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# Protein interaction cloning in yeast: Identification of mammalian proteins that react with the leucine zipper of Jun

(transcription factors/coiled-coils/tropomyosin/TAXREB67)

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ABSTRACT **To identify proteins that interact with Jun or Fos we have used the protein interaction cloning system developed by S. Fields and O.-K. Song [(1989) *Nature (London)* 340, 245–246] to clone mammalian cDNAs encoding polypeptides that interact with the dimerization and DNA-binding motif (bZIP; basic domain leucine zipper motif) of Jun. For this purpose, yeast cells lacking GAL4 activity but expressing a GAL4 DNA-binding domain–Jun bZIP fusion protein were transformed with a mouse embryo cDNA plasmid library in which the cDNA was joined to a gene segment encoding the GAL4 transcriptional activation domain. Several transformants exhibiting GAL4 activity were identified and shown to harbor plasmids encoding polypeptides predicted to form coiled-coil structures with Jun and/or Fos. One of these is a bZIP protein of the ATF/CREB protein family—probably the murine homolog of TAXREB67. Two others encode polypeptides with predicted potential to form coiled-coil structures, and seven other isolates encode segments of α- or β-tropomyosin, classical coiled-coil proteins. The tropomyosin polypeptides were found to interact in the yeast assay system with the bZIP region of Jun but not with the bZIP region of Fos. Our results illustrate the range of protein interaction cloning for discovering proteins that bind to a given target polypeptide.**

Jun and Fos are sequence-specific DNA-binding proteins that regulate transcription. Each protein has a bipartite DNA-binding domain [bZIP; basic domain leucine zipper motif (1)] consisting of an amphipathic helix that mediates dimerization through formation of a short coiled-coil structure (2), termed the "leucine zipper" (3), and an adjacent basic region that contacts DNA (4–6). Dimerization of monomers in a parallel orientation positions each basic region to interact with a half site of the DNA target sequence. In principle, a given bZIP transcription factor could interact with a variety of bZIP proteins with compatible amphipathic helices, yielding dimers with different DNA-binding and transcriptional properties (for review, see ref. 7). In the case of the known members of the Jun and Fos families, all pairwise combinations capable of forming dimers bind to the same dyad symmetric core sequence (8) (for reviews, see refs. 9 and 10), but their transcriptional properties often vary (11, 12). Moreover, the transcriptional activities of Jun can be modulated by interactions with other proteins, such as the glucocorticoid receptor (13–15), the inhibitory protein IP-1 (16), and a cell-specific inhibitor (17).

The goal of the present study is to identify additional mammalian proteins that interact with Jun or Fos, some of which may form DNA-binding dimers with activities different from those of Jun–Fos heterodimers. For this purpose, we have used a yeast genetic system described by Fields and coworkers (18, 19) in which expression of a *GAL1–lacZ* reporter gene under the control of a GAL4-activated promotor depends on reconstitution of GAL4 activity via protein–protein interaction. This is accomplished by apposition of the GAL4 DNA-binding [GAL4(DB)] and transcriptional activation [GAL4(TA)] domains via interaction of polypeptides fused to each domain. Colonies containing interacting polypeptides are detected with a chromogenic substrate for β-galactosidase. In our initial experiments, described in this report, we have used this method to clone from a mouse embryo cDNA library the coding sequences for proteins that interact with the bZIP region of c-Jun (bzJUN).* Among the interacting polypeptides identified were the bZIP region of a transcription factor of the ATF/CREB family, two previously unidentified polypeptides, and segments of the cytoskeletal coiled-coil proteins, α- and β-tropomyosin.

