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The Minimal Transactivation Region of *Saccharomyces cerevisiae* Gln3p Is Localized to 13 Amino Acids

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Regulated nitrogen catabolic gene transcription in *Saccharomyces cerevisiae* is mediated by four positive (Gln3p and Gat1p/Nil1p) and negative (Dal80p/Uga43p and Deh1p/Nil2p/GZF3p) regulators which function in opposition to one another. All four proteins contain GATA-type zinc finger domains, and three of them (Gln3p, Dal80p, and Deh1p) have been shown to bind to GATA sequences situated upstream of genes whose expression is sensitive to nitrogen catabolite repression (NCR). The positive regulators, Gln3p and Gat1p, are able to support transcriptional activation when tethered by LexAp to the promoter of a reporter gene whose upstream activation sequences have been replaced with one or more *lexA* operator sites. Existing data suggest that these four proteins regulate transcription by competing with one another for binding to the GATA sequences which mediate NCR-sensitive gene expression. We show that the minimal Gln3p domain mediating transcriptional activation consists of 13 amino acids with a predicted propensity to form an α-helix. Genetic analysis of this region (Gln3p residues 126 to 138, QQNGEIAQLWDFN) demonstrated that alanine may be substituted for the aromatic and acidic amino acids without destroying transcriptional activation potential. Similar substitution of alanine for the two hydrophobic amino acids, isoleucine and leucine, however, destroys activation, as does introduction of basic amino acids in place of the acidic residues or introduction of proline into the center of the sequence. A point mutation in the Gln3p activation region destroys its in vivo ability to support NCR-sensitive *DAL5* expression. We find no convincing evidence that NCR regulates Gln3p function by modulating the functioning of its activation region.

Four GATA factors mediate regulation of nitrogen catabolic gene expression in *Saccharomyces cerevisiae*: Gln3p, Gat1p/Nil1p, Dal80p/Uga43p, and Deh1p/Nil2p/Gzp3p (3, 10–12, 16, 19–21, 26–29, 31, 48, 49, 53, 54, 56, 64, 65, 71). Two of these proteins (Gln3p and Gat1p) are positive regulators required for nitrogen catabolite repression (NCR)-sensitive gene expression (14–16, 19, 29, 48, 49, 65). When fused to LexAp, Gln3p and Gat1p support transcriptional activation of a heterologous reporter gene in which a *lexA* operator site(s) replaces the upstream activation sequences, albeit at different levels (15, 28). In contrast, the other two GATA factors (Dal80p and Deh1p) are functionally negative regulators because deletions of their cognate genes result in increased expression of some, but not all, NCR-sensitive genes (12, 21, 26–28, 31, 56, 64, 71). All four proteins contain GATA binding motifs, and three of them have been shown by electrophoretic mobility shift assays to bind directly to GATA sequences upstream of NCR-sensitive genes (16, 26–29). In some cases, Dal80p, Deh1p, and Gln3p bind to the same GATA sequences, suggesting that the positively and negatively acting GATA factors regulate gene expression by competing with one another for binding to GATA sequences (28, 31, 64).

The yeast GATA factors are related not only in their DNA binding characteristics and antagonistic properties. The genes encoding them (*GLN3*, *GAT1*, *DAL80*, and *DEH1*) are highly cross-regulated (13–16, 26, 31, 64). *GLN3* expression is unique among the four because it does not appear to be regulated at transcription (less than threefold). In contrast, GAT1 expression is Gln3p dependent, Dal80p regulated, and in some strains and growth conditions, Deh1p and Gat1p regulated (14–16, 26, 56, 64). *DAL80* expression is Gln3p and Gat1p dependent as well as Dal80p and Deh1p regulated (14–16, 26, 64). *DEH1* expression is the least dependent of the four GATA factor genes on Gln3p, but it is partially Gat1p dependent and highly regulated by Dal80p (16, 56, 64).

Identifying the biochemical mechanisms by which Gln3p- and Gat1p-dependent transcription responds to the quality of nitrogen source available to the cell is an important step toward understanding NCR-sensitive gene regulation (18). When readily used nitrogen sources (e.g., asparagine, glutamine, and in some strains, ammonia) are present, GATA factor-mediated transcription occurs at barely detectable levels (17, 31, 73). In contrast, when poorly used nitrogenous compounds are available, Gln3p- and Gat1p-mediated transcription occurs at high levels. Little is known about the events and molecules that effect this regulation. This response to nitrogen source quality and quantity is known as NCR (17, 73). Drillien et al. identified Ure2p as a negative regulator of NCR-sensitive genes (34, 35). This phenotype and the epistasis of *gln3* mutations to those at *ure2* have led to multiple speculations concerning how Ure2p directly regulates Gln3p activity (23). Limited homology between Ure2p and glutathione *S*-transferase led to the proposition that Ure2p posttranscriptionally modifies Gln3p (22). Antibodies against Gln3p were reported to weakly precipitate Ure2p from crude cell extracts overexpressing both proteins, leading to the proposition that Gln3p and Ure2p form a stable complex during growth on glutamine (9). However, the biochemical function of Ure2p, a prion-type protein, and how it transmits a nitrogen regulatory signal to Gln3p remain to be convincingly demonstrated (74, 75). Moreover, Ure2p is not unique in its ability to transmit a nitrogen regulatory signal to the NCR-sensitive transcriptional apparatus; NCR-sensitive

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TABLE 1. Strains used in this work

Strain	Genotype	Reference or source
E. coli		
BMH 71-18 mutS	*thi supE* Δ(*lac-proAB*) *mutS*::Tn*10* [F′ *proA*$^+$*B*$^+$ *lacI*^{q}ZΔM15]	
DH5α	ϕ80d*lacZ*ΔM15 *recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^+) *deoR relA1 supE44* Δ(*lacZYA-argF*) *U169*	
ES1301 mutS	*lacZ53 mutS201*::Tn*5 thyA36 rha-5 metB1 deoC* IN(*rrnD-rrnE*)	
S. cerevisiae		
EGY48	*MAT*α *3lexAop*::*leu2 ura3 trp1 his3*	39
InvSc1	*MAT*α *his3-1 leu2 trp1-289 ura3-52*	Invitrogen, Carlsbad, Calif.
M1682-19b	*MAT***a** *ura3-52 trp1-289*	
PB200	*MAT***a** *ura3-52 trp1-289 dal81*Δ::*hisG*	
TCY1	*MAT*α *lys2 ura3*	
RR91	*MAT*α *lys2 ura3 gln3*::*hisG*	
RR911-1*	*MAT*α *lys2 ura3 gln3*::*hisG trp1*Δ::*hisG*	

production of steady-state *GAT1* mRNA can be clearly demonstrated in a *gln3 ure2* double-deletion mutant (15).

To further understand the operation of NCR-sensitive gene expression, we have begun a systematic study of the 730-amino-acid Gln3p. The relative abundance of acidic amino acids in the N-terminal portion of Gln3p prompted Minehart and Magasanik to designate this region an acidic activation region (48). Unlike DNA binding or protein dimerization regions, transcriptional activation regions do not possess easily identified structures (41). As a result, they are classified mainly by the relative abundance of particular amino acids in the peptide that supports activation (8). Such classes of activation regions include those rich in glutamine (69), glutamine/glycine (32), proline (2, 4), isoleucine (5), leucine (37), serine/threonine (24, 32), and acidic (6, 7, 25, 38, 58, 70) amino acids. These regions reportedly range in size from compact (23 amino acids in the case of p53) to the full length of the activator protein (50, 67). In some cases (e.g., Gal4p), isolated activation regions retain some or all of their characteristic physiological responses.

