MOLECULAR AND CELLULAR BIOLOGY, Mar. 1993, p. 1666–1674
0270-7306/93/031666-09$02.00/0

Vol. 13, No. 3

Conservation of Transcriptional Activation Functions of the NF-κB p50 and p65 Subunits in Mammalian Cells and *Saccharomyces cerevisiae*

PAUL A. MOORE, STEVEN M. RUBEN, AND CRAIG A. ROSEN*

Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110

Received 14 September 1992/Returned for modification 4 November 1992/Accepted 22 December 1992

The NF-κB transcription factor complex is composed of a 50-kDa (p50) and a 65-kDa (p65) subunit. Both subunits bind to similar DNA motifs and elicit transcriptional activation as either homo- or heterodimers. By using chimeric proteins that contain the DNA binding domain of the yeast transcriptional activator GAL4 and subdomains of p65, three distinct transcriptional activation domains were identified. One domain was localized to a region of 42 amino acids containing a potential leucine zipper structure, consistent with earlier reports. Two other domains, both acidic and rich in prolines, were also identified. Of perhaps more significance, the same minimal activation domains that were functional in mammalian cells were also functional in the yeast *Saccharomyces cerevisiae*. Coexpression of the NF-κB inhibitory molecule, IκB, reduced the transcriptional activity of p65 significantly, suggesting the ability of IκB to function in a similar manner in *S. cerevisiae*. Surprisingly, while the conserved *rel* homology domain of p65 demonstrated no transcriptional activity in either mammalian cells or *S. cerevisiae*, the corresponding domain in p50 was a strong transcriptional activator in *S. cerevisiae*. The observation that similar domains elicit transcriptional activation in mammalian cells and *S. cerevisiae* demonstrates strong conservation of the transcriptional machinery required for NF-κB function and provides a powerful genetic system to study the transcriptional mechanisms of these proteins.

Nuclear factor κB (NF-κB), originally described as a factor involved in tissue-specific control of the mouse κ light-chain enhancer (65, 66), is now known to be involved in the inducible expression of a wide range of genes (2, 6, 44). The κB DNA binding site has been identified in the regulatory elements of cytokine, cytokine receptor, major histocompatibility complex class I and II antigens, inflammatory and acute-phase response genes, and several viral enhancer elements (2, 6, 44). DNA binding activity appears to be constitutive in mature B cells (66) and in activated macrophages (24). However, in most other cells NF-κB is complexed with an inhibitory molecule (IκB) in the cytoplasm (3, 4, 21). Sequestration in the cytoplasm is thought to involve masking of the nuclear localization signal (8). Upon activation by a variety of stimuli, including mitogens, cytokines, and various viral proteins (2, 6, 44), NF-κB shuttles to the nucleus, a process thought to involve phosphorylation of IκB (21, 68). Initial studies demonstrated that IκB inhibits binding of the p65 homodimer and the p50/p65 heterodimer through interaction with p65 (5, 21, 54, 78). However, recent studies suggest that both subunits can interact with IκB (8).

Biochemical studies have shown that the major form of NF-κB is composed of a heterodimer of a 50-kDa (p50) and a 65-kDa (p65) subunit (5). Both subunits of NF-κB have been cloned, and sequence analyses demonstrate strong similarity in the amino-terminal 300 amino acids to the *rel* proto-oncogene product (22, 38, 54, 58), originally identified in the avian reticuloendotheliosis virus, *rev* T (73). It appears that the p50 subunit is processed from a p105 precursor protein (22, 38). The *rel* homology domain, which is also conserved in the *Drosophila* maternal morphogen *dorsal* (70), is required for DNA binding, multimerization, and nuclear localization (2). In contrast to the extensive similarity of these proteins at the amino terminus, the carboxy terminus of the *rel*-related family members is quite divergent, except for the precursors of p50 and the related protein p49 (1, 11, 63). The carboxy terminus of p105 and the p49 precursor p100 both contain a glycine-rich region and repeats of the ankyrin motif, which is found in proteins playing a role in cell growth and differentiation (47) as well as mediating heterodimer formation (40, 74). This motif is also present in the inhibitory molecule IκB (15, 27).

In earlier studies performed with cellular NF-κB complexes, it was suggested that the p50 subunit confers DNA binding, whereas the p65 subunit confers transcriptional activation functions (38, 78). However, recent studies using both native (76, 77) and cloned (54, 60) p65 protein indicate that it can associate with the κB motif in the absence of p50. In support of the transcriptional activation potential of p65, cotransfection experiments using p65 or GAL4p65 chimeric proteins demonstrated strong activation that was localized to the carboxy-terminal domain. Similar transfection studies with p50 have failed to demonstrate transcriptional activation functions (7, 57, 60, 64). Other studies have demonstrated that both c-*rel* and *dorsal* have activation domains within the C-terminal region (12, 20, 26, 29, 30, 57). In contrast, by using an in vitro transcription assay, it has been shown that the *rel* homology domain of p50 is capable of transcriptional activation (19, 39). Similar findings have yet to be demonstrated in an in vivo system.

To further delineate the minimal transcriptional activation domains of p65, an extensive deletion analysis of the carboxy terminus was performed. Transfection experiments in COS-7 cells revealed the presence of at least three distinct transcriptional activation domains. One domain was found to depend on the intactness of a potential leucine zipper, while the others were both acidic and proline rich. As observed with several other mammalian transcription factors (18, 42, 52, 62, 71), p65 was found to be a potent transcrip-

* Corresponding author.

tional activator in *Saccharomyces cerevisiae*. Most important, transcriptional activation in *S. cerevisiae* was dependent upon the same three activation domains identified in mammalian cells. In addition, IκB was able to inhibit the activity of p65 in *S. cerevisiae* through an interaction with the *rel* domain, similar to its mode of action in mammalian cells. Studies with the p50 subunit in *S. cerevisiae* revealed that the *rel* homology domain was able to induce expression from a promoter dependent on a κB motif for activity. This is in contrast to in vivo studies conducted in mammalian cells but is consistent with recent in vitro transcription studies demonstrating the ability of p50 to activate transcription. The observation that the same minimal activation domains are functional in mammalian cells and *S. cerevisiae* implies strong conservation between the transcriptional machinery required for NF-κB function in these two systems and provides a genetic system to characterize the mechanism of *trans* activation by NF-κB.

MATERIALS AND METHODS

Plasmids. The GALp65 chimeric proteins were constructed by first amplifying the fragment of p65 corresponding to the desired amino acids by the polymerase chain reaction (PCR) (see Fig. 1), restricting the amplified fragment with *Bam*HI and *Xba*I, and cloning the fragments in frame with the GAL4 sequence corresponding to amino acids 1 to 147 of the GAL4 DNA binding domain in plasmid pSG424 (61). Mutagenesis of the leucine zipper within amino acids 416 to 458 of p65 was performed by amplification of the respective region from CMV-p65L440/P and CMV-p65ΔL 442 (60). Sequence analyses of the resulting GALp65 fusion showed that these were the only changes in the DNA sequence. The GALVP16 chimeric was created by amplifying the terminal 78 amino acids of VP16 (amino acids 413 to 490) from the vector pMSVP16 (75), digesting with *Bam*HI and *Xba*I, and cloning in frame with the GAL4 DNA binding domain in the same manner as for the GALp65 chimeric proteins. The reporter plasmid used throughout for COS cell transfections was GAL4TKCAT, which contains five copies of the GAL4 binding site (GAL_{UAS}) cloned upstream of the herpesvirus thymidine kinase promoter (TK) in the eukaryotic expression vector pBL2CAT (67).