## MATERIALS AND METHODS

**Yeast Strains and Methods.** Yeast strain PCY2 (*MATα Δgal4 Δgal80 URA3::GAL1–lacZ lys2-801$^{amber}$ his3-Δ200 trp1-Δ63 leu2 ade2-101$^{ochre}$*) is the product of a cross between GGY1::171 (20) and YPH499 (21). PCY7 was constructed by integrating a 5.3-kilobase (kb) *Sal* I fragment, which encodes GAL4(DB)–bzJUN and the *LEU2* gene product, at the genomic *leu2* locus of PCY2 by one-step gene replacement (22). (PCY7 also has a 7.4-kb fragment containing a *GAL1–HIS3* reporter gene and *LYS2* integrated at the *lys2* locus.) Yeast strains were grown in yeast extract/peptone/dextrose or supplemented synthetic dextrose medium (23). Transformation was by the lithium acetate method (23) except that 50 μl of competent yeast cells was transformed with 5 μg of plasmid DNA without carrier DNA. β-Galactosidase activity of liquid yeast cultures was quantitated as described (23). Yeast colonies were assayed for β-galactosidase activity by plating transformants on supplemented synthetic dextrose medium lacking tryptophan and transferring the resulting colonies directly to a nitrocellulose filter, which was then treated as described (24), except that 5-bromo-4-chloro-3-indolyl β-D-galactoside was used at 1 mg/ml, and incubation was at 37°C. To recover centromere plasmids from yeast into *Escherichia coli*, plasmid DNA was isolated from spheroplasts (23) using the bacterial alkaline lysis minipreparation procedure (25) and used to transform competent *E. coli* DH5α (GIBCO/BRL). Typically, $10^3$ transformants were obtained from centromeric plasmid DNA derived from 0.75 ml of a saturated yeast culture grown in supplemented synthetic dextrose medium selective for the plasmid.

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Abbreviations: bZIP, basic domain leucine zipper motif; GAL4(DB), GAL4 DNA-binding domain (amino acids 1–147); GAL4(TA), GAL4 transcriptional activation domain (amino acids 768–881); bzJUN, bZIP region of c-JUN; NLS, nuclear localization signal; bzFOS, bZIP region of c-FOS; JZA, Jun zipper associated.

*The complete nucleotide sequence of the mTR67 cDNA clone described in this paper has been deposited in the GenBank data base (accession no. M94087).

**Construction of GAL4 Fusion Vectors.** Construction of GAL4 fusion vectors involved several intermediate plasmids, whose structures were verified by restriction mapping and sequencing across recombinant joints. The starting plasmid for construction of GAL4(DB) fusion vectors was pPC2, constructed by ligating the 4.8-kb *Bam*HI fragment carrying *ADC1* gene promoter ($P_{ADC1}$)–GAL4–*ADC1* gene terminator ($T_{ADC1}$) from pCL2 [which is identical to pCL1 (18) except that the orientation of this fragment is reversed] into the *Bam*HI site of pRS313 (21) such that $T_{ADC1}$ was adjacent to the *HIS3* gene. The final vectors were pPC62 (Fig. 1) and pPC16, which carries the *HIS3* marker in place of the *LEU2* marker in pPC62. The starting plasmid for the GAL4(TA) fusion vectors was pPC6, the product of a three-piece ligation of pBSKS(−) (Stratagene) linearized with *Bam*HI and treated with calf intestinal alkaline phosphatase, to the 1.4-kb *Bam*HI/*Hin*dIII $P_{ADC1}$ fragment and the 0.45-kb *Hin*dIII/*Bam*HI $T_{ADC1}$ fragment, both from pPC2, such that $T_{ADC1}$ was adjacent to the *Sac* I site of pBSKS(−). The final vector, pPC86 (Fig. 1), was derived from pPC51 and differs only in the polylinker sequence between the unique *Sal* I and *Sac* II restriction sites. In some of the early experiments (reported in Table 1), GAL4(TA) fusions were constructed in vector pPC12, which encodes the simian virus 40 large tumor antigen nuclear localization signal (NLS) (Met-Pro-Lys-Lys-Lys-Arg-Lys-Val) fused to a polylinker encoding Ser-Arg-Ser-Thr-Val-Ser-Ile-Ser-Leu-Ile-Ser-Asn-Ser-Cys-Ser-Pro followed by GAL4(TA) and a termination codon in that order. Details of the construction of the various plasmids will be provided on request.