The precise structure of acidic activators has been controversial (41). Early studies demonstrated that an engineered oligopeptide, containing abundant acidic residues and predicted to fold into an amphipathic α-helix, could, when fused to a DNA binding region, act in vivo as a yeast transcriptional activator (38). This model was challenged by the observations that functional acidic activator regions were tolerant of point mutations and small internal deletions and that many acidic peptides, irrelevant to eucaryal transcription, were capable of acting as activators (25, 45). These findings suggested that acidic activation regions might not have a precise secondary structure encoded in their sequence, leading to the term "acidic blob" (61). The demonstration that Gal4p and Gcn4p activation regions were largely unstructured at neutral pH but folded into predominantly β-sheets at acidic pH led to the suggestion of induced-fit conformational changes taking place during interaction between acidic activators and their target proteins in vivo (55, 59, 60, 70). The universality of the β-sheet model was, in turn, questioned as a result of the findings that (i) Gal4p derivatives unable to assume a β-sheet conformation retained significant activation potential (72), (ii) peptides from other transcription factors, including NF-κB, though unstructured in aqueous solution, assumed an α-helical conformation in the presence of trifluoroethanol (30, 57), and (iii) introduction of helix-breaking proline residues into some activation regions diminished their activation potential (29). Finally, a high-resolution (2.6-Å) structure of the 15-amino-acid p53-derived activation region, cocrystallized in a complex with the MDM2 oncoprotein, revealed that the p53 activation region was bound, as an amphipathic α-helix, in a hydrophobic cleft on the surface of MDM2 (43). In sum, acidic activation regions do not seem to consist of a unique, identifiable sequence or a characteristic secondary structure that can be predicted with certainty from deduced amino acid sequences.

The objective of the present work was to precisely localize and genetically characterize the putative activation region of Gln3p and to determine whether its operation responds to the quality of nitrogen source available. We localized the Gln3p activation region to a 13-amino-acid region with a predicted propensity to form an α-helix. In addition, amino acid residues important to its function were genetically identified. The operation of this activation motif cannot account for the in vivo response of NCR-sensitive gene expression to nitrogen source quality.

MATERIALS AND METHODS

Strains and media. The bacterial and yeast strains used in this work are listed in Table 1. The plasmids constructed for this work are listed in Fig. 1 and Table 2. LB broth was used for the propagation of bacterial strains. When required, it was supplemented with ampicillin (50 to 100 μg/ml) or tetracycline (12.5 μg/ml). YPD (10 g of yeast extract, 20 g of peptone, and 20 g of D-glucose per liter) was used as the rich medium for growth of yeast strains. YNB (Difco yeast nitrogen base [1.7 g/liter] with 0.1% nitrogen [0.5% when ammonia sulfate was used]) and 2% carbon sources were used in our experiments. The medium was supplemented as needed to meet the auxotrophic requirements of the strains used.

Plasmid constructions. Plasmids pVS32, pVS316, pVS316-2, pVS316-3, pVS32-3, pVS32-4, pVS32ΔXho, and pTSC511 were described by Cunningham et al. (29); pEG202, pSH18-34, p1840, pSH17-4, pRFHM1, and pJK101 were described by Estojak et al. (36) and Golemis et al. (39); pRS314 and pRS316 were described by Sikorski and Hieter (62); pJCD52 was described by Coffman et al. (16). The LexA-Put3 fusion plasmid (pVS3PG) was constructed by subcloning the 2.8-kb *Sal*I-*Pst*I fragment from cosmid c8157 (ATCC 70914), using

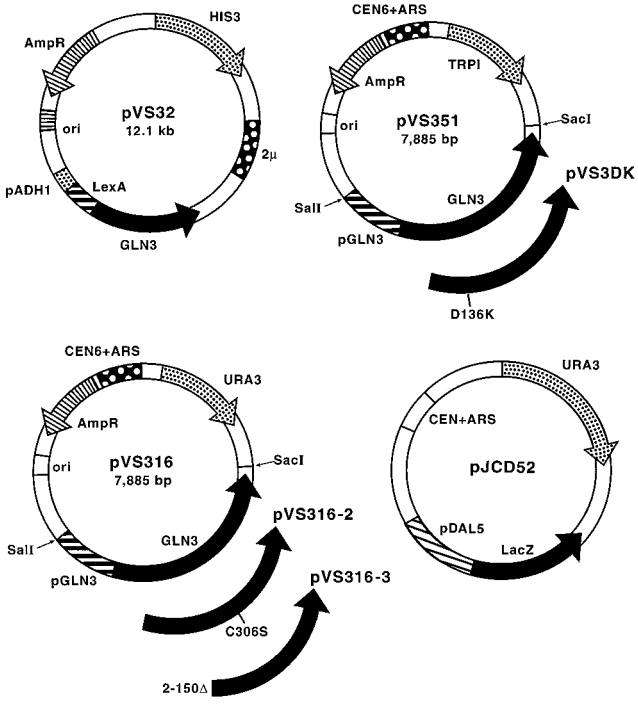

FIG. 1. Essential features of the LexA-fusion, Gln3p expression, and Gln3p-dependent reporter plasmids used in this work.

TABLE 2. Plasmids used in this work

Plasmid	Brief description[a]
LexA fusion plasmids based on the pBTM116 vector	
pVS3BTM	Full-length Gln3p-LexA fusion
pVS3ABTM	LexA fusion of the Gln3p fragment [1–210]
LexA fusion plasmids based on the pEG202 vector	
pVS32	Full-length Gln3p-LexA fusion
pVS32ΔXho	LexA fusion of the Gln3p fragment [1–149]
pVS32-2	LexA fusion of the Gln3p fragment [1–730Δ (471–669)]
pVS32-3	LexA fusion of the Gln3p fragment [1–470]
pVS32-4	LexA fusion of the Gln3p fragment [1–730Δ (2–150)]
pVS3449	LexA fusion of the Gln3p fragment [1–149]
pVS3417	LexA fusion of the Gln3p fragment [10–149]
pVS3371	LexA fusion of the Gln3p fragment [26–149]
pVS3349	LexA fusion of the Gln3p fragment [34–149]
pVS3281	LexA fusion of the Gln3p fragment [56–149]
pVS3250	LexA fusion of the Gln3p fragment [67–149]
pVS3230	LexA fusion of the Gln3p fragment [73–149]
pVS3192	LexA fusion of the Gln3p fragment [86–149]
pVS3168	LexA fusion of the Gln3p fragment [94–149]
pVS3133	LexA fusion of the Gln3p fragment [106–149]
pVS3415	LexA fusion of the Gln3p fragment [1–138]
pVS3364	LexA fusion of the Gln3p fragment [1–121]
pVS3328	LexA fusion of the Gln3p fragment [1–109]
pVS3285	LexA fusion of the Gln3p fragment [1–95]
pVS3231	LexA fusion of the Gln3p fragment [1–77]
pVS3176	LexA fusion of the Gln3p fragment [1–59]
pVS3215	LexA fusion of the Gln3p fragment [73–138]
pVS3115	LexA fusion of the Gln3p fragment [94–138]
pVS3HH	LexA fusion of the Gln3p fragment [126–210]
pVS3151	LexA fusion of the Gln3p fragment [151–210]
pVS373A	F73A mutation in the acidic activation domain
pVS376A	F76A mutation in the acidic activation domain
pVS37376AA	F73A,F76A mutation in the acidic activation domain
pVS3110A	F110A mutation in the acidic activation domain
pVS3128NA	N128A mutation in the acidic activation domain
pVS3128NK	N128K mutation in the acidic activation domain
pVS3129GK	G129K mutation in the acidic activation domain
pVS3129GP	G129P mutation in the acidic activation domain
pVS3130EA	E130A mutation in the acidic activation domain
pVS3130EK	E130K mutation in the acidic activation domain
pVS3130EP	E130P mutation in the acidic activation domain
pVS3130ER	E130R mutation in the acidic activation domain
pVS3131IA	I131A mutation in the acidic activation domain
pVS3131IK	I131K mutation in the acidic activation domain
pVS3134LA	L134A mutation in the acidic activation domain
pVS3135WA	W135A mutation in the acidic activation domain
pVS3135WP	W135P mutation in the acidic activation domain
pVS33537AA	W135A,F137A mutation in the acidic activation domain
pVS3136DA	D136A mutation in the acidic activation domain
pVS3136DK	D136K mutation in the acidic activation domain
pVS3137FA	F137A mutation in the acidic activation domain
Vectors for *gln3* complementation	
pVS316	Full-length *GLN3* in pRS316
pVS316-2	pVS316 derivative with Δ2-150 deletion in Gln3p
pVS316-3	pVS316 derivative with C306S mutation in Gln3p
pVS351	Full-length *GLN3* in pRS314
pVS3DK	pVS351 derivative with D136K mutation in Gln3p