For expression in *S. cerevisiae*, the GALp65 gene fusions, the GALVP16 gene fusion, and the GAL4 DNA binding domain alone were excised from the pSG424-based vectors as *Hin*dIII-*Xba*I fragments and subcloned into the *Hin*dIII-*Xba*I site of pVU-100 between the *ADH1* promoter and terminator sequences. pVU-100 is a multicopy vector and contains the *URA3* gene for selection in *S. cerevisiae* (79).

Full-length p65 and p65Δ, a naturally occurring spliced derivative of p65 (60) were amplified by PCR as *Bgl*II fragments from BLp65 (58) and BLp65Δ (60) and subcloned into the *Bam*HI site of pMA91 between the constitutive *PGK1* promoter and its terminator sequences to generate pY65 and pY65Δ. pMA91 is a multicopy plasmid which contains the *LEU2* gene for selection (50). The p65 *rel* homology domain (amino acids 1 to 310) was isolated as a *Hin*dIII-*Xba*I fragment following PCR of BLp65 and then was subcloned into the corresponding restriction sites of pVU-100 to generate pY65R. The p50 *rel* homology domain (amino acids 1 to 386) was amplified from BLp50 (58) as a *Bgl*II fragment and subcloned into the *Bam*HI site between the *PGK1* promoter and terminator sequences in pY316P to give pY50R and pY50RX (p50 in the opposite orientation). pY316P was constructed by inserting the 3.1-kb *Hin*dIII cassette from pMA91, containing the *PGK1* promoter and terminator sequences, into the *Hin*dIII site of pRS316 (69), a low-copy-number plasmid. The fusion between the *rel* homology domain of p65 and the terminal 78 amino acids of VP16 (amino acids 413 to 480) was carried out by inserting the resulting amplicon of a two-step PCR into the *Hin*dIII and *Xba*I sites of pVU-100 to give pY65VP16. IκB was isolated as a *Bam*HI-*Xba*I fragment from BL-IκB and subcloned into the corresponding sites of pVL-102 (79) to generate pYIκB, a multicopy plasmid dependent on the *LEU2* allele for selection. I-Rel was isolated as a *Hin*dIII-*Xba*I fragment from BL-I-Rel (59) and inserted into the *Hin*dIII-*Xba*I sites of pVL-100 to generate pYI-Rel.

Two κB reporter plasmids were used for studies in *S. cerevisiae*. The first plasmid, pYκB*lacZ*, is a multicopy plasmid containing three copies of the immunoglobulin κ light-chain κB motif upstream of the *Escherichia coli* β-galactosidase gene *lacZ*. This reporter was constructed by inserting the *TRP1* gene into the *URA3* gene of Igκ3 (34). The full-length *TRP1* gene, including 200 bp of flanking sequence on either side, was amplified by PCR from pRS314 (69), digested by *Apa*I, and inserted into the *Apa*I site of Igκ3, thus disrupting the *URA3* gene and rendering plasmid selection in *S. cerevisiae* dependent on the *TRP1* gene.

The second κB reporter used, pYκB*HIS*, is an integrated reporter bearing four copies of the immunoglobulin κ light-chain enhancer κB motif upstream of the yeast *HIS3* gene. First, a cassette containing a *GAL1* promoter deleted for its UAS sequence fused to the *HIS3* gene was isolated as a *Bam*HI-*Sal*I fragment from HR307a (81) and inserted into the corresponding restriction sites of pRS304 (69) to generate pY*HIS3*. Oligonucleotides containing four copies of the κB motif were then subcloned upstream of the *GAL* promoter between the *Bam*HI and *Sac*I sites to generate pYκB*HIS3*. The sequence of the sense oligonucleotide was 5′-CGGGG GAATCTCCAGAGGGGAATCTCCTCGAGGGGAATCT CCACTGGGGAATCTCC-3′.

Cell culture and transfection. COS-7 cells were maintained in Dulbecco's modified Eagle medium plus 10% fetal calf serum supplemented with 4.5 mg of glucose per ml and 50 μg of gentamicin (GIBCO) per ml. For transient transfection assays, 2×10^5 cells were plated on 35-mm plates, and a modified DEAE-dextran protocol was used to transfect the cells the following day as previously described (16). Cells were transfected with 0.5 μg of the reporter plasmid and 1 μg of the pSVGALp65 chimeric expression vectors. Cells were harvested 48 h after transfection, and chloramphenicol acetyltransferase (CAT) assays were performed as previously described (23).

Yeast strains, media, and transcriptional assays. For studies involving the analyses of GAL4 DNA binding domain protein fusions in *S. cerevisiae*, the strain YJ0-Z (*gal4Δ gal80Δ ura3-52 leu2-3, 112 ade1 his3 MEL-1 GAL1-lacZ*) was used (45). This strain contains an integrated copy of the *E. coli* β-galactosidase (*lacZ*) gene under the control of the yeast *GAL1* promoter. The yeast strain W3031b (**a** *his3 trp1 ura3 leu2 ade1*) was used as the recipient strain for studies involving κB reporter plasmids. W3031b:κB*HIS* contains an integrated copy of pYκB*HIS* at the *trp-1* locus. Integration at the *trp-1* locus was achieved by digesting pYKB*HIS* prior to transformation with *Eco*RV, which cuts uniquely within the *TRP1* gene of pYKB*HIS*.

Yeast strains were grown at 30°C and manipulated by standard procedures (25). Transformations were carried out by a variation of the lithium acetate procedure previously described (32), and transformants were grown on selective

minimal media containing 2% glucose. For studies involving YJ0-Z it was necessary to transfer transformants to selective minimal media containing 2% glycerol and 3% lactic acid to induce *lacZ* expression. β-Galactosidase activity was determined by sodium dodecyl sulfate-chloroform lysis (43), and units of activity were calculated by the method of Miller (53). β-Galactosidase assays performed on different days were normalized to values obtained for the reporter alone. At least three independent transformants for each plasmid were tested for activity.

RESULTS

p65 contains multiple transcriptional activation domains. It has recently been established that the carboxy-terminal portion of p65 confers the transcriptional activation properties and contains at least two activation domains (7, 60, 64). To establish more precisely the nature of these activation domains, progressive deletions within the carboxy-terminal portion of p65 were tested for transcriptional activity. As mammalian cells contain endogenous proteins which can bind to κB sites, the transcriptional activity of p65 was assessed via construction of chimeric proteins containing the DNA binding domain of the yeast transcriptional activator protein GAL4. Figure 1 depicts the regions of p65 analyzed for transcriptional activity and summarizes the results obtained.

Initially, successive carboxy-terminal (Fig. 2A) and amino-terminal (Fig. 2B) deletions of p65 were fused to the DNA binding domain of GAL4 (amino acids 1 to 147) in the pSG424 vector (61). The GALp65 constructs were transiently transfected into COS-7 cells together with a reporter construct that contains five copies of the GAL_{UAS} sequence upstream of the CAT gene. The activity of the various GALp65 fusion proteins was compared to the transcriptional activity induced by a GAL4 fusion with the herpesvirus VP16 transcriptional activator (GALVP16). This preliminary qualitative analyses revealed the presence of at least two distinct transcriptional activation domains (Fig. 2). One domain resides between amino acids 295 and 458 (Fig. 2A). Within this domain it appears that amino acids 402 to 458 are essential, as deletion of these residues completely abolishes *trans* activation (GALp65 295-402). Taken together, these results show that the *trans*-activation potential of p65 lies within its terminal 150 amino acids. The second activation domain lies between amino acids 491 and 550 (Fig. 2B). Both domains direct a level of transcriptional activation comparable to that obtained with GALVP16, a potent transcriptional activator (56, 75).

