The JUN oncoprotein, a vertebrate transcription factor, activates transcription in yeast

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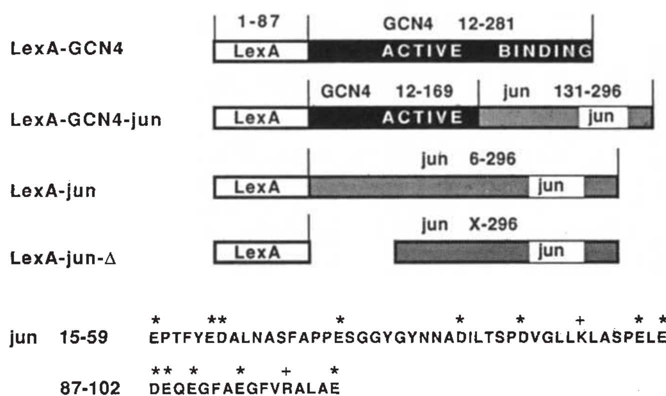
Transcriptional activation of RNA polymerase II in eukaryotic organisms ranging from yeasts to mammals has many common features such as enhancer elements, TATA elements, and activator proteins that bind specifically to promoter DNA (reviewed in refs (1, 2). The JUN oncoprotein, which causes sarcomas in chickens[3], shows significant homology to the DNA-binding domain of GCN4, a yeast protein that stimulates transcription of the amino acid biosynthetic genes[4]. The GCN4 and JUN proteins bind the same DNA sequences[5], consensus ATGA(C/G)TCAT (ref. 6), even though the DNA-binding domains are only 45% identical in amino acid sequence. The JUN protein almost certainly represents the oncogenic version of the normal AP-1 transcription factor[7], suggesting an evolutionary relationship between yeast and vertebrate activator proteins. Here, I demonstrate that JUN efficiently activates transcription in yeast either through its own or a heterologous DNA-binding domain. As is the case for yeast activator proteins, transcriptional stimulation by JUN requires an acidic activation region distinct from the DNA-binding domain. The functional interchangeability between yeast and vertebrate transcription factors strongly suggests a basic similarity in the molecular mechanism of eukaryotic transcriptional activation.

GCN4 binds specifically to the promoters of many genes involved in amino acid biosynthesis and coordinately induces their transcription[8,9]. GCN4 contains 281 amino acids and binds as a dimer[10] with optimal binding to the 9 base pair dyad ATGA(C/G)TCAT (ref. 6). The 60 C-terminal amino acids of GCN4 are sufficient both for specific DNA-binding[11] and for dimerization[10]. Although the GCN4 DNA-binding domain is necessary for recognizing the appropriate promoters, a short acidic region in the centre of the region is required for transcriptional activation[11]. This acidic activation region is also capable of transcriptional stimulation when fused to a heterologous DNA-binding domain, the *E. coli* LexA repressor. The resulting LexA-GCN4 hybrid protein is a bifunctional activator because it activates transcription from promoters containing either GCN4 or LexA binding sites upstream of TATA sequences[11].

Previously, I described a LexA-GCN4-JUN hybrid protein (originally called LGJ-1) in which the entire GCN4 DNA-binding domain (the C-terminal 112 amino acids) was replaced by the 166 C-terminal amino acids of JUN[5]. When introduced into yeast cells lacking the entire *GCN4* gene, this protein induced the transcription of *HIS3* and other amino acid biosynthetic genes, thus indicating that the JUN and GCN4 DNA-binding domains are functionally homologous. However, because the LexA-GCN4-JUN hybrid protein contained the intact GCN4 activation region, this experiment did not bear on the issue of whether the JUN protein itself was capable of transcriptional activation in yeast.

To address this question, I constructed a LexA-JUN hybrid protein in which essentially the entire JUN coding region was fused directly to the LexA DNA-binding domain (Fig. 1). A plasmid capable of expressing this protein was introduced into KY330, a yeast strain lacking the entire *GCN4* gene and harbouring a separate plasmid that contains the *E. coli* lexA [illegible]ator fused upstream of a yeast TATA element and β-[illegible]osidase structural gene. In this way, transcriptional activa[illegible] the LexA DNA-binding domain could be quanti[illegible]-galactosidase activity, and activation through the