**Construction and Screening of GAL4(TA) cDNA Library.** Poly(A) RNA from 14.5-day-old CD-1 mouse embryos was prepared as described (27). Double-stranded, blunt-ended cDNA was synthesized with 5 μg of poly(A) RNA essentially as described (28), except that Moloney murine leukemia virus reverse transcriptase and a primer adaptor containing a *Not* I site {5′-d[p$(GA)_{10}$GCGGCCGCCA$(T)_{18}$]-3′} were used. An equimolar mixture of two different *Sal* I adaptors [5′-d(TCGACCCCGGG)-3′, Stratagene; 5′-d(TCGACCCACGCGTCCG)-3′, GIBCO/BRL] was ligated to the blunt-ended cDNAs followed by digestion with *Not* I. The size-purified cDNAs were then ligated directionally into *Sal* I- and *Not* I-digested pPC51 and used to transform *E. coli* ElectroMAX DH10B cells (GIBCO/BRL) by electroporation (29). Plasmid DNA was then isolated directly from $2 \times 10^6$ transformed colonies. Plasmids from 12 random colonies all contained inserts, which ranged in size from 0.3 to 2 kb. Yeast PCY7 transformed with the plasmid library was plated at a density of $2 \times 10^3$ colonies per $100 \times 15$ mm$^2$ plate of supplemented synthetic dextrose lacking tryptophan. Transformants in which GAL4 activity had been reconstituted were identified by the colony filter β-galactosidase assay procedure described above.

**DNA Sequencing.** cDNAs were cloned into pPC51, pPC86, or pBluescript II SK(−) (Stratagene), and in some cases nested deletions were made (30) before sequencing both strands of the resulting double-stranded plasmids with Sequenase (United States Biochemical) according to the manufacturer's protocol.

## RESULTS

**Detection of Jun–Fos Leucine Zipper Interactions.** We first tested the suitability of the yeast protein interaction cloning system for detecting leucine zipper interactions. Yeast strain

