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Characterization of *NGG1*, a novel yeast gene required for glucose repression of GAL4p-regulated transcription

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The *GAL1-10* genes of *Saccharomyces cerevisiae* are regulated by the interaction of *cis-* and *trans-*acting factors which facilitate activated transcription in galactose but not in glucose medium. By selecting mutations that allow expression of a defective *gal1-10–his3* hybrid promoter, we have identified a novel gene, *NGG1*, which is required for glucose repression of the *GAL10*-related *his3*-G25 promoter. *ngg1* was identified as a recessive null mutation that in the presence of a *gal80* background resulted in a 300-fold relief of glucose repression for the *his3*-G25 promoter. This compared with a 20-fold and negligible relief of repression in *gal80* and *ngg1* strains, respectively. Deletion analysis of the *his3*-G25 promoter showed a correlation between the number of GAL4p binding sites and the relative level of NGG1p activity. Relief of glucose repression by *NGG1* was dependent on the presence of *GAL4*, but was independent of the *GAL4* promoter. In addition, NGG1p activity was seen for a promoter construct containing independent GAL4p binding sites. These results suggest that NGG1p acts to inhibit GAL4p function in glucose medium. We have cloned *NGG1* by complementation and found that it contains an open reading frame of 2106 bp which could encode a protein with a molecular weight of 79 230.

*Key words: ADA3/GAL4/GAL80/*glucose repression/transcription regulation

Introduction

Expression of the genes required for galactose metabolism in *Saccharomyces cerevisiae* is closely regulated by the available carbon source. In galactose, gene expression is induced through upstream activator sequences (UAS_G) that bind the regulatory protein, GAL4p (Johnston, 1987). In the absence of galactose, GAL4p binds to UAS_G but is prevented from activating transcription by interaction with GAL80 (Johnston *et al.*, 1987; Lue *et al.*, 1987; Ma and Ptashne, 1987a; Chasman and Kornberg, 1990; Parthun and Jaehning, 1990). In galactose this GAL4p–GAL80p complex becomes a potent activator in a process that requires the *GAL3* gene product (Torchia and Hopper, 1986; Bhat *et al.*, 1990). Recent data suggest that inactive GAL3p is converted to active GAL3p which conformationally alters, but not necessarily disrupts (Leuther and Johnston, 1992), the GAL4p–GAL80p complex to allow the activation of gene expression (Bhat and Hopper, 1992). Superimposed on this regulation is the global regulatory circuit of carbon catabolite repression. Growth on glucose represses the *GAL* genes and imposes the selective utilization of glucose in preference to other carbon sources (Gancedo, 1992; Johnston and Carlson, 1992; Trumbly, 1992). The *GAL* genes thus serve as a model to study the interplay of regulatory factors in a dynamic gene activation/repression system.

Four mechanisms have been described for the direct repression of *GAL* gene expression by glucose. The first operates through glucose responsive negative regulatory elements (URS_G elements) found within the *GAL* genes. Several such elements have been characterized in the divergently transcribed *GAL1-10* promoter (Struhl, 1985a; West *et al.*, 1987; Finley *et al.*, 1990; Flick and Johnston, 1990; Lamphier and Ptashne, 1992). In addition, several gene products have been characterized which are necessary for the activity of these URS_G elements. MIG1p (Nehlin and Ronne, 1990) binds to the *GAL1* proximal element URS_G A (Nehlin *et al.*, 1991; Flick and Johnston, 1992; Lamphier and Ptashne, 1992) and probably, by functioning in a complex with SSN6p and TUP1p (Rothstein and Sherman, 1980; Carlson *et al.*, 1984; Trumbly, 1986; Schultz and Carlson, 1987; Williams and Trumbly, 1990; Williams *et al.*, 1991; Keleher *et al.*, 1992), mediates the repression observed through this site. Three *URR* genes (*URR1, URR3* and *URR4*) are also required for repression mediated through URS_G A and a second site, URS_G C (Flick and Johnston, 1990, 1992).

The three additional mechanisms for direct repression of the *GAL* genes by glucose act through the regulation of GAL4p activity or expression, and can be grouped under the term UAS_G repression. The first of these results from the downregulation of the *GAL4* promoter ~5-fold in glucose medium (Griggs and Johnston, 1991; Nehlin *et al.*, 1991), which is magnified to result in a decrease in *GAL* gene transcription of up to 100-fold (Griggs and Johnston, 1991; Nehlin *et al.*, 1991; Lamphier and Ptashne, 1992). Similar to URS_G repression, this repression of the *GAL4* promoter is mediated through a MIG1p binding site and also requires SSN6p and TUP1p (Griggs and Johnston, 1991; Nehlin *et al.*, 1991). Although controversial, a second mechanism appears to act via GAL80p. Lamphier and Ptashne (1992) observed a 25-fold derepression of *GAL1* expression in glucose medium in a strain lacking *gal80*, and where transcriptional regulation of *GAL4* was eliminated. Similar to the two mechanisms discussed above, this GAL80p-dependent repression is regulated in a pathway that includes the SNF1p protein kinase (Celenza and Carlson, 1986; Schuller and Entian, 1987; Schultz and Carlson, 1987; Griggs and Johnston, 1991; Flick and Johnston, 1992; Lamphier and Ptashne, 1992). Recently, an additional UAS_G repression mechanism has been described which involves the direct inhibition of GAL4p (Stone and Sadowski, 1993). The central region of the GAL4 protein, located

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between the two characterized activation domains (Ma and Ptashne, 1987b), contains a glucose response domain and three inhibitory domains which confer glucose-responsive inhibition of GAL4p activity (Stone and Sadowski, 1993).

Many additional genes have been characterized which are required for the glucose repression of the *GAL* genes as well as their release from repression (reviewed in Johnston and Carlson, 1992). Some of these almost certainly play a general signalling function. Potential candidates for such roles are *REG1* and *HXK2,* since mutations in these genes also influence glucose repression of the *SUC2, MAL* and *CYC1* genes (reviewed in Johnston and Carlson, 1992; Entian and Barnett, 1992; Gancedo, 1992; Trumbly, 1992). This role is also supported by genetic studies which have shown that mutations within the *snf* genes are epistatic to both *hxk2* and *reg1* (Entian and Zimmerman, 1982; Neigeborn and Carlson, 1984, 1987). Other genes such as *GAL82* and *GAL83* appear to be specific for the glucose repression of the *GAL* genes (Matsumoto *et al.*, 1981, 1983; Flick and Johnston, 1990). Interestingly, the recent cloning of *GAL83* (Erickson and Johnston, 1993) has shown that GAL83p has homology to SIP1p (Yang *et al.*, 1992) which directly interacts with SNF1p.

Previously, we characterized a chimeric *gal1-10–his3* (*his3*-GG1) promoter which contains a GCN4 binding site located at the approximate position of the *GAL10* TATA element (Chen and Struhl, 1989; Brandl and Struhl, 1990; Brandl *et al.*, 1992). Unlike related *gal1-10–his3* chimeras (Chen and Struhl, 1988) that lack the GCN4 binding site, *his3*-GG1 is expressed in glucose medium. Expression in glucose medium requires the GCN4 binding site, an upstream REB1p binding site and a cryptic TATA element located proximal to the GCN4 binding site (Brandl and Struhl, 1990; Brandl *et al.*, 1992). The latter two elements are found within *GAL1-10* sequences. We have constructed derivatives of *his3*-GG1 that contain strong TATA elements (for example, *his3*-GZ-J18, see Figure 1; Brandl *et al.*, 1992). These derivatives are expressed in glucose medium because of their relationship to *his3*-GG1, but are also expressed at a high level in galactose medium because of the upstream GAL4p binding sites and their structural similarity to the *GAL10* promoter. This would suggest that while the *his3*-GG1 and *his3*-GZ-J18 promoters are expressed in glucose medium, they are also subject to glucose repression. Indeed, through the analysis of suppressor mutations that facilitate the expression of a nonfunctional *his3*-GZ-J18 derivative, we have characterized a novel gene, *NGG1,* which is required for the glucose repression of GAL4p-regulated genes. Our results support a model whereby NGG1p acts to limit transcriptional activation by GAL4p in glucose medium.

