Yeast *YAP1* encodes a novel form of the jun family of transcriptional activator proteins

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The jun family of transcriptional activators includes mammalian AP-1 as well as the yeast regulatory protein GCN4. Recently, an additional transcriptional activator has been found in yeast that recognizes the TGACTCA sequence element common in GCN4/AP-1 sites. This factor was designated yAP-1. The structural gene for yAP-1 has now been isolated and characterized. The deduced amino acid sequence predicts a protein of 650 residues, considerably larger than GCN4 or c-Jun. The amino terminus of yAP-1 is homologous to the carboxy-terminal DNA-binding domains of GCN4 and c-Jun. Disruption of the *YAP1* gene demonstrates this gene is not essential but is required for AP-1 recognition element-dependent transcriptional activation. DNA-affinity blots of proteins from *YAP1* cells suggest the presence of additional TGACTCA-binding proteins other than GCN4 and yAP-1. Furthermore, expression of at least one of these related DNA-binding proteins appears to be under control of yAP-1.

[*Key Words*: Yeast AP-1; transcription factor; DNA-binding domain; jun family]

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The use of a single DNA-binding site that can be recognized by multiple transcription factors is emerging as a common theme in eukaryotic transcriptional regulation. There are at least three different types of CCAAT-binding proteins from humans that recognize distinct subsets of the central CCAAT sequence (Graves et al. 1986; Dorn et al. 1987; Jones et al. 1987; Chodosh et al. 1988a). The SV40 'core' or TGTGGAAAG site can be recognized by the AP-3 factor as well as C/EBP (Mitchell et al. 1987; Jones et al. 1988; Landschulz et al. 1988b). The use of a common binding site allows for multiple transcriptional effects to be mediated through a single recognition element.

This motif of multiple factors for a single binding site introduces a further level of complexity to the analysis of purified DNA-binding proteins. It is possible that factors purified on the basis of binding may, in fact, consist of distinct proteins that recognize the same site. An example of this situation is the mammalian transcription factor AP-1 (Lee et al. 1987a).

AP-1 binds to sites containing the sequence TGACTCA (Angel et al. 1987; Lee et al. 1987b). Purified AP-1 can stimulate in vitro transcription of the SV40 early promoter and human metallothionein IIA gene (Lee et al. 1987a). In addition, AP-1 binding sites were found to mediate phorbol-ester inducibility through a heterologous promoter (Angel et al. 1987; Lee et al. 1987b).

Recent work has indicated that the protein product of the c-*jun* proto-oncogene has DNA-binding properties that are identical to those of AP-1 (Bohmann et al. 1987; Angel et al. 1988). Detailed analysis of the polypeptides from HeLa nuclear extracts, purified by use of an AP-1 recognition element (ARE) DNA-affinity column, indicates that there are several different proteins capable of binding the ARE (Franza et al. 1988; Rauscher et al. 1988). The deduced protein sequence of a serum-induced protein from mouse cells (Jun-B) shows homology with v-Jun (Ryder et al. 1988). These findings suggest that a family of related proteins exist that will bind to DNA sites containing the conserved pentameric element.

Members of the jun family also have been identified in yeast. Both c-Jun and v-Jun as well as Jun-B have homology at the carboxyl terminus of their deduced protein sequences with the DNA-binding domain of the positively-acting yeast transcription factor GCN4 (Bohmann et al. 1987; Vogt et al. 1987; Ryder et al. 1988). The GCN4 recognition element (GCRE: GTGACTCAC) is also bound by c-Jun (Bohmann et al. 1987). Additional functional homology comes from the observation that a lexA–v-Jun fusion protein can bind to the GCRE upstream from the *HIS3* promoter and activate transcription of that gene (Struhl 1987).

We have reported the isolation and characterization of an additional factor, distinct from GCN4, present in yeast cells, that binds the ARE and activates transcription (Harshman et al. 1988). This protein was named yAP-1 by virtue of the identical footprint formed on the ARE by this yeast factor and human AP-1. Analysis of the molecular weights of the polypeptide components present in the yeast AP-1 preparations revealed a major species of ~90 kD as well as several smaller proteins.

The presence of lower-molecular-weight proteins might be the result of proteolysis of the larger species or of the presence of a family of proteins that can recognize the same conserved sequence analogous to the situation in mammalian cells.

To address this and related issues more fully, we have cloned the gene that encodes the 90-kD species of yAP-1. This gene has been designated *YAP1*. The structure of the deduced yAP-1 protein is quite different from that of c-Jun or GCN4. Although all three proteins have homology within their DNA-binding domains, the location of this region is at the carboxyl terminus for both c-Jun and GCN4, but at the amino terminus for yAP-1. Disruption of the gene that encodes yAP-1 is not lethal but results in the loss of yAP-1 expression as well as that of several other proteins that can bind selectively to the ARE and GCRE. These findings suggest that yAP-1 is a member of a family of transcription factors, which are interrelated at the level of regulation as well as recognizing similar sequence motifs.

Results

Isolation of the YAP1 gene

We used monoclonal antibodies generated against yAP-1 protein (Harshman et al. 1988) to clone the *YAP1* gene from a λgt11 expression library constructed from yeast genomic DNA (Young and Davis 1983). The screening of $\sim 10^6$ phage (of which 30% were recombinant) yielded eight positive signals. Five of these clones reacted reproducibly with the monoclonals and were taken to plaque purity. Phage DNA preparations of these recombinants were made and cleaved with *Eco*RI. Two different classes of phage were observed on the basis of the *Eco*RI digestion products (Fig. 1). One class, a representative of which we designated λAC2, contained two *Eco*RI fragments of 1.9 kb and 0.22 kb. The other, represented by λAC5, also contained two *Eco*RI fragments, but of 2.5 kb and 0.8 kb. All these *Eco*RI fragments were subcloned individually into pUC19 (Yanisch-Perron et al. 1985) for further analysis.

