GAL4-I κ B α and GAL4-I κ B γ activate transcription by different mechanisms

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Received December 28, 1992; Revised and Accepted March 19, 1993

ABSTRACT

I κ B proteins regulate Rel/NF- κ B transcription complexes through a direct protein–protein interaction. In addition, we have previously shown that certain I κ B proteins (I κ B α and I κ B γ) can act as activators of transcription when fused to the DNA-binding domain of GAL4. We now show that a mutant chicken I κ B α protein that cannot interact with Rel proteins *in vitro* did not activate transcription when fused to GAL4 in chicken embryo fibroblasts (CEF) and *Saccharomyces cerevisiae*, and did not inhibit growth in yeast; in contrast, an I κ B α mutant that can still interact *in vitro* with Rel proteins activated transcription in both CEF and yeast and inhibited growth in yeast. In CEF, GAL4-I κ B α mediated transcription activation was inhibited by co-transfection with an expression vector for a RelA (p65) protein that contained sequences needed for interaction with I κ B α but that was deleted of its transcription activation domain. Therefore, it appears that GAL4-I κ B α activates transcription by interacting with an endogenous Rel family protein in CEF. In contrast, the activation domain from I κ B γ behaved as a genuine acidic activator of transcription and did not inhibit growth when expressed in yeast. Since transcription activation and growth inhibition by GAL4-I κ B α mutants in yeast correlated with their ability to interact with vertebrate Rel proteins, our results suggest that these activities of GAL4-I κ B α are mediated through interaction with a Rel-like protein in yeast, which is important for cell growth.

INTRODUCTION

I κ B proteins are specific inhibitors of the Rel/NF- κ B family of transcription factors. The Rel/NF- κ B family includes, among others, the vertebrate NF- κ B transcription complex (a p50-RelA [p65] heterodimer), the *Drosophila* embryonic morphogen Dorsal and the proto-oncoprotein cRel (1–4). All Rel proteins contain a highly conserved N-terminal region of approximately 350 amino acids (aa), called the Rel Homology (RH) domain, which has sequences involved in dimerization, DNA binding, I κ B binding and nuclear targeting. The C-terminal halves of Rel proteins are generally not conserved, but usually contain transcription

activation domains (5). One class of Rel proteins, including p50 and p52, are synthesized as larger precursor proteins (p105 and p100, respectively) that are thought to be proteolyzed to their final forms. A second class of Rel proteins, including cRel, RelA, RelB and Dorsal, are synthesized as mature proteins that are not processed.

I κ B is believed to associate with the RH domain of dimeric Rel protein complexes, and in many cases this association inhibits the ability of the Rel protein complex to bind DNA *in vitro* and to localize to the nucleus (3). For example, I κ B can bind to the NF- κ B p50-RelA heterodimer, inhibit DNA binding by this complex and mask the nuclear targeting sequence of RelA. Various stimuli lead to release of I κ B from Rel/NF- κ B protein complexes, whereupon the Rel/NF- κ B complex can enter the nucleus and bind DNA.

I κ B proteins are structurally related in that they all contain 5 to 8 copies of an approximately 30 to 35 aa repeat known as an ankyrin-like or cell-cycle repeat. Although first identified in two yeast proteins involved in cell-cycle control (6), the ankyrin-like repeats have now been found in a wide variety of proteins, including many transcription factors, and are believed to be involved in protein–protein interactions (7).

Several types of I κ B are known. I κ B α , which includes chicken p40, human MAD-3 and rat RL/IF-1, is an approximately 37 kilodalton protein specific for RelA and cRel (8–10). I κ B β is a 43 kilodalton protein, which can also inhibit RelA and cRel. I κ B γ corresponds to the C-terminal portion of NF- κ B p105 and is synthesized from an alternatively spliced mRNA (11); I κ B γ specifically interacts with p50 and cRel. Cactus is a *Drosophila* I κ B-like protein that specifically interacts with Dorsal (12, 13). Bcl-3 is a unique vertebrate I κ B protein that appears to be a specific inhibitor of p50 homodimers and a transcriptional activator when associated with p52 (14, 15, 15a).

