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Genetics

Evidence for translational regulation of the activator of general amino acid control in yeast

(*GCN4* gene/long 5′ leader/upstream ATG codons/*lacZ* fusion)

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ABSTRACT **The *GCN4* gene encodes a positive regulator of unlinked amino acid biosynthetic genes in yeast. I present evidence that the *GCN4* gene is itself regulated by amino acid availability and that the regulation occurs at the translational level. A *GCN4-lacZ* fusion was used as a measure of the expression of *GCN4* gene product. Starvation for histidine leads to derepression of the fusion enzyme in the wild type but not in a *gcn2*$^-$ strain. The *gcn2*$^-$ mutation does not reduce fusion transcript levels relative to wild type, suggesting that the product of *GCN2* functions as an activator of GCN4 translation. The *GCN4* transcript has a 5′ leader that is ≈600 nucleotides long and contains four small open reading frames. A deletion of the small open reading frames results in constitutive derepression of fusion enzyme levels as the result of an ≈10-fold increase in the efficiency of translation of the fusion transcript. The deletion suppresses the requirement for *GCN2* function. These results suggest that the *GCN4* 5′ leader acts in *cis* to repress GCN4 translation and that GCN4 translation increases in response to amino acid starvation as the result of GCN2 antagonism of the repressing sequences in the *GCN4* 5′ leader.**

A large number of unlinked genes encoding amino acid biosynthetic enzymes in yeast are under the control of a cross-pathway regulatory system known as general amino acid control. Starvation of yeast for any one of several amino acids leads to increased transcription of each of these coregulated genes (reviewed in ref. 1). The short nucleotide sequence, -T-G-A-C-T-C-, found nontandemly repeated upstream of *HIS3*, *HIS4*, and other coregulated genes, functions as a *cis*-acting site of positive regulation in the general control (2–5). *trans*-acting factors have been identified by the isolation of unlinked regulatory mutations. Recessive mutations in *GCD** genes lead to constitutive derepression of general control structural genes and thus identify negative regulatory elements; mutations in *GCN* genes prevent derepression and thus define positive regulatory elements (reviewed in ref. 1; see refs. 6 and 7). Genetic evidence suggests that, with the exception of GCN4, all other *GCN* gene products act as indirect activators by antagonism of *GCD*-mediated repression. In contrast, the *GCN4* product is a more direct activator and is expected to be negatively regulated by the *GCD1* gene product (7). The *GCN4* product is currently the best candidate for the activator that interacts with the sites of positive regulation that have been identified at general control structural genes.

GCN genes have been cloned by complementation of the corresponding mutations in yeast (7, 9). I present a structural and functional analysis of the *GCN4* gene in *Saccharomyces cerevisiae*. I present evidence that the expression of this gene is itself regulated by amino acid availability and the *GCN2* gene product. Whereas regulation of general control structural genes occurs at the level of transcription, regulation of *GCN4* appears to have a translational component.

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MATERIALS AND METHODS

Yeast transformations were performed by the method of Hinnen *et al.* (10). Plasmid preparation, DNA sequence analysis, total RNA isolation, preparation of poly(A)$^+$ RNA, and nuclease protections were done as described (4), except that in nuclease-protection experiments 12.5 μg of poly(A)$^+$ RNA was hybridized in 17-μl reaction mixtures, and lower nuclease S1 concentrations were used (see Fig. 4). In DNA·RNA hybridization blot analysis, parallel blots were probed with *lacZ* plus *GCN4* DNA and with pyruvate kinase-encoding DNA. The latter served as an internal control for the level of mRNA in different RNA samples. Hybridization blots were prepared (4) and transcript levels were measured by scanning densitometry of lightly exposed autoradiograms. Details of plasmid constructions are provided in Figs. 1 and 5.

RESULTS

Deletion Mapping of *GCN4*. The boundaries of the *GCN4* functional unit were determined by testing fragments of a cloned 2800-base-pair (bp) *GCN4* fragment for complementation of a *gcn4*$^-$ mutation. Fig. 2*A* shows the restriction map of a *GCN4* fragment described previously (7). Two sets of deletion fragments were constructed (Fig. 2 *B* and *C*); in each, progressively larger amounts of DNA were removed from one end of the fragment and replaced with the same pBR322 sequences (see Fig. 1 for details). The deletion fragments were carried on yeast 2-μm episomal plasmids containing the yeast *URA3* gene as a selectable marker. A *ura3*$^-$ *gcn4*$^-$ yeast strain was transformed with each deletion plasmid, and the resulting Ura$^+$ transformants were tested for complementation of the chromosomal *gcn4*$^-$ mutation. The results are listed to the right of each deletion fragment in Fig. 2 *B* and *C*.