Restriction mapping suggested, and Southern blotting confirmed, that the 1.9-kb and 2.5-kb fragments were homologous. Western analysis of protein extracts from *Escherichia coli* strains bearing the pUC19 subclones of the 1.9-kb and 2.5-kb fragments indicated that immunoreactive yAP-1 was being produced in these cells (data not shown). Both the 1.9-kb and 2.5-kb fragments were found to hybridize to a single 6.1-kb *Eco*RI fragment present in the yeast genome (Fig. 6B). Further Southern blotting (data not shown) indicated that the 2.5-kb fragment extends from an authentic yeast genomic *Eco*RI site to a random shear end point 2.5-kb upstream. The 800-bp fragment is an authentic genomic *Eco*RI fragment and lies next to the 2.5-kb fragment in the genome.

DNA sequence of the YAP1 *gene*

Western analyses of bacterially produced proteins encoded by subclones of the 2.5-kb fragment suggested a large portion of the yAP-1 coding sequence was contained within this region (S. Moye-Rowley, unpubl.). The DNA sequence of this fragment was determined (Fig. 2). The sequence predicts one long open reading frame (ORF) of 650 amino acid residues with no introns. The deduced molecular weight of this protein would be 72.5 in contrast to the observed molecular weight of 90 kD from SDS–polyacrylamide gels (Harshman et al. 1988). This discrepancy between the observed and predicted molecular weights has been seen for several yeast transcription factors including GCN4 (Hope and Struhl 1985), RAP1 (Shore and Nasmyth 1987), and HSTF (heat shock transcription factor) (Wiederrecht et al. 1988). The cause of this difference is unknown but may reflect some unusual structural features in these proteins.

Although yAP-1 is predicted from the deduced amino acid sequence to be an acidic protein, the charge distribution indicates that the amino terminus is basic but the carboxy-terminal region of the protein has an acidic nature. As we show later, the amino terminus of yAP-1 corresponds to the DNA-binding domain.

S1 nuclease analysis of the 5′ end of the *YAP1* mRNA was performed to localize the start points of *YAP1* gene transcription (Fig. 3). The major 5′ terminus is designated position +1, with several other minor start points at positions −7, +10, +15, and +38. Remarkably, all these start points map upstream of an ATG at position +81 that is not predicted to be included in the *YAP1* coding sequence. The ATG is followed 15 bp downstream by a TAG terminator in frame. Because eukaryotic ribosomes generally initiate at the first ATG encountered in an mRNA (Kozak 1984), this ATG upstream from the *YAP1* ORF would be expected to have an effect on downstream *YAP1* expression. Although we

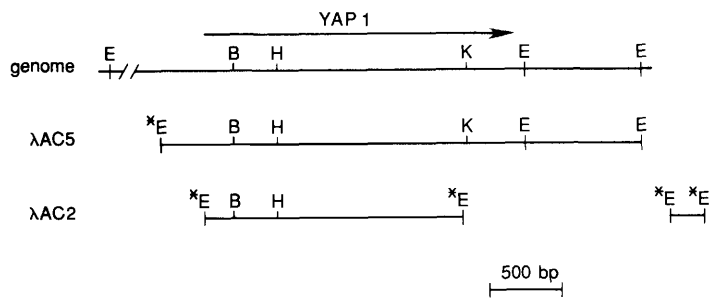

Figure 1. Restriction map of *YAP1* genomic region and λgt11 subclones. The arrow indicates the bounds and polarity of the yAP-1 coding sequence. Restriction sites are indicated as: (B) *Bam*HI; (E) native yeast genomic *Eco*RI site; (*E) synthetic *Eco*RI linker; (H) *Hpa*I; (K) *Kpn*I.

Figure 2. DNA sequence of the *YAP1* gene. The numbering is relative to position +1 being the major 5′ end for *YAP1* mRNA. The one-letter code is used for the amino acid sequence.

have no evidence of a role for the short upstream ORF, other yeast transcription factor genes, such as *GCN4* (Hinnebusch 1984) and *PPR1* (Kammerer 1984), have been found to have short potential coding sequences upstream of the main ORF that encodes the transcription factor. It will be of interest to determine the type of role the upstream ORF has in gene expression of *YAP1*.

yAP1 is homologous to GCN4 and jun

Previous work has demonstrated that the recognition sequences for yAP-1, AP-1, and GCN4 are very closely related (Bohmann et al. 1987; Harshman et al. 1988). We compared our deduced amino acid sequence for yAP-1 to those for c-Jun and GCN4 to ascertain if the known DNA-binding regions of the GCN4 (Hope and Struhl 1986) and c-Jun (Bohmann et al. 1987) proteins were conserved in yAP-1. Figure 4A shows a computer-generated alignment of the DNA-binding domains of GCN4 and c-Jun with a homologous region of the amino terminus of yAP-1. A comparison of the relative positioning of the DNA-binding domains of GCN4, c-Jun, and yAP-1 is shown in Figure 4B. The region of homology of yAP-1 contains 36% and 27% sequence identities with GCN4 and c-Jun, respectively. Over the same stretch of amino acids, c-Jun and GCN4 have 30% sequence identities. This strong sequence conservation prompted us to test the ability of a restriction fragment of *YAP1* encodes the c-Jun–GCN4 homology region to produce yAP-1 DNA-binding activity.

Identification of the YAP1 *DNA-binding domain*

The production of high levels of yAP-1 activity in *E. coli* was accomplished by placing fragments of the *YAP1* gene under control of the strong, heat-regulated λPL promoter contained in the vector pOTSV (Shatzman and Rosenberg 1987). Initial experiments used extracts prepared from bacteria carrying a pOTSV derivative that contained a *Bam*HI–*Eco*RI fragment that expresses 90% of the full-length yAP-1 protein. This construction was designated pPL-*YAP1*. A *Bam*HI–*Hpa*I fragment that encoded 93 amino acids of the amino terminus of yAP-1 was also inserted into pOTSV to form pBH1. Crude extracts were prepared from appropriate *E. coli* transformants bearing these two plasmids and assayed for yAP-1 binding activity. Control experiments established that *E. coli* cells contained no specific ARE-binding activity (data not shown).