We have previously shown that GAL4-I κ B α and GAL4-I κ B γ fusion proteins can activate transcription from promoters containing GAL4-binding sites in avian, mammalian and yeast cells (16). We have now investigated the mechanism by which these fusion proteins activate transcription. We present evidence that indicates that I κ B γ contains a genuine activation domain, whereas GAL4-I κ B α appears to activate transcription by interaction with endogenous Rel transcription factors. Our results suggest that Rel- and I κ b-like proteins are present in *Saccharomyces cerevisiae*.

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Figure 1. Transcription activation by GAL4-p40 fusion proteins in chicken embryo fibroblasts (CEF). In each panel, plasmids expressing the indicated proteins (top) were co-transfected into CEF with GAL4 reporter plasmid pG5BCAT and a CAT assay (bottom) was performed 2 days later as described in Materials and Methods. The approximate positions of the ankyrin-like repeats in p40 are indicated by black bars and numbered with roman numerals. Numbers above the protein structures indicate the aa from chicken p40 that are included in the fusion proteins. Mutations in ankyrin-like repeats III and IV of p40 (20) are indicated by stars. Each fusion protein has been tested at least three times and a representative CAT assay is shown at the bottom. CAT activity is reported as percent acetylation (% ACET.). (A) Activation by deletion mutants of p40. (B) Activation by GAL4-p40 proteins with site-directed mutations and by GAL4-Rel fusion proteins. Samples marked 1/10 were performed with ten-fold dilutions of cell extracts.

MATERIALS AND METHODS

Plasmids

All recombinant DNA techniques were performed according to standard procedures (17). Plasmid pSG424, for the expression of aa 1–147 of GAL4, and the reporter plasmid pG5BCAT, which contains five GAL4-binding sites upstream of a TATA box and the chloramphenicol acetyltransferase (CAT) gene, have been described previously (18, 19). Plasmids for the expression in avian cells of fusion proteins containing aa 1–147 of GAL4 and sequences from mouse cRel (pSG-3'Mc), from mouse p105 (pSG-Mp105[727–806]), and from chicken p40 (pSG-Chp40; pSG-5'Chp40; and pSG-3'Chp40) have been described previously (16). GAL4-p40 deletion plasmids were constructed as follows: pSG-Chp40Δ(60–281), a *Pst*I to *Pst*I deletion in pSG-Chp40; pSG-Chp40Δ(181–281), *Pst*I to *Pst*I deletion in pSG-Chp40; and pSG-Chp40(180–272), a *Pst*I to *Pvu*II fragment put into pSG424. pSG-ANK3 and pSG-ANK4 were made by subcloning *Eag*I to *Acc*I fragments from BSIkBANK3 and BSIkBANK4 (20), respectively, into pSG-Chp40 that had been digested with *Eag*I and *Acc*I. pSG-3'Chc was made by subcloning a *Hinc*II to *Xba*I fragment of chicken *c-rel* into pSG424 that had been digested with *Bam*HI, treated with Klenow, and then digested with *Xba*I. The retroviral vector JDG was made by subcloning a *Hind*III–*Dra*I fragment (containing codons for GAL4 aa 1–147) from pSG424 into the spleen necrosis virus vector JD214 (21) which had been digested with *Hind*III and *Cla*I/Klenow-treated.

The JDG expression vectors (JDG-Chp40, JDG-Mp105[727–806] and JDG-3'Mc) were made by subcloning the *Eco*RI fragments from the corresponding pSG vectors into the *Eco*RI-digested JDG vector.

Plasmid JDChp40, a retroviral vector for the expression of p40, has been described previously (16). Plasmids JDANK3 and JDANK4, for the expression of mutant p40 proteins, were made by replacing the sequences of wild-type p40 in JDChp40 with the corresponding mutant sequences. Plasmid JDHRelA, which expresses full-length human RelA from retroviral vector JD214BS+ (22), was made by cloning an *Xba*I to *Nae*I fragment of plasmid BLp65-RES (a gift of C. Rosen) into JD214BS+ that had been cleaved with *Xba*I and *Hinc*II; JD5'HRelA, which expresses aa 1–329 of human RelA, was made by cloning an *Xba*I to *Taq*I/Klenow-treated fragment of RelA into JD214BS+ that had been digested with *Xba*I and *Hinc*II. JDChp50 was made by removing the sequence downstream of the *Nsi*I site in JDChp105 (A.J. Capobianco and T.D. Gilmore, unpublished). JD214c-rel (JDChcRel) and JDV/C Cl–Hc_{del} have been described (23).