To further delineate the boundaries of the activation domains, additional deletions in p65 were constructed and tested for *trans*-activation properties (Fig. 3). Progressive amino-terminal deletions between amino acids 295 and 458 resulted in a gradual decrease in activity. While the amino acids between 373 and 416 appear to contribute to the *trans*-activation potential of this region of p65 (and perhaps define a domain of their own), merely the 42 amino acids present between residues 416 and 458, when fused to the DNA binding domain of GAL4, directed a strong level of transcriptional activation (Fig. 3A). The activity conferred by this domain appears to reside within a putative leucine zipper structure, as a point mutation which deletes a leucine at position 440 abolishes activity (compare GALp65 416-458 and 416-458 ΔL). Furthermore, substitution of a proline for a leucine at position 442, within this minimal domain, also abolished activity (data not shown). These results correlate

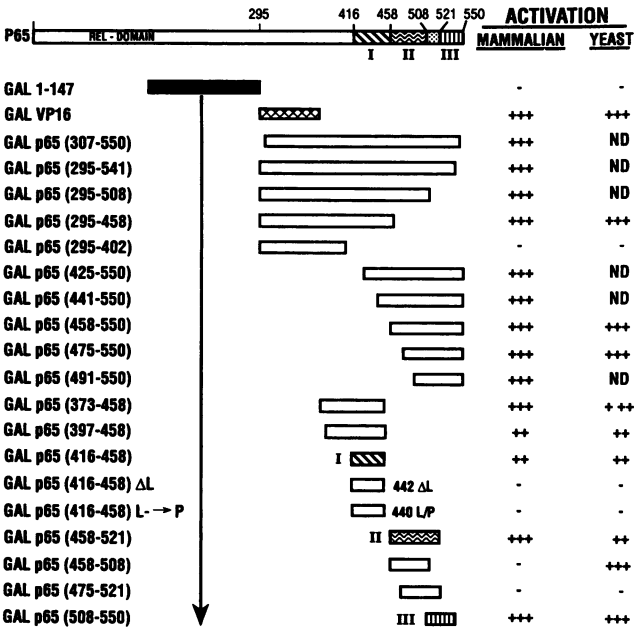

FIG. 1. Schematic representation of the p65 protein and its deletion derivatives. The *rel* domain of p65 lies within its N-terminal 295 amino acids. The three activation domains identified in this study lie between amino acids 416-458 (I), 458-521 (II), and 508-550 (III). Each of the p65 deletions was fused to the GAL4 DNA binding domain (black box) with the amino acids present within each deletion shown in parenthesis. The transcriptional activity of the GALp65 fusions was determined by transfection analyses in mammalian cells by using a CAT reporter (Fig. 2 and 3) and in *S. cerevisiae* by using a *lacZ* reporter (Table 1). +++, activation within a 50% range of the activation observed for GALVP16; ++, activation between 10 and 50% of the activation observed for GALVP16; −, activation equivalent to the basal level obtained with GAL1-147; ND, not determined. GALp65 chimeric proteins demonstrating no transcriptional activity in either mammalian cells or *S. cerevisiae* were shown by gel shift analyses to be normally expressed.

with previous studies obtained with the same two mutations in the full-length protein (60). Figure 3B demonstrates that the carboxy-terminal 42 amino acids of p65 are also sufficient for a high level of transcriptional activation and define the boundaries of a second activation domain (GALp65 508-550). This region has previously been shown to confer strong transcriptional activity (64). Inspection of the amino acid sequence of this domain demonstrates a preponderance of both proline residues and of acidic amino acids. Interestingly, a third transcriptional activation domain, which spans the region between the other two (GALp65 458-521), was also identified. Deletion analyses of this domain (Fig. 3B, GALp65 475-521) demonstrated that the transcriptional activity relies on amino acids between residues 458 and 475. In addition, the stretch of proline residues between 508 and 521 is required, as deletion of these sequences abolishes activation (GALp65 475-508).

The amino acids present within each of these *trans*-activation domains are presented in Fig. 4. The activation domains are designated AD_1 (GALp65 416-458), AD_2 (GALp65 458-521), and AD_3 (GALp65 508-550). While there is an overlap of amino acids between AD_2 and AD_3, the sequences of these domains are quite distinct, suggesting the possibility for different mechanisms of action.

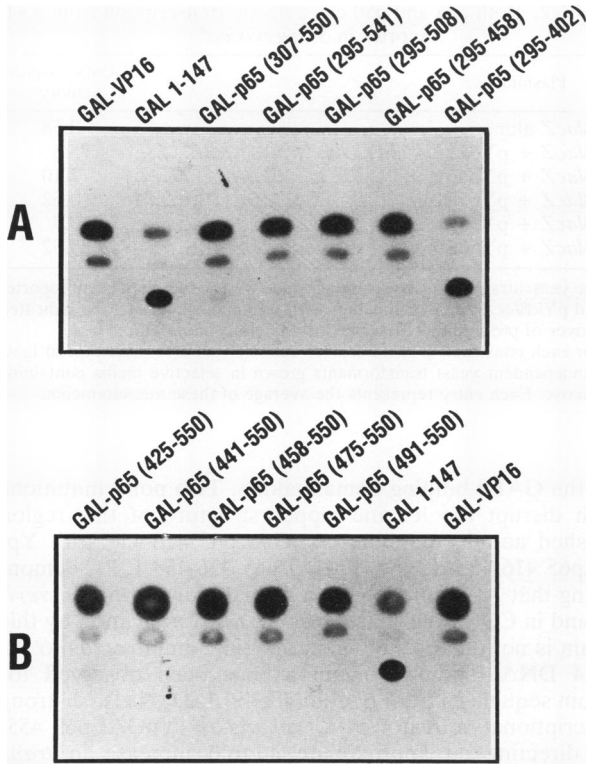

FIG. 2. Mapping the boundaries of the p65 transcriptional activation domains. Carboxy-terminal (A) and amino-terminal (B) deletions of the carboxy-terminal half of p65 were fused to the GAL4 DNA binding domain and tested for transcriptional activity. The plasmid DNAs indicated were cotransfected into COS-7 cells together with a CAT reporter plasmid containing five copies of the GAL4-responsive UAS sequence upstream of the thymidine kinase promoter (GAL4TKCAT). Each construction was assayed by COS cell transfection three to five times. Cells were harvested 48 h posttransfection, and CAT assays were performed. CAT activities of the GALp65 fusions are compared with that of GALVP16 (a potent trans-activator) and with the basal level of activation exhibited by GAL1-147.

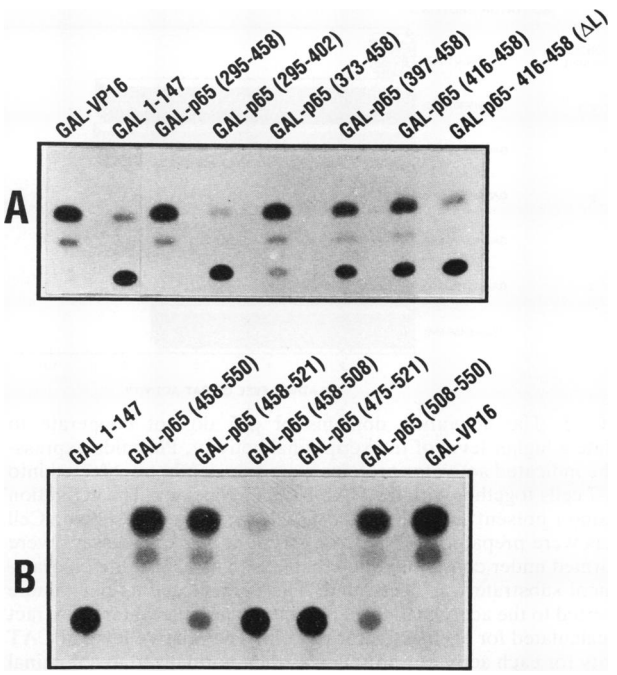

FIG. 3. Identification of three transcriptional activation domains within p65. The indicated GALp65 fusions, the GALVP16 fusion, and GAL1-147 plasmids were transiently transfected into COS-7 cells together with the reporter plasmid GAL4TKCAT. CAT assays were prepared from cell lysates harvested 48 h posttransfection. Each construction was assayed by COS cell transfection three to five times. (A) Activities of the GALp65 fusions which map the activation domain present within amino acids 295 and 458. (B) Activities of the two activation domains between 458 and 550.