DNase I footprinting demonstrates that the protection of the ARE seen in yAP-1 samples from yeast (Fig. 5, lane 2) or from the bacterially produced protein (Fig. 5, lanes 3–7) are indistinguishable. This result confirms that we have isolated the gene that encodes the yAP-1

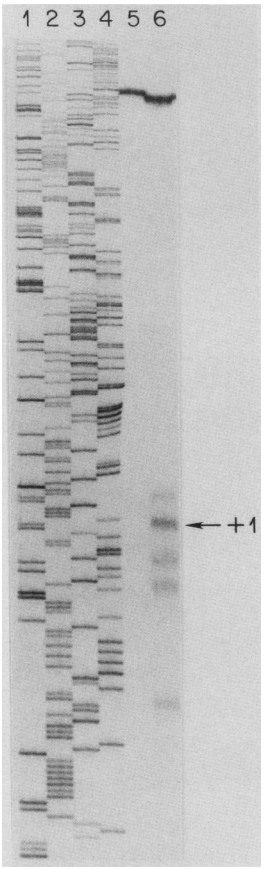

Figure 3. S1 nuclease mapping of the 5′ end of the *YAP1* mRNA. The same *YAP1*-specific oligonucleotide used to synthesize the S1 probe was also employed to generate a dideoxy-sequence ladder from the M13 template used for probe synthesis. (Lanes *1–4*) Dideoxy G,A,C,T reactions on M13 mp18 : RI/Bam 500 clone; (lane *5*) S1 digestion of probe annealed to yeast tRNA; (lane *6*) S1 digestion of probe annealed to yeast poly(A)$^+$ RNA. The major 5′ terminus (position +1) is indicated.

protein. In addition, the ability of the extract from the pBH1 transformants to produce a wild-type yAP-1 footprint indicates that the DNA-binding domain of yAP-1 is encoded by this fragment and is located between amino acid residues 63 and 156.

The DNA template used for footprint analysis also contains a GCRE that is labeled in Fig. 5. The yAP-1 sample purified from yeast gives a partial footprint on this site while neither of the bacterially derived extracts shows any detectable protection. This is consistent with the belief that other proteins exist in yeast that are capable of binding to sequences related to the TGACT repeat core of the ARE and GCRE. A large body of evidence has accumulated that implicates the presence of multiple factors capable of recognizing the ARE in mammalian cells (Rauscher et al. 1988). To examine the possibility that an analogous situation existed in yeast, we constructed a yeast strain that lacked a functional *YAP1* gene.

Characterization of a yeast yap1 *mutant*

We employed the method of one-step gene disruption (Rothstein 1983) to generate a mutant containing a nonfunctional yAP-1 protein. The 1.7-kb *HIS3 Bam*HI fragment was inserted into the *YAP1* gene between amino acid residues 156 and 157. The resulting fragment was introduced into a homozygous *his3* diploid strain as shown in Figure 6A and *HIS+* transformants were selected. Correct integration was verified by Southern blot analysis (Fig. 6B). Selected heterozygotes were sporulated, and the resulting tetrads were dissected by standard techniques (Sherman et al. 1979).

Analysis of spores of representative tetrads indicated that *HIS+* : *his−* segregants were recovered in a 2 : 2 fashion, thus indicating that the *yap1* :: *HIS3* insertion is not a lethal mutation. We could detect no gross phenotypic change in segregants bearing the insertion mutant. Direct examination of the mutant yAP-1 protein was next undertaken. An extract prepared from a *yap1* :: *HIS3* segregant was chromatographed through a heparin–agarose column and assayed for GCRE and ARE binding activity by DNase I footprinting. A control extract was prepared using a *YAP1*, *his3* segregant from the same tetrad. HSTF activity was assayed also in both extracts as a control DNA-binding protein.

Fractionation of a yeast nuclear extract over heparin agarose results in the elution of yAP-1 and HSTF DNA-binding activities in the 0.8 M KCl step (Harshman et al. 1988; Wiederrecht et al. 1987). We recovered equal levels of HSE binding activity in the *yap1* and *YAP1* extracts (data not shown). DNA-affinity blotting (Miskimins et al. 1985) was performed to determine the type of polypeptide chains in the two extracts that is capable of recognizing the GCRE and ARE (Fig. 7).

A DNA-affinity blot of the heparin–agarose fraction from the *YAP1* strain, with the ARE as the DNA probe, detects the characteristic set of bands, starting with full-length yAP-1 at 90 kD as well as a set of proteins from 50 kD to 30 kD (Harshman et al. 1988). However, the fraction from the *yap1* strain gives a distinctly different result. The 90-kD band is absent entirely while the lower-molecular-weight species are reduced in intensity or absent. Remarkably, a strong signal at ~45 kD remains. It is unlikely that this binding activity is the result of a novel yAP-1 fusion protein formed by the *HIS3* insertion because the deduced molecular weight from the known DNA sequence (Struhl 1985) of the fusion junction predicts a primary translation product of only 27 kD.

A distinct set of signals is observed when the GCRE is used to probe a parallel DNA-affinity blot. Strong binding is seen from polypeptides of 69, 65, and 55 kD in the *YAP1* extract. Only the 55 kD signal is seen in the *yap1* extract. GCN4 protein is unlikely to be detected in this assay because the cells from which the extracts were made were grown under conditions which repress GCN4 synthesis (Thireos et al. 1984; Hinnebusch 1984). The 55-kD polypeptide is larger than the observed molecular weight for GCN4 (Hope and Struhl 1985; Arndt and Fink 1986) and may represent some other TGACT-

A

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                                          *       *       *       *       *
yAP1 dldpetkqkrtaqNraAqrafReRKerkmkeLEkKVqsLesiqqqneveatfLrdqlitlvn 122
GCN4 pessdpaalkrarNteAarrsRaRKlqrmkqLEdKVeeLlsknyhlenevarLkklvger   281
     . .    . .  .:  :  . : :: ...  ::.:::..: . . .       :.. . .  .
CJUN sqerikaerkrmrNriAaskcRkRKleriarLEeKVktLkaqnselastanmLreqvaqlkq 319
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B