Results

The promoter of the chimeric gene, *his3*-GZ-J18, contains *GAL1-10* regulatory sequences flanked downstream by a GCN4p binding site (Figure 1; Chen and Struhl, 1989; Brandl and Struhl, 1990; Brandl *et al.*, 1992). Expression of this promoter in glucose medium is independent of GAL4p but requires three regulatory elements: a GCN4p binding site, a REB1p binding site and a TATA element located upstream of the GCN4 binding site (Brandl and Struhl, 1990; Brandl *et al.*, 1992). Surprisingly, when we converted the GCN4p binding site from the *HIS3* wild-type sequence (TGACTCT) to the optimal binding site (TGACTCA; *his3*-GG579), transcription dropped to an undetectable level (see Figure 2). This is unlike the *HIS3* wild-type promoter where transcription is enhanced ~3-fold when the GCN4p binding site is optimized (Hill *et al.*, 1986). We chose to identify the molecules relevant to this phenomenon by selecting for mutants that would facilitate expression of *his3*-GG579. Yeast strain CY579 (Table I), which contains

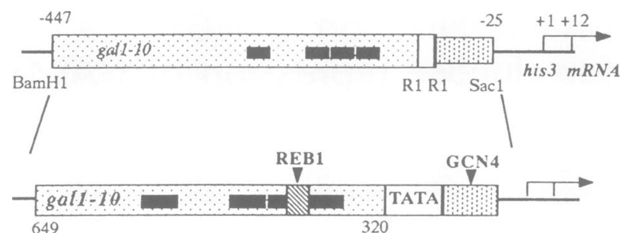

Fig. 1. Structure of *his3*-GZ-J18 and *his3*-GG579. *his3*-GZ-J18 and *his3*-GG579 contain *GAL1-10* sequences from 649 to 320 (Johnston and Davis, 1984) with four GAL4p binding sites (Giniger *et al.*, 1985; Bram *et al.*, 1986; dark boxes). This region has been fused between −447 and −25 of the *his3* promoter which has +1 and +12 start sites of transcription (Struhl, 1985b; shown numbered in the upper portion of the figure). *his3*-GZ-J18 contains the wild-type *his3* GCN4 binding site (TGACTCT) which activates transcription of this promoter in glucose medium; *his3*-GG579 contains the optimal GCN4p binding site (TGACTCA) which is nonfunctional in the context of this promoter. Both alleles also contain an *Eco*RI fragment directly upstream of the GCN4p binding site which includes a functional TATA element sequence TATAAA (Brandl *et al.*, 1992). This TATA element enables the GAL4p induced transcription of both *his3*-GZ-J18 and *his3*-GG579 promoters in galactose and transcription of *his3*-GZ-J18 in glucose medium. The REB1p binding site (striped box) is also required for transcription of *his3*-GZ-J18 in glucose medium. This element overlaps the second most proximal GAL4p binding site and is positioned ~130 bp from +1.

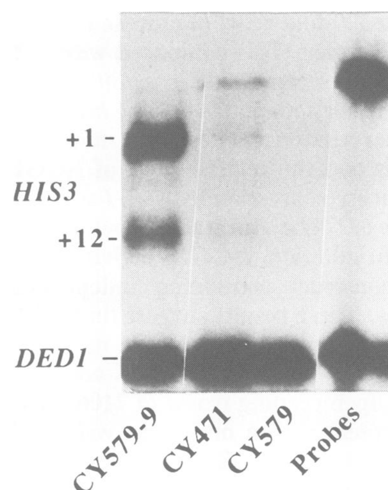

Fig. 2. RNA analysis from wild-type and mutant strains. RNA was prepared from the indicated yeast strain grown in minimal medium containing 2% glucose. For each sample, 25 μg of total RNA were hybridized to *HIS3* and *DED1* 5′ end-labelled oligonucleotide probes (Hill *et al.*, 1986; Chen and Struhl, 1988), digested with S1 nuclease and after separation on a denaturing acrylamide gel, subjected to autoradiography. Strains CY579-9 and CY579 contain the *his3*-GG579 allele with the optimal GCN4p binding site in mutant and wild-type backgrounds, respectively. CY471 contains the *his3*-GZ-J18 allele *his3* GCN4p binding site in the wild-type KY322 background. The probes lane is a 1/100 dilution of the undigested *HIS3* and *DED1* probes.

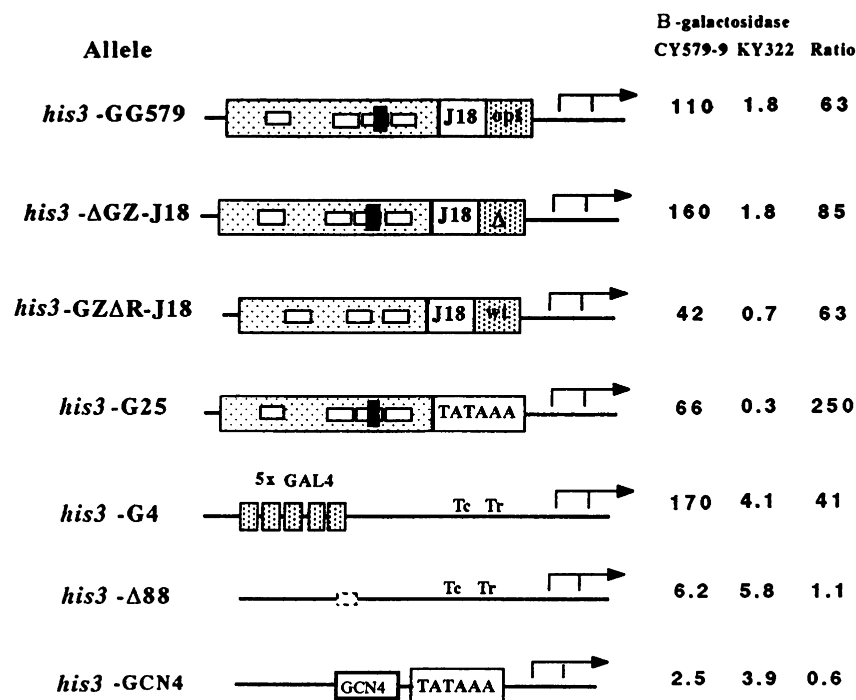

Fig. 3. Promoter specificity of gene expression in CY579-9. The structure of each promoter is shown with the elements shaded in the same scheme as in Figure 1. *his3*-GG579 and *his3*-GZΔR-J18 contain optimal (opt) and *his3* wild-type (wt) GCN4p binding sites, respectively. This latter derivative contains a linker disruption of the REB1p binding site. *his3*-ΔGZ-J18 contains a mutation of the GCN4p binding site (Δ) that converts it to the nonfunctional sequence, GGACTCT. These three J18 alleles all contain the J18 Z element sequence which includes the TATA element sequence, TATAAAA (Brandl *et al.*, 1992). *his3*-G25 lacks the downstream GCN4p binding sites and contains *GAL1-10* sequences from 299 to 649 (Johnston and Davis, 1984) as well as a TATA element sequence, TATAAA. *his3*-Δ88 is a derivative of the wild-type *his3* promoter that contains an insertion of an *Eco*RI–*Sac*I linker in the place of the GCN4 binding site (Hill *et al.*, 1986). *his3*-G4 contains five GAL4 binding sites inserted at this position. *his3*-GCN4 contains the *his3* promoter upstream of −80, including the GCN4 binding site, fused directly to a TATA element. Centromeric plasmids containing these promoters as *lacZ* fusions were transformed into yeast strains CY579-9 and KY322 and the β-galactosidase activity determined. The ratio of β-galactosidase activity (CY579-9/KY322) is also shown.

the *his3*-GG579 allele, was mutagenized and plated on minimal plates containing glucose and 10 mM aminotriazole (AT), a competitive inhibitor of the *HIS3* gene product, to select for colonies expressing elevated levels of *HIS3*. Twenty-four strains were isolated and transformed with a *lacZ* fusion derivative of the *his3*-GG579 allele to verify that growth on 10 mM AT was the result of an unlinked mutation that gave rise to increased transcription. One strain, CY579-9, was identified which upon S1 analysis was confirmed to have significantly elevated transcription of *his3*-GG579 when grown in glucose medium (Figure 2).