jun 15-59 EPTFYEDALNASFAPPESGGYGYNNADILTSPDVGLLKLASPELE

87-102 DEQEGFAEGFVRALAE

Fig. 1 Structures of LexA hybrid proteins, composed of the LexA DNA-binding domain (open box), the GCN4 coding region (black box with transcriptional activation and DNA-binding domains indicated), and the JUN coding region (shaded box with the DNA-binding domain indicated by JUN). The constructions of LexA-GCN4[11] and LexA-GCN4-JUN[5] have been described previously. To generate the DNA molecule encoding LexA-JUN, *jun* plasmid DNA was partially cleaved with *Fnu*dII, ligated to an octanucleotide *Sal*I linker, cleaved with *Sal*I and *Eco*RI, and the appropriate fragment fused to DNA encoding the LexA domain. Due to the cloning procedure, there are five additional amino acids located at the junction between the *LexA* and *JUN* coding regions. LexA-JUN deletion mutants were generated similarly except that *JUN* plasmid DNA was treated with *Bal*31 nuclease prior to ligating the *Sal*I linker. The end-points within the *JUN* coding sequence were determined by DNA sequencing. All protein coding regions are cloned in YCp88, a vector containing the *URA3* marker, *ARS1* and *CEN3* elements for maintenance as a minichromosome at one copy per cell, and the *DED1* promoter for expression of the hybrid protein[11]. The acidic regions of JUN are shown, acidic residues being marked * and basic residues +.

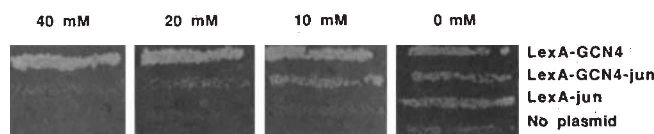


Fig. 2 Phenotypic analysis. YCp88 plasmids encoding the LexA hybrid proteins (Fig. 1) or no protein were introduced into yeast strain KY330 by selecting for Ura[+] colonies. Total *GCN4* function was assayed by complementation of the *gcn4*-Δ1 allele by plating the resulting transformants in minimal media lacking leucine and containing the indicated concentrations of aminotriazole; a competitive inhibitor of the *HIS3* gene product. The degree of aminotriazole resistance is directly related to the level of transcription of *HIS3* and other amino acid biosynthetic genes[6], normally regulated by GCN4.

JUN DNA-binding domain could be assessed by complementation of the *gcn4* deletion. The complementation assay was based on the facts that *gcn4* deletion strains grow slowly on minimal medium lacking amino acids and fail to grow in the presence of aminotriazole, a competitive inhibitor of the *HIS3* gene product. These phenotypes reflect the inability of *gcn4* deletion strains to induce transcription of *HIS3* and other amino acid biosynthetic genes above the basal level.

The basic result is that LexA-jun activates transcription thus indicating that the JUN oncogene contains a sequence(s) that functions as a transcriptional activation region in yeast. LexA-JUN can functionally replace GCN4 even though this hybrid protein contains none of the *GCN4* coding region. KY330 cells expressing LexA-JUN grew in the presence of 10 or 20 mM aminotriazole, and grew essentially at wild-type rates in minimal medium lacking amino acids (Fig. 2). In addition, these cells

also show a high level of β-galactosidase activity (Table 1). Thus, LexA-JUN activates transcription through either its own DNA-binding domain or the heterologous LexA DNA-binding domain.

The level of transcriptional activation by LexA-JUN was compared with that achieved by LexA-GCN4-JUN, which contains the GCN4 transcriptional activation region and the JUN DNA-binding domain, and LexA-GCN4, which contains essentially the entire GCN4 coding region (Fig. 1). When examined for activation through the LexA domain, all three proteins confer comparable levels of β-galactosidase (Table 1). This suggests that JUN and GCN4 contain transcriptional activation regions that are equally functional when fused to a heterologous DNA-binding domain.

When assayed for activation through the JUN DNA-binding domain, LexA-JUN appears to be slightly less effective than LexA-GCN4-JUN in that cells grow more slowly in the presence of aminotriazole (Fig. 2). This effect is rather subtle: using the well-established correlation between the degree of aminotriazole resistance and the level of *HIS3* messenger RNA[6], it can be estimated that the level of activation by these proteins differs by a factor of <2. Both proteins containing the JUN DNA-binding domain complement the *gcn4* deletion less efficiently than LexA-GCN4; that is, they activate transcription of the amino acid biosynthetic genes to lower levels. As discussed previously[5], this probably reflects the relative affinities of the GCN4 and JUN DNA-binding domains to their target sites *in vivo*. Nevertheless, in terms of the transcriptional activation function, the JUN oncoprotein is nearly as functional in yeast cells as GCN4, a native yeast activator protein.