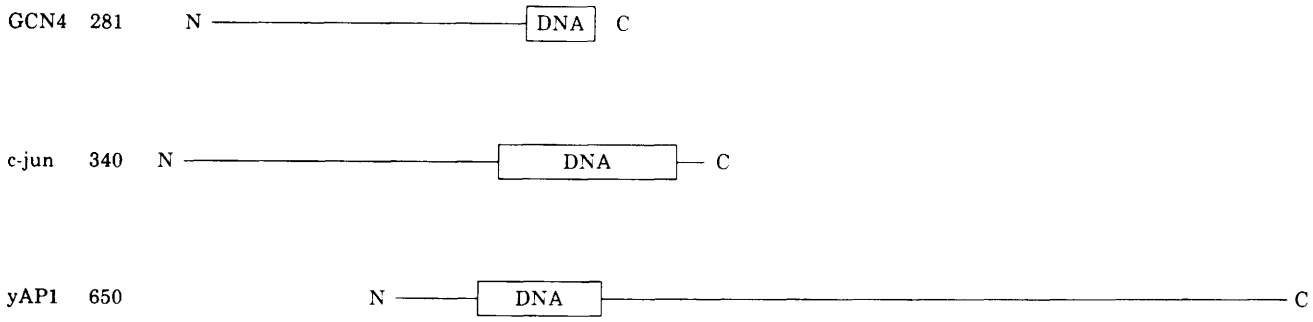
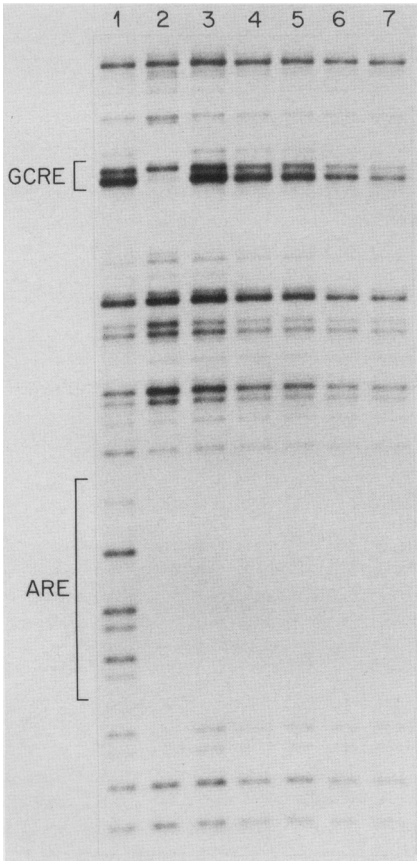


Figure 4. (*A*) Amino acid sequence alignment of c-Jun, GCN4, and yAP-1. Only the region corresponding to the DNA-binding domains of the respective protein is shown. The positions corresponding to the repeating pattern of leucines in yAP-1 are designated with asterisks. This alignment represents the best fit for the three proteins. Amino acid identities are indicated by uppercase letters and two dots while conservative replacements are shown by a single dot. The one-letter amino acid code is used throughout. The numbers refer to the amino acid position in each factor where the alignment stops. (*B*) Location of DNA-binding domains of c-Jun, GCN4, and yAP-1. Representation of the position of the region encoding the DNA-binding domain relative to the rest of the protein sequence. The numbers refer to the length of the respective factor. (*N*) Amino terminus of the protein; (*C*) carboxyl terminus; (DNA) DNA-binding domain.



recognizing protein. The 69-kD band, which is reduced in intensity, and the 65-kD band, which is not seen in the *yap1* mutant, are probably not forms of yAP-1 because they are not detected by the ARE. Functional yAP-1 may be required for the expression of the 69- and 65-kD proteins. However, further experiments are required to confirm this.

Transcriptional activation by cloned YAP1

Further evidence that the gene we isolated encodes yAP-1 comes from an analysis of the ability of the cloned gene to stimulate ARE-dependent transcription in a *yap1* mutant background. We employed *CYC1–lacZ* gene fusions in which β-galactosidase activity is dependent on the presence of a functional ARE. Expression of *YAP1* was placed under control of the inducible galactose promoter (Johnston and Davis 1984). The results are shown in Table 1.

In a wild-type *YAP1* cell, 130 U/mg of β-galactosidase is produced when glucose is present as the carbon source while 70 U/mg is generated during growth on galactose.

Figure 5. DNase I footprint comparison of yeast and bacterially produced yAP-1. The ARE and GCRE are denoted by brackets. Footprint reactions were performed as described by Wiederrecht et al. (1987) using the indicated amount of extract. (Lane *1*) No protein; (lane *2*) 10 μl of affinity-purified yAP-1; (lanes *3–5*) 1, 5, and 10 μl of crude extract from AR68/pPL-*YAP1*; (lanes *6,7*) 1, and 5 μl of crude extract from AR68/pBH1.

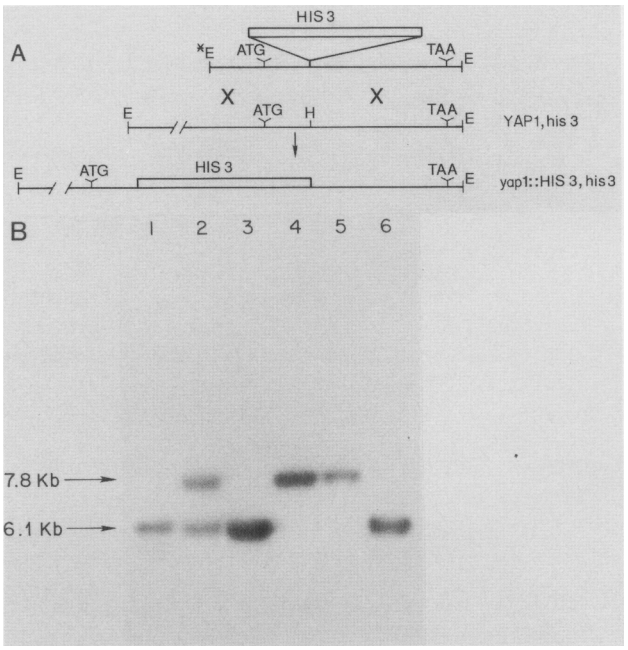


Figure 6. Gene disruption of *YAP1*. (*A*) Schematic showing the integration of the *HIS3* insertion mutant into the wild-type 6.1-kb *Eco*RI fragment. Restriction sites are indicated as in Fig. 1. (ATG) Start codon for *YAP1* ORF; (TAA) termination codon. (*B*) Southern blot of yeast strains used in disrupting *YAP1*. All DNA preparations (Winston et al. 1983) were cleaved with *Eco*RI. The blot was probed with the nick-translated 2.5-kb *Eco*RI fragment. The sizes of the detected fragments are shown. Lanes *3–6* represent DNA preparations from each segregant of the same tetrad. (Lane *1*) SEY6210.5; (lane *2*) SM81 (heterozygous *YAP1* disruption in SEY6210.5); (lane *3*) *YAP1*, *his3* segregant; (lane *4*) *yap1* :: *HIS3*, *his3* segregant; (lane *5*) *yap1* :: *HIS3*, *his3* segregant; (lane *6*) *YAP1*, *his3* segregant.