The allele specificity of the enhanced expression seen in CY579-9 in glucose medium was examined with *lacZ* fusion derivatives of *his3* alleles differing in their upstream activator sequences (Figure 3). Enhanced expression in CY579-9 compared with the wild-type strain KY322 was observed for all derivatives containing GAL4p binding sites, including an allele which contained five GAL4p binding sites in the context of the *his3* promoter (*his3*-G4). Enhanced expression was independent of GCN4p (compare *his3*-GG579 with *his3*-ΔGZ-J18 and *his3*-G25) and REB1p binding sites (compare *his3*-GG579 with *his3*-GZΔR-J18), both of which are required for *his3*-GZ-J18 expression. Expression was assayed for two promoters that lack GAL4p binding sites, *his3*-Δ88 and *his3*-GCN4. The latter of these closely resembles the *his3*-GG579 and *his3*-G25 promoters, but is activated by GCN4p rather than GAL4p. No change in expression was observed for *his3*-Δ88; but interestingly, in contrast to the GAL4p-activated promoters, expression of *his3*-GCN4 was slightly reduced in the mutant strain.

Structurally and functionally, the *his3*-GG579 promoter closely resembles the *GAL10* promoter. In galactose medium the *his3*-GG579 promoter is activated by GAL4p in conjunction with the downstream TATA element (Brandl *et al.*, 1992). In wild-type strains this promoter should also be subject to the negative regulatory mechanisms imposed on the *GAL10* promoter. Expression of the *his3*-GG579 allele in yeast strain CY579-9 in glucose and in raffinose media approached the level observed for the promoter in the wild-type strain, KY322, in galactose medium (65 and 70%, respectively). This, and the lack of a requirement for GCN4p and REB1p binding sites, strongly suggested that we had selected a mutation(s) that allowed the normal regulatory mechanisms imposed by glucose and galactose on the *GAL10*-like *his3*-GG579 promoter to be bypassed, rather than selecting for a mutation that suppressed the repression due to the optimal GCN4p binding site.

To determine the number of genetic loci involved with the growth of CY579-9 on plates containing 10 mM AT and glucose (as a measure of increased expression of *his3*-GG579), this strain was backcrossed to wild-type (CY323) and the diploid was subjected to tetrad analysis. The analysis of spores for growth on 10 mM AT plates showed that AT resistance segregated 22:40:22 or ~1:2:1 (growth:poor growth:no growth). Analysis of 21 tetrads yielded the following ascal types: (parental ditype, PD) = 5 (nonparental ditype, NPD) = 4, (total, T) = 12. These distributions are indicative of two unlinked mutations

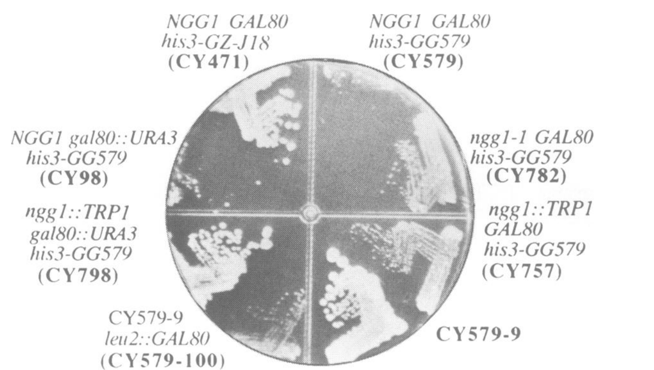

Fig. 4. Growth of wild-type and mutant yeast strains containing *his3*-GG579. The indicated yeast strains were streaked onto minimal plates containing 10 mM AT and 2% glucose and grown at 30°C for 5 days. All strains contain *his3*-GG579 (GCN4 binding site sequence, TGACTCA) except for CY471 which contains *his3*-GZ-J18 (GCN4p binding site sequence, TGACTCT) in the wild-type background. Relevant genotypes of the strains are: CY579 (wild-type), CY782 (*ngg1-1*), CY757 (*ngg1::TRP1*), CY798 (*ngg1::TRP1 gal80::URA3*), CY98 (*gal80::URA3*). CY579-100 has been constructed from the mutant strain CY579-9 by integration of *GAL80* at *leu2*.

contributing to the phenotype. Both mutations are recessive; diploids from the backcross of CY579-9 and CY323 did not grow on plates containing 10 mM AT and glucose.

GAL80 will complement one of the mutations in CY579-9

The virtually complete transcriptional activation of *his3*-GG579 in yeast strain CY579-9 in glucose medium suggested that GAL80p was not inhibiting GAL4p function in this strain. To test whether there was a defect in GAL80p or in its synthesis, we introduced *GAL80* into CY579-9 by gene replacement at *leu2*. The resulting strain CY579-100 grew significantly slower on plates containing 10 mM AT and glucose than CY579-9 (Figure 4). The rate of growth was in fact very similar to that of CY757 and CY782, strains containing null *ngg1* alleles (see below). This strongly suggests that a *gal80* mutation is one of the two mutations that facilitates growth of CY579-9 on 10 mM AT.

Cloning *NGG1*

To clone the additional gene responsible for the CY579-9 phenotype, this strain containing the reporter plasmid *his3*-GG579LacZ, was transformed with a library of yeast genomic sequences in the centromeric vector pINT2 (Percival-Smith and Segall, 1986) and replica plated onto plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). Eight white colonies were isolated and found to contain the identical plasmid, pB736. The active gene, *NGG1*, was localized to a 2.7 kb *Sac*I–*Pst*I fragment of pB736 by subcloning into YCplac33 (Gietz and Sugino, 1988). This plasmid inhibited growth of CY579-9 on 10 mM AT plates containing glucose and reduced expression of the *his3*-G25 allele (*GAL1-10* UAS plus TATA element; see Figure 3) 4-fold as determined from β-galactosidase assays (data not shown). The nucleotide sequence of the 2.7 kb fragment was determined and found to contain an open reading frame of 2106 bp that could encode a protein of 79 230 starting with a 5′ proximal ATG codon at nucleotide 166 (Figure 5). The equivalent fragment was cloned from a genomic library made from the mutant strain CY579-9.