The results show that DNA sequences bounded by the endpoints of deletion fragments 5′Δ+874 and 3′Δ+1470 are essential for *GCN4* function. However, the results obtained with 5′Δ+711 suggest that sequences further upstream, in the vicinity of the *Bam*HI site, are also required for a wild-type level of *GCN4* function. In fact, insertion of *Escherichia coli lacZ*-coding DNA into the *Bam*HI site leads to a Gcn4$^-$ phenotype (see below).

A Long Open Reading Frame (ORF) Spans the *GCN4* Functional Unit. Fig. 2*D* summarizes the strategy employed to determine the sequence of 1827 bp in the *GCN4* region. A long ORF of 281 codons is present in the DNA sequence, beginning at position +591 in Fig. 3. The predicted amino acid sequence of the ORF is shown beneath the DNA se-

Abbreviations: ORF, open reading frame; bp, base pair(s).

*A new nomenclature for general amino acid control regulatory genes was recently adopted. The *GCD1* gene was known previously as *TRA3* (8), and *gcn2-1* and *gcn4-101* were known as *aas1-3* and *aas3-1* (7).

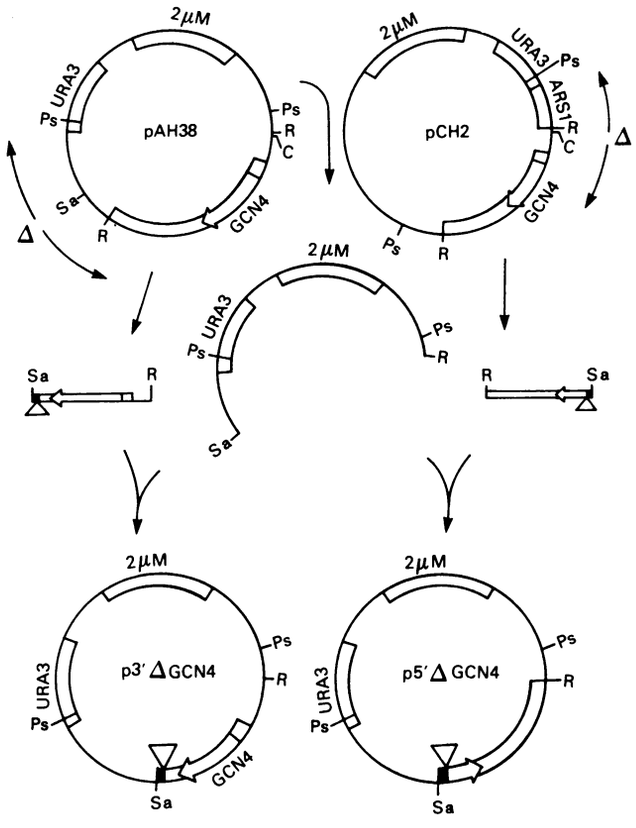

FIG. 1. Construction of plasmids with 5′ and 3′ deletions (Δ) in the *GCN4* region. R, *Eco*RI; Ps, *Pst* I; C, *Cla* I; Sa, *Sal* I. Thin lines are pBR322 sequences. 3′-deletion plasmids were constructed by BAL-31 nuclease treatment of pAH38 which had been linearized by digestion at the unique *Sal* I site; 5′-deletion plasmids were constructed beginning with *Cla* I-digested pCH2. *Sal* I octanucleotide linkers were attached to BAL-31-digested fragments according to Maniatis *et al.* (11). After digestion with *Sal* I, *Eco*RI, and *Pst* I and NACS-52 chromatography (Bethesda Research Laboratories) to remove free linkers, fragments were ligated with the large *Sal* I/*Eco*RI fragment of pAH38 and used to transform *E. coli* (monitored by ampicillin resistance). Transformants were screened for the presence of two *Sal* I/*Eco*RI fragments by gel electrophoresis, and deletion endpoints were determined by sizing of the smaller of the two fragments and by testing of the remaining unique restriction sites in the *GCN4* region. In a number of cases (see Fig. 3), the deletion junctions were determined by DNA sequence analysis, using 20% gels (12), of the small *Sal* I/*Eco*RI fragment 3′ end-labeled at the *Sal* I site. The sequences between the *Sal* I and *Ava* I sites of pBR322 are adjacent to the deletion junction in each construct.