pMA241 and pMA210 are low copy plasmids in which expression of GAL4 1–147 and full-length GAL4, respectively, is controlled by the ADH1 promoter (24). Plasmids pMA-Chp40 and pMA-MAD3 have been described previously (16). Plasmids pMA-ANK3 and pMA-ANK4 were made by subcloning *Eco*RI fragments from pSG-ANK3 and pSG-ANK4 respectively, into

Figure 2. Association of GAL4-p40 with Rel family proteins (A) Co-immunoprecipitation of GAL4-p40 and vRel. vRel (lane 1), GAL4-p40 (lane 2) and vrel plus GAL4-p40 (lane 3) were translated *in vitro* (TOTAL). A portion of these samples was also immunoprecipitated (IMMUNOPPT) with anti-vRel antiserum (lanes 4, 5 and 6 correspond to samples in lanes 1, 2 and 3, respectively). (B) Co-immunoprecipitation of GAL4-p40 and human RelA. GAL4-p40 (lane 1), GAL4-ANK3 (lane 2), GAL4-ANK4 (lane 3) and wild-type human RelA (lane 4) were translated *in vitro*. These four samples were immunoprecipitated with anti-p40 antiserum (lanes 1–4 correspond to lanes 5–8, respectively). In lanes 9–11, human RelA was mixed with GAL4-p40 (lane 9), GAL4-ANK3 (lane 10) or GAL4-ANK4 (lane 11), prior to immunoprecipitation with anti-p40 antiserum. Samples were analyzed on a 10% SDS–polyacrylamide gel, and the dried gel was subjected to fluorography. Arrows indicate the positions of vRel or RelA and GAL4-p40.

pMA241. pMA-Bcl3 was made by subcloning the *EcoRI* fragment from pSG-Bcl3 into *EcoRI*-digested pMA424. pSG-Bcl3 was constructed by inserting an *NaeI* fragment from *bcl-3* cDNA plasmid cLK2 (14) into pSG424 that had been digested with *SmaI*.

CG129 (vRel), pGEMHRelA and pGEMGChp40 (GAL4-p40 fusion) were used for *in vitro* transcription/translation. CG129 has been described previously (25). pGEMHRelA was made by subcloning an *XbaI* to *HindIII* fragment containing the entire coding region of human RelA into pGEM4 that had been digested with *XbaI* and *HindIII*. pGEMGChp40 was made by subcloning a *HindIII*–*XbaI* fragment from pSG-Chp40 into pGEM3 digested with *HindIII* and *XbaI*. pGEMG-ANK3 and pGEMG-ANK4 express mutant GAL4-p40 proteins and were made by subcloning appropriate fragments into pGEMGChp40.

Cell culture and CAT assays

Chicken embryo fibroblasts (CEF) were prepared and cultured as described previously (23). All transfections were performed by the DMSO-polybrene method (23, 26).

CAT assays were performed as described previously (16). Briefly, CEF were transfected with 5 μ g each of producer and reporter plasmids. For competition experiments, CEF were transfected with 1 μ g each of producer and reporter plasmid DNA and 10 μ g of competitor plasmid. In all transfections, 1 μ g of plasmid MSV- β gal was included to normalize for transfection efficiency. Forty-eight hrs after transfection, cells were lysed and CAT activity was determined on samples that had been normalized according to β -galactosidase activity. CAT activity is presented as the percentage of radioactivity in acetylated chloramphenicol out of the total radioactivity in the acetylated and nonacetylated forms of chloramphenicol.

In vitro transcription/translation

Five μ g of plasmid DNA was transcribed *in vitro* using SP6 RNA polymerase. The RNA was then used for *in vitro* translation in a rabbit reticulocyte lysate according to the manufacturer's specifications (Promega) in the presence of [35 S]-labelled methionine and cysteine (>1000 Ci/mmol; Tran 35 S-label, Amersham). Portions of the samples were resuspended directly in SDS sample buffer and analyzed by SDS–PAGE. For protein complexing experiments, vRel and GAL4-p40 were co-translated and immunoprecipitated with anti-vRel antiserum as described (27); RelA was mixed with GAL4-p40, GAL4-ANK3 and GAL4-ANK4 in TBS buffer (25 mM Tris–HCl, pH 8; 150 mM NaCl; 2.5 mM KCl), incubated at 30°C for 30 min and immunoprecipitated with anti-p40 antiserum. The radiolabelled proteins were separated on a 10% SDS polyacrylamide gel, which was then soaked in sodium salicylate for 1 hr, dried and subjected to fluorography.