To determine whether any co-operativity exists between the three activation domains, CAT assays were quantitated under conditions of linearity (Fig. 5). The entire carboxy terminus of p65, containing all three of the minimal activation domains, directed a level approximately 8.5-fold greater than the basal level. When present independently, both AD_1 and AD_3 were found to direct a similar level of transcriptional activation. Therefore, the presence of either of these activation domains alone appears sufficient to direct maximal levels of *trans* activation. This demonstrates that at least in COS cells, the individual activation domains do not appear to synergize to mediate a higher level of transcriptional activation. AD2 appears to be a weaker *trans*-activation domain than AD1 and AD3, directing a level of activation approximately fourfold greater than the basal level. Interestingly, when AD2 is present in combination with either AD1 or AD3 it appears to lower the level of transcriptional activation obtained by AD1 and AD3 individually (Fig. 5).

p65 activation domains are functional in the yeast *S. cerevisiae*. Several studies have established that certain mammalian transcriptional activators, including both the human glucocorticoid (62) and estrogen (52) receptors, the *fos* and *jun* proto-oncogene products (42, 71), and mammalian p53 (18), can function to activate transcription in *S. cerevisiae*. However, this is not a universal phenomenon, as certain mammalian transcriptional activators do not function in *S. cerevisiae* (e.g., Sp1 [10]). To determine whether any of the domains of p65 which activate transcription in mammalian cells can also function in *S. cerevisiae*, the series of GALp65 fusion proteins examined in COS cells were subcloned into a yeast expression plasmid under control of the constitutively expressed *ADH-1* promoter. The resultant plasmids were transformed into a *gal4* mutant yeast strain, which contains an integrated copy of a GAL_{UAS}/*lacZ* reporter, and the

```
AD1   416 - PPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTN - 458
                                435   442   449

AD2   458 - PAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPLMEYPEAITRLVTGAQRPPDPPAPAPLGAPGL - 521

AD3   508 - PPDPAPAPLGAPLGLPNGLLSGDEDFSSIADMDFSALLSQISS - 550
```

FIG. 4. Amino acid sequence of the activation domains present in p65. The amino acid sequences of the three activation domains identified in this study are presented. The residues bearing a positive charge (R and K) are marked +, while those bearing a negative charge (D and E) are marked −. Prolines (P) are underlined, and the triad of leucines (L) separated by seven amino acids (amino acids 435, 442, and 449) and predicted to form a potential leucine zipper structure are in boldface.

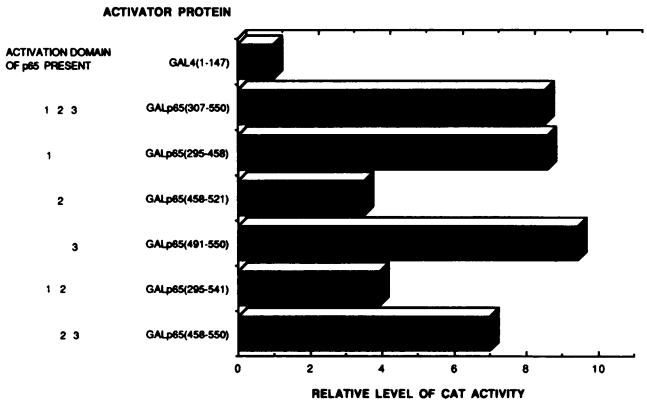

FIG. 5. The activation domains of p65 do not cooperate to mediate a higher level of transcriptional activity. Plasmids expressing the indicated activator proteins were transiently transfected into COS-7 cells together with the GALTKCAT reporter. The activation domain(s) present in each of the GALp65 fusions is shown. Cell lysates were prepared 48 h posttransfection, and CAT assays were performed under conditions such that less than 40% of the chloramphenicol substrate was acetylated. The percent counts per minute converted to the acetylated form per minute per microliter of extract was calculated for each activator protein. The relative level of CAT activity for each activator protein was then normalized to a nominal value of one given to the basal level of expression exhibited by GAL4(1-147).

trans-activation potential of the various GALp65 fusion proteins was determined. Remarkably, each of the minimal p65 activation domains (AD_1, AD_2, and AD_3) functional in COS cells also induced strong transcriptional activation in *S. cerevisiae* (Table 1).

Analyses of AD_1 in *S. cerevisiae* demonstrated that as observed in mammalian cells, the activation domain mapped to be between amino acids 295 and 458 can be restricted to the amino acids residing between 416 and 458 (Table 1). This region directed a 120-fold level of *trans* activation greater than the GAL4 binding domain alone. Two point mutations which disrupt the leucine zipper structure of this region abolished activity (compare YpGALp65 416-458 with YpGALp65 416-458ΔL and YpGALp65 416-458 L/P), demonstrating that AD_1 functions in a similar manner in *S. cerevisiae* and in COS cells. Furthermore, *trans* activation by this domain is not due to the fusion of acidic amino acids to the GAL4 DNA binding domain as has been observed for random sequences from *E. coli* (48, 49). AD_2 is also a strong transcriptional activator in *S. cerevisiae* (YpGALp65 455-521) directing an approximate 40-fold increase in *trans* activation. In accord with results from COS cells, the activity of AD_2 is dependent on the amino acids between 458 and 475, as deletion of these amino acids abolishes activity (YpGALp65 475-521). However, in contrast to activity in mammalian cells, the activity of AD_2 in *S. cerevisiae* is not dependent on the amino acids between 508 and 521, a proline-rich stretch. Indeed, deletion of this stretch of amino acids creates a more powerful activator in *S. cerevisiae* (YpGALp65 458-508). Finally, AD_3 also directs a high level of *trans* activation in *S. cerevisiae* (YpGALp65 508-550) directing a 70-fold increase in transcriptional activity. Hence, in accord with activity in mammalian cells, AD1 and AD3 are also slightly stronger transcriptional activators than AD_2 in *S. cerevisiae* (Fig. 5).

TABLE 1. Transcriptional activity of the GALp65 chimeric proteins in *S. cerevisiae*[a]

Plasmid	β-Galactosidase activity[b]
YpGAL(1-147)	0.83
YpGALVP16	98.8
YpGALp65(295-458)	100.0
YpGALp65(295-402)	0.33
YpGALp65(373-458)	112.6
YpGALp65(397-458)	81.7
YpGALp65(416-458)[c]	61.4
YpGALp65(416-458ΔL)	0.91
YpGALp65(416-458L/P)	1.33
YpGALp65(458-550)	53.9
YpGALp65(458-521)[c]	30.1
YpGALp65(458-508)	60.7
YpGALp65(475-521)	2.10
YpGALp65(508-550)[c]	57.3

[a] Plasmids expressing the indicated GALp65 fusion proteins, GAL(1-147), and the GALVP16 fusion were transformed into the yeast strain YJ0Z which contains an integrated copy of the *E. coli lacZ* gene under the control of the *GAL-1* promoter. To induce β-galactosidase activity, transformants were grown in 3% glycerol and 2% lactic acid.

[b] β-Galactosidase activity was determined for at least three independent transformants, and each entry represents the average of these measurements.

[c] The minimal activation domains of p65 functional in mammalian cells.

TABLE 2. Both p65 and p50 can activate transcription from a κB reporter in *S. cerevisiae*[a]

Plasmid	β-Galactosidase activity[b]
pYκB*lacZ* alone	0.58
pYκB*lacZ* + pY65	75.8
pYκB*lacZ* + pY65Δ	2.10
pYκB*lacZ* + pY65R	0.62
pYκB*lacZ* + pY50R	17.7
pYκB*lacZ* + pY50RX	0.92

[a] The yeast strain W3031b was transformed with the κB-dependent reporter plasmid pYκB*lacZ* alone or together with plasmids expressing the indicated derivatives of p65 and p50 (described in Materials and Methods).

