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The DNA-Binding Domains of the jun Oncoprotein and the Yeast GCN4 Transcriptional Activator Protein Are Functionally Homologous

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Summary

The jun oncoprotein, which causes sarcomas in chickens, and the DNA-binding domain of yeast GCN4, which coordinately regulates the expression of amino acid biosynthetic genes, show significant homology. In yeast cells deleted for the *GCN4* gene, *GCN4* function can be conferred by a hybrid protein in which the GCN4 DNA-binding domain is replaced by the homologous region of jun. Moreover, in strains containing various mutations of the GCN4 binding site in the *HIS3* promoter, *HIS3* expression is affected similarly by the hybrid protein and by GCN4. These results indicate that the jun oncoprotein binds the same DNA sequences as GCN4, and strongly suggest that jun is derived from a normal cellular transcription factor (possibly AP-1, which recognizes similar sequences). This provides direct evidence for the idea that alterations in the machinery for proper gene expression can lead to the oncogenic state.

Introduction

Although numerous oncogenes have been identified over the past several years, it appears that the encoded oncoproteins act by a relatively small number of molecular mechanisms (reviewed by Weinberg, 1985; Bishop, 1987). Most oncoproteins are cytoplasmically located or membrane-associated and can be described as protein kinases, GTP-binding signal transducers, growth factors, receptors, or some combination thereof. Presumably, these oncoproteins confer their effects by altering normal cellular metabolism or intercellular signaling. In addition, there is a small class of oncoproteins that are localized to the nucleus (e.g., myc, myb, fos, E1A), and it is presumed that these transform cells by altering normal gene regulation (reviewed by Kingston et al., 1985). However, despite the demonstration that myc and E1A can alter the expression of specific cellular genes (Nevins, 1982; Green et al., 1983; Kaddurah-Daouk et al., 1987), the molecular mechanism by which these proteins act is unknown.

Recently, Maki et al. (1987) have identified a new oncogene, *jun*, that was originally derived from a defective avian sarcoma virus (ASV 17). ASV 17 induces progressively growing fibrosarcomas in chickens and transforms cultured chick embryo fibroblasts into elongated, refractile neoplastic cells (Cavalieri et al., 1985). DNA sequence analysis revealed that the transforming protein of ASV 17 is a gag-jun fusion, and that *jun* lacked homology to any other known oncogene (Maki et al., 1987). Surprisingly, however, the C terminus of the predicted jun protein showed 45% amino acid identity to the 66 C-terminal amino acids of GCN4, a transcriptional activator protein from the yeast Saccharomyces cerevisiae (Vogt et al., 1987; see Figure 1).

GCN4 binds specifically to the promoter regions of many genes involved in amino acid biosynthesis and coordinately induces their transcription (Hope and Struhl, 1985; Arndt and Fink, 1986). GCN4 binds as a dimer (Hope and Struhl, 1987) to target sites whose consensus sequence is the 9 bp palindrome ATGA(C/G)TCAT; this consensus sequence also represents the optimal GCN4 binding site (Hill et al., 1986). Extensive deletion analysis of GCN4 indicates that the 60 C-terminal amino acids are sufficient both for specific DNA-binding (Hope and Struhl, 1986) and for dimerization (Hope and Struhl, 1987). Although the GCN4 DNA-binding domain is necessary for recognizing the appropriate promoters, a short acidic region in the center of the protein is required for transcriptional activation (Hope and Struhl, 1986; Struhl, 1987). GCN4 is synthesized only during conditions of amino acid starvation by virtue of a novel translational control mechanism (Thireos et al., 1984; Hinnebusch, 1984), thus accounting for the transcriptional regulation of the amino acid biosynthetic genes.

From the homology between the jun oncoprotein and the GCN4 DNA-binding domain, Vogt et al. (1987) suggested that jun might bind to DNA in a sequence-specific manner. If this were the case, it might be imagined further that the jun protein would recognize the same sequences as GCN4. In support of this hypothesis, the consensus sequence for binding by AP-1, a mammalian transcription factor that interacts with phorbol ester–inducible promoter elements (Angel et al., 1987; Lee et al., 1987), is very similar to the GCN4 recognition site.