When an isogenic *yap1* :: *HIS3* transformant is assayed under the same growth conditions, 0.2 and 0.11 units are formed during glucose and galactose growth, respectively. Introduction of a *GAL1–YAP1* gene fusion into this mutant background leads to no change in the level of β-galactosidase activity during glucose growth. However, induction of the *GAL1* promoter by growth on galactose leads to a 1000-fold elevation in ARE-dependent β-galactosidase activity. These data support the conclusion that the gene we have isolated indeed encodes the positively acting transcription factor yAP-1. Disruption of the coding sequence of this gene leads to an inability to activate transcription through the ARE, while expression of the clone under *GAL* promoter control can correct this defect.

Discussion

YAP1 *encodes transcription factor yAP-1*

We have employed monoclonal antibodies directed against the yAP-1 protein (Harshman et al. 1988) to isolate from a λgt11 library (Young and Davis 1983) the yeast gene that encodes this factor. Three independent lines of evidence lead us to conclude we have cloned the *YAP1* gene: (1) the footprint of the bacterially expressed protein is identical to that of authentic yeast factor; (2) the deduced protein sequence of the DNA-binding region of the clone has homology with the analogous region from GCN4 and c-Jun; and (3) disruption of the *YAP1* ORF prevents ARE-dependent transcriptional activation.

An unexpected result is the loss of ARE-dependent activation upon disruption of the *YAP1* gene (Table 1). In these cells, the *GCN4* gene is still intact and under these growth conditions will activate transcription from the GCRE (Harshman et al. 1988). Previous work has demonstrated that GCN4 binds as well to the ARE as to a GCRE from the *HIS3* promoter (Harshman et al. 1988) and that transcriptional activation through the GCRE is dependent on GCN4 binding (Hill et al. 1986). These data support the idea that GCN4 should be able to bind to the ARE and to activate transcription. With this in mind, it is surprising that the level of β-galactosidase activity produced in the *GCN4, yap1* cell from the *ARE–CYC1–lacZ* gene fusion is no higher than the same fusion lacking an upstream activation site (S. Moye-Rowley, unpubl.). This suggests that authentic yeast GCN4 protein can discriminate between the ARE and yeast GCRE. Alternatively, yAP-1 may be involved in expression of the *GCN4* gene.

yAP-1: a representative of a new class of jun-type transcriptional activator?

Our original observations (Harshman et al. 1988) of the AP-1-like footprint on and transcriptional activation by

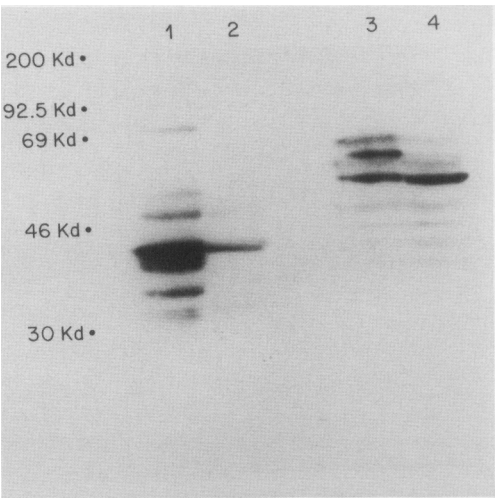


Figure 7. DNA-affinity blot of *YAP1* and *yap1* heparin–agarose fractions. Equal amounts of protein derived from either the *YAP1* or *yap1* strain were loaded onto a 10% SDS–polyacrylamide gel and processed for a DNA-affinity blot as described (Harshman et al. 1988). Lanes *1* and *2* were probed with the ARE while lanes *3* and *4* were probed with the GCRE. (Lanes *1* and *3*) Protein from the *YAP1* cell; (lanes *2* and *4*) contain protein from the *yap1* strain. Molecular weight standards (Rainbow Markers, Amersham) are indicated.

Table 1. *Transcriptional activation in yeast by cloned* YAP1

YAP1 allele	β-Galactosidase activity	
	glucose	galactose
YAP1	130	70
yap1 :: *HIS3*	0.2	0.11
GAL–YAP1, yap1 :: *HIS3*	0.15	103

The transformed yeast strains were grown with either 2% glucose or 3% galactose as the carbon source. β-Galactosidase activities are expressed as nmoles/min mg protein and were performed as in (Harshman et al. 1988). The *YAP1* alleles are described in the text.

the ARE in yeast led us to believe we had identified the yeast AP-1 factor. However, the isolation of a human c-Jun genomic clone that predicts a primary translation product of only 340 residues (Bohmann et al. 1987; Angel et al. 1988) and comparison to our yAP-1 sequence indicates yAP-1 is a protein with related properties to c-Jun but quite different in structure. yAP-1 recognizes the same sequence as c-Jun but has its DNA-binding domain located at the amino terminus of the protein while the c-Jun DNA-binding domain is at the carboxy terminus (Fig. 4B). In this regard, GCN4 protein structure is much closer to that of c-Jun with both proteins having their DNA-binding domains at the carboxy end.

Even though the DNA-binding domains of AP-1 and c-Jun–GCN4 are located at different ends of the respective proteins, the primary sequences of these regions are homologous (Fig. 4A). Within this region of homology, a recently described potential structure is conserved between c-Jun, GCN4 and yAP-1, the 'leucine zipper' (Landschulz et al. 1988a). This structure is characterized by the motif (leu–6 residues) repeating at least four times. An interesting feature of the leucine repeats in yAP-1 is the presence of an asparagine residue in place of a leucine at the start of the third repeat (Fig. 4A). Although, at present, there is no experimental evidence to support the importance of this repeating pattern of leucines, the availability of the cloned genes will permit this hypothesis to be tested.