[b] For each construct, β-galactosidase activity was determined for at least three independent yeast transformants grown in selective media containing 2% glucose. Each entry represents the average of these measurements.

To determine whether the GALp65 fusion proteins unable to direct *trans* activation in either COS or yeast cells were being made, high-salt cell extracts were prepared from both COS and yeast cells and analyzed by gel shift analyses for GAL4 specific DNA binding. Each of the inactive GALp65 fusion proteins were able to bind the GAL_{UAS} sequence as efficiently as active GALp65 fusions. There was no significant difference in binding efficiencies to the GAL_{UAS} DNA, suggesting no difference in levels of expression (data not shown). Thus, the inability of certain GALp65 fusion proteins to *trans* activate is not due to abnormal expression.

Full-length p50 and p65 can *trans* activate a κB reporter in *S. cerevisiae*. To examine the function of the NF-κB transcriptional activation domains within their natural context, the ability of full-length p65 and p50 to function in *S. cerevisiae* was examined. A yeast vector expressing p65 or p50 was cotransfected into *S. cerevisiae* together with a reporter plasmid containing three copies of an NF-κB DNA binding motif upstream of the *lacZ* gene. Significant transcriptional stimulation was observed when the reporter construct was cotransformed with p65 alone (Table 2). Therefore, full-length p65 is able to bind to the κB motif in *S. cerevisiae* in the absence of p50. This finding is consistent

with results obtained in mammalian cells, suggesting that p65 is a strong transcriptional activator in the absence of p50 (7, 60). Furthermore, p65Δ, a naturally occurring mutation of p65 that lacks residues within the dimerization domain (60), was unable to direct *trans* activation in *S. cerevisiae*. This suggests that transcriptional activity of p65 in *S. cerevisiae* also requires homodimer formation. The region of p65 responsible for *trans* activation lies within the carboxy terminus, as the *rel* homology domain alone (pY65R; Table 2) was unable to activate the κB reporter.

In contrast to results obtained with p65, transfection studies in mammalian cells have failed to demonstrate transcriptional activation with the p50 DNA subunit (38, 64). However, in an in vitro transcription system, the *rel* homology domain of p50 is a potent transcriptional activator of certain κB DNA binding motifs (19, 39). To examine the transcriptional activity of p50, experiments similar to those using p65 were performed with a yeast vector expressing p50 and a reporter plasmid containing three copies of the κB motif. Whereas the *rel* homology domain of p65 was unable to activate gene expression, the *rel* homology domain of p50 demonstrated strong transcriptional activity (p50R; Table 2). When expressed in the opposite orientation, p50 was unable to induce activity (p50RX; Table 2). Therefore, the domain of p50 identified as a transcriptional activator in a mammalian transcription assay also confers transcriptional activation in *S. cerevisiae*.

IκB functions in *S. cerevisiae* to repress p65 function through the *rel* homology domain. To analyze further the similarities of NF-κB function in mammalian cells and *S. cerevisiae*, the ability of IκB to repress p65 function in *S. cerevisiae* was examined. For these studies, which involved the interaction of two foreign gene products, it was necessary to use an integrated reporter system. Therefore, the yeast strain W3031b:κB*HIS*, which contains a copy of the κB:*HIS* reporter pYκB*HIS* integrated at the *trp-1* locus, was constructed (see Materials and Methods). This strain was transformed separately with plasmids expressing p65, p65Δ, or p65VP16 (an in-frame translational fusion of the amino-terminal half of p65 with the acidic activation domain of the herpesvirus VP16 protein). The ability of the resulting transformants to grow in the absence of histidine serves as a measure of transcriptional activation on the κB motif. pYκB*HIS* plus pY65 and pYκB*HIS* + pY65VP16 exhibited growth in the absence of histidine, whereas pYκB*HIS* alone and pYκB*HIS* + pY65Δ did not. Thus, as shown previously, p65 but not p65Δ was able to induce expression from the κB motif. Also, p65VP16, which contains only the *rel* homology domain of p65, was able to induce expression through the acidic activation domain of VP16, which is a potent transcriptional activator in *S. cerevisiae* (13).

As IκB is believed to inhibit p65 activity by interacting with its N-terminal *rel* homology domain (8), the ability of IκB to effect p65 function in *S. cerevisiae* was examined by cotransforming the pY65VP16 expression plasmid separately with pYIκB and pVL-102 into the κB*HIS* reporter strain. pVL-102 is the backbone expression plasmid present in pYIκB and serves as a negative control. Although cotransformants of pY65VP16 and pYIκB were still able to grow in the absence of histidine, their ability to grow in aminotriazole was blocked at a lower concentration than the negative control (Table 3). Aminotriazole is a competitive inhibitor of the *HIS3* gene product, and the ability of *S. cerevisiae* to grow at various concentrations of this compound serves as a direct measure of the level of *HIS3* expression (28). Thus, the presence of IκB reduces the level of activation of the *HIS3* expression achieved by p65VP16. In contrast, coexpression of p65VP16 with I-Rel (an inhibitor of p50 binding [58]) did not inhibit p65VP16 induction of the κB reporter. This suggests that the ability of IκB to partially block p65 activity in *S. cerevisiae* is conferred through an interaction with the *rel* homology domain.

TABLE 3. Inhibition of p65VP16 activation in *S. cerevisiae* by coexpression of IκB[a]

Plasmid	Growth phenotype				
	+HIS	−HIS	1 mM AT	2 mM AT	4 mM AT
pVL-100	+++	+++	+++	+++	++
pYIκB	+++	+++	++	+	−
pYI-Rel	+++	+++	+++	+++	+++

[a] The yeast strain W3031bκB*HIS* was transformed with the expression vector pY65VP16 together with either pVL-100, pYIκB, or pYI-Rel. Growth of the resulting transformants in the presence of histidine (+HIS) and in its absence (−HIS) was determined. The transformants were then tested for their ability to grow in the presence of increasing concentrations of aminotriazole (AT), a competitive inhibitor of the HIS3 gene product (29). Phenotypes are designated as follows: +++, indistinguishable from growth in the presence of histidine; ++, slightly poorer growth; +, weak but detectable growth; −, no growth.

DISCUSSION

Results of earlier studies have established that the carboxy-terminal domain of NF-κB p65 confers the transcriptional activity of the NF-κB complex. To gain further insight into the mechanism of p65 transcriptional activation, the domains of p65 responsible for *trans* activation were mapped. The activity of p65 was measured by using chimeric proteins consisting of the DNA binding domain of the yeast transcriptional activator GAL4 and subdomains of p65. Transcriptional activity was first assessed in cotransfection assays into COS cells with a GAL_{UAS}-driven CAT reporter plasmid. We chose to examine the transcriptional activity with the GAL4 fusion proteins as opposed to the full-length p65, for two principal reasons. First, with p65, a reporter system containing κB DNA binding motifs would be required. Thus, the presence of endogenous NF-κB as well as additional *rel*-related proteins, each of which can interact with a κB binding sequence on their own, could influence results. Second, the presence of the *rel* homology domain would also allow for multiple protein associations with endogenous *rel*-related proteins.