In this study I replace the GCN4 DNA-binding domain by the homologous region of the jun oncoprotein and investigate the DNA-binding properties of the resulting hybrid protein in yeast cells. The results indicate that jun recognizes the same sequences as GCN4, and they strongly suggest that *jun* is derived from a normal cellular transcription factor. Moreover, this provides evidence for the idea that alterations in the level or activity of a normal transcription factor can lead to the oncogenic state, and it identifies presumptive target genes whose expression is directly controlled by the oncoprotein.

Results

Replacing the GCN4 DNA-Binding Domain by the Homologous Region of jun

Since the purpose of the experiments was to determine if the jun oncoprotein would bind to the same DNA sequences as GCN4, molecules were constructed in which the region encoding the GCN4 DNA-binding domain was replaced by the homologous jun region (derived from a molecule that was kindly provided by Timothy Bos and Peter Vogt). Specifically, the molecules encoded LexA-

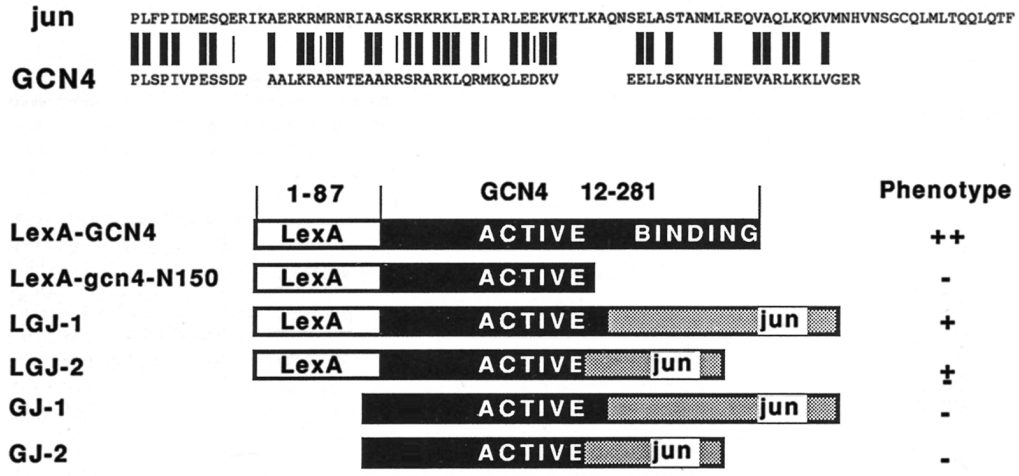

Figure 1. Protein Structures

Shown above is the alignment of the C-terminal sequences of jun (amino acids 206 to 296) and GCN4 (amino acids 216 to 281), with identical (thick lines) and similar (thin lines) residues indicated. Shown below are the structures of hybrid proteins, composed of the LexA DNA-binding domain (open box), GCN4 (black box with transcriptional activation and DNA-binding domains indicated), and jun (shaded box); the drawing is to scale. The GCN4 phenotypes (++, +, ±, or −) conferred by the various proteins are defined by growth in medium containing 10 mM aminotriazole; see text.

GCN4-jun (LGJ) hybrid proteins containing the LexA DNA-binding domain, the GCN4 transcriptional activation region, and the presumptive jun DNA-binding domain (Figure 1). The GCN4 activation region was included to avoid the potential problem of whether a protein containing the putative jun DNA-binding domain would activate transcription in yeast. Since the GCN4 activation region is capable of transcriptional stimulation even when fused to a heterologous DNA-binding domain such as LexA (Hope and Struhl, 1986), the LGJ hybrid proteins provide an in vivo DNA-binding assay for the *jun* region. The LexA DNA-binding domain was included to provide an internal control for the ability of the hybrid protein to stimulate transcription via the GCN4 activation region.