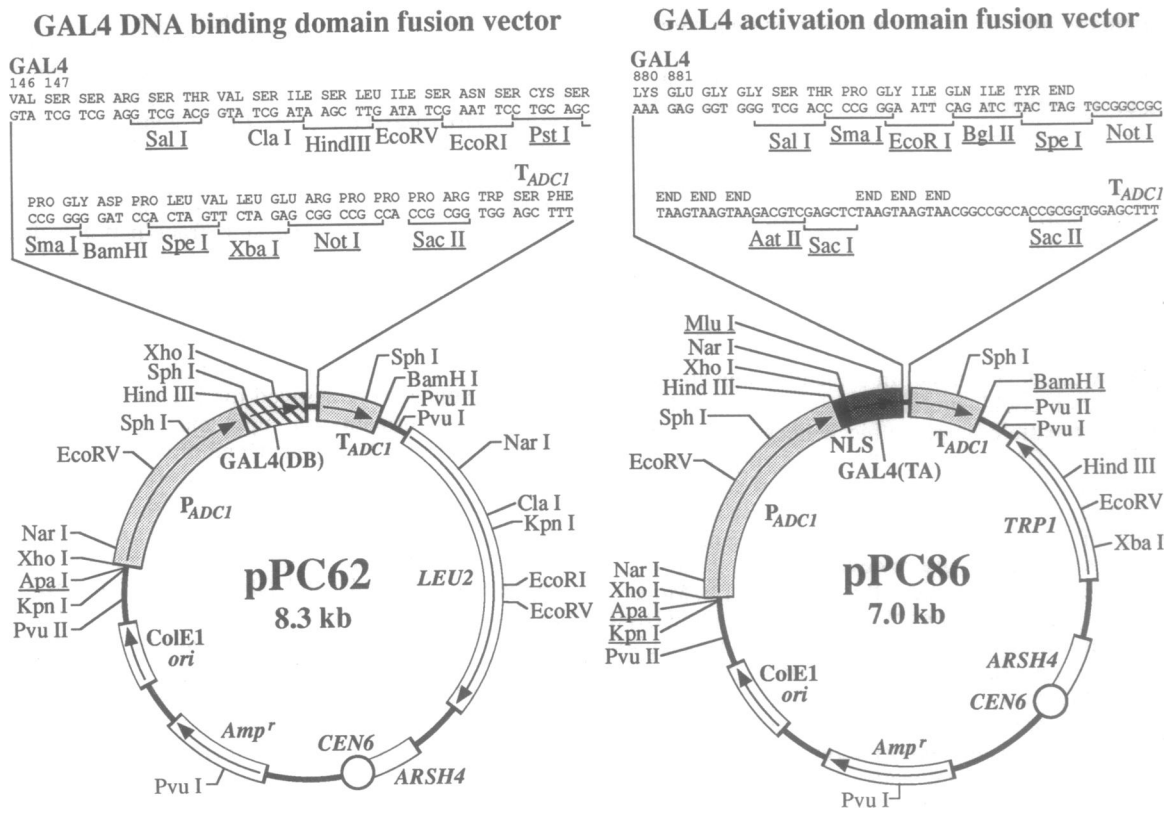


FIG. 1. Structure of GAL4 fusion vectors. (*Left*) pPC62 encodes GAL4(DB) expressed from a strong constitutive promotor ($P_{ADC1}$) followed by a polylinker and the terminator ($T_{ADC1}$) of the yeast *ADC1* gene (26). It carries the *LEU2* yeast selectable marker. (*Right*) pPC86 encodes GAL4(TA) and carries the *TRP1* marker. A sequence encoding a NLS is fused to the GAL(TA) codons, followed by a polylinker ending with termination codons (END) in all three reading frames. In addition, both shuttle plasmids carry a yeast centromere (*CEN6*), a yeast replication origin (*ARSH4*), a bacterial replication origin (ColE1 *ori*), and an ampicillin-resistance gene (*Amp$^r$*). The final two codons of each GAL4 domain are indicated, as is the beginning of the *ADC1* terminator sequences. Restriction enzyme sites that are unique within each plasmid are underlined.

GGY1::171 (20), in which the *GAL4* and *GAL80* genes are deleted and which contains a genomic *GAL1–lacZ* reporter gene, was cotransformed with single-copy plasmids expressing GAL4(DB) and GAL4(TA) fusion proteins. Double transformants in which GAL4 activity had been reconstituted via interaction of proteins fused to GAL4(DB) and GAL4(TA) were detected by a direct yeast colony filter β-galactosidase assay (see Fig. 2). The relative strengths of the interactions were quantitated by assaying liquid cultures of individual yeast transformants for β-galactosidase activity (Table 1).

Transformants expressing GAL4(DB) and GAL4(TA) alone, or bzJUN or the bZIP region of c-Fos (bzFOS) fused to one but not the other domain of GAL4, exhibited background levels of galactosidase activity (Table 1, rows 1–3, 5, and 6). As expected, full-length c-Jun, which contains transcriptional activation domains outside its bZIP region (31), induced β-galactosidase activity by itself when expressed as a fusion with GAL4(DB) but not when fused to GAL4(TA) (rows 4 and 7, respectively). The bzJUN homodimer interaction produced a signal ≈3-fold above background (row 8), whereas the heterodimer interaction between bzJUN and bzFOS activated transcription by 2–3 orders of magnitude (rows 9 and 10). This heterodimer interaction was detected when bzJUN was fused to GAL4(DB) and bzFOS was fused to GAL4(TA), as well as when the reciprocal fusions were tested (rows 9 and 10, respectively). In addition, these homo- and heterodimer interactions were detected when one bZIP domain was fused either to the N terminus of GAL4(TA) (in the vector pPC12; Table 1) or to the C terminus of GAL4(TA) (in the vector pPC51 or pPC86; Table 2 and unpublished observations). Finally, the bzFOS homodimer interaction was not detectable above background (row 11). These results are consistent with previous reports of the relative stabilities of Jun–Fos heterodimers, Jun homodimers, and Fos homodimers (33–35). We conclude that peptide pairs with the stability of Fos–Jun or Jun–Jun dimers can be readily detected by protein interaction cloning in yeast.