Deletion analyses of the yeast GCN4 and GAL4 activator proteins have shown that transcriptional activation regions are defined by short acidic regions with no primary sequence homology[1,11,12]. Indeed, JUN contains a 45 amino acid region between residues 15 and 59 with a net negative charge of -7 and a 16 amino acid region between residues 87 and 102 with a net charge of -4 (ref. 3, fig. 1). As shown in Table 1, deletions that remove more than 100 N-terminal residues of JUN and hence lack both acidic regions confer extremely low levels of activation. Deletions with end-points between residues 54 and 71, which remove one of the acidic regions show a 2-5 fold decrease in the level of expression. Thus, this initial deletion analysis of LexA-JUN indicates that these acidic regions are important for transcriptional activation in yeast.

The observation that approximately 1% of short *E. coli* sequences act as a transcriptional activation region when fused to a 147 amino acid GAL4 DNA-binding domain[13], raises the formal possibility that JUN activation in yeast cells may not reflect a meaningful functional relationship between yeast and vertebrate transcription factors but rather the presence of a sequence that fortuitously behaves as a yeast activation region. However, this possibility is very unlikely because JUN activates transcription through its own or the LexA DNA-binding domain at levels that are near those achieved by the intact GCN4 activation region. In contrast, essentially all the 'functional' *E. coli* segments activate transcription poorly through the GAL4 DNA-binding domain and not at all through the LexA domain[13]. In addition, after fusing random DNA sequences to the LexA DNA-binding domain, we were unable to select for transcriptional activators out of a population of approximately 10^4 LexA hybrid proteins (A. R. Oliphant and K.S., unpublished observations). Thus it is rare to obtain protein sequences that can stimulate transcription when fused to the LexA domain, especially at levels that are comparable to that obtained with a native yeast activation region. It is also worth noting that the 147-residue GAL4 DNA-binding domain used for identifying the *E. coli* activation segments contains about 70 additional residues beyond those necessary for DNA binding including an acidic region (net negative charge of -7 between residues 65 to 117). Thus the *E. coli* peptides might not be independent activation regions, but rather inactive or partially active segments that act in combination with the otherwise cryptic GAL4 acidic region. These considerations strongly support the idea that JUN activation in yeast reflects both an evolutionary and mechanistic similarity between yeast and vertebrate transcription factors.

It has been suggested that yeast activator proteins such as GCN4 and GAL4 stimulate transcription by contacting components of the basic transcription machinery[11-15], possibly proteins that bind to the highly conserved TATA elements[1]. The results presented here suggest that JUN, an oncogenic version of the normal AP-1 transcriptional factor of vertebrates[7], can interact functionally with the basic transcription machinery of yeast. The obvious implication is that the basic transcription machineries of eukaryotic organisms from yeast to man are evolutionarily conserved. In support of this idea, it has been shown very recently that GAL4 activates transcription in mammalian cells[16,17], and that the FOS oncoprotein, a hypothetical transcription factor, activates transcription in yeast cells[18]. Such functional interchangeability may make it fruitful to study the mechanism of transcriptional activation *in vivo* or *in vitro* using mixtures of yeast and mammalian components. Thus, contrary to some beliefs, it appears that mRNA transcriptional initiation in all eukaryotes may occur by a common molecular mechanism.

I thank Pehr A. B. Harbury for excellent technical assistance. This work was supported by a grant from the National Institutes of Health.

Table 1 Activation through the LexA DNA-binding domain.

Protein	Activation region	*jun* sequences	β-galactosidase
LexA-GCN4	GCN4	None	410
LexA-GCN4-JUN	GCN4	130–296	310
LexA-JUN	JUN	6–296	380
LexA-JUN-Δ1	JUN	22–296	300
LexA-JUN-Δ2	JUN	54–296	170
LexA-JUN-Δ3	JUN	68–296	110
LexA-JUN-Δ4	JUN	71–296	140
LexA-JUN-Δ5	JUN	101–296	10
LexA-JUN-Δ6	JUN	124–296	8
LexA-JUN-Δ7	JUN	136–296	5
LexA-JUN-Δ8	JUN	137–296	5
LexA-JUN-Δ9	JUN	147–296	2
LexA-gcn4-N77	None	None	<1
None	None	None	<1

Yeast strain KY330, a derivative of KY803 (*trp1*-Δ1 *ura3*-52 *leu2*-P1 *gcn4*-Δ1)[11] harbouring the YEp21-Sc3423 plasmid containing a *lexA* operator and *cyc1* TATA element upstream of the *lacZ* structural gene[11], was transformed by YCp88 plasmids capable of expressing various LexA hybrid proteins (see Figs. 1, 2). The resulting transformants were grown in minimal medium lacking leucine and assayed for β-galactosidase activity as described previously[11]. The listed values represent the averages of three independent determinations and have an error of approximately ±20%.

Received 19 January; accepted 3 March 1988.

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