[a] Numbers in brackets denote residues.

PCR-generated adapters restoring the full-length *PUT3* coding sequence into the *Eco*RI and *Xho*I sites of plasmid pEG202.

Plasmid pVS351 was constructed by subcloning a 3.1-kb *Sac*I-*Sal*I fragment from plasmid pTSC511 into plasmid pRS314. To construct plasmid pVS3DK, a desired mutation coding for the D136K substitution was introduced into plasmid pTSC511, using the Promega pALTER site-directed mutagenesis kit followed by similar subcloning of the 3.1 kb *Sac*I-*Sal*I fragment into plasmid pRS314.

Deletion plasmids. A set of nested deletions (plasmids listed in Fig. 3) was generated at either end of the Gln3p acidic activation region by using high-fidelity PCR amplification. To this end, a panel of 12 upper-strand (derived from the Watson strand of *GLN3*) and 8 lower-strand (derived from the Crick strand of *GLN3*) PCR primers were designed to specify amplification of the entire 149-amino-acid acidic activation region and progressive deletions from its ends. The resulting products were subcloned into the *Eco*RI and *Xho*I sites of plasmid pEG202.

Random mutagenesis. Random mutagenesis of the *GLN3* segment coding for amino acids 106 to 149 was achieved through error-prone PCR amplification of this coding sequence as described earlier (68). PCR products were subcloned as LexA fusions into the *Eco*RI and *Xho*I sites of plasmid pEG202. This recombinant DNA library was used to transform yeast strain EGY48 carrying reporter plasmid pSH18-34.

Site-directed mutagenesis with the pSelect system. Site-directed mutagenesis was used to introduce point mutations into the *GLN3* gene. The single-stranded template method was used to introduce the C306S mutation in the Gln3p Zn finger motif (oligonucleotide GTCTTGAAAGTTTTACAATTGAAACTTTGT ATCAGAGGTTT) (Promega pSelect/pAltered), whereas the D136K substitution was constructed by the double-stranded template method in combination with the mutagenic oligonucleotide TTGGTCCACGTTAAACTTCCAAAGTT GTGC (Promega pSelect/pAltered).

PCR-mediated site-directed mutagenesis of the Gln3p acidic activation region. Derivatives of the Gln3p acidic activation region fused to LexA were constructed essentially as described above for deletions within the 149-amino-acid-long N-terminal segment of Gln3p (Gln3[1-149]p). After gel purification, PCR products were cloned into plasmid pEG202 as LexA fusions, using conventional cloning techniques. The presence of substitutions was confirmed by sequencing.

Transcriptional activation and repression assays. Transcriptional activation and repression assays were performed as described by Golemis et al. (39). For the transcriptional complementation experiments, Gln3p-dependent reporter plasmid pJCD52 (16) replaced the LexA-dependent reporter plasmid pSH18-34.

Detection of β-galactosidase in yeast cells. Yeast plasmids containing the *Escherichia coli lacZ* gene flanked by yeast transcriptional signals were used to evaluate the expression patterns and transcriptional potentials of native and chimeric yeast transcription factors. The intracellular levels of β-galactosidase were determined by using a modification of the method of Guarente and Mason (40) as follows. Up to four independent colonies from each transformation were inoculated each into 20 ml of appropriate growth medium and incubated at 26°C with agitation to a cell density of A_{600} of 0.3 to 0.8 prior to assay. Data are expressed in Miller units.

Translation and charge calculations. Conceptual translation and charge calculations were performed by using the EditSeq module of DNASTAR (DNASTAR, Inc., Madison, Wis.) software. Oligonucleotides were designed by using the Oligo 4.0 program from National BioSciences, Inc., Plymouth, Minn.

RESULTS

Requirements of the N-terminal acidic region and Zn finger motif for Gln3p function. To evaluate the requirement of the GATA-zinc finger and N-terminal acidic regions for Gln3p function in vivo, we constructed plasmids that express wild-type (pVS316) and mutant (pVS316-2 and pVS316-3) *GLN3* alleles under control of their native promoter (Fig. 1). Plasmids pVS316-2 and pVS316-3 directed synthesis of mutant Gln3 proteins which contained a C306S substitution of the conserved cysteine residue required for the integrity of the GATA-type Zn finger motif (pVS316-2) or lacked the hypothesized acidic activation region (amino acids 2 to 150 deleted; pVS316-3). The vector plasmid control (pRS316) contained no *GLN3*-related sequences (62). These plasmids were used to transform *gln3* deletion strain RR91, and the growth of these transformants in glucose-asparagine medium was measured (Table 3); the *gln3* phenotype is slow growth under these conditions (49). Deletion of the chromosomal *GLN3* locus (strain RR91) increases the doubling time in glucose-asparagine medium six- to sevenfold compared to wild-type strain TCY1, consistent with earlier reports for *gln3* strains containing point

TABLE 3. Doubling times of *S. cerevisiae* strains carrying various *GLN3* alleles

Strain (pertinent genotype)	Plasmid (genotype)	Doubling time (min)[a]
TCY1 (*GLN3*)	None	110
RR91 (*gln3*Δ)	None	727
	pRS316 (vector)	720
	pVS316 (*GLN3*)	236
	pVS316-2 (*gln3* [C306S])	704
	pVS316-3 (*gln3* [deletion of amino acids 2–150])	711

[a] Determined as described by Cunningham et al. (29, 49). We cannot explain the twofold-slower growth observed when complementation with a plasmid-borne *GLN3* gene is compared to that of a chromosomal copy (strain TCY1). This might have resulted, however, from changing the *Aat*II site in the *GLN3* promoter to a *Sal*I site, which was required for cloning this large gene into the centromeric vector.

mutations (49). Transformation of strain RR91 with centromeric plasmid pVS316 containing wild-type *GLN3* decreased its doubling time about threefold relative to transformation with the vector (plasmid pRS316). In contrast, the growth rate was not significantly increased when RR91 cells were transformed with parent vector pRS316 or either of the two *gln3* mutants (plasmids pVS316-2 and -3) (Table 3). These data demonstrated that Gln3p motifs, tentatively identified by sequence homologies, were essential for the protein's biological role, and strengthened the designation of Gln3p as a positive transcriptional regulator.