Table 1. Mammalian leucine zipper interactions detected by GAL4 activity in yeast

| | Double transformants | | β-Gal activity |
|---|---|---|---|
| | GAL4(DB) fusion | GAL4(TA) fusion | |
| 1. | GAL4(DB) | NLS–GAL4(TA) | <0.01 |
| 2. | GAL4(DB)–bzJUN | NLS–GAL4(TA) | <0.01 |
| 3. | GAL4(DB)–bzFOS | NLS–GAL4(TA) | <0.01 |
| 4. | GAL4(DB)–JUN | NLS–GAL4(TA) | 5.2 |
| 5. | GAL4(DB) | NLS–bzJUN–GAL4(TA) | <0.01 |
| 6. | GAL4(DB) | NLS–bzFOS–GAL4(TA) | <0.01 |
| 7. | GAL4(DB) | NLS–JUN–GAL4(TA) | <0.01 |
| 8. | GAL4(DB)–bzJUN | NLS–bzJUN–GAL4(TA) | 0.03 |
| 9. | GAL4(DB)–bzJUN | NLS–bzFOS–GAL4(TA) | 18.0 |
| 10. | GAL4(DB)–bzFOS | NLS–bzJUN–GAL4(TA) | 3.8 |
| 11. | GAL4(DB)–bzFOS | NLS–bzFOS–GAL4(TA) | <0.01 |
| 12. | Wild-type GAL4 | pRS315* | 100.0 |

Yeast strain GGY1::171 was transformed with centromere plasmids expressing the indicated GAL4 fusion genes as described in the text. β-Galactosidase (β-Gal) activity of double transformants is reported as a percentage of β-galactosidase activity induced by wild-type GAL4 (row 12; 1560 units). GAL4(DB) fusions were derived from pPC16 and encode GAL4(DB) fused to Ser-Arg-Ser-Ser-Gly followed by either JUN (murine amino acids 1–334), bzJUN (murine amino acids 250–334), or bzFOS (human amino acids 132–216) each followed immediately by a termination codon. GAL4(TA) fusions were derived from pPC12 and encode the simian virus 40 large tumor antigen NLS fused to Ser-Ser-Gly followed by JUN (murine amino acids 1–325) or bzJUN (murine amino acids 250–325), both followed by Cys-Ser-Pro-GAL4(TA) and a termination codon, or by bzFOS (human amino acids 132–206) followed by Ser-Thr-Val-Ser-Ile-Ser-Leu-Ile-Ser-Asn-Ser-Cys-Ser-Pro-GAL4-(TA) and a termination codon. Wild-type GAL4 was expressed from plasmid pPC2.

*This plasmid is the backbone of all the NLS–GAL4(TA) fusion vectors and does not express a GAL4-related product (21).

Table 2. Interaction of the bZIP regions of JUN and FOS with polypeptides encoded by cDNAs isolated by protein interaction cloning

| | NLS–GAL4(TA) cDNA fusion | Interaction with GAL4(DB) fused to | | | |
|---|---|---|---|---|---|
| | | bzJUN | bzFOS | pPC62* | pRS315† |
| 1. | bzJUN | ++ (0.03) | +++ (3.3) | – | – |
| 2. | JZA-3 | ++ | + | + | + |
| 3. | JZA-20 | + | – | – | – |
| 4. | bzmTR67 | ++ | + | – | – |
| 5. | α-Tropomyosin | +++ (0.09) | – | – | – |
| 6. | β-Tropomyosin | +++ (3.8) | – | – | – |
| 7. | pPC86‡ | – | – | – | – |

Yeast strain PCY2 was transformed with centromere plasmids expressing the indicated GAL4 fusion genes. The relative strength of interactions was assessed by the yeast colony filter β-galactosidase assay and is reported as + to +++. – indicates no color development after an overnight incubation. Values in parentheses are the mean β-galactosidase activity determined from two independent transformants and are reported as percentage wild-type GAL4-induced β-galactosidase activity (2300 units). Transformants with no reported value gave <0.01% β-galactosidase activity. GAL4(TA) fusions to bzmTR67 (amino acids 271–349) and bzJUN (murine amino acids 250–334) were derived from pPC86 (Fig. 1) and encode GAL4(TA) fused to Gly-Gly-Ser-Ser followed by the insert ending with a termination codon. GAL4(TA) fusions to JZA-3, JZA-20, and α- and β-tropomyosin are the largest original isolates from the library screen and were cloned in pPC51. GAL4(DB) fusions were derived from pPC62 (Fig. 1) and encode GAL4(DB) fused to Ser-Arg-Ser-Ser followed by either bzFOS [murine amino acids 132–211 derived from pWBFos (32)] or bzJUN (murine amino acids 250–334) each followed immediately by a termination codon. JZA, Jun zipper associated.