Existence of a jun-type family of proteins in yeast

Compelling evidence has emerged to indicate that a family of proteins that recognize the ARE exists (Rauscher et al. 1988) in mammalian cells. Southern blots with a c-*jun* probe (Bohmann et al. 1987) detect several different fragments that indicate the presence of other genes related to c-*jun*. DNA sequencing of a mouse cDNA that encodes the serum-stimulated jun-B protein demonstrates this factor has homology with v-Jun although this protein is not mouse c-Jun (Ryder et al. 1988).

Yeast contains an ARE-binding family, with no less than two members, GCN4 and yAP-1. The availability of the *YAP1* gene in cloned form combined with the manipulations possible with yeast genetics, have allowed us to construct a genetic background depleted of yAP-1 activity. DNA-affinity blots of protein from the disrupted *yap1* strain suggest that another ARE-binding protein of 45 kD is present in yeast. Confirmation of this suggestion requires analysis of a *yap1* deletion mutant. In addition, the GCRE detects three more distinct proteins of 69, 65, and 55 kD. A recent report demonstrates the presence of an 80-kD ARE-binding protein in mammalian cells as well as the previously observed set of proteins that migrate from 39 to 47 kD (Rauscher et al. 1988). Experiments are under way to test the relationship of the 90-kD yAP-1 factor with factors in mammalian cells as well as to characterize further the novel ARE and GCRE binding activities we have detected. The presence in yeast cells of multiple TGACT-recognizing proteins may provide an explanation as to the nonessentiality of the *YAP1* gene. Loss of the yAP-1 protein may be compensated for by other proteins with overlapping DNA-binding specificity. This compensation would have to be a subtle effect since ARE-dependent β-galactosidase activity remains low in an *yap1* strain. It will be of interest to examine the phenotype of an *yap1, gcn4* double mutant to assess the effect of the loss of the two known TGACT-recognizing proteins on yeast physiology.

A number of examples have appeared that demonstrate that the transcriptional machinery is more similar between yeast and mammals than previously thought. Proteins analogous to mammalian CAAT factors (Chodosh et al. 1988b) as well as a TATA-binding protein (Buratowski et al 1988; Cavallini et al. 1988) have been detected in yeast. This conservation suggests that the basic eukaryotic process of transcription by RNA polymerase II may be studied in yeast to yield results that can be extrapolated to higher cells. Bearing in mind the functional conservation between yeast and mammalian transcription, the analysis of transcription factors binding the ARE and GCRE in yeast may further the understanding of the role played in mammalian cells by c-Jun and related factors.

Materials and methods

Bacterial and yeast strains

All M13 subclones and most plasmids were constructed and propagated in JM101 (Messing et al. 1981). DH5 (Hanahan 1985) was used as the host for construction of the *GAL* fusion vector. MM294 (*cI*$^+$) was employed to construct the fusion between the λpL promoter and *YAP1* in pOTSV while AR68 (*cI857, htpR*) was used to permit heat-inducible expression as described (Shatzman and Rosenberg 1987).

Yeast strains SEY6210 (MATα, *leu2-3,-112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, Mel*$^-$) and SEY6211 (*Mat***a**, *leu2-3,-112, ura3-52, his3-Δ200, trp1-Δ901, ade2-101, Mel*$^-$) were obtained from S. Emr (California Institute of Technology). The diploid formed by crossing these two strains was designated SEY6210.5. This diploid strain was employed for the initial gene disruption experiments. To avoid any spurious results from the effects of the backgrounds of the two strains used to construct the diploid, we also disrupted the *YAP1* gene in SEY6210, thus generating an isogenic pair of *YAP1* : *yap1* strains. The *yap1* strain was designated SM9. Standard yeast genetic techniques were employed (Sherman et al. 1979). Plasmids were introduced into yeast cells by the method of

Beggs (Beggs 1978) or by a modification of the Lithium acetate technique (Ito et al. 1983; R. Aroian, California Institute of Technology).

DNA manipulations

DNA fragments were cloned into plasmid and M13 vectors by standard techniques (Maniatis et al. 1982). λDNA was prepared by the use of LambdaSorb (Promega Biotec). The *YAP1* gene disruption plasmid, pSM25, was constructed by inserting a *Bgl*II linker (New England Biolabs, CAGATCTG) into the *Hpa*I site of the 2.5-kb *Eco*RI fragment subcloned in pUC19. The 1.7-kb *Bam*HI *HIS3* fragment was then inserted into this *Bgl*II site. pSM25 was cleaved with *Eco*RI prior to yeast transformation.

The *GAL–YAP1* gene fusion was constructed by attaching *Bgl*II linkers to the 1.9-kb *Eco*RI fragment of *YAP1* and inserting this fragment into the *Bam*HI site of pSEYC68-GAL (Emr et al. 1986) to form pSEYC68-GALR1.9. Next, a *Bam*HI–*Sal*I fragment that contained the complete carboxyl terminus was inserted into *Bam*HI–*Sal*I-cleaved pSEYC68-GALR1.9 to reconstitute the 3′ end of *YAP1*. This construct was named pGAL19. Transformants bearing pGAL19 could be selected on the basis of the *URA3* gene in the pSEYC68 backbone (Emr et al. 1986). The *ARE–lacZ* fusion gene was carried on a derivative of pLGΔBS (Harshman et al. 1988) containing *LEU2*. The *Stu*I–*Sal*I fragment of pLGΔBS was replaced by the 2.0-kb *Hpa*I–*Sal*I LEU2 fragment to form pCS10. Two ARE oligonucleotides were inserted into the *Bgl*II site to construct pSM38. pCS10 was constructed by C. Schoenherr of this laboratory. Transformants containing both pGAL19 and pSM38 could be detected by the *Ura*$^+$, Leu$^+$ phenotype.