Assay of Gln3p transcriptional activation potential in the LexA fusion assay system. We examined the putative acidic activation region of Gln3p by first analyzing a full-length LexA-Gln3 fusion protein (plasmid pVS32) (Fig. 1). This plasmid was transformed into host strain EGY48, containing plasmid pSH18-34, a LexA activator-dependent reporter plasmid (Fig. 1). Consistent with its role as a positive regulator of NCR-sensitive gene expression, the Gln3p-LexA fusion directed high-level expression of the reporter gene compared to LexAp alone (plasmid pEG202) or fused to the transcriptionally inert moiety of LexA-bicoid protein (plasmid pRFHM1) (Fig. 2A).

Before proceeding with analysis of the Gln3p transcriptional activation region, we tested its operation relative to the quality of nitrogen source available in the growth medium. A subset of yeast transcriptional activators (e.g., Gal4p and Put3p) have been found to retain their sensitivity to environmental regulatory signals when fused to a LexA- or Gal4p-derived DNA binding module (reviewed in reference 67). Therefore, we assayed the transactivation potential of LexA-Gln3p in the presence of repressing (asparagine or glutamine) or derepressing (proline) nitrogen sources. The ability of LexA–full-length Gln3p to transactivate expression of the reporter gene in strain EGY48 responded weakly (less than twofold) if at all to nitrogen source quality (Fig. 2B). A similar result was observed with a strain derived from Σ1278b. This response cannot account for the NCR sensitivity of Gln3p-dependent gene expression (50- to 200-fold response) observed in vivo (reviewed in reference 17, 18, and 73).

Similarly constructed LexA-Put3 and LexA-Gal4 fusion proteins were assayed, as controls, for their response to the quality of nitrogen source provided. As expected from previously reported data (33), reporter gene expression supported by Put3p increased about 16-fold when the environmental signal (proline) was present (Fig. 2C). Moreover, the equivalent basal levels of reporter gene expression observed in minimal γ-aminobutyric acid (GABA) and minimal asparagine media argued that Put3p operation in transcriptional activation did not respond to NCR. Gal4p is not regulated by nitrogen source quality, and the levels of transcriptional activation supported by LexA-Gal4p in cells provided with proline, glutamine, or asparagine were equivalent (Fig. 2D). The normal proline inducibility of Put3p-mediated activation and lack of NCR sensitivity for Gal4p-mediated activation argued that the inability of LexA-Gln3p to respond to environmental signals (i.e., nitrogen source quality) was not a pervasive characteristic of the transactivation assay that we used.

The putative Gln3p activation region was first localized by creating several truncated LexA-Gln3p derivatives of parental plasmid pVS32 (Fig. 1). C-terminal truncation of Gln3p did not adversely affect its activation potential (Fig. 2A, plasmids pVS32, pVS32-3, and pVS32ΔXho). In fact, the LexA-tagged 149 Gln3p amino-terminal residues (expressed from plasmid pVS32ΔXho) supported almost threefold-greater transcriptional activation than the full-length Gln3p derivative (Fig. 2A). In contrast, a *gln3* mutant lacking the N-terminal 149 amino acids, corresponding to the hypothetical acidic activation region (48) (plasmid pVS32-4), possessed approximately 1/10

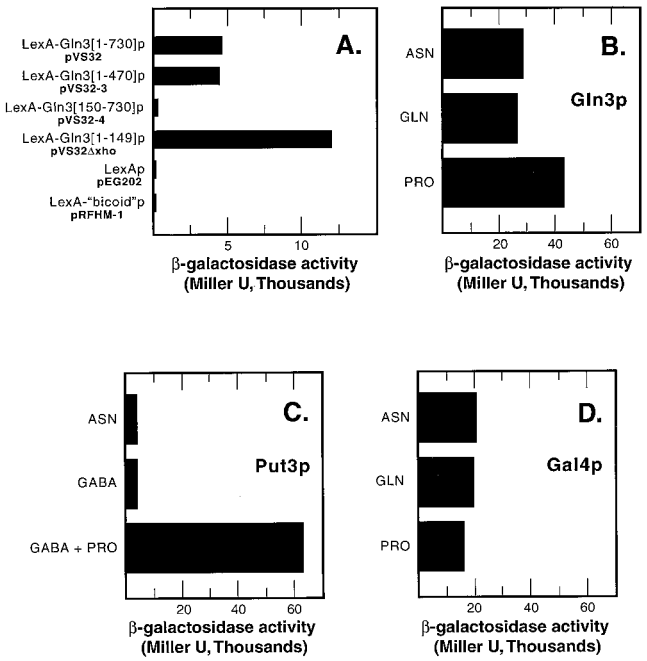

FIG. 2. (A) Transactivation mediated by wild-type and truncated Gln3-LexA fusion proteins. Host strain EGY48 was grown in CM Ura-His-glucose-glutamine medium. The reporter was plasmid p1840. (B) Transactivation mediated by full-length Gln3-LexA fusion protein in cells grown with a repressive (asparagine or glutamine) or derepressive (proline) nitrogen source. Host strain InvSc1 was grown in minimal medium supplemented as needed for auxotrophies and with 0.1% asparagine (ASN), glutamine (GLN), or proline (PRO) as sole nitrogen source. The reporter plasmid was pSH18-34, a reporter plasmid different from that used for panel A; this and the strain differences account for the difference observed in the wild-type activation levels for the experiments in panels A and B. (C) Transactivation by a full-length Put3-LexA fusion protein in cells provided with a repressive (asparagine) or derepressive (GABA) nitrogen source as well as with a medium that induces *PUT* gene expression (GABA + PRO). Host strain InvSc1 was grown in the presence of GABA, asparagine, or GABA plus proline as nitrogen source as described by des Etages et al. (33). The reporter was plasmid pSH18-34. (D) Transactivation by the Gal4[74-881]-LexA fusion protein assayed in cells provided with repressive and derepressive nitrogen sources. Host strain InvSc1 was grown in YNB-glucose medium supplemented as needed to meet auxotrophic requirements of the strain with 0.1% asparagine, glutamine, or proline as nitrogen source. The reporter plasmid was pSH18-34.

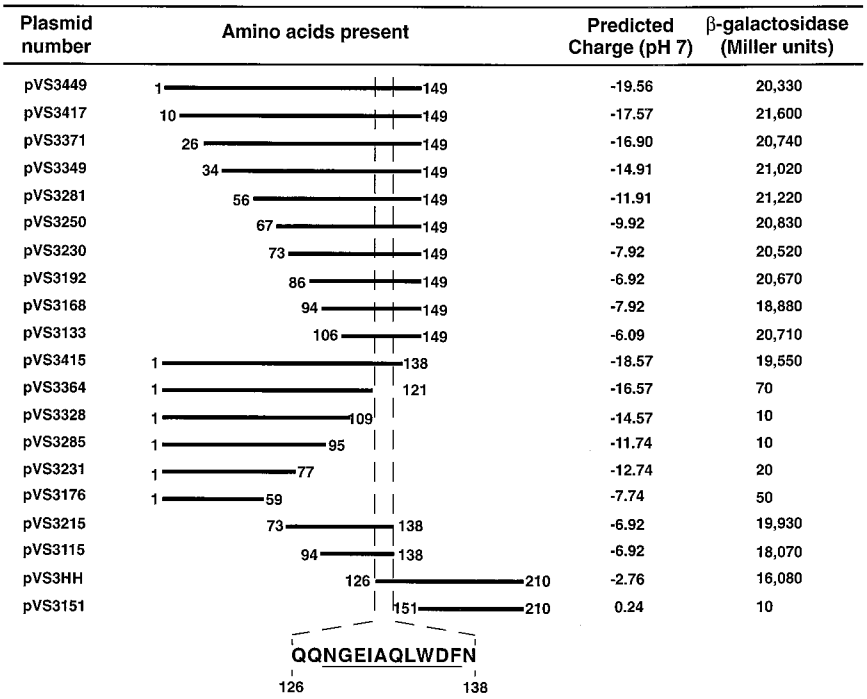

FIG. 3. Transactivation mediated by fragments of the Gln3p acidic region (residues 1 to 149) were constructed and expressed as LexA fusions in strain EGY48. β-Galactosidase production was measured by using reporter plasmid pSH18-34. The transformants were grown in CM Ura-His-glucose-ammonia medium. Amino acids predicted to possess a propensity to form an α-helix are underlined.

of the transactivation potential observed for the full-length Gln3p fusion (Fig. 2A).