The transfection studies with the GALp65 fusion proteins in COS cells revealed the presence of at least three functional transcriptional activation domains. The first activation domain was localized to a region of 42 amino acids that contains a potential leucine zipper structure. This finding is consistent with earlier studies demonstrating the importance of this region for NF-κB activity (7, 60, 64). The importance of this domain was further evidenced by the observation that deletion of a single leucine abolished activity. Thus, while this region is acidic, it appears that the predicted amphipathic structure is essential for activation functions. The classical leucine zipper structure was first identified as a motif important for dimerization and concomitant binding to DNA (41). However, functional leucine zipper structures have been identified in the motifs of proteins not involved in DNA binding. For example the proto-oncogene *myb* has been shown to contain a leucine zipper structure that is not involved in DNA binding but interacts with a negative regulatory element (36). Likewise, we predict that the

leucine zipper-like structure in p65, although not involved in DNA binding, is involved in protein-protein interaction. In addition to this domain, two other regions of p65 that impart transcriptional activation were identified. The first resides within amino acids 458 to 521 and stretches of amino acids containing both acidic and proline residues appear to be important for the activity of this domain. The second domain between 508 and 550 also contains a high density of proline residues and acidic amino acids. The presence of a high content of both acidic (28, 33, 74) and proline-rich (51, 80) residues is a hallmark of many of the well-characterized transcriptional activators. The presence of multiple transcriptional activation domains may have important implications for NF-κB function. First, as each of these regions appears to be quite distinct, with respect to amino acid composition, they are likely to elicit their effects via different mechanisms. Therefore, it might be envisioned that each domain forms associations with different regulatory partners such as coactivator or adaptor proteins (9, 17). The ability of NF-κB to regulate transcription through association with numerous cellular components, some of which are likely to be cell and tissue specific, provides a mechanism by which NF-κB can differentially regulate the numerous genes containing κB-responsive sites.

A second and perhaps most significant aspect of this study is the observation that NF-κB can function in yeast cells. Although several mammalian transcriptional activators have a demonstrated transcriptional function in yeast cells (18, 42, 52, 62, 71) and it is believed that the transcriptional apparatus is highly conserved between higher eukaryotes and yeast cells (56, 72), in many instances the domains required for this activity are quite dissimilar to the regions required for corresponding functions in mammalian cells. For example, the glutamine-rich *trans* activator Sp1 has been reported not to function in yeast cells (10). Previously it has been demonstrated that two *rel*-related proteins, c-*rel* and *dorsal*, can also activate transcription in yeast cells (12, 34, 35). However, as neither the activation domains of c-*rel* or *dorsal* resemble those of p65, it remained to be shown that p65 could activate in yeast cells. Full-length p65 was found to be a potent transcriptional activator of a κB-motif dependent reporter in yeast cells. Furthermore, the observation that each of the three regions of NF-κB p65 found to be important for activity in mammalian cells was also a strong transcriptional activator in yeast cells suggests there is a strong conservation between the transcriptional machinery in mammalian and yeast cells required for NF-κB function. It might thus be envisioned that factors which associate with the activation domains in mammalian cells have homologous counterparts in yeast cells. This is perhaps somewhat surprising, as an NF-κB-like activity has not been demonstrated in yeast extracts (53a). Further evidence for conservation in the NF-κB pathway comes from analysis of the NF-κB p50 protein in *S. cerevisiae*. Transfection studies in mammalian cells have as yet failed to demonstrate transcriptional activity with this subunit (38, 60, 63, 64). In contrast, in vitro analysis suggests that p50 is indeed a transcriptional activator and that activation is dependent upon the κB sequence used (19, 39). In accord with results obtained with mammalian in vitro transcription systems, the p50 subunit was found to be a strong transcriptional activator in *S. cerevisiae*. It would therefore appear that the results obtained in the yeast system mimic more closely those obtained with the mammalian in vitro transcription assay. Considering the fact that *S. cerevisiae* is not believed to contain any *rel*-related proteins, it is unlikely that p50 activates transcription in *S. cerevisiae* following heterodimerization with another *rel*-related protein. The observation that p50 homodimers which are identical to the factor KBF-1 (38) have been implicated in controlling the expression of the murine major histocompatibility complex class I genes (31) and of tumor necrosis factor α (14) suggests that such a complex may play a role in controlling the expression of specific genes. The fact that p50 homodimers can activate in yeast cells but not in transient transfection of mammalian cells may be due to the absence of inhibitor proteins in yeast cells which are likely present in mammalian cells. For example, IκBγ is known to specifically inhibit p50 binding (46), I-Rel is believed to regulate NF-κB complexes through an interaction with p50 (59), and a protein present in nontransformed $CD4^+$ T lymphocytes causes sequestration of the p50-p50 complex (37).

In further agreement with the results of in vitro transcription experiments, the sequences of p50 mediating activation in *S. cerevisiae* were located within the *rel* homology domain. In contrast, the p65 *rel* homology domain did not appear to activate transcription, again mimicking results of the in vitro analyses. However, it remains to be determined whether on specific κB motifs the *rel* homology domain of p65 can activate transcription as has been seen in vitro for p50 (19, 39). One could envisage that depending on the κB motif and the surrounding sequence, the NF-κB complex may adopt different conformations exposing or concealing activation domains, thus adding a further level of control.

As a final analysis of NF-κB function in *S. cerevisiae*, we chose to examine the effect of the inhibitory molecule, IκB, on p65 function. IκB is the cytosolic repressor that when bound to NF-κB elicits cytoplasmic sequestration of the complex (2). Upon mitogenic stimulation, NF-κB shuttles to the nucleus, a process thought to reflect phosphorylation of IκB. To examine the effect of IκB on NF-κB function, a yeast strain containing an integrated copy of a κB-dependent *HIS3* reporter gene was transformed with the expression vector pY65VP16 (a fusion protein between the *rel* homology domain of p65 and the acidic activation domain of VP16) alone or together with pYIκB. Alone, pY65VP16 was capable of activating the κB*HIS3* reporter and thus allowing the resulting yeast transformants to grow in the absence of histidine. Cotransformation of pYIκB reduced the ability of pYVP16 transformants to grow in the absence of histidine with increasing concentrations of aminotriazole, a competitive inhibitor of the *HIS3* gene product (28). This observation strongly suggests that IκB functions in *S. cerevisiae* and provides further evidence that *S. cerevisiae* serves as a credible model to study the interplay between the various components of the NF-κB complex.

Our findings demonstrate the possibility of multiple independent transcriptional activation functions for the NF-κB p65 and p50 subunits. The observation that transcriptional activity of the individual domains is conserved between mammalian cells and yeast cells should aid in further dissection of the NF-κB activation pathway, as studies in this system will not be complicated by the presence of endogenous host factors that interact with either the κB DNA motif or the *rel* homology domain. Therefore, the power of yeast genetics may prove to be a useful tool for the isolation of putative cofactors of p65 and p50, as has recently been achieved for the herpes simplex *trans* activator VP16 (10).

ACKNOWLEDGMENTS

We thank Jon Swaffield and Stephen Johnson for the yeast strain YJO-Z, Sue Kingsman for the plasmid pMA91, Joanne Kamens and Roger Brent for the plasmid Igκ-3, Thomas Wilson and Jeffrey

Millbrandt for the plasmid HR307a, and Tina Rose for preparation of the manuscript.

S.M.R. is a recipient of a fellowship from the Leukemia Society of America.