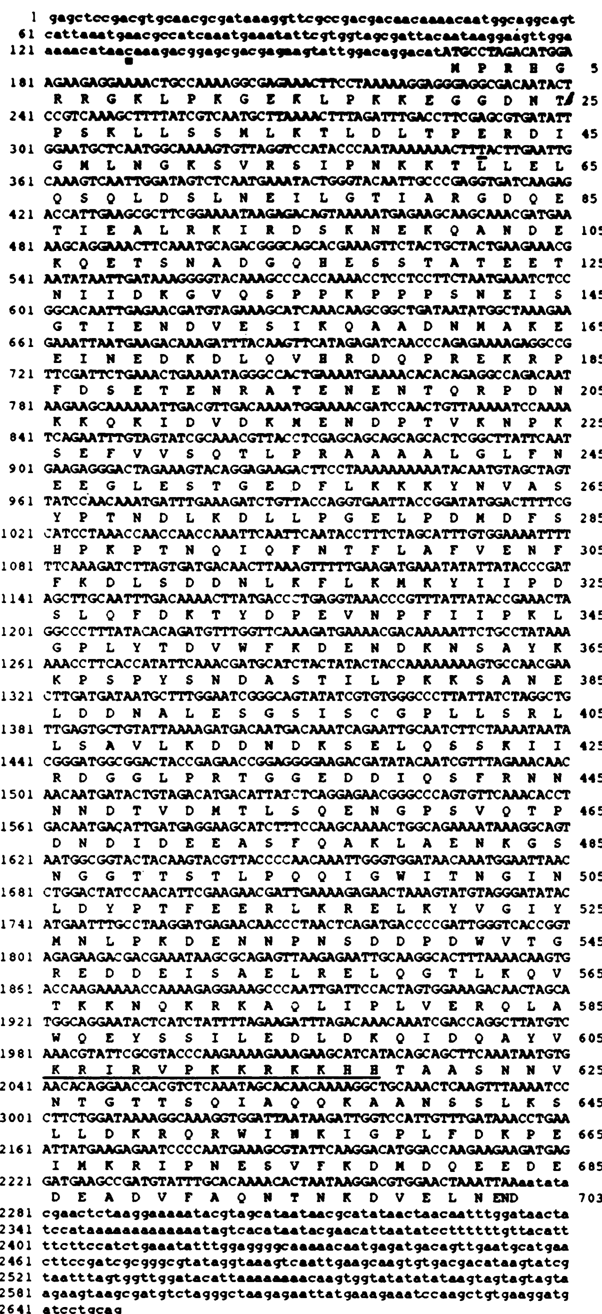

Fig. 5. *NGG1* sequence. The nucleotide sequence of the 2650 bp *Sac*I–*Pst*I fragment which contains the *NGG1* gene product is shown numbered on the left side from the *Sac*I site along with the putative protein product (numbered on the right) which begins with the ATG initiation codon at nucleotide position 166. The 5′ end of the major transcript at nucleotide 130 is underlined as is the A to T transversion which converts Leu62 to a stop codon in the *ngg1-1* and a putative nuclear localization signal from amino acids 606–618. The sequence has been deposited in the GenBank database under the accession number L12137.

As expected, this allele *ngg1-1* was nonfunctional in complementation assays and sequence analysis identified a T to A transversion at nucleotide 350 which converts Leu62 to a stop codon. Two additional experiments were performed to support the conclusion that the functional protein is contained within the indicated coding region. First, disrupting the reading frame at the *Hin*dIII site at nucleotide 247 resulted in a molecule with no NGG1p activity. Second, we subcloned the fragment containing the initiator ATG to

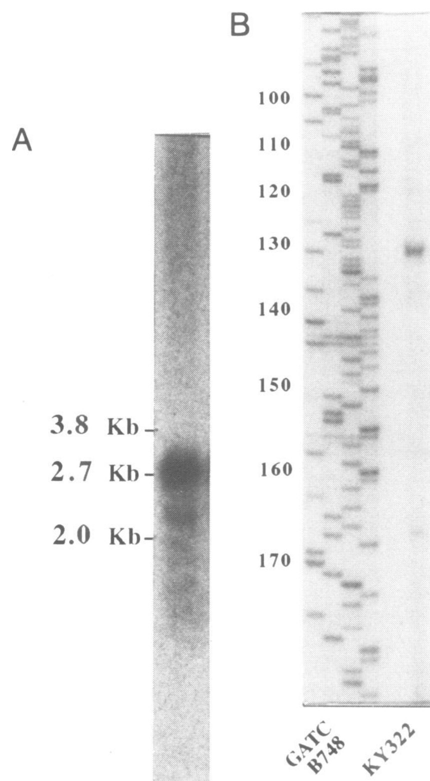

Fig. 6. Molecular analysis of *NGG1*. (**A**) Northern analysis. Total RNA from yeast strain KY322 was electrophoretically separated on a denaturing RNA gel and after transfer to nitrocellulose, hybridized to a radiolabelled fragment containing *NGG1* sequences from 560 to 918. Ribosomal RNAs were used as size markers and are indicated as 3.8 and 2.0 kb as is the major transcript of 2.7 kb. (**B**) Primer extension. Total RNA from yeast strain KY322 was annealed to a 5′ end-labelled primer of 20 bases beginning at nucleotide 242 and extended with reverse transcriptase. The cDNA products were separated on a denaturing acrylamide gel and their mobility compared with a sequencing ladder on *NGG1* (B748) synthesized from the same primer. The numbering scheme indicates the nucleotide position as in Figure 5.

the *Sna*BI site (nucleotide 2000) downstream of the *DED1* promoter in the centromeric plasmid YCP88 (Hope and Struhl, 1986). This molecule prevented growth of CY579-9 on plates containing 10 mM AT and 2% glucose. Furthermore, a similar construct cloned in the inverse orientation and a molecule with a deletion of 140 amino acids at the carboxyl-terminus of the protein did not repress growth of CY579-9 on 10 mM AT plates.

To verify the linkage of the cloned DNA to that responsible for the CY579-9 phenotype, the *ngg1-1* allele was cloned into YIplac211 (Geitz and Sugino, 1988) and integrated into CY579-9 to generate a strain with the endogenous *ngg1-1* allele linked to *URA3*. A Ura^+ transformant was crossed with the wild-type strain CY323. Tetrad analysis of the resulting diploid showed that all spores that grew at a rate equivalent to CY579-9 on plates containing 10 mM AT and glucose were Ura^+.

Molecular analyses of NGG1

Primer extension analysis identified the principal transcriptional start site for *NGG1* to be at nucleotide 130 (Figure 6A). Additional minor starts (or pause sites) were identified at nucleotide 166 (the A of the initiator Met) and downstream of the translational start site. To analyze the transcripts from *NGG1* by Northern analysis (Figure 6B), hybridization probes were prepared from the *Rsa*I−*Rsa*I restriction fragment contained in nucleotides 560−918 (Figure 7B). A major transcript of ~2.7 kb and a minor transcript of ~2.1 kb were identified under conditions of high stringency. Both messages are sufficient in length to code for the intact protein. Whether the two messages represent 5′ or 3′ heterogeneity in the message is currently being evaluated. The additional message is probably not the result of a related gene, as genomic blots performed at moderate stringency detect only a single hybridizing band (C.J.Brandl, unpublished result).

A database search through the GenBank and EMBL libraries identified the 5′ end of *UBC1* (Seufert *et al.*, 1990) at the 3′ end of *NGG1*. The initiator Met of *UBC1* is located 317 bp downstream of the *NGG1* stop codon. We have localized these genes to chromosome IV by hybridization to the mapped genomic clones of Olson *et al.* (1986). This agrees with a tentative localization of *UBC1* to chromosome IV (W.Seufert, personal communication). *NGG1* hybridizes to ATCC clone number 70352 which positions it between *ARO1* and *RAD9* (L.Riles, personal communication). A further search of the database with the Blast program (Altschul *et al.*, 1990) revealed no obvious relationship between NGG1p and other proteins or translated DNA sequences.

Disruption of NGG1

To assess more directly the physiological role of NGG1p, we generated DNA molecules in which a 659 bp region within the amino-terminal half of *NGG1* was replaced with *TRP1*. This DNA was introduced into yeast cells by gene replacement to generate strains lacking *NGG1* (confirmed by PCR). CY757 (*ngg1* deletion strain with the *his3*-GG579 allele), grew at approximately one-third the rate of the wild-type strain KY322 on rich media containing either glucose, galactose or raffinose. Therefore, excluding the possibility that a functional domain of the protein remains and is expressed, NGG1p is not essential for cell viability. This had been suspected due to the nature of the *ngg1-1* allele. We also noticed that liquid cultures of CY579-9 and CY757 were difficult to form pellets during low speed centrifugation. Comparison of these strains by light microscopy revealed that the mutant cells were generally found in clusters and were slightly enlarged relative to the wild-type strain KY322.

In support of the genetic analysis showing a *gal80* and *ngg1* component in the original strain (CY579-9), an *ngg1 gal80* double mutant (CY798) grew at a rate identical to CY579-9 on 10 mM AT plates containing 2% glucose (Figure 4). Furthermore, the allele specificity for enhanced expression in CY798 was identical to that for the original mutant strain CY579-9 (see Figure 3). Disruption of *ngg1* alone (strains CY757 and CY782) allowed for a slightly enhanced rate as compared with the wild-type equivalent CY579. Interestingly, while disruption of *gal80* alone (CY98) had little effect on growth, spontaneous suppressor mutations were reproducibly found at high abundance when CY98 was plated on 10 mM AT. This suggests that many genes can function with *gal80* to relieve the glucose repression on this promoter (see Griggs and Johnston, 1991; Nehlin *et al.*, 1991; Lamphier and Ptashne, 1992).