quence. The long ORF spans the *GCN4* functional unit defined by deletion analysis. Deletion fragment 3′Δ+1306 has the smallest 3′ deletion that results in noncomplementation of *gcn4-101* (Fig. 2*C*), and it also has the smallest 3′ deletion that removes sequences from the ORF (Fig. 3). All deletions that extend further into the ORF result in Gcn4$^-$ transformants, whereas 3′ deletions that terminate outside the ORF (e.g., 3′Δ+1470, Fig. 3) result in Gcn4$^+$ transformants. All 5′ deletions that affect *GCN4* function also remove ORF sequences. However, it is remarkable that deletions 5′Δ+711 and 5′Δ+874 remove the beginning of the ORF but are not Gcn4$^-$. In fact, 5′Δ+874 lacks the first 95 codons of the ORF but yields transformants indistinguishable in phenotype from those containing the wild-type plasmid. These data indicate a general correspondence between the *GCN4* functional unit and the ORF in the DNA sequence, suggesting that the long ORF encodes the *GCN4* gene product. However, they also imply that the NH_2-terminal third of the predicted protein is unnecessary for GCN4 function.

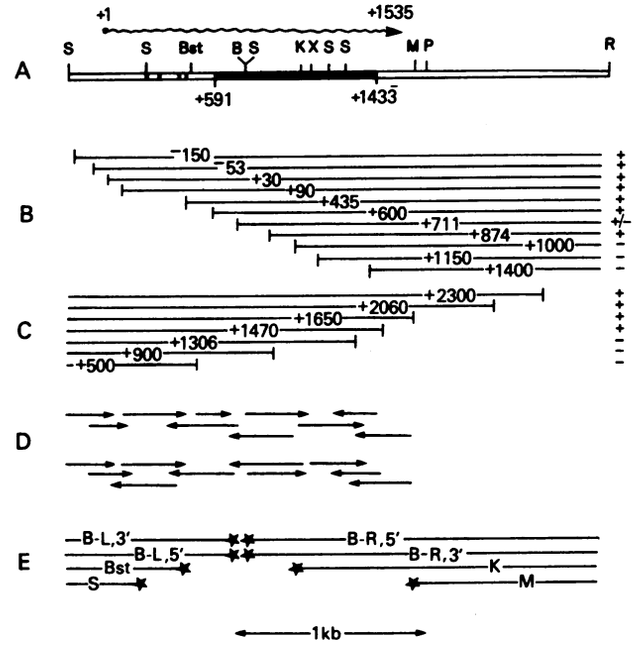

FIG. 2. (*A*) Restriction map of the *GCN4* region. S, *Sau*3A; Bst, *Bst*EII; B, *Bam*HI; K, *Kpn* I; X, *Xba* I; M, *Mlu* I; P, *Pvu* II; R, *Eco*RI. The transcription unit is shown by the wavy arrow; ORFs are shaded (see below). Coordinates are relative to the 5′ end of the transcript (+1). (*B* and *C*) Deletions from the 5′ and 3′ ends, respectively. Deleted sequences are represented by blank regions and deletions are designated by the coordinates of the last deleted nucleotides. Exact deletion endpoints were determined for those deletions also shown in Fig. 3; endpoints of other deletions are correct to within approximately 50 bp. +, −, and +/− are the Gcn4 phenotypes determined by replica printing transformants of strain H24(α *gcn4-101 ura3-52*) from minimal medium to minimal medium supplemented with 10 mM 3-aminotriazole and monitoring their growth. 3-Aminotriazole inhibits histidine biosynthesis, and the nonderepressibility of the histidine biosynthetic enzymes in *gcn*$^-$ mutants results in a marked reduction in their growth rate, relative to Gcn$^+$ strains, in the presence of this compound (8). (*D*) Sequencing strategy. Upper arrows designate sequences of the top strand that were determined. Arrows pointing left to right correspond to 5′ labeled fragments; right to left indicates 3′ labeling. The lower set of arrows corresponds to analysis of the bottom strand. (*E*) Probes used in nuclease protection mapping of the transcription unit. Labeled positions are shown with stars. B-L and B-R refer to the left and right *Bam*HI-terminated fragments, respectively, labeled at the 5′ or 3′ end. Other letters correspond to restriction endonucleases as described in *A*. kb, Kilobase.

An independent assessment of the location of the *GCN4* gene is provided by deletion mapping of the *gcn4-101* mutation in the chromosome. Ura$^+$Gcn4$^-$ transformants of a *gcn4-101* strain, each containing a deletion plasmid that fails to complement *gcn4-101*, were examined for their ability to give rise to Gcn$^+$ recombinants. Transformants harboring deletion plasmids 5′Δ+1000, 5′Δ+1150, and 3′Δ+1306 all spontaneously give rise to Gcn$^+$ colonies. These Gcn4$^+$ cells remain Gcn$^+$ after loss of the plasmid (becoming Ura$^-$) during nonselective growth in complete medium, showing that in each case, the chromosomal *gcn4*$^-$ mutation was replaced by a *GCN4*$^+$ allele. However, deletion plasmids 5′Δ+1400 and 3′Δ+900 fail to give rise to Gcn4$^+$ recombinants. It follows that the *gcn4-101* mutation maps within or very near the interval +1150 to +1306, which is within the region defined above as required for *GCN4* function.