Assays in yeast

For assaying transcription activation in yeast, 5 μ g of plasmid DNA was transformed into yeast strain YM335:RY171 (28) using the lithium acetate method (29). Transformants were selected on His[–] plates. After 2–4 days at 30°C, individual colonies from each transformation were picked and streaked on histidine-free plates. Colonies from these plates were picked and grown in selective liquid medium to an OD₆₀₀ of about 0.5. These cells were then used for β -galactosidase assays. Assays for β -galactosidase activity were performed as described previously (30).

RESULTS AND DISCUSSION

Activation of transcription by GAL4-I $\times$ B α proteins in chicken cells

Chicken p40 (I $\times$ B α) contains five ankyrin-like repeats and an acidic C-terminal domain (8). We have previously shown that a GAL4-p40 fusion protein (encoded by pSG-Chp40; Figure 1) can activate transcription from reporter plasmids containing GAL4-binding sites (16). In order to map the sequences in GAL4-p40 involved in transcription activation, we created a series of deletions in the plasmid encoding GAL4-p40. These deletion plasmids were assayed in transient transfection assays in CEF for their ability to activate transcription from GAL4 reporter plasmid G5BCAT. Most deletions in the ankyrin-like repeat region of GAL4-p40 completely abolished its ability to activate transcription (Figure 1A). As we have shown previously (16), pSG-5'Chp40, a plasmid encoding the first four ankyrin-like repeats and part of the fifth ankyrin-like repeat of p40, was still able to activate transcription, albeit to a lower extent than a GAL4-p40 protein containing five intact ankyrin-like repeats and the acidic C terminus (pSG-Chp40). These results indicated that an intact structure of the p40 ankyrin-like repeats was necessary for transcription activation by GAL4-p40.

Since ankyrin-like repeats in p40 have been shown to be essential for interaction with Rel proteins (20), we reasoned that GAL4-p40 might activate transcription by interacting with endogenous Rel proteins containing transcription activation domains (see ref. 31). To test this hypothesis, we assayed the ability of GAL4-p40 proteins containing point mutations in ankyrin-like repeat 3 (pSG-ANK3) or 4 (pSG-ANK4) to activate transcription. Inoue *et al.* (20) have shown that these mutations

Figure 3. Competition for GAL4-IxB mediated activation in CEF. JDG-Chp40 (B), JDG-Mp105(727-806) (C) and JDG-3'Mc (D) were co-transfected with pG5BCAT and a ten-fold excess of the competitor plasmids shown in (A). Black boxes indicate the approximate positions of the ankyrin-like repeats in p40 and the stars indicate mutant ankyrin-like repeats. The numbers above the drawings indicate the aa included in each protein. The long terminal repeats (LTR) are indicated on the JDG producer plasmids. The plasmid names above each lane indicate the competitor plasmid (COMP) used in that sample; JD214 is a control retroviral vector with no inserted gene. A representative CAT assay for each producer plasmid is shown; each competition experiment has been performed at least two times.

in ankyrin-like repeat 4 abolish the ability of p40 to interact with Rel proteins, whereas similar mutations in ankyrin-like repeat 3 do not affect the ability of p40 to bind to Rel proteins. We found that pSG-ANK3 activated transcription as well as wild-type pSG-Chp40, whereas pSG-ANK4 did not activate transcription above basal levels (Figure 1B). This result is consistent with activation by GAL4-p40 being due to interaction with an endogenous Rel protein in CEF; however, we cannot exclude the possibility that GAL4-ANK4 has a general conformational change that renders it inactive in our assays.