To quantitate the effect of NGG1p on the transcription of *GAL10*, *his3*-G25 (*GAL1-10* UAS) as a *lacZ* fusion was introduced into strains containing single or double mutations

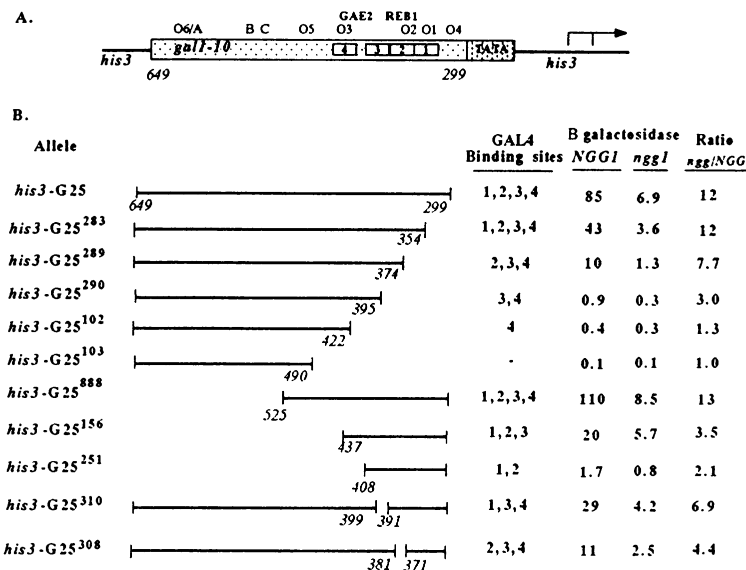

Fig. 7. NGG1p functions in the absence of a URS_G element. (**A**) Structure of the *his3*-G25 allele. The four GAL4p binding sites are boxed and numbered consecutively from *GAL10* proximal to *GAL10* distal. The approximate positions of operator sequences defined by West *et al.* (O1–O6; West *et al.*, 1987; Finley *et al.*, 1990) and Flick and Johnston (URS A, B, C; Flick and Johnston, 1990, 1992) are indicated as is the REB1p binding site (Finley and West, 1989; Brandl and Struhl, 1990; Chasman *et al.*, 1990) and the galactose-independent activating element GAE_2 (Finley and West, 1989). The deletion derivatives shown below have 5′ and/or 3′ endpoints within *GAL1-10* sequences at the positions indicated in italics. The numbering scheme is that of Johnston and Davis (1984). (**B**) Expression of *lacZ* fusion alleles in *ngg1:TRP1* (CY798) and *NGG1* (CY98) strains. Centromeric plasmids containing the indicated promoters as *lacZ* fusion were transformed into yeast strains CY798 and CY98 and the β-galactosidase activity determined. The ratio of β-galactosidase activity (CY798/CY98) as a measure of NGG1p repression for each allele is shown. To assist in localizing the endpoints of each deletion, the GAL4p binding sites contained within each promoter is also indicated.

Table I. Expression of *gal/his3* alleles in *ngg1* and *gal80* strains

		β-galactosidase activity				
Allele	Carbon source	KY322 (*NGG1 GAL80*)	CY757 (*ngg1 GAL80*)	CY98 (*NGG1 gal80*)	CY798 (*ngg1 gal80*)	Ratio (CY798/CY98)
his3-G25	Glucose	0.3	0.3	6.4	88	14
his3-G25	Galactose	199	360	230	620	2.6
his3-G25	Glu plus gal	0.3	0.3	9.2	110	12
his3-G4	Glucose	1.5	0.4	290	640	2.2
his3-G4	Galactose	780	950	940	870	0.9

Centromeric plasmids containing the *his3*-G25 and *his3*-G4 promoters as *lacZ* fusions were transformed into yeast strains KY322 (wild-type), CY757 (*ngg1::TRP1*), CY98 (*gal80::URA3*) and CY798 (*ngg1::TRP1*; *gal80::URA3*). Cells were grown in medium containing 2% glucose, 2% galactose or 1% glucose plus 1% galactose and the β-galactosidase activity determined. Values presented represent the averages of two experiments performed in triplicate with a standard error of <30%. The far right column is the ratio of β-galactosidase activity in strains CY798 versus CY98. The structures of *his3*-G25 and *his3*-G4 are shown in Figure 3.

of *ngg1* and *gal80* (Table I). Disruption of *ngg1* in the presence of *GAL80* (CY757) did not result in a significant increase in expression of *his3*-G25 in glucose or glucose plus galactose media. This contrasts with the apparent increase for the similar *his3*-GG579 allele in CY757, as noted from growth on 10 mM AT plates (Figure 4) and suggests that differences in strain background can affect growth rate on 10 mM AT. In a *gal80* background, disruption of *ngg1* resulted in an increase in transcription of >10-fold in glucose or glucose plus galactose media (compare CY798 with CY98). Therefore, the effects of *ngg1* mutant alleles on the *his3*-G25 promoter, are only observable under conditions in which GAL4p is active. Since disruption of *gal80* alone contributed >20-fold to the relief of glucose repression, the total relief of glucose repression in the double mutant strain was ~300-fold (compare CY798 with the wild-type strain KY322). In galactose medium, for both *gal80* (CY798 versus CY98) and *GAL80* (CY757 versus KY322) backgrounds, disruption of *ngg1* resulted in a 2- to 3-fold increase in expression of *his3*-G25. The effects of NGG1p are thus not strictly glucose-specific for the *his3*-G25 promoter.

NGG1p activity acts through GAL4p binding sites

Mechanisms of glucose repression of *GAL*-regulated genes has been divided into two pathways, URS_G and UAS_G (Flick and Johnston, 1990). In its simplest terms, UAS_G repression results from inhibition of GAL4p activity and should be detected by reduced expression from independent GAL4p binding sites. URS_G repression requires specific operator sequences that can be mapped and function independently to mediate repression. To determine whether NGG1p repression could be mediated through isolated GAL4p binding sites, an allele (*his3*-G4; see Figure 3) that contains five GAL4p binding sites in the context of the *HIS3* TATA elements (Struhl, 1986), was also examined in the mutant backgrounds in glucose and galactose media (Table I); comparison with the results for the *his3*-G25 promoter is somewhat complicated because the *his3*-G4 promoter is

subject to significantly less *GAL80*-independent glucose repression than is *his3*-G25, 3.2-fold versus 36-fold (compare ratios of CY98 in glucose with galactose for *his3*-G4 and *his3*-G25). However, since the *his3*-G4 promoter showed a 2.2-fold increase in expression in the *nggl gal80* background (CY798) compared with the *gal80* background (CY98), relief of 61% of the total repression, we conclude that NGG1p exerts its effects through the *GAL4* UAS. In contrast to *his3*-G25, no significant effect was seen for *his3*-G4 in galactose medium. Interestingly, the expression of *his3*-G4 was decreased ~4-fold in glucose medium in the *nggl* mutant strain CY757 compared with the wild-type strain KY322. This decrease requires the presence of the GAL4p binding sites since it was not observed in the related promoter *his3*-Δ88 (Figure 3; data not shown) and suggests that *NGG1* may play a more complex role in *GAL* gene regulation than glucose repression of *GAL1-10*.

To eliminate further the possibility that NGG1p acts through a specific URS_G but rather through UAS_G, deletion derivatives of *his3*-G25 fused to *lacZ* were assayed for expression in the *gal80* strains CY98 (*NGG1*) and CY798 (*nggl::TRP1*), in an attempt to localize an NGG1p-responsive operator sequence. The structure of the mutant alleles and the expression seen in CY798 and CY98 is shown in Figure 7. Rather than defining a precise region required for NGG1p we have found that the GAL4p binding sites are critical for NGG1p function. In this analysis disruption of *nggl* resulted in a 12-fold relief of repression for an intact *GAL1-10* region. A 3′ deletion of *GAL1-10* to nucleotide 354 (*his3*-$G25^{283}$; numbering as in Johnston and Davis, 1984) did not alter this ratio. However, deletion into the first GAL4p binding site resulted in a reduction in the *nggl* response (*his3*-$G25^{289}$). Progressive loss of GAL4p binding sites 2, 3 and 4 resulted in an increasingly diminished effect of *nggl* (*his3*-$G25^{290}$, $G25^{102}$ and $G25^{103}$). Similarly, the deletion of 5′ *GAL1-10* sequences to nucleotide 525 (*his3*-$G25^{888}$), including the upstream MIG1p binding site (Griggs and Johnston, 1991; Nehlin *et al.*, 1991), did not alter the *nggl* response; but the loss of GAL4p binding site 4 (*his3*-$G25^{156}$) resulted in a decrease in the NGG1p response. Furthermore, the disruption alleles *his3*-$G25^{308}$ and *his3*-$G25^{310}$, which delete only GAL4p binding sites 1 and 2, respectively, result in a significant decrease in the *nggl* response. While not totally eliminating the possibility that a NGG1p responsive operator exists, these results show that deletion or disruption of any of the GAL4p binding sites diminishes the *nggl* effect. This agrees with GAL4p being the target for NGG1p action.