Bacterial production of yAP-1

Appropriate AR68 transformants were grown at 30°C in 2XYT media and 100 μg/ml ampicillin until the A_{600} = 0.6. An equal volume of 65°C media was added, and the cultures were placed at 42°C for 80 min. After harvesting, the pellet was resuspended in 2 ml of B buffer [0.1 M Tris (pH 7.5), 0.2 M KCl, 10% glycerol, 10 mM β-mercaptoethanol] per gram cells. PMSF was added to 1 mM and the cell suspension was digested with 750 μg/ml lysozyme for 30 min at 4°C. The cells were then sonicated six times for 10 sec each time at 350 watts on ice. Debris was removed by centrifugation. Solid ammonium sulfate was added to 0.38 gm/ml original volume with mixing for 30 min. The precipitated proteins were pelleted, resuspended in 2 volumes per gram original cell weight of 0.05 HGKE [25 mM HEPES (pH 7.6), 10% (vol/vol) glycerol, 50 mM KCl, 0.1 mM EDTA] buffer (Harshman et al. 1988), and dialyzed against the same buffer overnight. This extract could be used directly for footprinting.

S1 mapping

Yeast RNA was prepared from SEY6210.5 by the method of Hinnebusch and Fink (1983). This RNA was chromatographed on oligo(dT)-cellulose to enrich for poly(A)$^+$ RNA as described (Maniatis et al. 1982). The 5′-end-labeled probe was prepared by labeling an oligonucleotide corresponding to +183 to +203 in *YAP1* with T.4 polynucleotide kinase and [γ-^{32}P]ATP. This labeled oligonucleotide was annealed to an M13mp18 clone of the 500-bp *Eco*RI–*Bam*HI fragment from the 5′ end of *YAP1*. The primer was extended with cold dNTPs and the Klenow fragment as described (Greene 1987). The radiolabeled strand was isolated from a strand separation gel (Maxam and Gilbert 1980). Hybridization and S1 nuclease treatment were performed as in Moye et al. (1985).

Yeast biochemistry

Selected segregants were grown in a 10-liter New Brunswick fermenter in yeast minimal media (Sherman et al. 1979) supplemented with 1% yeast extract. Yeast nuclear extracts were made and heparin–agarose columns were run as in Wiederrecht et al. (1987). The footprinting template used contains an ARE as well as a GCRE and is derived from pARE/GCRE (Harshman et al. 1988). DNA-affinity blots were performed as described (Harshman et al. 1988) with radiolabeled and ligated oligonucleotides as probes.

Other methods

All gene disruptions and segregants were verified by Southern blotting (Maniatis et al. 1982). DNA sequencing was carried out by the dideoxy-nucleotide method of Sanger (1977) with the [α-^{35}S]dATP as the label (Biggin et al. 1983). Antibody screening of the λgt11 library was performed using standard protocols (Mierendorf et al. 1987) that employ a mixture of anti-yAP-1 monoclonal supernatants (a representative of which is Mab5C11). β-Galactosidase assays were carried out as before (Harshman et al. 1988).

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Note

Sequence data described in this paper have been submitted to the EMBL/GenBank Data Libraries.

References

Angel, P., M. Imagawa, R. Chiu, B. Stein, R.J. Imbra, H.J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin. 1987. Phorbol ester-inducible genes contain a common *cis* element recognized by a TPA-modulated *trans*-acting factor. *Cell* **49:** 729–739.

Angel, P., E.A. Allegretto, S.T. Okino, K. Hattori, W.J. Boyle, T. Hunter, and M. Karin. 1988. Oncogene *jun* encodes a sequence-specific *trans*-activator similar to AP-1. *Nature* **332:** 166–171.

Arndt, K. and G. Fink. 1986. GCN4 protein, a positive transcription factor in yeast, binds general control promoters at all 5′TGACT3′ sequences. *Proc. Natl. Acad. Sci.* **83:** 8516–8520.

Beggs, J.D. 1978. Transformation of yeast by a replicating hybrid plasmid. *Nature* **275:** 682–688.

Biggin, M., T. Gibson, and G. Hong. 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. *Proc. Natl. Acad. Sci.* **80:** 3963–3965.

Bohmann, D., T.J. Bos, A. Admon, T. Nishimura, P.K. Vogt, and R. Tjian. 1987. Human proto-oncogene c-*jun* encodes a DNA-binding protein with structural and functional properties of transcription factor AP-1. *Science* **238:** 1386–1392.

Buratowski, S., S. Hahn, P.A. Sharp, and L. Guarente. 1988.

Function of a yeast TATA element-binding protein in a mammalian transcription system. *Nature* **334:** 37–42.

Cavallini, B., J. Huet, J.-L. Plassat, A. Sentenac, J.-M. Egly, and P. Chambon. 1988. A yeast activity can substitute for the HeLa cell TATA box factor. *Nature* **334:** 77–80.

Chodosh, L.A., A.S. Baldwin, R.W. Carthew, and P.A. Sharp. 1988a. Human CCAAT-binding proteins have heterologous subunits. *Cell* **53:** 11–24

Chodosh, L.A., J. Olesen, S. Hahn, A.S. Baldwin, L. Guarente, and P.A. Sharp. 1988b. A yeast and human CCAAT-binding protein have heterologous subunits that are functionally interchangeable. *Cell* **53:** 25–35.

Dorn, A., J. Bollekens, A. Staub, C. Benoist, and D. Mathis. 1987. A multiplicity of CCAAT box-binding proteins. *Cell* **50:** 863–872.

Emr, S., Vassarotti, A., Garrett, J., Geller, B., Takeda, M., and Douglas, M. 1986. The amino terminus of the yeast F_1-ATPase β-subunit functions as a mitochondrial import signal. *J. Cell Biol.* **102:** 523–533.

Franza, B.R., Jr., F.J. Rauscher, S.F. Josephs and T. Curran. 1988. The fos complex and fos-related antigens recognize sequence that contain AP-1 binding sites. *Science* **239:** 1150–1153.

Graves, B.J., P.F. Johnson, and S.L. McKnight. 1986. Homologous recognition of a promoter domain common to the MSV LTR and the HSV tk gene. *Cell* **44:** 565–576.

Greene, J.M. 1987. S1 analysis of mRNA using single-stranded DNA probes. *In Current protocols in molecular biology.* (ed. F.M. Ausubel, R. Brent, R. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl), pp. 4.6.1.–4.6.9. John Wiley and Sons, New York.

Hanahan, D. 1985. Techniques for Transformation of *E. coli*. *In DNA cloning: volume I* (ed. D.M. Glover), pp. 109–135. IRL Press, Oxford, England.

Harshman, K.D., W.S. Moye-Rowley, and C.S. Parker. 1988. Transcriptional activation by the SV40 AP-1 recognition element in yeast is mediated by a factor similar to AP-1 that is distinct from GCN4. *Cell* **53:** 321–330.