To determine which Rel protein might interact with GAL4-p40 to activate transcription we compared the relative strength of activation by various Rel activation domains. A GAL4-RelA protein (containing C-terminal sequences from human RelA/p65, encoded by pSG-3'HRelA) was the strongest activator of transcription in CEF: pSG-3'HRelA was about 25-fold stronger than pSG-3'Mc (C-terminal sequences from mouse cRel) and about seven-fold stronger than pSG-3'Chc (C-terminal sequences from chicken cRel) and more than two-fold stronger than pSG-Chp40 (aa 49-318 of chicken IxB α) (Figure 1B). Because activation by GAL4-p40 is higher than the activation seen with GAL4-cRel proteins; it is unlikely that GAL4-p40 activates

transcription solely through cRel; more likely, GAL4-p40 interacts with multiple Rel proteins, including RelA, to activate transcription in CEF. However, it is also possible that activation by a GAL4-p40:cRel complex is synergistic or that association with other strongly activating proteins is involved in activation by GAL4-p40.

To demonstrate directly that the GAL4-p40 could interact with Rel family proteins, in a manner similar to wild-type p40, we performed co-precipitation experiments with *in vitro* translated GAL4-p40 and vRel or human RelA (Figure 2). In the experiment shown in Figure 2A, GAL4-p40 and vRel were co-translated *in vitro* and immunoprecipitated with anti-vRel antiserum: GAL4-p40 by itself was not directly immunoprecipitated with anti-vRel antiserum, but GAL4-p40 was co-precipitated with vRel, presumably due to its association with vRel. Similarly, GAL4-p40 and GAL4-ANK3 complexed with human RelA *in vitro*, but GAL4-ANK4 did not (Figure 2B). Therefore, the addition of GAL4 sequences to p40 proteins does not appear to affect their ability to interact with Rel family proteins. Furthermore, when expressed in mouse cells, a similar GAL4-IxB α fusion protein is found in a complex with endogenous mouse RelA (R.Taub, personal communication).

PLASMID	PROTEIN STRUCTURE	Relative β -Gal Activity
pMA241		<0.1
pMA210		100
pMA-Chp40		185
pMA-ANK3		213
pMA-ANK4		0.2
pMA-MAD3		104
pMA-Bcl3		4.2

Figure 4. Transcription activation by GAL4-I κ B fusion proteins in *S. cerevisiae*. Transcription activation by the indicated plasmids was determined in *S. cerevisiae* strain YM335:RY171, which contains a chromosomal copy of a reporter locus containing a *GAL1-lacZ* fusion gene and which lacks an endogenous *GAL4* gene (28). All values are relative to the β -galactosidase activity seen with wild-type GAL4 (100%). Each value is the average of values obtained from at least three independent transformants. Standard deviations ranged from 8 to 15%.

Squelching of GAL4-p40 mediated activation

If association with a Rel family protein is responsible for transcription activation by GAL4-p40, then co-expression of a Rel protein missing its C-terminal activation domain should reduce activation by GAL4-p40 since the overexpressed truncated Rel protein should displace the endogenous wild-type Rel protein bound to the GAL4-p40 fusion protein. To determine whether overexpression of a truncated Rel protein could reduce activation by GAL4-p40, CEF were co-transfected for reporter CAT assays with a spleen necrosis virus (SNV) expression plasmid for GAL4-p40 (JDG-Chp40) and a ten-fold excess of a Rel expression plasmid or control plasmids (Figure 3). (The SNV vector was used to express the GAL4 fusion proteins since the SNV long terminal repeat is not affected by expression of Rel family proteins, whereas the simian virus 40 promoter of pSG424 has κ B sites to which Rel proteins can bind.) Co-transfection with an expression plasmid for a mutant RelA protein (JD5'HRelA) or a mutant chicken cRel protein (JDV/C Cl-Hc_{del}), each truncated just after the RH domain and missing the C-terminal sequences necessary for activation (32–35), greatly reduced activation by GAL4-p40 (Figure 3B). This result is consistent with the deleted Rel proteins displacing the endogenous, activation competent, Rel protein(s); alternatively, association of a C-terminally-deleted Rel protein with GAL4-p40 may prevent interaction of GAL4-p40 with a non-Rel-like activator, or may change the conformation of GAL4-p40 in a way that interferes with its activating function.