The *GCN4* Transcript Has a Long 5′ Leader. A 1.5-kilobase transcript homologous to *GCN4* ORF sequences was detected in nuclease protection experiments. Fig. 2*E* shows the single-stranded, end-labeled DNA probes used in these ex-

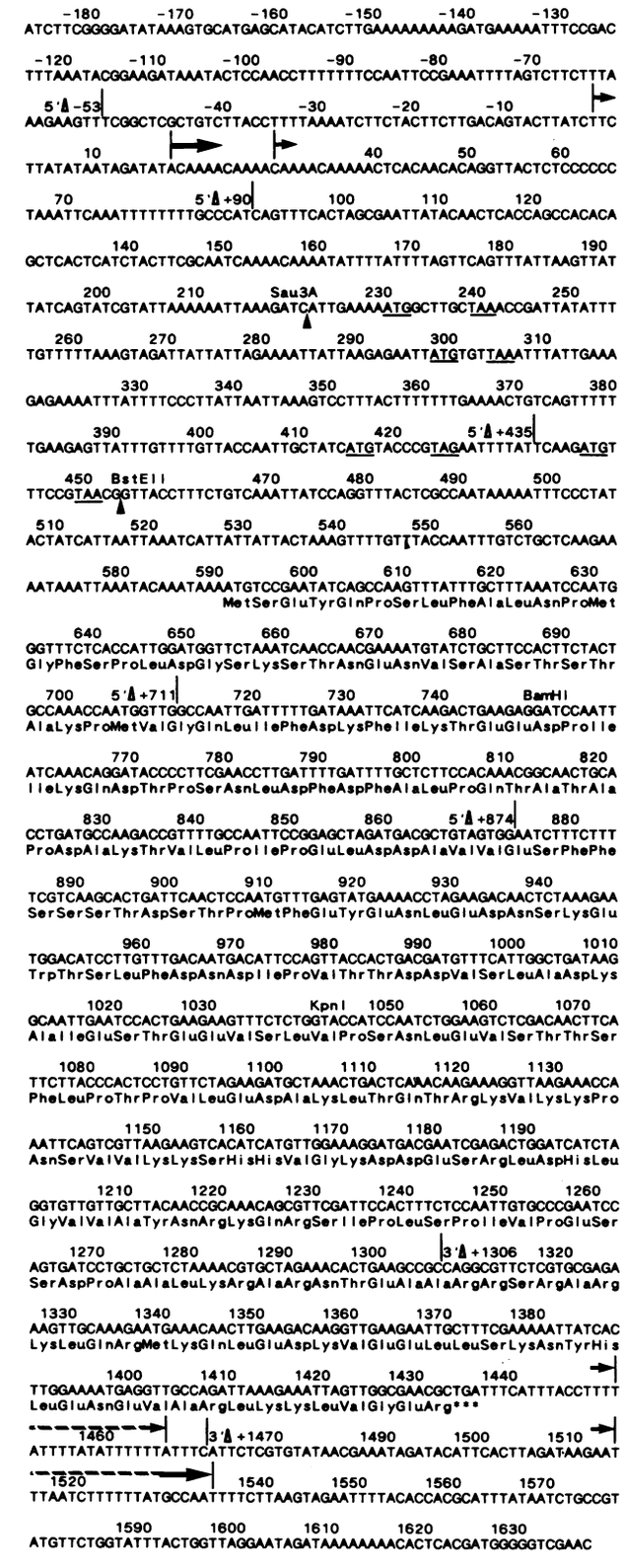

FIG. 3. DNA sequence of the *GCN4* region. Numbering is relative to the furthest upstream 5′ mRNA terminus. Large arrows represent major transcript termini; small arrows mark minor termini. Small ORFs in the 5′ leader are indicated by underlining of their 5′ (ATG) and 3′ (stop) codons. The breakpoints of the sequenced 5′ and 3′ deletion fragments are indicated by vertical lines, and the two arrowheads beneath the sequence in the 5′ leader mark the junctions of the internal *Sau*3A/*Bst*EII deletion.