The starting molecule for the experiments, YCp88-*lexA-GCN4*, contains coding sequences for a LexA-GCN4 hybrid protein that have been cloned into the YCp88 vector (Hope and Struhl, 1986). YCp88 contains the *URA3* selectable marker, the *ARS1* and *CEN3* elements for stable maintenance as a minichromosome at one copy per cell, and the *DED1* promoter for expression of the hybrid protein. The LexA-GCN4 hybrid protein is composed of the LexA DNA-binding domain (amino acids 1–87) at the N terminus and essentially the entire GCN4 coding sequences (amino acids 12–281) at the C terminus (Figure 1). This hybrid protein acts as a bifunctional activator since it stimulates transcription from promoters containing either GCN4 or LexA binding sites upstream of TATA promoter elements (Hope and Struhl, 1986).

The hybrid protein used in most of the experiments, LGJ-1, was identical to the starting LexA-GCN4 protein except that the 112 amino acid C-terminal domain of GCN4 (i.e., the entire DNA-binding domain) was replaced by the 166 C-terminal amino acids of jun (Figure 1). For LGJ-2, the 140 C-terminal amino acids of GCN4 (the entire DNA-binding domain and 6 out of the 60 amino acids of the acidic activation region) were replaced by the 99 C-terminal amino acids of jun. In addition, derivatives of LGJ-1 and LGJ-2 lacking the LexA DNA-binding domain (termed GJ-1 and GJ-2 respectively) were also generated (Figure 1).

The LGJ Protein Functionally Substitutes for GCN4 *In Vivo*

The GCN4 function of the LGJ hybrid proteins was assayed by the standard method of in vivo complementation of a *gcn4* mutation. YCp88 plasmids encoding various proteins were introduced into yeast strain KY803, which contains a total deletion of the native *GCN4* gene (Hope and Struhl, 1986). This parental strain displays two distinct phenotypes. First, in minimal medium lacking amino acids, the growth rate of KY803 is about 50% of that of a corresponding wild-type strain. Second, KY803 is unable to grow in the presence of 10 mM 3-amino-1,2,4-triazole (aminotriazole), a competitive inhibitor of the *HIS3* gene product that causes histidine starvation because the cells fail to induce *HIS3* transcription above the basal level. Indeed, the degree of aminotriazole resistance is directly related to the level of *HIS3* mRNA (Hill et al., 1986).

The growth properties of KY803 derivatives expressing the various proteins are indicated in Figure 1. As expected, LexA-GCN4 restores the wild-type growth phenotypes, whereas LexA-gcn4-N150 (which is deleted for the GCN4 DNA-binding domain) fails to have any effect. Interestingly, expression of LGJ-1 allowed cells to grow in the presence of 10 mM aminotriazole, although at a slightly slower rate than cells containing LexA-GCN4. In addition, such cells grow at normal rates in the absence of amino acids. Thus LGJ-1 functionally substitutes for GCN4 in its ability to induce the expression of *HIS3* and other amino acid biosynthetic genes.

Expression of LGJ-2 allows cells to grow at normal rates in the absence of amino acids, and at very slow rates in