On the other hand, co-transfection with an expression plasmid for full-length RelA (JDHRelA) slightly increased activation by GAL4-p40, consistent with RelA interacting with GAL4-p40. Co-transfection with a chicken NF- κ B p50 expression plasmid reduced activation by GAL4-p40 by two-fold, possibly because p40 can interact weakly with p50 (36). JDChp40 and JDANK3, expression plasmids for wild-type chicken p40 and the ANK3

Figure 5. GAL4-p40 inhibits growth when expressed in *S. cerevisiae*. Yeast strain YM335:RY171 was transformed with expression plasmids for wild-type GAL4 (pMA210), GAL4 1–147 (pMA241), GAL4-p40 (pMAChp40), GAL4-ANK3 (pMA-ANK3), GAL4-ANK4 (pMA-ANK4), GAL4-Mouse p105 aa 727–806 (pMA-Mp105[727–806]), and GAL4-Bcl3 (pMA-Bcl3), and cells were plated on His[−] selective plates. A single colony from each of these transformations was picked and streaked on His[−] plates and incubated at 30°C for 2 days. A representative plate is shown.

mutant, respectively, greatly reduced the transcription activation seen with JDG-Chp40, presumably by titrating out (squelching) the endogenous Rel proteins responsible for activation by GAL4-p40 (Figure 3B). JDANK4, expressing the p40 mutant that cannot interact with Rel proteins, did not decrease activation by GAL4-p40.

By deletion mapping, we previously identified an 80 aa, highly acidic sequence located near the last ankyrin-repeat of mouse I κ B γ (the C terminus of NF- κ B p105) that can activate transcription when fused to GAL4 (16). To determine the mechanism by which the GAL4-I κ B γ protein activated transcription, a similar series of competition experiments were performed using JDG-Mp105(727–806), an expression plasmid for a GAL4 fusion protein containing aa 727–806 of mouse p105, the minimal transcription activation sequence that we previously identified in the C-terminal half of mouse p105 (16). In this case, none of the p40 expression vectors could compete effectively for activation by the GAL4-mouse p105 fusion protein (Figure 3C). However, JDRelA (expressing wild-type human RelA) reduced activation by GAL4-Mp105(727–806) by 10-fold. The expression plasmids for the RelA protein deleted immediately after the RH domain (JD5'HRelA) and for p50 (JDChp50) did not significantly inhibit activation by JDG-Mp105(727–806). These results are consistent with the C-terminal activation sequences from mouse p105 containing a gene activation domain that interacts with a co-activator that is identical to that with which the RelA activation sequences interact. Our finding of sequences in the C terminus of p105 that behave like a genuine transcription activation domain and the recent report that full-length p105 expressed in baculovirus can bind DNA (D. Baltimore, personal communication) support our previous proposal (16) that p105 may not always require processing to p50 before being involved in transcription activation.

As a control, we tested transcription activation by a GAL4 fusion protein containing the mouse cRel C-terminal activation domain (JDG-3'Mc) in a similar series of competition

experiments (Figure 3D). Similar to the results with GAL4-3'Mp105, co-transfection with plasmids encoding full-length Rel proteins containing intact transcription activation domains (JDHRelA and JDChcRel) greatly diminished activation by JDG-3'Mc, whereas a RelA protein lacking a strong activation domain (JD5'HRelA) did not. JDChp40 and JDANK3 slightly increased activation by JDG-3'Mc, whereas the non-interacting ANK4 protein did not appreciably affect activation by JDG-3'Mc.

Transcription activation and growth inhibition by GAL4-I κ B fusion proteins in yeast

Both chicken I κ B α and mouse I κ B γ (C-terminal sequences of p105) can activate transcription in *S.cerevisiae* when fused to GAL4 (16). Since no Rel protein has yet been identified in yeast, we decided to reexamine this effect in light of our results with GAL4-p40 in CEF. As found previously (16), GAL4-p40 activated transcription almost twice as strongly as full-length GAL4 from a reporter gene containing a GAL4-binding site in yeast (Figure 4). Similar to the results we obtained in CEF, GAL4-ANK3 activated transcription as strongly as wild-type GAL4-p40, whereas GAL4-ANK4 was inactive. These results are consistent with GAL4-p40 activating transcription in yeast through interaction with a Rel-like protein. As we have shown previously (16), GAL4-MAD3 (human I κ B α) also behaved as a strong activator of transcription in yeast.