Dissection of the Gln3p activation region. To more precisely localize the Gln3p region mediating transcriptional activation, we analyzed plasmid pVS3449, which expressed the N-terminal 149 amino acids of Gln3p shown in Fig. 2A to support transcriptional activation. We prepared a series of nested deletions and fused them to LexA as described in Materials and Methods. These truncated Gln3p derivatives were assayed for the ability to transactivate transcription. Deletions removing amino acids from the N terminus to residue 106 were as active in transactivation as the full-length 149-amino-acid peptide (Fig. 3, plasmids pVS3449 to pVS3133). A similar result was observed upon deleting 11 C-terminal amino acids from the peptide (Fig. 3, plasmid pVS3415). However, deleting the next C-terminal 17 amino acids decreased transactivation potential 200- to 300-fold (Fig. 3, plasmid pVS3364). These results localized a strong transactivation capability to the C-terminal fifth of the 149-amino-acid Gln3p fragment (residues 121 to 138). A similar loss of activation capability was observed with further C-terminal deletions (Fig. 3, plasmids pVS3328, pVS3285, pVS3231, and pVS3176). Four additional constructs (plasmids pVS3215, pVS3115, pVS3HH, and pVS3151, containing residues 73 to 138, 94 to 138, 126 to 210, and 151 to 210, respectively) further localized the transactivation potential to a 13-amino-acid Gln3p segment, $_{126}$QQNGEIAQLWDFN$_{138}$ (Fig. 3). This segment contains hydrophobic (I, L), aromatic (W, F), and acidic (E, D) amino acids and is predicted by the PHD secondary structure prediction program (55a, 55b; phd @EMBL-Heidelberg.de) to possess a propensity to form an α-helix. Comparison of truncated Gln3p derivatives able to support transcriptional activation failed to demonstrate a correlation between the predicted negative charge of active polypeptides (calculated at neutral pH) and their transactivation potentials (Fig. 3). In fact, amino acids required for Gln3p-mediated transcriptional activation (residues 126 to 138) were situated well beyond the highly acidic N-terminal region (residues 3 to 48) of Gln3[1-149]p.

To test the assumption that loss-of-function phenotypes did not result from the loss of the mutant proteins' abilities to occupy LexA operator sites, activation mutants were subjected to the repression assay of Golemis et al. (39). The data obtained, i.e., results of analyses of the transcriptional interference, indicated that all of the mutant proteins tested above were able to occupy the *lexA* operator sites to an extent that was comparable to that of LexA protein alone (Fig. 4).

Difficulties that occasionally occur as a result of deletion mutations prompted us to perform a second experiment to localize the activation region. DNA encoding a peptide (amino acids 106 to 149) that extended beyond the minimal 13-amino-acid activation region (residues 126 to 138) was heavily mutagenized, and the resulting mutants were scored by using a color-based plate assay of activation (see Materials and Methods). To limit mutagenesis to the activation region coding sequence, we performed error-prone PCR amplification of the *GLN3* fragment before cloning it into the LexA fusion vector. After random mutagenesis and screening for loss-of-function mutations, their phenotypes on plates were confirmed by liquid β-galactosidase assays (all had activity of less than 200 Miller units, compared to a wild-type value of approximately 20,000), and the mutant sequences were determined (Table 4). In agreement with the deletion mapping results reported above, all plasmids supporting less than 10% of wild-type transactivation potential possessed alterations of the sequence from amino acids 126 to 138. Thus, both deletion mapping and random mutagenesis suggested that the minimal activation region of Gln3p is localized to a putative α-helical segment of the protein located between amino acids 126 and 138.

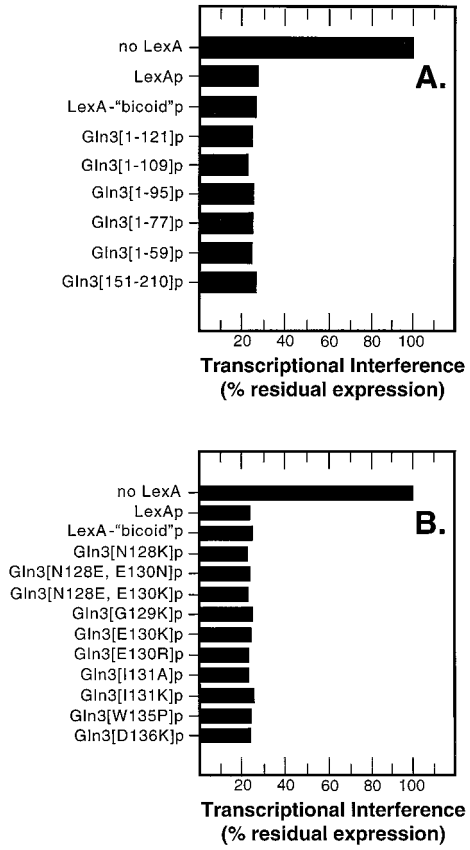

FIG. 4. Transcriptional interference/repression assays of inert or loss-of-function deletion (A) and point (B) mutants in the Gln3p acidic region. The repression assay was performed as described by Golemis et al. (39). Details of the plasmids used in this experiment are given in Fig. 3 and 5.

Mutational analysis of the Gln3p activation region. Using the foregoing data together with information on acidic activator structures available from the literature (e.g., the provisional significance of acidic, aromatic, and branched hydrophobic amino acids and the detrimental effects of helix-breaking and basic residues), we introduced specific point mutations into the Gln3p activation region and determined their effects on transactivation potential (Fig. 5). Alanine substitutions for aromatic amino acids (F73, F76, F110, W135, and F137) did not lead to substantial loss of transactivation potential, even when both aromatic residues (F73 and F76 or W135 and F137) present in the minimal wild-type activation module (residues 1 to 149; plasmid pVS3449) were substituted (Fig. 5, mutants F73A, F76A and W135A,F137A). Likewise, an alanine substitution of the acidic D136 residue had little effect on the transactivation potential (Fig. 5, D136A). Substitution of alanine for the remaining acidic residue (E130) resulted in a mutant that retained one-third of the wild-type activation potential. In contrast, introduction of lysine or arginine at either position was very deleterious (Fig. 5, E130K, E130R, and D136K). An N128K substitution (this substitution was also obtained by random mutagenesis in Table 4) similarly destroyed activation capability of the region, though this result is less straightforwardly interpreted because an alanine substitution at position N128 diminished activation potential to about 10% of the wild-type level (Fig. 5). Together, these data argue that incorporation of basic residues into the Gln3[126-138]p segment invariably resulted in loss-of-function, indicating that the presence of lysine or arginine within the activation module is incompatible with its operation. Although alanine substitutions of the aromatic amino acids that we tested were well tolerated, replacement of either of the other two hydrophobic residues (I131 and L134) with alanine eliminated and nearly eliminated transactivation potential, respectively (Fig. 5, I131A and L134A). Critical dependence on isoleucyl and/or leucyl residues has been reported for other native and artificial eucaryal activation regions as well (5, 37, 58). Finally, the helix-breaking W135P

TABLE 4. Deduced amino acid sequences of Gln3[106-149] proteins exhibiting less than 10% of wild-type activity[a]