REFERENCES

1. **Antonino, N., C.-C. Chang, L. Lombardi, M. Salina, P. Corradini, A. Maiolo, R. S. K. Chaganti, and R. Dalla-Favera.** 1991. B cell lymphoma-associated chromosomal translocation involves candidate oncogene lyt-10, homologous to NF-κB p50. Cell **67:**1075–1087.
2. **Baeuerle, P.** 1991. The inducible transcription activator NF-κB: regulation by distinct protein subunits. Biochem. Biophys. Res. Commun. **1072:**63–80.
3. **Baeuerle, P. A., and D. Baltimore.** 1988. IκB: a specific inhibitor of the NF-κB transcription factor. Science **242:**540–546.
4. **Baeuerle, P. A., and D. Baltimore.** 1988. Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-κB transcription factor. Cell **53:**211–217.
5. **Baeuerle, P. A., and D. Baltimore.** 1989. A 65 kD subunit of active NF-κB is required for inhibition of NF-κB. Genes Dev. **3:**1689–1698.
6. **Baeuerle, P. A., and D. Baltimore.** 1991. The physiology of the NF-κB transcription factor. Mol. Aspects Cell. Regul. **6:**409–432.
7. **Ballard, D., E. Dixon, N. Peffer, H. Bogerd, S. Doerre, B. Stein, and W. Greene.** 1992. The 65-kDa subunit of human NF-κB functions as a potent transcriptional activator and a target for v-Rel-mediated repression. Proc. Natl. Acad. Sci. USA **89:** 1875–1879.
8. **Beg, A., S. M. Ruben, R. I. Scheinman, S. Haskill, C. A. Rosen, and A. S. Baldwin, Jr.** 1992. IκB interacts with the nuclear localization sequences of the subunits of NF-κB: a mechanism for cytoplasmic retention. Genes Dev. **6:**1899–1913.
9. **Berger, S. L., W. D. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61:**1199–1208.
10. **Berger, S. L., B. Pina, N. Silverman, G. Marcus, J. Agapite, J. Regier, S. Triezenberg, and L. Guarente.** 1992. Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell **70:**251–265.
11. **Bours, V., P. Burd, K. Brown, J. Villalobos, S. Park, R.-P. Ryseck, R. Bravo, K. Kelly, and U. Siebenlist.** 1992. A novel mitogen-inducible gene product related to p50/p105-NF-κB participates in *trans*-activation through a κB site. Mol. Cell. Biol. **12:**685–695.
12. **Bull, P., K. L. Morley, M. F. Hoekstra, T. Hunter, and I. M. Verma.** 1990. The mouse c-*rel* protein has an N-terminal regulatory domain and a C-terminal transcriptional *trans*-activation domain. Mol. Cell. Biol. **10:**5473–5485.
13. **Chasman, D., J. Leatherwood, M. Carey, M. Ptashne, and R. Kornberg.** 1989. Activation of yeast polymerase II transcription by herpesvirus VP16 and GAL4 derivatives in vitro. Mol. Cell. Biol. **9:**4746–4749.
14. **Collart, M., P. Baeuerle, and P. Vassalli.** 1990. Regulation of tumor necrosis factor α transcription in macrophages: involvement of four κB-like motifs and of constitutive and inducible forms of NF-κB. Mol. Cell. Biol. **10:**1498–1506.
15. **Davis, N., S. Ghosh, D. L. Simmons, P. Tempst, H.-C. Liou, D. Baltimore, and H. R. Bose, Jr.** 1991. Rel-associated pp40: an inhibitor of the Rel family of transcription factors. Science **253:**1268–1271.
16. **Dillon, P. J., P. Nelbock, A. Perkins, and C. A. Rosen.** 1990. Function of the human immunodeficiency virus types 1 and 2 Rev proteins is dependent upon their ability to interact with a structured region present in the *env* gene mRNA. J. Virol. **64:**4428–4437.
17. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66:**563–576.
18. **Fields, S., and S. K. Jang.** 1990. Presence of a potent transcription activating sequence in the p53 protein. Science **249:**32768.
19. **Fujita, T., G. Nolan, S. Ghosh, and D. Baltimore.** 1992. Independent modes of transcriptional activation by the p50 and p65 subunits of NF-κB. Genes Dev. **6:**775–787.
20. **Gelinas, C., and H. M. Temin.** 1988. The *v-rel* oncogene encodes a cell-specific transcriptional activator of certain promoters. Oncogene **3:**349–355.
21. **Ghosh, S., and D. Baltimore.** 1990. Activation *in vitro* of NF-κB by phosphorylation of its inhibitor IκB. Nature (London) **344:** 678–682.
22. **Ghosh, S., A. M. Gifford, L. B. Riviere, P. Tempst, G. P. Nolan, and D. Baltimore.** 1990. Cloning of the p50 DNA binding subunit of NF-κB: homology to *rel* and *dorsal*. Cell **62:**1019–1029.
23. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
24. **Griffin, G. E., D. Leung, T. M. Folks, S. Kunkel, and G. J. Nabel.** 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. Nature (London) **339:**70–73.
25. **Guthrie, C., and G. R. Fink.** 1991. Guide to yeast genetics and molecular biology. Methods Enzymol. **194.**
26. **Hannink, M., and H. M. Temin.** 1989. *trans*-Activation of gene expression by nuclear and cytoplasmic *rel* proteins. Mol. Cell. Biol. **9:**4323–4336.
27. **Haskill, S., A. A. Beg, S. M. Tompkins, J. S. Morris, A. D. Yurochko, A. Sampson-Johannes, K. Mondal, P. Ralph, and A. S. Baldwin, Jr.** 1991. Characterization of an immediate-early gene induced in adherent monocytes that encodes IκB-like activity. Cell **65:**1281–1289.
28. **Hill, D. E., I. A. Hope, J. P. Macke, and K. Struhl.** 1986. Saturation mutagenesis of the yeast *HIS3* regulatory site: requirements for transcriptional induction and for binding by GCN4 activator protein. Science **234:**451–457.
29. **Inoue, J.-I., L. D. Kerr, L. J. Ransone, E. Bengal, T. Hunter, and I. M. Verma.** 1991. *c-rel* activates but *v-rel* suppresses transcription from κB sites. Proc. Natl. Acad. Sci. USA **88:** 3715–3719.
30. **Ip, Y. T., R. Kraut, M. Levine, and C. Rushlow.** 1991. The dorsal morphogen is a sequence-specific DNA-binding protein that interacts with a long-range repression element in Drosophila. Cell **64:**439–446.
31. **Israel, A., O. LeBail, D. Hata, J. Piette, M. Kieran, F. Logeat, D. Wallach, M. Fellous, and P. Kourilsky.** 1989. TNF stimulates expression of mouse MHC class I genes by inducing an NF-κB like enhancer binding activity which displaces constitutive factors. EMBO J. **8:**3793–3800.
32. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
33. **Johnston, M.** 1987. A model fungal gene regulatory mechanism: the GAL genes of *Saccharomyces cerevisiae*. Microbiol. Rev. **51:**458–476.
34. **Kamens, J., and R. Brent.** 1991. A yeast transcription assay defines distinct *rel* and *dorsal* DNA recognition sequences. New Biol. **3:**1005–1013.
35. **Kamens, J., P. Richardson, G. Mosialos, R. Brent, and T. Gilmore.** 1990. Oncogenic transformation by v-*rel* requires an amino-terminal activation domain. Mol. Cell. Biol. **10:**2840–2847.
36. **Kanei-Ishiii, C., E. M. MacMillan, T. Nomura, A. Sarai, R. Ramsay, S. Aimoto, S. Ishii, and T. J. Gonda.** 1992. Transactivation and transformation by Myb are negatively regulated by a leucine-zipper structure. Proc. Natl. Acad. Sci. USA **89:**3088–3092.
37. **Kang, S.-M., A.-C. Tran, M. Grilli, and M. J. Lenardo.** 1992. NF-κB subunit regulation in nontransformed CD4+ T lymphocytes. Science **256:**1452–1457.
38. **Kieran, M., V. Blank, F. Logeat, J. Vandekerchove, F. Lottspeich, O. LeBail, M. B. Urban, P. Kourilsky, P. A. Baeuerle, and A. Israel.** 1990. The DNA binding subunit of NF-κB is identical to factor KBF1 and homologous to the *rel* oncogene product. Cell **62:**1007–1018.
39. **Kretzschmar, M., M. Meisterenst, C. Scheidereit, G. Li, and R.**