Recently, it has been shown that Bcl-3 can interact with a DNA-bound NF- κ B p52 dimer and function as a transcription activator (15a). In assays in yeast, a GAL4-Bcl-3 protein containing aa 59–446 (the seven ankyrin-like repeats and a proline- and serine-rich C terminus) was only very weakly activating (Figure 4). This result is consistent with results that show that both N-terminal and C-terminal sequences of Bcl-3 are needed for full transcription activation in mouse cells (15a).

The expression of certain vertebrate and viral transcription factors, such as p53 and Tax (37, 38), in yeast inhibits cell growth, presumably because these factors interact with essential yeast proteins. Similarly, we noticed that yeast cells expressing GAL4-p40 grew more slowly than cells expressing wild-type GAL4 or GAL4 1–147. As shown in Figure 5, GAL4-p40 and GAL4-ANK3 inhibited the growth of *S.cerevisiae*, whereas GAL4-ANK4 expressing cells grew as well as yeast cells expressing GAL4 (1–147) or wild-type GAL4. These results suggest that the activating protein(s) with which GAL4-p40 interacts in yeast is important for cell growth and that titration of this protein(s) by GAL4-p40 is detrimental to the cell. In contrast, GAL4 fusion proteins containing aa 727–806 of mouse p105 or aa 59–446 of Bcl-3 did not affect growth in yeast (Figure 5), indicating that these I κ B-like sequences do not titrate a factor essential for cell growth and that they are likely to activate transcription in yeast by a mechanism that is different from GAL4-p40.

Our results indicate that GAL4-I κ B α proteins activate transcription by interaction with endogenous Rel-like proteins in vertebrate and yeast cells. In contrast, mouse I κ B γ appears to contain sequences that can act as a genuine, probably acidic, activator of transcription when fused to GAL4. We are currently attempting to isolate a gene that encodes the factor that mediates both GAL4-p40 transcription activation and growth inhibition in *S.cerevisiae*. Furthermore, since the ability of chicken I κ B α proteins to interact with a putative yeast Rel-like protein has been conserved, I κ B-like regulatory proteins may also exist in yeast.

ACKNOWLEDGEMENTS

We thank T. McKeithan for the *bcl-3* cDNA, Cetus Corporation for the MAD-3 cDNA, L. Kerr and I. Verma for cDNAs containing the ANK3 and ANK4 mutations in p40, S. Ghosh and D. Baltimore for the murine p105 cDNA, C. Rosen for the human p65 cDNA, N. Davis and H. R. Bose Jr for the chicken p40 cDNA and anti-p40 antiserum, and A. Capobianco for plasmid JDChp50. We thank K. Piffat for help with the construction of RelA expression plasmids. We thank E. Loechler and members of our laboratory for critical reading of the manuscript. This research was supported primarily by National Cancer Institute grant CA47763 and small parts of this research were supported by a Biomedical Research Support Grant (SO7RR07043–26) from Boston University. P.J.M. was supported by a graduate scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC). G.S.S. is a participant in the undergraduate research program in the Biology Department at Boston University. T.D.G. was partially supported by a Faculty Research Award from the American Cancer Society.