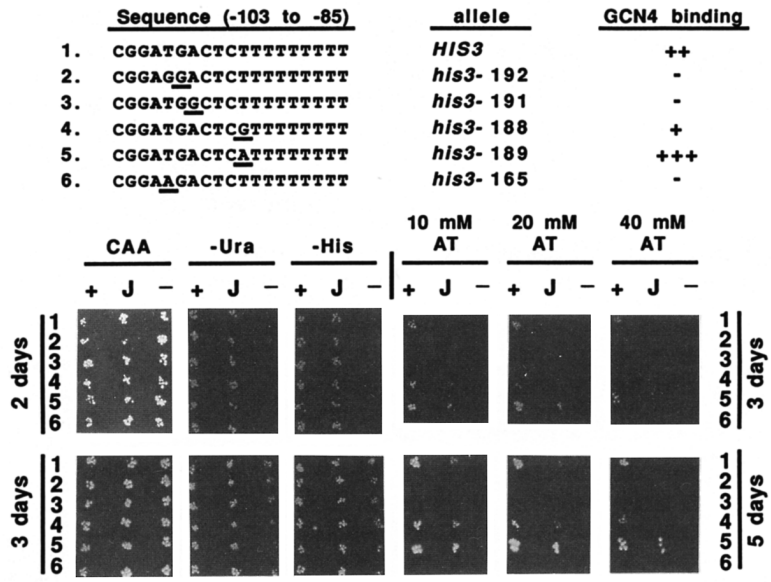

Figure 2. Phenotypic Analysis of *his3* Strains Expressing Hybrid Proteins

For the six *his3* alleles used in the experiments, the top part shows the *his3* DNA sequences between nucleotides −103 and −85 (deviations from the wild-type sequence are underlined) and the GCN4-binding properties as determined by Hill et al. (1986). Shown below are the growth properties of strains containing these six *his3* alleles and expressing LexA-GCN4 (+), LGJ-1 (J), or LexA-gcn4-N150 (−). The growth medium contained 1% casamino acids plus tryptophan (CAA), minimal supplements without uracil (−Ura), minimal supplements without histidine (−His), or aminotriazole (AT) at concentrations of 10, 20, or 40 mM, as indicated. Growth was for the number of days indicated. See text and Experimental Procedures for details.

medium containing 10 mM aminotriazole. When such cells are streaked on plates containing aminotriazole, variants that grow at more rapid rates are observed at an approximate frequency of 10^{-6}; these have yet to be characterized further. Thus LGJ-2 can functionally substitute for GCN4, but it is less efficient than LGJ-1. Surprisingly, derivatives lacking the LexA DNA-binding region—GJ-1 and GJ-2—do not complement the *gcn4* deletion, although rare variants growing in aminotriazole medium can be isolated. Explanations for these observations will be considered in the Discussion.

The GCN4 and jun DNA-Binding Domains Behave Homologously

The observation that the homologous jun region can functionally replace the GCN4 DNA-binding domain strongly suggests that these two proteins recognize the same DNA sequences. To investigate this further, plasmids encoding LGJ-1, LexA-GCN4, and LexA-gcn4-N150 were introduced into a set of isogenic *gcn4* deletion strains that differ solely at the GCN4 binding site within the *HIS3* promoter. The DNA sequences and GCN4 binding properties of the various *his3* alleles have been determined previously (Hill et al., 1986) and are shown in Figure 2. Three of the alleles, *his3-192*, *his3-191*, and *his3-165*, are defective in binding by GCN4 and hence fail to induce *HIS3* transcription. Two of the alleles are altered at −93, the one position where the native *HIS3* binding site deviates from the consensus sequence. When compared with the wild-type allele, *his3-189* binds GCN4 more tightly and it induces *HIS3* transcription to higher levels. In contrast, *his3-188* shows detectable but weaker GCN4 binding and it confers relatively weak induction of *HIS3* transcription.

To determine the phenotypes, approximately 5–10 cells from cultures grown in nonselective medium were spotted onto plates containing various concentrations of aminotriazole or combinations of amino acids. In this way, all 18 strains could be compared on the same plate for any given growth condition. Moreover, individual cells from the same culture represent independent determinations of the growth rate. Indeed, the time it takes to form a colony of an arbitrary size represents an endpoint determination of the doubling time, which in turn can be directly related to the level of *HIS3* induction (Hill et al., 1986).

Table 1. Phenotypic Analysis of *his3* Strains Expressing Hybrid Proteins

	Hybrid Protein Expressed		
his3 Allele	LexA-GCN4	LGJ-1	LexA-gcn4-N150
HIS3	+ +	+[a]	−
his3-192	−	−	−
his3-191	−	−	−
his3-188	+	+[a]	−
his3-189	+ + +	+ +	−
his3-165	−	−	−

Phenotypes for strains containing a given *his3* allele and hybrid protein were determined from the relative degree of aminotriazole resistance as shown in Figure 2. Entries are listed as + + (wild-type resistance), + + + (more resistance), + (less resistance), or − (no resistance).

[a] For strains expressing LGJ-1, *his3-188* cells grow slightly faster than *HIS3* cells.