Assay	Charge (pH 7.0)	Peptide sequence[b] (126 … 138)	Charge (pH 7.0)
A		DIAMFDSLATTQPIDIAASNQQNGEIAQLWDFNVDQFNMTPSNS[c]	−6.09
	1-1	DIAMFDSLATTQPIDIAASNQQNvklhnfgtltwtnat	−1.92
	1-2	DIAMFDSLAiTQPIDIAASNQQNvklhnfgtltwtnat	−1.92
	1-3	DIAMFDShATTQPlglAASNQQNvklhnfgtltwtnst	−0.76
	1-4	DIAMFDSLAqlspstqllgvmlnwstlksqscris	−0.12
	1-5	DIAMFDSLATTQPIDIAhptnimvklhnfgtl	−3.59
	1-6	DIAMFDSLvTTQPIDIAASNQQNGENA	−4.09
	1-7	DIAMFDSLATTQPnvmAASNQQNveta	−3.09
	1-8	DIAMFDSLATTQPIDaAASNQ	−3.09
	1-9	DIAMFDSLAqlspst	−3.92
	1-10	DIAMFDSLAqpsssi	−2.09
	1-11	DIAMFDSLprlgpst	−1.09
	1-12	DIAMFDSLATT	−2.09
B		DIAMFDSLATTQPIDIAASNQQNGEIAQLWDFNVDQFNMTPSNS[c]	−6.09
	2-1	DIAMFDShATTQPIDIAASNQQNGklAQLsDFNVDQINMTPSNS	−3.92
	2-2	DIAMFDSLATTQPIDIAASNQQNGglAQLrgFNmDkFNMTPSNS	−2.09
	2-3	DIAMFDSLAaTQPIDIAASNQliGEIAQLWDFNVDQFNMTPSNS	−6.09
	2-4	DIAMFDSLATTQPIDIAASNQQkGEIAQLWDFNVDQFNMTPSNS	−5.09

[a] Transcriptional activation in cells transformed with a wild-type Gln3p-encoding gene fragment carried by plasmid pVS3133 (Gln3[106-149]p) and its mutant alleles. Assays were conducted in yeast strain EGY48 transformed with reporter (*lexA-lacZ*) plasmid pSH18-34 as described earlier (39). The medium contained proline as the sole nitrogen source. Electrostatic charge was calculated by using conceptual translation of the mutated nucleotide sequences.

[b] Mutant residues are indicated in lowercase.

[c] Wild-type sequence.

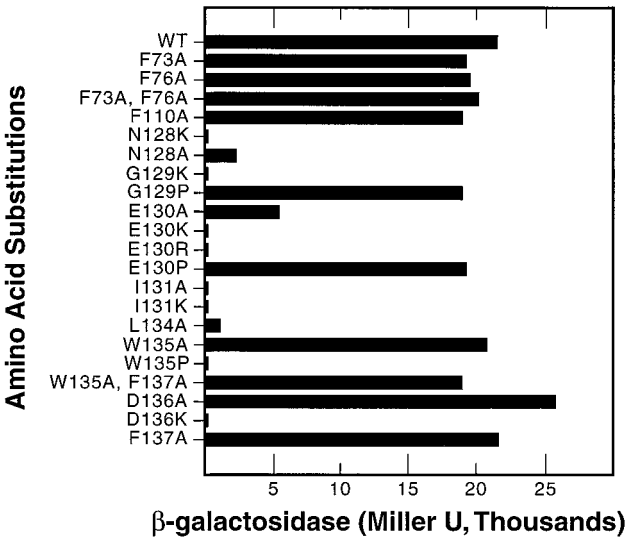

FIG. 5. Effects of alterations in the Gln3p acidic region on transactivation. Point mutations causing single amino acid substitutions in the putative acidic activation region were constructed and expressed as LexA fusion proteins in yeast strain EGY48, using plasmid pSH18-34 as the *lacZ* reporter. The medium was CM Ura-His-glucose-ammonia medium. Plasmids used: wild type (WT), pVS3449; F73A, pVS373A; F76A, pVS376A; F37A,F76A, pVS37376AA; F110A, pVS3110A; N128K, pVS3128NK; N128A, pVS3128NA; G129K, pVS3129GK; G129P, pVS3129GP; E130A, pVS3130EA; E130K, pVS3130EK; E130R, pVS3130ER; E130P, pVS3130EP; I131A, pVS3131IA; I131K, pVS3131IK; L134A, pVS3134LA; W135A, pVS3135WA; W135P, pVS3135WP; W135A, F137A, pVS33537AA; D136A, pVS3136DA; D136K, pVS3136DK; F137A, pVS3137FA.

substitution resulted in an essentially loss-of-function phenotype. However, a W135A substitution, which is compatible with α-helix formation, did not lead to substantially diminished transcriptional activation. Proline substitutions at positions G129 and E130 were not deleterious (Fig. 5, G129P and E130P). In this context, it is pertinent to recall that proline substitutions have been reported to be tolerable when situated at the beginning of a helical motif (50, 55).

Effect of the *gln3* D136K mutation on *DAL5* transcriptional activation. To assess the effect of an activation region point mutation on Gln3p-dependent transcription in vivo, the D136K mutation (Fig. 1, plasmid pVS3DK), which leads to loss of transactivation by the Gln3p-derived activation region, was tested for its ability to complement a *gln3* deletion (strain RR91-1). Wild-type plasmid pVS351 (Fig. 1) was used as a positive control. The plasmid-borne *GLN3* gene (pVS351) fully complemented the genomic *gln3* deletion; i.e., *DAL5-lacZ* expression was the same as observed in a wild-type control. Moreover, this *DAL5* expression exhibited normal NCR sensitivity (Fig. 6). Mutant plasmid pVS3DK expressing a *gln3* mutant protein (Fig. 1, D136K substitution) was similarly tested. This mutation resulted in significantly diminished DAL5 expression (Fig. 6), which is consistent with its detrimental effect on activation by the cognate LexA fusion. Residual *DAL5* transcription, however, remained NCR sensitive.

DISCUSSION

In agreement with earlier conclusions, based on sequence homology (48), our data demonstrate the necessity of the Gln3p GATA-type zinc finger motif and putative transcriptional activation region for its operation. A single-point mutation in a conserved cysteine residue of the zinc finger motif (C306S) failed to complement a *gln3* deletion for growth. A similar result was found when the N-terminal acidic region of Gln3p was deleted. Genetic analysis of the Gln3p N-terminal acidic region (amino acids 1 to 149) identified the 13-residue peptide QQNGEIAQLWDFN (Gln3p amino acids 126 to 138) as the minimum region required for transcriptional activation in the LexA assay system. This peptide contains acidic and hydrophobic amino acids and is predicted to possess a propensity to fold into an α-helix (residues 128 to 137), thus correlating with the characteristics of other eucaryal acidic activation regions (41). Although negatively charged amino acid clusters have served to predict the existence and gross positions of activation motifs including that of Gln3p (6, 25, 38, 48, 58, 70), the Gln3p region shown in this work to be required for transcriptional activation contains only two negatively charged amino acids, both of which may be converted to alanine without total loss of activity. There is also an absence of correlation between the overall negative charge and magnitude of transactivation directed by particular Gln3p derivatives fused to LexA. These findings are further evidence demonstrating the dispensability of most negatively charged amino acids for activation by typical acidic activators such as Gal4p or Gcn4p (44). Although contained in an acidic region, the Gln3p transactivation region is situated 50 or more amino acids C terminal to the region of greatest acidity (residues 3 to 48). Analysis of point mutations generated within the Gln3p activation region demonstrate the dispensability of aromatic and acidic amino acids as well as the importance of branched amino acids I131 and L134. We finally demonstrate the detrimental effects of introducing basic (Lys and Arg) or α-helix-breaking residues into the minimal activation motif of Gln3p.