periments. Each of the probes was hybridized with poly(A)$^+$ RNA isolated from wild-type cells cultured in minimal medium (general control repressed) and in the presence of 3-aminotriazole (general control derepressed.) Considering first the probes labeled at the *Bam*HI (B) site in the long ORF, only the 5′-labeled B-L and the 3′-labeled B-R probes (Fig. 2*E*) were protected from nuclease S1 digestion by hybridization to poly(A)$^+$ RNA (Fig. 4). The lengths of the protected fragments are the same using RNA from repressed (R) and derepressed (DR) cells. These lengths map the 5′ and 3′ termini of the *GCN4* transcript 700 bp to the left and 750 bp to the right of the *Bam*HI site, respectively. (Minor, smaller fragments are evident in the derepressed RNA samples. These fragments were not observed reproducibly, nor were they detected using other probes containing the sequences corresponding to the termini of the minor fragments.) Results with other probes and a different nuclease (exonuclease VII) confirmed these results and also mapped the transcript termini more precisely. A probe end-labeled at the *Mlu* I site gave no protected fragments (data not shown).A

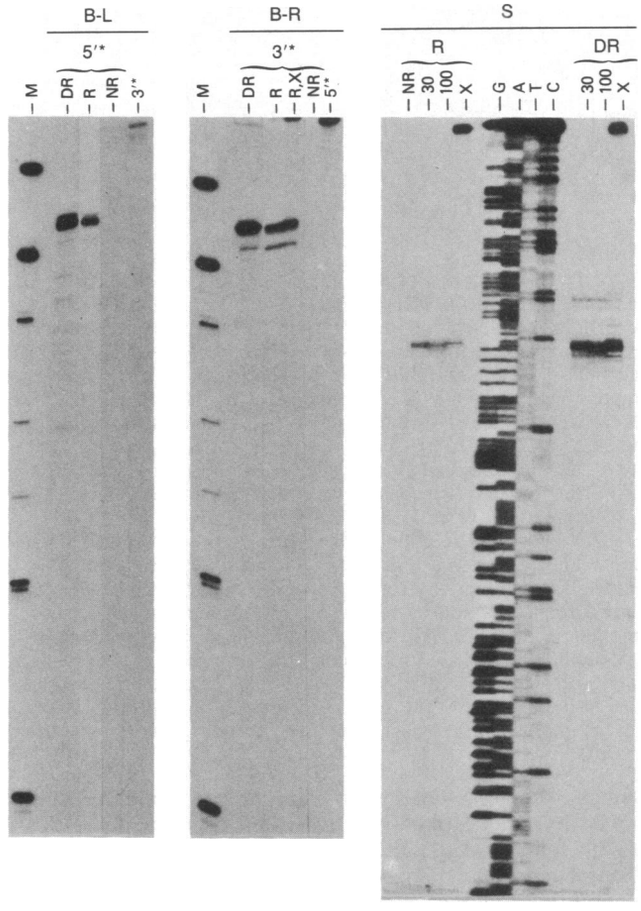

FIG. 4. Nuclease protection mapping of the *GCN4* transcript. DNA probes used are listed above each panel (see Fig. 2*E*) along with the source of RNA (R, repressed cells; DR, derepressed cells) and the nuclease employed (X, exonuclease VII; all other digestions were with nuclease S1). Strain S288C was the source of RNA; repressed and derepressed culture conditions were described previously (5). Digestions at two nuclease S1 concentrations (30 and 100, 30 and 100 units/ml, respectively) are shown for the *Sau*3A (S) probe. NR refers to mock hybridizations with *E. coli* tRNA; G, A, T, and C lanes are Maxam–Gilbert sequencing reactions of the probe; M lanes contain end-labeled *Hin*fI fragments of pBR322 for size markers. (Exonuclease VII digests exhibit considerable amounts of the full-length probes. At least for the 5′ probes this is the result of incomplete digestion since the upstream terminal 375 nucleotides of these probes are pBR322 sequences.)

probe 3′ end-labeled at the *Kpn* I site (data not shown) mapped the major 3′ terminus about 100 bp downstream from the 3′ end of the long ORF (Fig. 3). Two additional 5′ end-labeled probes, labeled at the *Sau*3A site (S, Fig. 4) and the *Bst*EII site (data not shown), both in the 5′ leader, mapped the major 5′ end of the *GCN4* transcript 573 bp upstream from the ATG codon of the long ORF (Fig. 3). All 5′ probes detected minor 5′ termini spanning a 30-bp region around the major 5′ end. The furthest upstream minor 5′ terminus is designated +1; the predominant 5′ terminus maps at +18.