NGG1 does not regulate *GAL4* expression

Glucose reduces the expression of *GAL4* ~5-fold (Griggs and Johnston, 1991; Nehlin *et al.*, 1991). This, in turn, results in up to a 100-fold reduction in *GAL* gene expression (Griggs and Johnston, 1991; Nehlin *et al.*, 1991; Lamphier and Ptashne, 1992). To determine whether NGG1p functions in the regulation of *GAL4* expression, we have analyzed the effect of disrupting *nggl* in a strain in which *GAL4* is expressed from a promoter which is not downregulated in glucose medium. Expression of the *his3*-G25 allele was assayed in yeast strains YT6 (*gal4 gal80*) and CY899 (isogenic to YT6 except *nggl::TRP1*) which had been transformed with a plasmid, pPHO5G4 (Stone and Sadowski, 1993), in which *GAL4* is expressed from the *PHO5* promoter

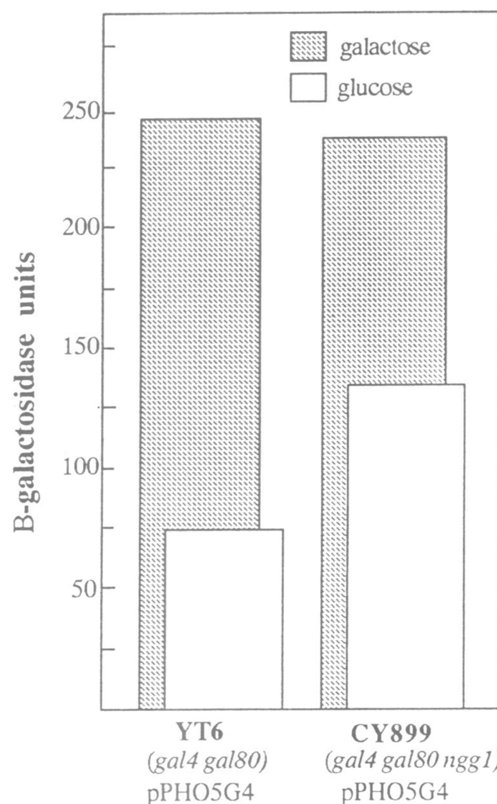

Fig. 8. NGG1p function does not require the native *GAL4* promoter. Transcription of *his3*-G25 (as a *lacZ* fusion) was assayed in yeast strains YT6 (*gal4 gal80*) and CY899 (isogenic to YT6 except *nggl::TRP1*) that had been transformed with pPHO5G4 (Stone and Sadowski, 1993). pPHO5G4 is a centromeric plasmid in which *GAL4* is expressed from the *PHO5* promoter. Cells were grown in minimal medium containing 2% glucose (open boxes) or 2% galactose (stippled boxes). β-galactosidase assays were performed after disruption of the cells with glass beads as described by Himmelfarb *et al.* (1990). Unit activity has been standardized to the protein concentration in each extract.

(Meyhack *et al.*, 1982). Under these conditions glucose repression on the *his3*-G25 promoter is reduced dramatically to a level of ~3-fold (Figure 8, compare YT6 in glucose and galactose media). Disruption of *nggl* resulted in a 1.8-fold increase in expression of *his3*-G25 in glucose medium (CY899 versus YT6). This represents a relief of ~60% of the glucose repression, a level that is comparable with that seen earlier in strains expressing *GAL4* from its native promoter. Since NGG1p does not require the native *GAL4* promoter to repress expression of *GAL* genes in glucose medium, its primary function must not be to regulate expression of *GAL4*. In addition, we have found no difference in expression of *his3*-G25 in YT6 and CY899 in the absence of pPHO5G4 (data not shown). This further supports the conclusion that NGG1p acts by regulating GAL4p function.

Discussion

Transcription of the *GAL1-10* genes is acutely responsive to changes in the available carbon source. As such, it is not surprising that this promoter region contains >12 *cis*-acting elements and is regulated by >15 genes. Through the analysis of suppressor mutations that facilitate the expression

of a defective *gal–his3* fusion promoter (*his3*-GG579), we have characterized *NGG1* as a novel gene involved with *GAL* gene regulation. This gene was identified as a recessive null mutation, which in the presence of a *gal80* background, facilitated a 300-fold increase in transcription of the *GAL10*-related promoters, *his3*-GG579 and *his3*-G25, in glucose medium. The magnitude of this response indicates that *NGG1* has a significant role in glucose repression. In retrospect, we were fortunate that our original strain acquired a *gal80* mutation since the increase in expression of *his3*-GG579 in a strain containing only a null *ngg1* allele is insufficient to allow rapid growth on 10 mM AT selective plates.

Four genes, which have been described to affect glucose repression of *GAL1-10*, remain uncloned: *URR1, URR3, URR4* (Flick and Johnston, 1992) and *GAL82* (Matsumoto *et al.*, 1981, 1983). *NGG1* is not allelic with *URR1* as this gene maps to chromosome XI (Flick and Johnston, 1992). We believe that *NGG1* is not *URR3* or *URR4* because a centromeric plasmid containing *NGG1* did not alter the expression of a UAS_{LEU2}–$GAL1_{UAS}$–*his3* fusion in *urr3* and *urr4* strains (kindly provided by M.Johnston). Furthermore, we believe that *NGG1* is not *GAL82* because, unlike mutations in *gal82* (J.Erickson and M.Johnston, personal communication), mutations in *ngg1* are not suppressed by overexpression of *REG1*.

Mechanism of NGG1p-mediated repression

The structure of NGG1p provides few clues to its mechanism of action. Comparison of the amino acid sequence of NGG1p with known protein sequences and translated sequences found in the GenBank or EMBL databases revealed no obvious homologies. Notably, there is a highly positively charged region, from amino acids 606 to 618, which resembles a nuclear localization signal (Dingwell and Laskey, 1991). Deletions through this region of the protein result in a loss of function (A.M.Furlanetto and C.J.Brandl, unpublished observation), thus suggesting that NGG1p functions in the nucleus.

We currently believe that NGG1p functions in glucose repression by inhibiting the DNA binding of GAL4p (Giniger *et al.*, 1985; Selleck and Majors, 1987) or its ability to activate transcription. GAL4p is the most likely target for NGG1p because the relief of repression required the GAL4p binding sites of *his3*-G25 and could be observed for a promoter that contained independent GAL4p binding sites, *his3*-G4. In addition, disruption of *ngg1* and *gal80* had no effect on transcription of *his3*-G25 in strains lacking *GAL4*. Furthermore, both of these models are consistent with the observation that the *ngg1* response was most evident in a *gal80* background, conditions in which functional GAL4p is available to activate transcription. One of the principal mechanisms for glucose repression of the *GAL* genes is the inhibition of *GAL4* expression in glucose (Griggs and Johnston, 1991; Nehlin *et al.*, 1991). While all of the findings mentioned above are consistent with a model whereby NGG1p is a negative regulator of *GAL4* expression, we believe that this is less likely because relief of repression due to disruption of *ngg1* was seen when *GAL4* transcription was under the control of the *PHO5* promoter. The recent identification of *ADA3*, a potential general co-activator, as *NGG1* also supports a model in which NGG1p acts to regulate the function of GAL4p (Berger *et al.*, 1992; L.Guarente, personal communication, see below).

