglG exists as a dimer. The molecular size of the phosphorylated form of BglG, determined with the same method, was ~30,000 daltons (Fig. 1B), which suggests that phosphorylated BglG exists as a monomer.

We obtained direct evidence that dimeric BglG binds to its RNA target although monomeric phosphorylated BglG does not by probing the proteins in their native state with RNA (Fig. 2). Extracts prepared from bgl^0 and bgl^+ *E. coli* strains, enriched respectively for either the dimer or the phosphorylated monomer, were fractionated on native gels, blotted onto nitrocellulose filters, and probed with labeled target RNA. Conditions that allowed the separation of the two forms of BglG were chosen on the basis of their known relative mobilities (R_m) in native gels (Fig. 1). Our results indicate that the RNA probe bound to the dimeric nonphosphorylated form of BglG but did not bind to the monomeric phosphorylated form (Fig. 2A). By probing the extracts, after fractionation by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), with anti-BglG antibodies (Fig. 2B), we were able to show that the two extracts contained the same total amount of phosphorylated and nonphosphorylated BglG.

To further establish that BglG functions as a dimer, we used the NH_2-terminal DNA binding domain of the bacteriophage λ repressor as a reporter for dimerization (*13*). The NH_2-terminal domain of the λ repressor does not dimerize and thus requires a dimerization domain to allow it to bind strongly to its operator and repress transcription. To test the ability of BglG to serve as a dimerization domain, we constructed a gene fusion between the NH_2-terminal coding sequence of the λ repressor and the complete *bglG* gene (λ-*bglG*). The regulatory properties of the chimeric λ-BglG protein were compared to the properties of intact λ repressor (*ind1*), λ-zip, a λ-leucine zipper chimera that behaves like an intact λ repressor (*13*), the DNA binding domain of the λ repressor (amino acids 1 to 131), and λ-zip interrupted by truncat-