A transcript homologous to the *GCN4* ORF with the length predicted from nuclease protection experiments (1500 bp) was also detected by DNA·RNA hybridization blot analysis, using as a probe the 291-bp *Bam*HI/*Kpn* I fragment from within the *GCN4* long ORF (Fig. 5*C*). This transcript is present at high levels in transformants containing the multicopy 5′- and 3′-deletion plasmids (described above) with deletion endpoints outside the *GCN4* transcription unit (data not shown). In contrast, plasmids with 5′ deletions that terminate within the *GCN4* transcription unit defined by nuclease mapping (e.g., 5′Δ+90, 5′Δ+435, and 5′Δ+711) give rise to shorter *GCN4*-homologous transcripts with new 5′ termini mapping in the remaining leader sequences or in the adjacent plasmid sequences (data not shown). Likewise, 3′ deletions 3′Δ+1470 and 3′Δ+1306, in which the normal 3′ terminus is deleted, produce longer transcripts with 3′ ends mapping 700 bp downstream from the normal 3′ end (data not shown). The occurrence of these new transcripts supports the mapping of the *GCN4* transcription unit described above. Moreover, the generation of new 5′ ends helps to explain the Gcn4$^+$ phenotype of transformants containing either of two 5′ deletion plasmids that lack the normal *GCN4* mRNA 5′ terminus: 5′Δ+90 and 5′Δ+600. For 5′Δ+711, which also lacks the long ORF initiation codon and yet still gives Gcn4$^+$ transformants, I presume that a new, in-frame ATG codon in the adjacent plasmid sequences permits translation of the remaining ORF sequences. In fact, in-frame ATG codons in upstream pBR322 sequences are present in new 5′Δ+711 transcripts.

The *GCN4* ORF Is Expressed *in Vivo* and Subject to Amino Acid Regulation. An in-frame fusion between the beginning of the long ORF and *E. coli lacZ*-protein-coding sequences was constructed by inserting a 3000-bp *lacZ Bam*HI fragment into the *Bam*HI site in *GCN4* (Fig. 5*A*). When inserted in the right orientation, the NH_2-terminal 55 codons of the *GCN4* long ORF are fused in-frame to the COOH-terminal 1016 codons of *lacZ*. This construct directs synthesis of β-galactosidase activity in yeast, and the level of enzyme activity derepresses about 6-fold in response to histidine starvation in a wild-type (wt) strain (*GCN4*$^+$-*lacZ*, Fig. 5*B*). (Insertion of the *lacZ* fragment in the opposite orientation results in no detectable enzyme activity.) Derepression of enzyme activity from *GCN4*$^+$-*lacZ* is abolished by the *gcn2-1* mutation (Fig. 5*B*). In addition, deletion of the 231-bp *Sau*3A/*Bst*EII fragment, containing all four small ORFs in the 5′ leader (Fig. 5*A*), leads to ≈40-fold constitutive derepression of fusion enzyme levels both in the wild type and in a *gcn2*$^-$ strain (*GCN4*Δ-*lacZ*, Fig. 5*B*).

The levels of the fusion transcripts in the same cultures assayed for β-galactosidase activity were measured by DNA·RNA hybridization blot analysis (Fig. 5*C*). The level of the *GCN4*$^+$-*lacZ* transcript derepresses in a wild-type transformant about 4-fold (relative to pyruvate kinase mRNA; see *Materials and Methods*), in parallel with derepression of the fusion enzyme. In *gcn2*$^-$ transformants, derepression of the *GCN4*$^+$-*lacZ* transcript still occurs, reaching 2-fold higher levels than in wild-type transformants; however, no increase in fusion enzyme levels is detected. For *GCN4*Δ-*lacZ*, the fusion transcript is constitutively elevated, but only to the derepressed level of the *GCN4*$^+$-*lacZ* transcript—an increase ≈10% of the 40-fold increase in the fusion enzyme resulting from this deletion.