Stone and Sadowski (1993) have shown that the central region of GAL4p mediates a rapid inhibition of GAL4p activity by glucose. As we have found for the disruption of *ngg1*, the extent of the repression due to the central region varies depending on the total level of glucose repression for the promoter, but for the derivatives tested it is in the same range of magnitude as that due to *ngg1*. Stone and Sadowski (1993) suggest that the central region effect is mediated by a protein directly interacting with GAL4p thus inhibiting its cooperative binding (Kang *et al.*, 1993) and/or its activation potential. NGG1p is a potential candidate to work in this regulatory pathway.

It should be emphasized that in these models NGG1p may not directly interact with GAL4p. Several factors that are required for full GAL4p activity have been characterized which could act as the target for NGG1p. GAL11p (Suzuki *et al.*, 1988; Himmelfarb *et al.*, 1990) has been identified as a factor that can enhance the activity of GAL4p and PPR1p. Although its mechanism of action has not been fully characterized, GAL11p is thought to interact directly with GAL4p (Himmelfarb *et al.*, 1990). *SUG1* has been identified as a second gene that can influence GAL4p activity (Swaffield *et al.*, 1992). A recessive mutation, *sug1-1*, can enhance the activity of GAL4p deletion derivatives lacking the carboxyl-terminal activation domain (Swaffield *et al.*, 1992). Since the *sug1-1* mutation also partially enhanced the activity of full-length GAL4p, it may also represent a co-activator required for optimal GAL4p function. Full activity of GAL4p also requires a complex of proteins that include SWI1p, SWI2p and SWI3p (Peterson and Herskowitz, 1992). These factors are thought to function by enabling activator proteins to contend with the repressive effects of chromatin structure (Winston and Carlson, 1992). Rather than interacting with GAL4p, NGG1p may repress or disrupt the necessary interactions between these factors and GAL4p in glucose medium.

Interestingly, *NGG1* (*ADA3*) has recently been identified in a selection for mutations which suppress the toxic effects of high level expression of *GAL4–VP16* chimeras (Berger *et al.*, 1992; L.Guarente, personal communication). Null mutations in *ngg1/ada3* result in reduced transcriptional activation by GAL4–VP16 which parallels their loss of toxicity. The Guarente laboratory has also shown that other activators, including GAL4p and GCN4p, show reduced expression in *ngg1* mutant strains (L.Guarente, personal communication). This suggests that NGG1p/ADA3p may be a general adaptor or co-activator required for transcriptional activation. In this respect, NGG1p may be similar to GAL11p, SUG1p and the SWI complex. We have also found that under certain conditions disruption of *ngg1* can result in reduced transcriptional activation. Expression of the *his3*-G4 promoter in glucose medium was repressed ~4-fold by disruption of *ngg1* (Table I; expression of *his3*-G4 in CY757 versus KY322). This effect did require the GAL4p binding sites, but whether it represents an alteration in GAL4p function is unclear as it was not apparent upon induction of the promoter in galactose medium, nor was it evident for the *his3*-G25 promoter. We have also observed NGG1p to function as an activator under conditions in which *GAL4* is overexpressed from the *ADH1* promoter. Under these conditions, expression of *NGG1* from a multicopy

Table II. *S.cerevisiae* strains

Strain	Relevant genotype	Reference or source[a]
KY322	*MATa ura3-52 ade2-101 trp-Δ1 GCN4-p39 lys2-801 his3-Δ200 leu2::PET56*	Chen and Struhl (1988)
CY579	Isogenic to KY322 except *his3-GG579*	Chen and Struhl (1988) Brandl and Struhl (1990)
CY471	Isogenic to KY322 except *his3-GZ-J18*	Brandl *et al.* (1992)
CY579-9	Isogenic to CY579 except *nggl-1 gal80*	TW
CY579-100	Isogenic to CY579-9 except *leu2::GAL80*	TW
CY323	Isogenic to CY579 except *MATα*	TW
CY756	Isogenic to KY322 except *nggl::TRP1*	TW
CY757	Isogenic to CY579 except *nggl::TRP1*	TW
CY98	Isogenic to CY579 except *gal80::URA3*	TW
CY798	Isogenic to CY579 except *nggl::TRP1 gal80::URA3*	TW
CY782	Isogenic to CY579 except *nggl-1*	TW
CY765	Isogenic to CY757 except *MATα*	TW
YT6	*MATα gal4-542 gal80-538 ura3-52 his3-200 ade2-101 adel lys2-80 trp1-901 arol leu2-3-112 met*	Himmelfarb *et al.* (1990)
CY899	Isogenic to YT6 except *nggl::TRP1*	TW

[a]TW, strains constructed in this work.

plasmid resulted in a 3-fold increase in expression of *his3*-G25 (data not shown). We have also seen a slight reduction in GCN4p-activated transcription in *nggl* mutant strains (Figure 3). The exact role of NGG1p would thus seem dependent on the functional state and level of expression of the activator protein, as well as the structure of the reporter construct. The ability of NGG1p to either repress or stimulate potentially activated transcription suggests that it may play a role in the differential regulation of activator function, perhaps in response to changes in environmental signals.

Materials and methods

Yeast strains, media and growth conditions

The genotypes of the yeast strains used in these experiments are shown in Table II. Null mutations of *GAL80* were generated by the one step disruption method of Rothstein (Rothstein, 1983) with pKOG80 (kindly provided by I.Sadowski) which contains a Tn10LUK (Huisman *et al.*, 1987) of the *GAL80* coding sequences. Ura$^+$ colonies were selected and verified for disruption of *GAL80* by Southern blotting. The *gal4 gal80* mutant strain, YT6, was kindly provided by I.Sadowski.

CY782 was constructed by two-step gene replacement. First, a *Sac*I–*Pst*I fragment containing the *nggl-1* allele in the *URA3* integrating vector YIplac211 (Gietz and Sugino, 1988), B782, was restricted with *Sna*BI (nucleotide position 2299) and integrated into CY757 (*nggl::TRP1*). Gene replacement at *nggl* was selected for after growth on 5-fluoro-orotic acid (Boeke *et al.*, 1984) by loss of the CY757 *TRP1* marker. Similarly, *nggl-1* was integrated into CY579-9 for genetic analysis after restriction of B782 with *Sna*BI.

GAL80 was integrated at the *leu2::PET56* locus of CY579-9 to generate the Leu$^+$ strain, CY579-100, after restriction of YIpG80*leu* (kindly provided by I.Sadowski) with *Eco*RV.

In general, yeast strains were grown at 30°C in liquid suspension or on 2% Bacto-agar plates in YPD broth (1% yeast extract, 2% peptone and 2% glucose) or in minimal medium (0.67% yeast nitrogen base without amino acids, 2% glucose supplemented with 1% lysine, 1% leucine, 0.5% tryptophan, 0.25% adenine and uracil when required). Plasmid DNA was transformed into yeast cells treated with lithium acetate (Ito *et al.*, 1983) and recovered as described by Hoffman and Winston (1987).

Genetic analysis

MATa and *MATα* haploid strains were cross-streaked and diploids were selected on appropriate selective media. Sporulation was carried out for 3–7 days on plates containing potassium acetate. The sporulated culture was inoculated into 10% glusulase (Dupont) for 20–40 min at 25°C then placed on ice. Tetrads were dissected under a light microscope with a manipulator as described by Rose *et al.* (1990). Spore viability was strain-dependent being ~90% for wild-type strains but <40% for *nggl* strains. The frequency of sporulation of the *nggl* strains was also only ~10% of that seen for the wild-type strains.