The results of this experiment (Figure 2; Table 1) are summarized as follows. First, in medium containing all amino acids, the 18 strains behave indistinguishably, thus confirming that *GCN4* function is not needed under these conditions. Second, in minimal medium containing or lacking histidine, strains expressing LGJ-1 or LexA-GCN4 grow about 50% faster than a strain expressing the protein lacking either the GCN4 or jun DNA-binding domain; i.e., colonies form in 2 days instead of 3 days. As expected from the fact that the basal *HIS3* level is more than sufficient for wild-type growth rates in minimal medium (Struhl, 1982), the different *his3* alleles behave similarly. Third, in medium containing aminotriazole, LGJ-1 and LexA-GCN4 permit growth, but only in strains containing the wild-type,

his3-188, and *his3-189* alleles. In all cases, strains containing LexA-GCN4 grow somewhat faster than strains containing LGJ-1. Fourth, for both LGJ-1 and LexA-GCN4, growth in strains containing *his3-189* is faster than in strains containing the wild-type allele; this is particularly evident at high concentrations of aminotriazole. Fifth, comparison of strains containing *his3-188* versus the wild-type allele shows that LexA-GCN4 confers faster growth in combination with the wild-type allele, whereas LGJ-1 appears to confer slightly faster growth in combination with *his3-188*.

These results indicate that the jun DNA-binding domain activates *HIS3* transcription only if the promoter region contains a functional GCN4 binding site. Moreover, maximal *HIS3* induction mediated by the jun hybrid protein occurs in combination with the optimal GCN4 binding site. Thus the GCN4 and jun DNA-binding regions behave homologously on a set of target sites, suggesting that they recognize very similar DNA sequences.

Discussion

jun Oncoprotein and GCN4 Bind the Same DNA Sequences

The main result of this work is that the C terminus of the jun oncoprotein can functionally replace the GCN4 DNA-binding domain in vivo. The fact that LGJ-1, like GCN4, can confer aminotriazole resistance and normal growth rates in medium lacking amino acids indicates that this hybrid protein can induce the expression of *HIS3* and other amino acid biosynthetic genes. More important, *HIS3* induction by LGJ-1 or LexA-GCN4 requires the presence of a GCN4 binding site, and maximal induction by either protein occurs with the optimal binding site. This functional homology between the jun and GCN4 DNA-binding domains indicates that the two proteins recognize the same DNA sequences. Taken together, the homology between GCN4 and jun (Vogt et al., 1987) and the similar DNA sequence requirements for LGJ-1 and LexA-GCN4 action strongly suggest that jun and GCN4 have a common structural motif for DNA binding.

It appears that LexA-GCN4 activates *HIS3* transcription more efficiently than either of the jun hybrid proteins (Figure 2). By using the well-established correlation between the degree of aminotriazole resistance and the level of *HIS3* mRNA (Hill et al., 1986), it can be estimated that activation by LGJ-1 is about 20%–60% as efficient as by LexA-GCN4, and activation by LGJ-2 is about 5%–20% as efficient. This effect is unlikely to reflect transcriptional activation per se or differential amounts/stabilities of the proteins because the two proteins equally stimulate transcription via the LexA DNA-binding domain (data not shown). More likely the difference reflects the relative affinities of the proteins for the target sites in vivo, especially given that the level of GCN4 protein under these conditions is limiting (Hill et al., 1986; Hope and Struhl, 1986). Mechanistically, this difference could be due to the intrinsic affinity of the protein–DNA interaction or to the protein's ability to form proper structures (dimers in the case of GCN4).

One unexpected result was that both LGJ hybrid proteins confer *GCN4* function, whereas neither of the derivatives lacking the LexA DNA-binding domain (GJ-1 and GJ-2) were able to do so (Figure 1). In other words, jun-dependent transcriptional activation appears to require the LexA DNA-binding domain. This effect cannot be attributed to specific DNA-binding properties of the LexA region because the *HIS3* promoter does not contain a LexA DNA-binding site, and because LexA-gcn4-N150, which lacks both the GCN4 and jun DNA-binding regions, is unable to complement the *gcn4* deletion (Hope and Struhl, 1986; Figure 2).