Consistent with its role as a major positive regulator of yeast nitrogen metabolism, Gln3p, or even a small fraction of the protein, mediated strong transcriptional activation from a LexA-sensitive promoter when expressed in yeast cells as a LexA fusion. This transcriptional activity, however, was nearly insensitive to the quality of the nitrogen source present in the medium. The importance of the region that mediates transcriptional activation to the in vivo function of Gln3p is further emphasized by the finding that a single-point mutation in the activation motif (D136K) diminished significantly (11-fold) the ability of the mutant protein to complement a *gln3* deletion and support NCR-sensitive *DAL5* expression. The residual 9% of *DAL5* expression, however, remained NCR sensitive.

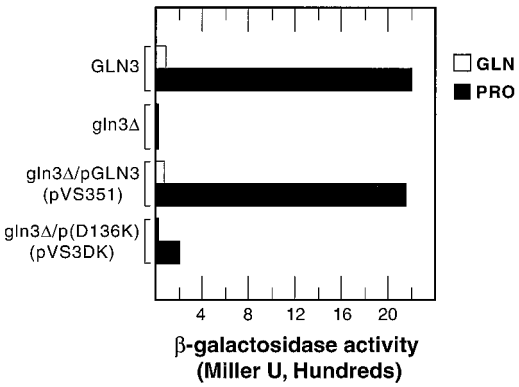

FIG. 6. Complementation of a *gln3*Δ mutation by a full-length wild-type *GLN3* gene (plasmid pVS351) and a single-point-mutant (pVS3DK) *gln3* allele. Wild-type and D136K mutant Gln3 proteins were expressed by using the native *GLN3* promoter, and their effects on *lacZ* expression supported by *DAL5-lacZ* fusion plasmid pJCD52 were assayed in *gln3* strain RR91-1 grown in YNB medium supplemented as needed to fulfill the strain's auxotrophies and using 0.1% glutamine (GLN) or proline (PRO) as sole nitrogen source.

Transcriptional activation potentials of Gln3[1-470]p and full-length Gln3p were two- to threefold lower than that observed for Gln3[1-149]p (Fig. 2A). Although this observation may be interpreted to mean that regions down-regulating the activation region are situated between residues 149 and 470, the physiological significance of this observation is yet to be demonstrated. Alternatively, the observation could derive simply from the fact that LexA–full-length Gln3p is sequestered by genomic GATA sequences (the protein contains a wild-type binding site), whereas LexA-Gln3[1-149]p is not. Arguing against this explanation, however, is the fact that Gln3p was highly overproduced in both instances.

The lack of strong nitrogen source-dependent transactivation supported by LexA-Gln3p is also consistent with data indicating that its in vivo function likely extends significantly beyond transcriptional activation of the NCR-sensitive genes during growth on a derepressing nitrogen source. For example, Gln3p is required for expression of *GNP1* (glutamine permease) regardless of the nitrogen source used (75). Significantly, the promoter of *GNP1* does not contain UAS_{NTR}-homologous GATA sequences. What is not clear in this instance, however, is whether or not the Gln3p dependence is primary or more indirect. Additionally, pleiotropic effects of *gln3* disruptions were observed as slow growth in a variety of nutritional and other growth conditions (63). Both of these reports suggest that Gln3p acts as a positive regulator for the expression of at least a subset of its genomic targets in the presence of glutamine or other repressing nitrogen source, thus calling for significant revision of the current models explaining the diminished (NCR-sensitive) transcription of some but not all Gln3p-dependent genes.