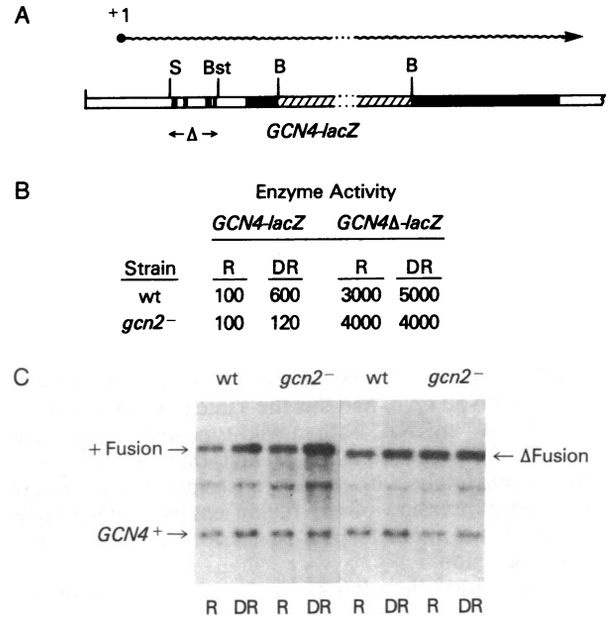

FIG. 5. Analysis of *GCN4-lacZ* fusions. (*A*) Fusion constructs. The diagram is labeled as in Fig. 2. The *lacZ Bam*HI fragment (from plasmid pMC1871, a gift from M. Casadaban) was ligated to a *Bam*HI-digested 5′-deletion plasmid (Fig. 1) lacking the pBR322 *Bam*HI site but retaining all *GCN4* sequences found in pAH38 (i.e., the deletion junction is still within pBR322 sequences; see Fig. 1). The orientation of the *lacZ* fragment was determined by mapping the *lacZ Eco*RI site with respect to pAH38 restriction sites. *GCN4*Δ-*lacZ* was constructed by isolating the large *GCN4*$^+$-*lacZ Sal* I/*Bst*EII fragment, which lacks all *GCN4* sequences upstream from the *Bst*EII site, attaching *Bgl* I linkers to the ends of the fragment, and ligating it to the −188 to +221 *GCN4 Sau*3A fragment (see Fig. 3) isolated from pAH38. Orientation of the *Sau*3A insert was determined by mapping the positions of *Sca* I and *Xmn* I sites in the *Sau*3A fragment with respect to the *GCN4 Bam*HI site at +749. (*B*) β-Galactosidase activities of *GCN4-lacZ* and *GCN4*Δ-*lacZ* transformants of TD28 (α *ura3-52 ino1*, wt) and H15 (α *gcn2-1 ura3-52 leu2-3 leu2-112*, gcn2$^-$) in minimal medium supplemented with 2 mM leucine, 0.5 mM isoleucine, 0.5 mM valine, and 0.2 mM inositol (R, repressed), and in the same medium containing 10 mM 3-aminotriazole (DR, derepressed). Growth conditions and enzyme assays were described previously (5). A *URA3-lacZ* fusion (5) shows no change in expression when assayed under the same growth conditions, both in a wild-type and in a *gcn2-1* strain (data not shown). (*C*) DNA·RNA hybridization blot analysis of *GCN4*$^+$ and fusion transcripts using 5 μg of total RNA isolated from the same cultures assayed for β-galactosidase activity. Fusion transcripts were probed with the *Bam*HI *lacZ* fragment, ^{32}P-labeled by "nick-translation" (11). Fusion transcripts show the expected 3000-bp increase in size due to insertion of the *lacZ* fragment into the *GCN4* transcription unit. The origin of the minor band between the fusion and *GCN4*$^+$ transcripts is not known; however, it has the mobility of large rRNA and therefore may be due to nonspecific hybridization of the probe to this abundant RNA. +Fusion, *GCN4*$^+$-*lacZ* fusion transcript; ΔFusion, *GCN4*Δ-*lacZ* fusion transcript.

DISCUSSION

The *GCN4* gene has an unusual DNA sequence organization that appears to underlie the regulation of its expression. An ORF of 843 nucleotides, which coincides with the *GCN4* functional unit defined genetically, is located at the 3′ end of a transcription unit 1500 nucleotides long. A *lacZ* fusion to this ORF is translated *in vivo*. There is no evidence for processing of the *GCN4* transcript during general control repressed or derepressed growth, suggesting that the GCN4

protein is made from a transcript containing a 5′ leader almost 600 nucleotides long. Four small ORFs of two or three codons are present in the 5′ leader, none of which are in-frame with the long ORF downstream, and numerous termination codons occur in all three reading frames. Deletion analysis indicates that the leader sequences are not required for *GCN4* function, at least when the remaining coding sequences are present in high copy; however, these results do not rule out a role for the leader in regulation of *GCN4* expression. In fact, an internal deletion in the leader, which eliminates the four small ORFs, abolishes regulation of the fusion enzyme and results in constitutively derepressed fusion enzyme levels ≈40-fold higher than the repressed *GCN4*$^+$-*lacZ* enzyme level. Nuclease S1 mapping has shown that the 5′ end of the *GCN4Δ-lacZ* transcript maps to within 50 bp of and has the same pattern of minor 5′ ends as *GCN4*$^+$ mRNA, both in wild-type and in *gcn2*$^-$ cells (unpublished observations). The fact that deletion of sequences completely within the transcription unit dramatically alters expression of the fusion enzyme suggests that these sequences mediate post-transcriptional regulation of *GCN4* expression.