*This plasmid expresses GAL4(DB) alone.

†This plasmid is the backbone of pPC62 and does not express a GAL4-related product (21).

‡This plasmid expresses GAL4(TA) alone.

**Isolation of Mammalian cDNAs Encoding Polypeptides That Interact with bzJUN.** Next, we sought to clone mammalian cDNAs encoding polypeptides that interact with bzJUN. For this purpose, we constructed the yeast strain PCY7, which expresses a GAL4(DB)–bzJUN protein from an integrated fusion gene and harbors the integrated *GAL1–lacZ* reporter gene as well as *GAL4* and *GAL80* deletions. PCY7 was transformed with a mouse embryo cDNA library constructed in the single-copy yeast expression vector pPC51. This plasmid is identical to pPC86 shown in Fig. 1 except for the sequences between the unique *Sal* I and *Sac* II restriction sites. Oligo(dT)-primed cDNAs were directionally cloned downstream of the NLS–GAL4(TA) fusion gene. Thus, the library cDNAs are expressed from the *ADC1* promotor as a NLS–GAL4(TA) cDNA fusion, with each insert providing its own stop codon. This orientation requires only one in-frame fusion junction. In addition, two glycine codons were engineered at the junction on the supposition that flexibility between GAL4(TA) and the test polypeptide might be beneficial. (The effect of glycine codons, however, was not tested.)

Yeast transformants were selected by plating on supplemented synthetic dextrose medium lacking tryptophan and subsequently screened for β-galactosidase activity by the yeast colony filter assay (Fig. 2). A screen of $10^5$ transformants yielded 12 positive colonies after incubations of 30 min to 12 hr. From these 12 colonies, 11 independent isolates were identified and shown to cosegregate the $Trp^+$ and galactosidase-positive phenotypes. Plasmids recovered from each of these 11 transformants were shown to confer both the

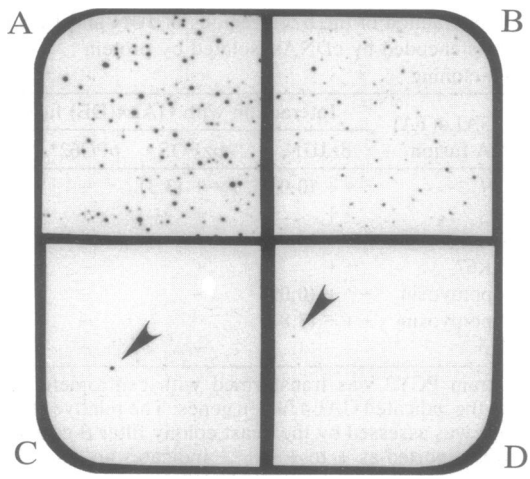


FIG. 2. Detection of yeast transformants by the colony filter β-galactosidase assay. Yeast strain PCY7, which expresses GAL4(DB)–bzJUN, was transformed with a derivative of pPC51 expressing GAL4(TA) fused to bzFOS (*A*), bzJUN (*B*), or polypeptides encoded by a mouse embryo cDNA library (*C* and *D*). $Trp^+$ transformants were grown into colonies on supplemented synthetic dextrose medium, transferred to nitrocellulose filters, and assayed for β-galactosidase activity. Arrowheads indicate two representative positive colonies detected among the ≈500 colonies present on each filter quadrant.

$Trp^+$ and galactosidase-positive phenotypes when reintroduced into PCY7.