The most likely explanation for this phenomenon is that the LexA domain facilitates dimerization (or formation of higher-order structures) of the jun domain. Given the palindromic nature of the optimal *HIS3* target site, it is extremely likely that jun like GCN4 (Hope and Struhl, 1987), must form dimers in order to bind DNA. Moreover, dimerization effects were proposed to explain a very similar phenomenon observed with other LexA hybrid proteins containing the entire transcriptional activation region (Hope and Struhl, 1986). In that case, expression mediated by a LexA DNA-binding domain that contained relatively weak dimerization contacts was increased 5-fold by the presence of an intact GCN4 DNA-binding domain. Interestingly, both LGJ-1 and LGJ-2 activate transcription of a "*lexA* promoter" at levels near that obtained with LexA-GCN4, not at levels observed with derivatives lacking the GCN4 DNA binding domain (data not shown); this suggests that the jun and LexA DNA-binding domains mutually aid proper subunit formation. In any event, neither the relative inefficiency of LGJ-1 nor the unexpected effect of the LexA DNA-binding domain influences the major conclusion that the jun and GCN4 DNA-binding domains behave homologously and hence recognize similar DNA sequences.

In addition to their relevance to the mechanism of oncogenesis, the results in this paper provide information about the amino acids that are important for directly contacting the DNA. It is likely that most if not all of the crucial residues are located in the 30 amino acid regions of jun and GCN4 in which there are 17 identical residues and 4 conservative differences (Figure 1). This region has considerable α-helical character (Hope and Struhl, 1986), but there is no similarity to either of the standard DNA-binding motifs for transcriptional regulatory proteins, helix-turn-helix or zinc finger (Pabo and Sauer, 1984; Miller et al., 1985). One interesting observation is that in induction of *his3-188* or the wild-type allele, both of which differ from the optimal sequence at position −93, LexA-GCN4 is more efficient with the wild-type allele, whereas LGJ-1 appears to prefer *his3-188*. Although these allele *specificities* are subtle, they are reminiscent of the situation with λ repressor and λ Cro proteins, which recognize the same basic sequence but with altered specificities (Hochschild and Ptashne, 1986).

Implications for the Mechanism of Oncogenesis

The *jun* oncogene, which was originally derived from an avian sarcoma virus, has normal cellular homologs in the chicken, Japanese quail, mouse, rat, and human (Maki

et al., 1987). The fact that the jun oncoprotein is a sequence-specific DNA-binding protein strongly suggests that the cellular homologs also recognize specific DNA sequences. More important, the specific DNA-binding activity of these proteins almost certainly means that they are transcriptional regulators that directly affect the expression of specific target genes whose promoters contain the recognition site. In principle, the jun oncoprotein and its cellular counterparts could activate and/or repress transcription.

One striking similarity is that the DNA sequence recognized by GCN4 and jun is essentially identical to the sequence that is recognized by AP-1, a mammalian transcription factor (Angel et al., 1987; Lee et al., 1987). AP-1 binding sites are found in the enhancer regions of the collagenase, stromelysin, metallothionein II_A, and SV40 genes, and these sites are crucial for transcription in vivo and in vitro. AP-1 DNA-binding activity is increased when cells are treated with phorbol esters, which accounts for the transcriptional induction of genes containing AP-1 binding sites that occurs under these conditions (Angel et al., 1987; Lee et al., 1987). The relationship between the GCN4, jun, and AP-1 binding sequences suggests the possibility that the jun oncoprotein is derived from the normal AP-1 transcription factor.

This paper presents the first demonstration that an oncoprotein contains a specific DNA-binding activity, and, by extension, it identifies presumptive target genes whose expression is likely to be controlled directly by jun. This provides direct evidence for the idea that alterations in the level or activity of a normal transcription factor, possibly AP-1, can lead to the oncogenic state. If jun is indeed derived from AP-1, it could be imagined that signals such as phorbol esters are received at the membrane and then transmitted to intracellular messengers such as protein kinase C. An activated protein kinase C could then modify the AP-1 transcription factor, thereby directly resulting in altered gene expression. In this view, the jun oncoprotein may differ from AP-1 in such a fashion as to escape the normal regulatory pathway.