DNA constructs

Molecules were constructed using standard cloning techniques and verified by restriction and sequence analysis. All of the *his3* alleles contain a 6.1 kb fragment of yeast chromosomal DNA containing *PET56*, *HIS3* and *DED1* genes (Struhl, 1985b). *his3*-GG579 is a derivative of *his3*-GZ-J18 (Brandl *et al.*, 1992) which contains an optimal GCN4 binding site (TGACTCA) rather than the *his3* wild-type GCN4 binding site.

his3-G25 is a derivative of *his3*-G17 (Chen and Struhl, 1988) with *GAL1-10* sequences from 299–649 (Johnston and Davis, 1984) cloned as a *Bam*HI–*Eco*RI fragment between −447 of *his3* and a 23 bp oligonucleotide which contains a TATA element, sequence TATAAA, positioned at −25 of *his3* (Struhl, 1985b). These alleles closely resemble the native *GAL10* promoter and are activated in galactose by GAL4. *his3*-G25^{888}, *his3*-G25^{156} and G25^{251} are derivatives of *his3*-G25 with 5′ truncations of the *GAL1-10* sequences to positions 525, 437 and 408, respectively. *his3*-G25^{283}, -G25^{289}, -G25^{290}, -G25^{102}, -G25^{103} have 3′ truncations of the *GAL1-10* sequences to 354, 374, 395, 422 and 409, respectively, *his3*-G25^{308} and *his3*-G25^{310} are related molecules with an internal deletion from nucleotides 371–381 and 391–399, respectively.

his3-Δ88 is a derivative of the wild-type *his3* promoter which contains an insertion of a *Eco*RI–*Sac*I linker in the place of the GCN4 binding site (Hill *et al.*, 1986). *his3*-G4 is a derivative of *his3*-Δ88, which contains five GAL4p binding sites (Carey *et al.*, 1989, 1990) cloned as a *Bam*HI–*Eco*RI fragment between −90 and −447 of *his3* (Struhl, 1985b). To facilitate the insertion of the GAL4p binding sites, a linker of 29 bp of pBR322 (*Eco*RI–*Hin*dIII) was included 3′ of the most proximal site. *his3*-GCN4 contains the *his3* promoter region from −80 to −447 (Struhl, 1985b), including the GCN4p binding site, cloned as a *Bam*HI–*Eco*RI fragment, directly upstream of the TATA element of *his3*-G25.

lacZ fusions were constructed as *his3*–*lacZ* fusions. The appropriate promoter regions from the upstream *Bam*HI site (−447) to the *Hin*dIII site at nucleotide 342 of *his3* were cloned into the equivalent sites of the Leu$^+$ centromeric plasmid YCp87 (kindly provided by K.Struhl).

NGG1 was expressed from the *DED1* promoter by introducing a *Bam*HI site 5′ of the initiator methionine using PCR mutagenesis and an oligonucleotide with the sequence 5′-GCGGATCCCATATGCCTAGA-CATGGAAG-3′. A second *Bam*HI site was introduced with a linker at the *Sna*BI site (nucleotide 2298). This 2.1 kb *Bam*HI (B765) fragment was cloned in both orientations into *Bam*HI-restricted YCp88-GCN4 (Pu and Struhl, 1991) to generate YCp88-NGG1 and YCp88-NGG1r.

Centromeric plasmids expressing *GAL4* from the *PHO5* promoter were kindly provided by I.Sadowski (Stone and Sadowski, 1993).

Phenotypic analysis

Growth rates of strains were compared on minimal plates containing 2% glucose and 10 mM AT. AT is a competitive inhibitor of the *HIS3* gene product (Klopotowski and Wiater, 1965) and as shown previously, growth rate in AT is proportion to the level of *HIS3* mRNA (Hill *et al.*, 1986; Chen and Struhl, 1988). Both plate and liquid culture assays of β-galactosidase activity (Guarente, 1983) were carried out as described by Ausubel *et al.* (1990) for KY322 derived strains. Yeast strains were grown in minimal media containing the appropriate carbon source to an optical density at 600 nm of 1.0−1.5. Cells were pelleted, washed in Z buffer and concentrated 2- to 10-fold prior to their assay. Activity was standardized to cell density. For yeast strains YT6 and CY899, β-galactosidase activity was determined after disruption of the cells with glass beads as described by Himmelfarb *et al.* (1990). For these assays, activity was standardized to protein concentration as determined from the Bio-Rad protein assay kit. To ensure the presence of GCN4p for the analysis of *his3*-GCN4, *GCN4* was expressed from the *DED1* promoter on the centromeric plasmid YCp88 (Hope and Struhl, 1986; Pu and Struhl, 1991).

Isolation of mutants

CY579 cells were grown to log phase in rich medium, pelleted and washed in water. The cells were suspended in their original volume and exposed to UV radiation at 254 nm. Cell viability was ~20%. Cells were plated at a density of 2×10^6 onto minimal media containing 10 mM AT and 2% glucose and grown at room temperature. Twenty-four fast growing clones were transformed with the equivalent *his3*-GG579 LacZ fusion on the Leu^+ centromeric plasmid YCp87 (YCp87-*his3*-GG579LacZ) and β-galactosidase levels determined on plates to confirm that AT resistance was the result of enhanced transcription.

Cloning the NGG1 gene

CY579-9 containing the reporter plasmid YCp87-*his3*-GG579LacZ, was transformed to Ura^+ with a wild-type genomic pool in the centromeric vector pINT2 (Percival-Smith and Segall, 1986; kindly provided by J.Archambault and J.Friesen) and plated on minimal plates containing 2% glucose and X-gal. Plasmids were recovered from white colonies and genomic fragments were subcloned into the Ura^+ centromeric plasmid YCplac33 (Gietz and Sugino, 1988) and tested for their ability to suppress the growth of CY579-9 on plates containing 10 mM AT. A 2.7 kb *Sac*I−*Pst*I fragment (B748; YCplac33-NGG1) repressed expression of both the integrated *his3*-GG579 allele and the equivalent *lacZ* fusion. The mutant allele, *nggl-1*, was isolated by colony hybridization (Ausubel *et al.*, 1990) of a genomic library constructed from appropriately sized *Sac*I−*Pst*I restriction fragments from CY579-9.

Null mutations of NGG1

Strains with *nggl* null mutations were generated by direct gene replacement with a *nggl* allele (*nggl::TRP1*) which contains an 860 bp *Xmn*I−*Bgl*II *TRP1* fragment from YCplac22 inserted into the equivalent sites of B748 at nucleotide positions 428 and 1087.

RNA analysis

Total RNA was prepared from log phase cells as described by Oettinger and Struhl (1985). S1 analyses with ^{32}P end-labelled probes from *HIS3* and *DED1* were performed as described by Chen and Struhl (1988). For Northern analysis of the *NGG1* transcript, 20 μg of RNA were separated by electrophoresis on a 1% agarose gel containing formaldehyde (Ausubel *et al.*, 1990). RNA was transferred to nitrocellulose and hybridized to a ^{32}P randomly labelled *Rsa*I−*Rsa*I fragment (nucleotides 561−919) of B748. The blot was washed with a maximum stringency of 0.2 × SSC at 65°C. Primer extension was performed as described by Furter-Graves and Hall (1990). Briefly, 50 μg of total cellular RNA were incubated with 1.0 pmol of oligonucleotide primer (5′-GGAGTATTGTCGCCTCCCTC-3′; nucleotides 242−223) end-labelled with ^{32}P in 1 × hybridization buffer (250 mM KCl, 62.5 mM Tris−HCl, pH 8.3, 0.25 mM EDTA) in a 12 μl volume. Annealing was carried out at 49°C for 30 min after heating to 70°C and slow cooling. The hybrids were diluted to a final volume of 33 μl containing 3.4 mM $MgCl_2$, 10 mM dithiothreitol, 500 μM dNTPs, 42 mM Tris−HCl (pH 8.3) and 300 U MMTV reverse transcriptase (BRL) and incubated at 49°C for 90 min. The extended DNA fragments were precipitated in ethanol and resuspended in 8 μl of formamide loading dye containing 0.15 M NaOH. The samples were heated to 90°C for 2 min and quickly chilled prior to loading on a 6% acrylamide sequencing gel.

DNA sequence analysis

DNA sequence was determined by the method of Sanger *et al.* (1977) using the T7 polymerase kit supplied by Pharmacia. Single-stranded DNA templates were prepared in the vectors pTZ18r and 19r by superinfection with M13KO7. Primers were constructed to facilitate the sequencing of both strands.

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