REFERENCES

- Gilmore, T.D. (1990) *Cell* **62**, 841–843.
- Baeuerle, P.A. (1991) *Biochim. Biophys. Acta* **1072**, 63–80.
- Schmitz, M.L., Henkel, T. and Baeuerle, P.A. (1991) *Trends Cell Biol.* **1**, 130–137.
- Bose, H.R. Jr (1992) *Biochim. Biophys. Acta* **1114**, 1–17.
- Gilmore, T.D. (1992) *Cancer Surveys* **15**, 69–87.
- Breedon, L. and Nasmyth, K. (1987) *Nature* **329**, 651–654.
- Blank, V., Kourilsky, P. and Israël, A. (1992) *Trends Biochem. Sci.* **17**, 135–140.
- Davis, N., Ghosh, S., Simmons, D.I., Tempst, P., Liou, H.-C., Baltimore, D. and Bose, H.R. Jr (1991) *Science* **253**, 1268–1271.
- Haskill, S., Beg, A.A., Tompkins, S.M., Morris, J.S., Yurochko, A.D., Sampson-Johannes, A., Mondal, K., Ralph, P. and Baldwin, A.S. Jr (1991) *Cell* **65**, 1281–1289.
- Tewari, M., Dobrzanski, P., Mohn, K.L., Cressman, D.E., Hsu, J.-C., Bravo, R. and Taub, R. (1992) *Mol. Cell. Biol.* **12**, 2898–2908.
- Inoue, J., Kerr, L.D., Kakizuka, A. and Verma, I.M. (1992) *Cell* **68**, 1109–1120.
- Geisler, R., Bergmann, A., Hiromi, Y. and Nüsslein-Volhard, C. (1992) *Cell* **71**, 613–621.
- Kidd, S. (1992) *Cell* **71**, 623–635.
- Ohno, H., Takimoto, G. and McKeithan, T.W. (1990) *Cell* **60**, 991–997.
- Franzoso, G., Bours, V., Park, S., Tomita-Yamaguchi, M., Kelly, K. and Siebenlist, U. (1992) *Nature* **359**, 339–342.
- Bours, V., Franzoso, G., Azarenko, V., Park, S., Kanno, T., Brown, K. and Siebenlist, U. (1993) *Cell* **72**, 729–739.
- Morin, P.J. and Gilmore, T.D. (1992) *Nucleic Acids. Res.* **20**, 2453–2458.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (2nd Edition). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Sadowski, I. and Ptashne, M. (1989) *Nucleic Acids Res.* **18**, 7539.
- Lillie, J.W. and Green, M.R. (1989) *Nature* **338**, 39–44.
- Inoue, J., Kerr, L.D., Rashid, D., Davis, N., Bose, H.R. Jr and Verma, I.M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4333–4337.
- Dougherty, J.P. and Temin, H.M. (1986) *Mol. Cell. Biol.* **6**, 4387–4395.
- Sif, S., Capobianco, A.J. and Gilmore, T.D. (1993) *Oncogene* in revision.
- Capobianco, A.J., Simmons, D.L. and Gilmore, T.D. (1990) *Oncogene* **5**, 257–265.
- Ma, J. and Ptashne, M. (1987) *Cell* **51**, 113–119.
- Capobianco, A.J. and Gilmore, T.D. (1991) *Oncogene* **6**, 2203–2210.
- Kawai, S. and Nishizawa, M. (1984) *Mol. Cell. Biol.* **4**, 1172–1174.
- Capobianco, A.J., Chang, D., Mosialos, G. and Gilmore, T.D. (1992) *J. Virol.* **66**, 3758–3767.
- Yocum, R., Hanley, S., West, R. Jr and Ptashne, M. (1984) *Mol. Cell Biol.* **4**, 1985–1998.
- Ito, H., Fukuda, Y., Murata, K. and Kimura, A. (1983) *J. Bacteriol.* **153**, 163–183.

30. Kamens,J., Richardson,P., Mosialos,G., Brent,R. and Gilmore,T. (1990) *Mol. Cell. Biol.* **10**, 2840–2847.
31. Fields,S. and Song,O. (1989) *Nature* **340**, 245–246.
32. Schmitz,M.L. and Baeuerle,P.A. (1991) *EMBO J.* **10**, 3805–3817.
33. Ruben,S.M., Narayanan,R., Klement,J.F., Chen,C.-H. and Rosen,C.A. (1992) *Mol. Cell. Biol.* **12**, 444–454.
34. Ballard,D.W., Dixon,E.P., Peffer,N.J., Bogerd,H., Doerre,S., Stein,B. and Greene,W.C. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1875–1879.
35. Richardson,P.M. and Gilmore,T.D. (1991) *J. Virol.* **65**, 3122–3130.
36. Beg,A.A., Ruben,S.M., Scheinman,R.I., Haskill,S., Rosen,C.A. and Baldwin,A.S.Jr (1992) *Genes Dev.* **6**, 1899–1913.
37. Bischoff,J.R., Casso,D. and Beach,D. (1992) *Mol. Cell. Biol.* **12**, 1405–1411.
38. Wu,L.-C.C., Tan,T.-H. and Shahied,S.I. (1992) *J. Virol.* **66**, 7253–7261.