In most eukaryotic mRNAs, including those analyzed in yeast, the 5′-proximal AUG is the initiation codon for protein synthesis (13) and internal AUGs generally do not function in translation initiation (14, 15). However, in a significant number of animal cell mRNAs, the initiator codon is not the 5′-proximal AUG (13), showing that internal AUGs can be recognized by higher eukaryotic ribosomes. The insertion of upstream AUGs can significantly reduce the efficiency of initiation downstream; conversely, the elimination of upstream AUGs can result in more efficient translation of a downstream ORF (reviewed in ref. 15).

An internal deletion of the multiple ATG codons in the 5′ leader of *GCN4* derepresses expression of both the fusion enzyme and the steady-state levels of the fusion transcript. However, the derepression of the GCN4Δ-lacZ enzyme is an order of magnitude greater than the increase in the *GCN4Δ-lacZ* transcript. This suggests that the upstream AUGs in GCN4 mRNA repress the efficiency of translation initiation at the long ORF downstream. This may provide a mechanism for regulating *GCN4* expression in response to amino acid availability if the degree of inhibition exerted by the upstream AUGs is lessened by amino acid starvation. The data in Fig. 5 suggest that the *GCN2* product mediates this increase in translation efficiency. However, two additional facts must be considered: (*i*) the increase in the *GCN4*$^+$-*lacZ* enzyme in the wild type is paralleled by a similar increase in the steady-state level of the fusion transcript; (*ii*) the *GCN4*$^+$ transcript is not regulated to the same degree as the *GCN4*$^+$-*lacZ* transcript (2-fold derepression vs. 4-fold derepression; Fig. 5 and unpublished data). One interpretation of these two facts is that the observed regulation of the GCN4$^+$-lacZ enzyme in the wild type only reflects changes in fusion transcript levels and that the inserted *lacZ* sequences preferentially increase the stability of the *GCN4*$^+$-*lacZ* transcript during starvation conditions. However, in a *gcn2*$^-$ strain, an even greater increase in the *GCN4*$^+$-*lacZ* transcript is not sufficient to derepress synthesis of the fusion enzyme, arguing that *GCN2* function is required for efficient translation of the *GCN4*$^+$-*lacZ* transcript irrespective of the transcript level. Although the underlying mechanism determining fusion transcript levels is not known, it is clear that roughly the same levels of fusion transcript produced by derepressed *GCN4*$^+$-*lacZ* and by *GCN4Δ-lacZ* give rise to substantially different amounts of enzyme. The *GCN4Δ-lacZ* transcript is translated ≈6-fold more efficiently than the derepressed *GCN4*$^+$-*lacZ* transcript. [It is not clear why derepressed GCN4$^+$-lacZ enzyme levels are so much lower than GCN4Δ-lacZ enzyme levels. Perhaps the starvation regime (6 hr in 10 mM 3-aminotriazole) is not sufficient to fully derepress *GCN4* expression.] Further experiments are required to determine if the *gcn2*$^-$ mutation and the *Sau*3A/*Bst*EII deletion have the same effects on expression of the wild-type GCN4 protein.

The pattern of regulation exhibited by *GCN4*$^+$-*lacZ* is consistent with the regulatory model suggested previously from genetic data (7), which predicts that the level of GCN4 activity is increased by amino acid starvation and that this increase is dependent on *GCN2* function. The data in Fig. 5 suggest that this regulation occurs by an increase in the synthesis of GCN4 protein. *GCN2* is believed to act indirectly by antagonism of the *GCD1* repressor function, which in turn is expected to negatively regulate *GCN4* (7). Our results suggest that the *GCD1* product acts via a *cis*-acting negative control site in the GCN4 mRNA 5′ leader to repress GCN4 translation, since removal of this site from the leader makes *GCN2* function dispensable for derepression of *GCN4* (Fig. 5).

The GCN4 protein is currently the best candidate for the activator that interacts with the sites of positive control at general control structural genes. Our data indicate that the NH_2-terminal portion of GCN4 protein is dispensable for this function (at least when deletion constructs are present in multiple copies). It is interesting that the COOH-terminal end of GCN4, which is essential for regulatory function, is rich in basic amino acids (≈30% lysine, arginine, and histidine)—a feature consistent with DNA binding.

Noted Added in Proof. Similar results concerning the regulatory role of the *GCN4* mRNA 5′ leader were recently obtained by Thireos *et al.* (16).

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