Roeder. 1992. Transcriptional regulation of the HIV-1 promoter by NF-κB *in vitro*. Genes Dev. **6:**761–774.
40. **LaMarco, K., C. C. Thompson, B. P. Byers, E. M. Wlaton, and S. L. McKnight.** 1991. Identification of Ets- and Notch-related subunits in GA binding protein. Science **253:**789–792.
41. **Landschulz, W. H., P. F. Johnson, and S. L. McKnight.** 1988. The leucine binding zipper: a hypothetical structure common to a new class of DNA binding proteins. Science **240:**1759–1764.
42. **Lech, K., K. Anderson, and R. Brent.** 1988. DNA-bound Fos proteins activate transcription in yeast. Cell **52:**179–184.
43. **Legrain, P., and M. Rosbach.** 1989. Some *cis* and *trans*-acting mutants for splicing target pre-mRNA to the cytoplasm. Cell **57:**573–583.
44. **Lenardo, M. J., and D. Baltimore.** 1989. NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell **58:**227–229.
45. **Leuther, K., and S. Johnston.** 1992. Non-dissociation of GAL4 and GAL80 *in vivo* after galactose induction. Science **256:**1333–1335.
46. **Liou, H.-C., G. P. Nolan, S. Ghosh, T. Fujita, and D. Baltimore.** 1992. The NF-κB p50 precursor, p105, contains an internal IκB-like inhibitor that preferentially inhibits p50. EMBO J. **11:**3003–3009.
47. **Lux, S. E., K. M. John, and V. Bennett.** 1990. Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. Nature (London) **344:**36–42.
48. **Ma, J., and M. Ptashne.** 1987. A new class of yeast transcriptional activators. Cell **51:**113–119.
49. **Ma, J., and M. Ptashne.** 1988. Converting a eukaryotic transcriptional inhibitor into an activator. Cell **55:**443–446.
50. **Mellor, J., N. A. Dobson, A. J. Kingsman, and S. M. Kingsman.** 1985. Factors affecting heterologous gene expression in *Saccharomyces cerevisiae*. Gene **33:**215–226.
51. **Mermod, N., E. A. O'Neil, T. J. Kelly, and R. Tjian.** 1989. The proline-rich transcriptional activator of CTF/NF-1 distinct from the replication and DNA binding domain. Cell **58:**741.
52. **Metzger, D., J. H. White, and P. Chambon.** 1988. The human oestrogen receptor functions in yeast. Nature (London) **334:**31–36.
53. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
53a. **Moore, P. A., and C. A. Rosen.** Unpublished results.
54. **Nolan, G. P., S. Ghosh, H.-C. Liou, P. Tempst, and D. Baltimore.** 1991. DNA binding and IκB inhibition of the cloned p65 subunit of NF-κB, a *rel*-related polypeptide. Cell **64:**961–969.
55. **Perkins, N., R. Schmid, C. Duckett, K. Leung, N. Rice, and G. Nabel.** 1992. Distinct combinations of NF-κB subunits determine the specificity of transcriptional activation. Proc. Natl. Acad. Sci. USA **89:**1529–1533.
56. **Ptashne, M.** 1988. How eukaryotic transcriptional activators work. Nature (London) **335:**683–689.
57. **Richardson, P. M., and T. D. Gilmore.** 1991. vRel is an inactive member of the Rel family of transcriptional activating proteins. J. Virol. **65:**3122–3130.
58. **Ruben, S. M., P. J. Dillon, R. Schreck, T. Henkel, C.-H. Chen, M. Maher, P. A. Baeuerle, and C. A. Rosen.** 1991. Isolation of a *rel*-related human cDNA that potentially encodes the 65-kD subunit of NF-κB. Science **251:**1490–1493.
59. **Ruben, S. M., J. Klement, T. Coleman, M. Maher, C.-H. Chen, and C. Rosen.** 1992. I-Rel: a novel *rel*-related protein that inhibits NF-κB transcriptional activity. Genes Dev. **6:**745–760.
60. **Ruben, S. M., R. Narayanan, J. F. Klement, C.-H. Chen, and C. Rosen.** 1992. Functional characterization of the NF-κB p65 transcriptional activator and an alternatively spliced derivative. Mol. Cell. Biol. **12:**444–454.
61. **Sadowski, I., and M. Ptashne.** 1989. A vector for expressing GAL4(1-47) fusions in mammalian cells. Nucleic Acids Res. **17:**7539.
62. **Schena, M., and K. Yamamoto.** 1988. Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. Science **241:**965–967.
63. **Schmid, R., N. Perkins, C. Duckett, P. Andrews, and G. Nabel.** 1991. Cloning of an NF-κB subunit which stimulates HIV transcription in synergy with p65. Nature (London) **352:**733–738.
64. **Schmitz, M. L., and P. A. Baeuerle.** 1991. The p65 subunit is responsible for the strong transcription activating potential of NF-κB. EMBO J. **10:**3805–3817.
65. **Sen, R., and D. Baltimore.** 1986. Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a post-translational mechanism. Cell **47:**921–928.
66. **Sen, R., and D. Baltimore.** 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell **46:**705–716.
67. **Shi, Y., E. Seto, L.-S. Chang, and T. Shenk.** 1991. Transcriptional repression by YY1, a human GL1-Krüppel-related protein, and relief of repression by adenovirus E1A protein. Cell **67:**377–388.
68. **Shirakawa, F., and S. B. Mizel.** 1989. In vitro activation and nuclear translocation of NF-κB catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. Mol. Cell. Biol. **9:**2424–2430.
69. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
70. **Steward, R.** 1987. *Dorsal*, an embryonic polarity gene in *Drosophila* is homologous to the vertebrate proto-oncogene, *c-rel*. Science **238:**692–694.
71. **Struhl, K.** 1988. The *Jun* oncoprotein, a vertebrate transcription factor, activates transcription in yeast. Nature (London) **332:**649–650.
72. **Struhl, K.** 1989. Molecular mechanisms of transcriptional regulation in yeast. Annu. Rev. Biochem. **58:**1051–1077.
73. **Theilen, G., and R. T. Zeigel.** 1966. Biological studies with RE virus (strain T) that induces reticuloendotheliosis in turkeys, chickens and Japanese quails. J. Natl. Cancer Inst. **37:**747–749.
74. **Thompson, C. C., T. A. Brown, and S. L. McKnight.** 1991. Convergence of Ets- and Notch-related structural motifs in a heteromeric DNA binding complex. Science **253:**762–768.
75. **Triezenberg, S., R. Kingsbury, and S. McKnight.** 1989. Functional dissection of VP16, the *trans*-activator of herpes simplex virus immediate early gene expression. Genes Dev. **2:**718–729.
76. **Urban, M., and P. Baeuerle.** 1991. The role of the p50 and p65 subunits of NF-κB in the recognition of cognate sequences. New Biol. **3:**279–288.
77. **Urban, M., R. Schreck, and P. Baeuerle.** 1991. NF-κB contacts DNA by a heterodimer of the p50 and p65 subunit. EMBO J. **10:**1817–1825.
78. **Urban, M. B., and P. A. Baeuerle.** 1990. The 65-kD subunit of NF-κB is a receptor for IκB and a modulator of DNA-binding specificity. Genes Dev. **4:**1975–1984.
79. **Vernet, T., D. Dignard, and D. Thomas.** 1987. A family of yeast expression vectors containing the phage f1 intergenic region. Gene **52:**225–233.
80. **Williams, T., A. Admon, B. Luscher, and R. Tijan.** 1988. Cloning and expression of AP-2, a cell-type-specific transcription factor that activates inducible enhancer elements. Genes Dev. **2:**1557.
81. **Wilson, T. E., T. J. Fahrner, M. Johnston, and J. Milbrandt.** 1992. Identification of the DNA binding site for NGFI-B by genetic selection in yeast. Science **252:**1290–1300.