Experimental Procedures

Constructing the LGJ Hybrid Proteins

DNA molecules were generated by standard techniques, and their structures were verified by DNA sequencing. All the DNA molecules derive from YCp88-*lexA-GCN4*, which has been described previously (Hope and Struhl, 1986). YCp88 contains the *URA3* selectable marker, the *ARS1* and *CEN3* elements for stable maintenance as a minichromosome at one copy per cell, and the *DED1* promoter for expression of the hybrid protein. The LexA-GCN4 hybrid protein is composed of the LexA DNA-binding domain (amino acids 1–87) at the N terminus and essentially the entire GCN4 coding sequence (amino acids 12–281) at the C terminus (Figure 1). The intracellular level of LexA-GCN4 protein is roughly equivalent to the level of GCN4 under full inducing conditions (Hope and Struhl, 1986). The DNA containing the *jun* oncogene, pG5-5-1, was constructed by and was the generous gift of Timothy Bos and Peter Vogt. This DNA contained the *gag-jun* fusion gene (Maki et al., 1987) cloned into the SmaI site of pGEM4 (Promega Biotec).

To obtain the molecules for expressing LGJ-1, the *jun* plasmid DNA was treated with NaeI, ligated to an octanucleotide XbaI linker, cleaved with XbaI and EcoRI, and cloned between the XbaI and EcoRI sites of YCp88-*lexA-GCN4*. For the plasmid expressing LGJ-2, the *jun* plasmid DNA was treated with RsaI, ligated to an octanucleotide SalI linker, cleaved with SalI and EcoRI, and cloned between the SalI and EcoRI sites of a derivative of YCp88-*lexA-GCN4* in which the region corresponding to amino acids 141 to 219 was replaced by a SalI linker. Plasmids GJ-1 and GJ-2, which lack sequences encoding the LexA DNA-binding domain, were made simply by replacing the BamHI–EcoRI fragment of YCp88-*GCN4* (Hope and Struhl, 1986) with the BamHI–EcoRI fragment of the above plasmids respectively expressing LGJ-1 and LGJ-2.

Phenotypic Analysis

The starting yeast strain, KY803 (*trp1-Δ1 ura3-52 leu-P1 gcn4-Δ1*), has been described previously (Hope and Struhl, 1986). The *gcn4* deletion mutation removes the entire protein coding region as well as part of the 5′ RNA leader and 3′ untranslated sequences. Isogenic derivatives of KY803 containing various point mutations of the GCN4 binding site in the *HIS3* promoter were obtained by two successive gene-replacement events. First, KY803 was transformed in one step to Trp^+ by the method of Rothstein (1983), with a linear DNA molecule in which part of the *HIS3* promoter and structural gene (nucleotides −106 to +479) were replaced by the intact *TRP1* gene; as expected, the resulting strain was His^-. Second, DNAs containing the various *his3* alleles were used to replace the *his3-TRP1* allele exactly as described by Hill et al. (1986).

The YCp88 DNAs encoding the various proteins were introduced into the *gcn4* deletion strains by selecting for Ura^+ transformants on glucose minimal medium containing 1% casamino acids and 2 mg per liter of tryptophan. The main assay for *GCN4* function in vivo was performed by growth on solid medium containing aminotriazole at concentrations of 10, 20, and 40 mM. Since the relative degree of aminotriazole resistance can be directly related to the level of *HIS3* mRNA (Hill et al., 1986), this test provides an excellent measure of *HIS3* expression in vivo. For assaying *GCN4* function in the absence of aminotriazole, strains were tested for growth on minimal medium lacking uracil or histidine. As the basal *HIS3* level is sufficient for wild-type growth rates under these conditions (Struhl, 1982), this test measures GCN4 activation of amino acid biosynthetic genes other than *HIS3*. In all experiments involving minimal medium, adenine, lysine, leucine, tryptophan, and uracil or histidine were added at concentrations of 5 mg per liter. The phenotypes were tested either by conventional streaking or by diluting cultures grown in the presence of casamino acids and tryptophan such that approximately 5–10 cells were spotted on plates containing the appropriate media (see Results and Figure 2).

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