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.

Hinnebusch, A.G. 1984. Evidence for translational regulation of the activator of general amino acid control in yeast. *Proc. Natl. Acad. Sci.* **81:** 6442–6446.

Hinnebusch, A. and G. Fink. 1983. Repeated DNA sequences upstream from HIS1 also occur at several other co-regulated genes in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **258:** 5238–5247.

Hope, I. and K. Struhl. 1985. GCN4 protein, synthesized in vitro, binds *HIS3* regulatory sequences: implications for general control of amino acid biosynthetic genes in yeast. *Cell* **43:** 177–188.

Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153:** 163–168.

Johnston, M. and R. Davis. 1984. Sequences that regulate the divergent *GAL1–GAL10* promoter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **4:** 1440–1448.

Jones, K.A., J.T. Kadonaga, P.J. Rosenfeld, T.J. Kelly, and R. Tjian. 1987. A cellular DNA-binding protein that activates eukaroytic transcription and DNA replication. *Cell* **48:** 79–89.

Jones, N.C., P.W.J. Rigby, and E.B. Ziff. 1988. *Trans*-acting protein factors and the regulation of eukaryotic transcription: lessons from studies of DNA tumor viruses. *Genes Dev.* **2:** 267–281.

Kammerer, B., A. Guyonvarch, and J.D. Hubert. 1984. Yeast regulatory gene PPR1. *J. Mol. Biol.* **180:** 239–250.

Kozak, M. 1984. Selection of initiation sites by eukaryotic ribosomes: effect of inserting AUG triplets upstream from the coding sequence for preproinsulin. *Nucleic Acids Res.* **12:** 3873–3893.

Landschulz, W.H., P.F. Johnson, and S.L. McKnight. 1988a. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. *Science* **240:** 1759–1764.

Landschulz, W., P. Johnson, E. Adashi, B. Graves, and S. McKnight. 1988b. Isolation of a recombinant copy of the gene encoding C/EBP. *Genes Dev.* **2:** 786–800.

Lee, W., A. Haslinger, M. Karin, and R. Tjian. 1987a. Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40. *Nature* **325:** 368–372.

Lee, W., P. Mitchell, and R. Tjian. 1987b. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell* **49:** 741–752.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Maxam, A. and W. Gilbert. 1980. Sequencing end labeled DNA. *Methods Enzymol.* **65:** 499–560.

Messing, J., R. Crea, and P. Seeburg. 1981. A system for shotgun DNA sequencing. *Nucleic Acids Res.* **9:** 309–321.

Mierendorf, R., C. Percy, and R. Young. 1987. Gene isolation by screening λgt11 libraries with antibodies. *Methods Enzymol.* **152:** 458–469.

Miskimins, W., M. Roberts, A. McClellend, and F. Ruddle. 1985. Use of a protein-blotting procedure and a specific DNA probe to identify nuclear proteins that recognize the promoter region of the transferrin receptor gene. *Proc. Natl. Acad. Sci.* **82:** 6741–6744.

Mitchell, P.J., C. Wang, and R. Tjian. 1987. Positive and negative regulation of transcription in vitro: Enhancer-binding protein AP-2 is inhibited by SV40 T-antigen. *Cell* **50:** 847–861.

Moye, W.S., N. Amuro, J.K.M. Rao, and H. Zalkin. 1985. Nucleotide sequence of yeast *GDH1* encoding NADP-dependent glutamate dehydrogenase. *J. Biol. Chem.* **260:** 8502–8508.

Rauscher, III, F.J., D.R. Cohen, T. Curran, T.J. Bos, P.K. Vogt, D Bohmann, R. Tjian, and B.R. Franza, Jr. 1988. Fos-associated protein p39 is the product of the *jun* proto-oncogene. *Science* **240:** 1010–1016.

Rothstein, R. 1983. One step gene disruption in yeast. *Methods Enzymol.* **101:** 202–211.

Ryder, K., L.F. Lau, and D. Nathans. 1988. A gene activated by growth factors is related to the oncogene v-*jun*. *Proc. Natl. Acad. Sci.* **85:** 1487–1491.

Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74:** 5463–5467

Shatzman, A. and M. Rosenberg. 1987 Expression, identification and characterization of recombinant gene products in *Escherichia coli*. *Methods Enzymol.* **152:** 661–673.

Sherman, F., G. Fink, and J. Hicks. 1979. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Shore, D. and K. Nasmyth. 1987. Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. *Cell* **51:** 721–732.

Struhl, K. 1985. Nucleotide sequence and transcriptional mapping of the yeast *PET56-HIS3-DED1* gene region. *Nucleic*

Acids Res. **13:** 8587–8601.

Struhl, K. 1987. The DNA-binding domains of jun oncoprotein and the yeast GCN4 transcriptional activator protein are functionally homologous. *Cell* **50:** 841–846.

Thireos, G., M.D. Penn, and H. Greer. 1984. 5′ untranslated sequences are required for the translational control of a yeast regulatory gene. *Proc. Natl. Acad. Sci.* **81:** 5096–5100.

Vogt, P.K., T.J. Bos, and R.F. Doolittle. 1987. Homology between the DNA binding domain of the GCN4 regulatory protein of yeast and the carboxy-terminal region of a protein encoded for by the oncogene *jun*. *Proc. Natl. Acad. Sci.* **84:** 3316–3319.

Wiederrecht, G., D.J. Shuey, W.A. Kibbe, and C.S. Parker. 1987. The Saccharomyces and Drosophila heat shock transcription factors are identical in size and DNA binding properties. *Cell* **48:** 507–515.

Wiederrecht, G., D. Seto, and C.S. Parker. 1988. Isolation of the gene encoding the *Saccharomyces cerevisiae* heat shock transcription factor. *Cell* **54:** 841–853.

Winston, F., F. Chumley, and G. Fink. 1983. Eviction and transplacement of mutant genes in yeast. *Methods Enzymol.* **65:** 211–228.

Yanisch-Perron, C., J. Viera, and J. Messing. 1985. Improved M13 phage cloning vectors and strains nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33:** 103–119.

Young, R. and R. Davis. 1983. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci.* **80:** 1194–1198.

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