To exclude the possibility that a clone was isolated simply because it encodes a polypeptide that interacts with the GAL4(DB) portion of the target protein and not the bzJUN portion, we cotransformed yeast strain PCY2 (which is identical to PCY7 except that it does not express any GAL4 fusion protein) with each positive library plasmid and pPC62 (Fig. 1), which expresses GAL4(DB) alone, and tested for galactosidase activity (Table 2). Only the clone we termed Jun zipper associated (JZA)-3 (see below) exhibited activity in this test. However the GAL4(TA)–JZA-3 fusion also gave a positive signal when expressed alone in PCY2 (Table 2), indicating that it is not dependent on GAL4(DB) to activate the *GAL1–lacZ* reporter gene. The most straightforward interpretation of this result is that the JZA-3-encoded polypeptide binds a DNA sequence present in the promoter of the *GAL1–lacZ* reporter gene. Since the GAL4(TA)–JZA-3 fusion displayed a stronger transcriptional signal in the presence of GAL4(DB)–bzJUN than when expressed alone or with GAL4(DB), it is possible that the JZA-3 peptide interacts with bzJUN.

**Nucleotide Sequence Analysis of cDNA Clones.** Comparison of the nucleotide sequences of each of the 11 isolates with each other and with the GenBank/EMBL nucleotide sequence data base revealed six different classes (Table 2). One clone encoded the C-terminal 185 amino acids of c-Jun, which encompasses its bZIP region (36). Two clones, JZA-3 and JZA-20, did not share significant similarity with any of the sequences in the data base or with each other. JZA-3 is a 2.4-kb partial cDNA that fuses a 411-amino acid open reading frame downstream of GAL4(TA). By assaying successive C-terminal deletions, we determined that removal of 14 codons (1105 nucleotides) had no effect on activity, whereas deletion of 135 additional codons (405 nucleotides) abolished activity. This internal 405-nucleotide region between the two deletion points encodes a 28-amino acid stretch (Fig. 3*A*) with a 62% probability of forming a coiled-coil structure (37). JZA-20 is a 358-nucleotide partial cDNA clone encoding a 72-amino acid open reading frame fused to GAL4(TA) (Fig. 3*B*). This protein coding sequence contains a 28-amino acid

**A** **JZA-3**

TETLCYVMPSSSARCAQFPRAQDKVHYYIKLKDLRDQLKGIERNTDVQEV
QYTFDLQLAQEDAKKMAVKEEKYDPGYEAAYGGAYGENPCNGEPCGIASN
GLTAHSAEPRGEATPGDVPNGQWMAQSFAEQIPSFNNCGTREQEEESHA*

**B** **JZA-20**

DPPQSPPDSESEQLLLETERLLGEASSNWSQAKRVLQEVRELRDLYRQMD
LQTPDSHLRQTSQHSQYRKSLM*

**C** **mTR67**

SDNDSGICMSPESYLGSPQHSPSTSRAPPDNLPSPGGSRGSPRPKPYDPP
GVSLTAKVKTEKLDKKLKKMEQNKTAATRYRQKKRAEQEALTGECKELEK
KNEALKEKADSLAKEIQYLKDLIEEVRKARGKKRVP*

FIG. 3. Derived amino acid sequence of partial cDNA clones encoding polypeptides that interact with bzJUN. JZA-3 (*A*), JZA-20 (*B*), and mTR67 (*C*) contain amino acid sequences (underlined) with 62%, 52%, and 99% probabilities, respectively, of adopting coiled-coil structures (37). Amino acids defining heptad repeats within the predicted coiled-coil regions are in boldface type. Termination codons are indicated by an asterisk. Each cDNA clone contained a poly(A) tail preceded by a putative polyadenylylation signal.

(four-heptad repeat) sequence with a 52% probability of adopting a coiled-coil structure (37).

A fourth clone encodes 136 amino acids that include a bZIP sequence homologous to those of the ATF/CREB family of transcriptional regulators (Fig. 3*C*) (38). This partial cDNA was used as a probe to identify a 1.7-kb mRNA present in mouse brain, heart, lung, liver, intestine, kidney, adrenal, spleen, pancreas, thymus, uterus, ovary, oviduct, testis, seminal vesicle, embryo, and placenta (unpublished data). A nearly full-length cDNA (called mTR67) isolated from an 8.5-day mouse embryo cDNA library (27) encoded a protein of 349 amino acids that has 86% amino acid sequence identity with a recently described human DNA binding protein, TAXREB67 (39), a member of the ATF/CREB family of transcription factors. Heterodimer formation between c-Jun and members of the ATF family has recently been reported (40).

The fifth and sixth classes consist of partial cDNAs for α-tropomyosin (four isolates) and β-tropomyosin (three isolates). In each class, the individual isolates encode tropomyosin polypeptides of variable length that include the normal C terminus of the protein. For α-tropomyosin, the peptides vary from 29% to 96% of the full-length protein; for β-tropomyosin, they vary from 42% to 66%. Since the tropomyosins form amphipathic helices throughout most of their length, each peptide can presumably form a coiled-coil with the leucine zipper of c-Jun.

**Interaction of Identified Peptides with the bZIP of Fos.** To determine whether the interactions of the various peptides with the bZIP of Jun are specific for Jun, we tested for associations in transformed yeast cells between the products of the cDNAs that we isolated and the bZIP region of c-Fos. Table 2 shows that the bZIP region of mTR67 interacts with bzJUN and weakly with bzFOS. JZA-20, on the other hand, displayed a weak interaction with bzJUN, whereas an interaction with bzFOS was not detectable by either the liquid culture β-galactosidase assay or the more sensitive yeast colony filter assay. Similarly, the tropomyosins interacted specifically with bzJUN. We conclude that some of the peptides, including α- and β-tropomyosins, interact with the bZIP of Jun with some specificity.

## DISCUSSION

In this report, we describe the initial results of a systematic screen for proteins that interact with Jun and Fos. For this purpose, we have used a yeast genetic system (18, 19) for directly cloning mammalian cDNAs encoding proteins that interact with a given target protein. In the experiments

reported here we have concentrated on Jun–bZIP interactions. First, we showed that the yeast system is sufficiently sensitive to detect Jun–Jun as well as Jun–Fos zipper interactions but not Fos–Fos interactions. Next, we cloned from a mouse embryonic cDNA library several different cDNAs encoding polypeptides that can interact with the bZIP region of Jun. Eleven of 12 initial positive isolates turned out to be bona fide Jun-interacting clones. In each case, the polypeptide was known or predicted to form a coiled-coil structure. The known proteins were c-Jun; a protein closely related to TAXREB67, a member of the ATF/CREB family; and $\alpha$- and $\beta$-tropomyosins. In addition, two other polypeptides were identified.

Based on analysis of a nearly full-length cDNA, the ATF-related protein is probably the murine homolog of TAXREB67 (38, 39). It had a weak interaction with bzJUN and was also found to interact with bzFOS. These data are consistent with recent evidence that Jun and Fos form heterodimers with ATF4 (40). It is interesting that such cross-family heterodimers have binding specificities different from their parental homodimers (40, 41), suggesting that a comprehensive screen for bZIP proteins that interact with Jun or Fos might turn up other bZIP proteins that form heterodimers with atypical DNA-binding specificities.

The two additional clones that we isolated encode polypeptides that interact only weakly with bzJUN and undetectably with bzFOS. Although each of them has a sequence predicted to be capable of forming coiled-coils, neither has an adjacent basic region typical of bZIP proteins. Whether the Jun interactions are physiologically significant is not known.

Our most provocative result is the finding that Jun, a transcriptional regulator, interacts with the cytoplasmic tropomyosins. The data in Table 2 showing that neither $\alpha$- nor $\beta$-tropomyosin reacts with bzFOS suggest that the selective interaction with bzJUN is not simply a nonspecific coiled-coil association but rather may represent a natural regulatory interaction. The possibility that c-Jun is reversibly sequestered in the cytoplasm by an interaction with tropomyosin remains to be investigated. Precedents for this type of regulation come from studies of the REL family of transcription factors, which are held in the cytoplasm by a family of cytoplasmic anchor molecules (42).

As noted earlier, regions of Jun or Fos other than the bZIP domains are sites of interaction with a number of proteins: kinases (43, 44), inhibitory proteins (16, 17), and, presumably, components of the transcriptional machinery. Since some segments of the non-bZIP regions differ considerably among the various Jun and Fos family members, some of these interactions may be specific for a given family member. Therefore, it will be of interest to extend the search for interacting proteins to unique domains of the individual Jun and Fos transcription factors.

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