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REFERENCES

1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of a *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:**541–545.
2. **Alevizopoulos, A., Y. Dusserre, M. Tsai-Pflugfelder, T. von der Weid, W. Wahli, and N. Mermod.** 1995. A proline-rich TGF-β-responsive transcriptional activator interacts with histone H3. Genes Dev. **9:**3051–3066.
3. **Andre, B., D. Talibi, S. S. Boudekou, C. Hein, S. Vissers, and D. Coornaert.** 1995. Two mutually exclusive regulatory systems inhibit UAS_{GATA}, a cluster of 5′GAT(A/T)A-3′ upstream from the *UGA4* gene of *Saccharomyces cerevisiae*. Nucleic Acids Res. **23:**558–564.
4. **Artandi, S. E., K. Merrell, N. Avitahl, K. K. Wong, and K. Calame.** 1995. TFE3 contains two activation domains, one acidic and the other proline-rich, that synergistically activate transcription. Nucleic Acids Res. **23:**3865–3871.
5. **Attardi, L. D., and R. Tjian.** 1993. *Drosophila* tissue-specific transcription factor NTF-1 contains a novel isoleucine-rich activation motif. Genes Dev. **7:**1341–1353.
6. **Blair, W. S., H. P. Bogerd, S. J. Madore, and B. R. Cullen.** 1994. Mutational analysis of the transcription activation domain of RelA: identification of a highly synergistic minimal acidic activation module. Mol. Cell. Biol. **14:**7226–7234.
7. **Blair, W. S., R. A. Fridell, and B. R. Cullen.** 1996. Synergistic enhancement of both initiation and elongation by acidic transcription activation domain. EMBO J. **15:**1658–1665.
8. **Blau, J., H. Xiao, S. McCracken, P. O'Hare, J. Greenblatt, and D. Bentley.** 1996. Three functional classes of transcriptional activation domains. Mol. Cell. Biol. **16:**2044–2055.
9. **Blinder, D., P. Coschigano, and B. Magasanik.** 1996. Interaction of the GATA factor Gln3p with the nitrogen regulator Ure2p in *Saccharomyces cerevisiae*. J. Bacteriol. **178:**4734–4736.
10. **Blinder, D., and B. Magasanik.** 1995. Recognition of nitrogen-responsive upstream activation sequences of *Saccharomyces cerevisiae* by the product of the *GLN3* gene. J. Bacteriol. **177:**4190–4193.
11. **Bysani, N., J. R. Daugherty, and T. G. Cooper.** 1991. Saturation mutagenesis of the UAS_{NTR} (GATAA) responsible for nitrogen catabolite repression-sensitive transcriptional activation of the allantoin pathway genes in *Saccharomyces cerevisiae*. J. Bacteriol. **173:**4977–4982.
12. **Chisholm, G., and T. G. Cooper.** 1982. Isolation and characterization of mutations that produce the allantoin-degrading enzymes constitutively in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **2:**1088–1095.
13. **Coffman, J. A., H. M. El Berry, and T. G. Cooper.** 1994. The URE2 protein regulates nitrogen catabolic gene expression through the GATAA-containing UAS_{NTR} element in *Saccharomyces cerevisiae*. J. Bacteriol. **176:**7476–7483.
14. **Coffman, J. A., R. Rai, and T. G. Cooper.** 1995. Genetic evidence for Gln3p-independent, nitrogen catabolite repression-sensitive gene expression in *Saccharomyces cerevisiae*. J. Bacteriol. **177:**6910–6918.
15. **Coffman, J. A., R. Rai, T. Cunningham, V. Svetlov, and T. G. Cooper.** 1996. Gat1p, a GATA-family protein whose production is sensitive to nitrogen catabolite repression, participates in transcription activation of nitrogen catabolic genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**847–858.
16. **Coffman, J. A., R. Rai, D. M. Loprete, T. Cunningham, V. Svetlov, and T. G. Cooper.** 1997. Cross regulation of four GATA factors that control nitrogen catabolic gene expression in *Saccharomyces cerevisiae*. J. Bacteriol. **179:**3416–3429.
17. **Cooper, T. G.** 1982. Nitrogen metabolism in *Saccharomyces cerevisiae*, p. 39–99. *In* J. N. Strathern, E. W. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Cooper, T. G.** 1996. Allantoin degradative system—an integrated transcriptional response to multiple signals, p. 139–169. *In* R. Brambl and G. Marzluf (ed.), Mycota. Springer-Verlag, Berlin, Germany.
19. **Cooper, T. G., D. Ferguson, R. Rai, and N. Bysani.** 1990. The *GLN3* gene product is required for transcriptional activation of allantoin system gene expression in *Saccharomyces cerevisiae*. J. Bacteriol. **172:**1014–1018.
20. **Cooper, T. G., R. Rai, and H. S. Yoo.** 1989. Requirement of upstream activation sequences for nitrogen catabolite repression of the allantoin system genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**5440–5444.
21. **Coornaert, D., S. Vissers, B. Andre, and M. Grenson.** 1992. The *UGA43* negative regulatory gene of *Saccharomyces cerevisiae* contains both a GATA-1 type zinc finger and a putative leucine zipper. Curr. Genet. **21:**301–307.
22. **Coschigano, P. W., and B. Magasanik.** 1991. The *URE2* gene product of *Saccharomyces cerevisiae* plays an important role in the cellular response to the nitrogen source and has homology to glutathione *S*-transferase. Mol. Cell. Biol. **11:**822–832.
23. **Courchesne, W. E., and B. Magasanik.** 1988. Regulation of nitrogen assimilation in *Saccharomyces cerevisiae*: roles of the *URE2* and *GLN3* genes. J. Bacteriol. **170:**708–713.
24. **Coustry, F., S. N. Maity, and B. de Crombrugghe.** 1995. Studies on transcription activation by the multimeric CCAAT-binding factor CBF. J. Biol. Chem. **270:**468–475.
25. **Cress, W. D., and S. J. Triezenberg.** 1991. Critical structural elements of the VP16 transcriptional activation domain. Science **251:**87–90.
26. **Cunningham, T. S., and T. G. Cooper.** 1991. Expression of the *DAL80* gene, whose product is homologous to the GATA factors and is a negative regulator of multiple nitrogen catabolic genes in *Saccharomyces cerevisiae*, is sensitive to nitrogen catabolite repression. Mol. Cell. Biol. **11:**6205–6215.
27. **Cunningham, T. S., and T. G. Cooper.** 1993. The *Saccharomyces cerevisiae DAL80* repressor protein binds to multiple copies of GATAA-containing sequences (URS_{GATA}). J. Bacteriol. **175:**5851–5861.
28. **Cunningham, T. S., R. A. Dorrington, and T. G. Cooper.** 1994. The *UGA4* UAS_{NTR} site required for *GLN3*-dependent transcriptional activation also mediates *DAL80*-responsive regulation and DAL80 protein binding in *Saccharomyces cerevisiae*. J. Bacteriol. **176:**4718–4725.
29. **Cunningham, T. S., V. Svetlov, R. Rai, and T. G. Cooper.** 1996. *Saccharomyces cerevisiae* Gln3p binds to UAS_{NTR} elements and activates transcription of nitrogen catabolite repression-sensitive genes. J. Bacteriol. **178:**3470–3479.
30. **Dahlman-Wright, K., H. Baumann, I. J. McEwan, T. Almlöf, A. P. H. Wright, J.-Å. Gustafsson, and T. Härd.** 1995. Structural characterization of a minimal functional transactivation domain from the human glucocorticoid receptor. Proc. Natl. Acad. Sci. USA **92:**1699–1703.
31. **Daugherty, J. R., R. Rai, H. M. ElBerry, and T. G. Cooper.** 1993. Regulatory circuit for responses of nitrogen catabolic gene expression to the GLN3 and DAL80 proteins and nitrogen catabolite repression in *Saccharomyces cerevisiae*. J. Bacteriol. **175:**64–73.
32. **DeFalco, J., and G. Childs.** 1996. The embryonic transcription factor stage specific activator protein contains a potent bipartite activation domain that interacts with several RNA polymerase II basal transcription factors. Proc. Natl. Acad. Sci. USA **93:**5802–5807.
33. **des Etages, S. A. G., D. A. Falvey, R. J. Reece, and M. C. Brandriss.** 1996. Functional analysis of the PUT3 transcriptional activator of the proline utilization pathway in *Saccharomyces cerevisiae*. Genetics **142:**1069–1082.
34. **Drillien, R., M. Aigle, and F. Lacroute.** 1973. Yeast mutants pleiotropically

impaired in the regulation of two glutamate dehydrogenases. Biochem. Biophys. Res. Commun. **53:**367–372.
35. **Drillien, R., and F. Lacroute.** 1972. Ureidosuccinic acid uptake in yeast and some aspects of its regulation. J. Bacteriol. **109:**203–208.
36. **Estojak, J. R., R. Brent, and E. A. Golemis.** 1995. Correlation of two-hybrid affinity data with in vitro measurements. Mol. Cell. Biol. **15:**5820–5829.
37. **Feng, B., and G. Marzluf.** 1996. The regulatory protein NIT4 that mediates nitrate induction in *Neurospora crassa* contains a complex tripartite activation domain with a novel leucine-rich acidic motif. Curr. Genet. **29:**537–548.
38. **Giniger, E., and M. Ptashne.** 1987. Transcription in yeast activated by a putative amphipathic α helix linked to a DNA binding unit. Nature **330:**670–672.
39. **Golemis, E. A., J. Gyurius, and R. Brent.** 1994. Two hybrid systems/interaction traps, p. 13.14.1–13.14.17. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. S. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley and Sons, New York, N.Y.
40. **Guarente, L., and T. Mason.** 1983. Heme regulatory transcription of the *CYC1* gene of *S. cerevisiae* via an upstream activation site. Cell **32:**1279–1286.
41. **Hahn, S.** 1993. Structure(?) and function of acidic transcription activators. Cell **72:**481–483.
42. **Kim, T. K., and R. G. Roeder.** 1993. Transcriptional activation in yeast by the proline-rich activation domain of human CTF1. J. Biol. Chem. **268:**20866–20869.
43. **Kussie, P. H., S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine, and N. P. Pavletich.** 1996. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science **274:**948–953.
44. **Leuther, K. K., D. A. Bushnell, and R. D. Kornberg.** 1996. Two-dimensional crystallography of TFIIB- and IIE-RNA polymerase II complexes: implications for start site selection and initiation complex formation. Cell **85:**773–779.
45. **Ma, J., and M. Ptashne.** 1987. A new class of transcriptional activators. Cell **51:**113–119.
46. **McEwan, I. J., K. Dahlman-Wright, J. Ford, and A. P. Wright.** 1996. Functional interaction of the c-*Myc* transactivation domain with the TATA binding protein: evidence for an induced fit model of transactivation domain folding. Biochemistry **35:**9584–9593.
47. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 403. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Minehart, P. L., and B. Magasanik.** 1991. Sequence and expression of GLN3, a positive nitrogen regulatory gene of *Saccharomyces cerevisiae* encoding a protein with a putative zinc finger DNA-binding domain. Mol. Cell. Biol. **11:**6216–6228.
49. **Mitchell, A. P., and B. Magasanik.** 1984. Regulation of glutamine-repressible gene products by the *GLN3* function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**2758–2766.
50. **Presta, L. G., and G. D. Rose.** 1988. Helix signals in proteins. Science **240:**1632–1641.
51. **Ptashne, M., and A. A. F. Gann.** 1990. Activators and targets. Nature **346:** 329–331.
52. **Qui, H. F., E. Dubois, and F. Messenguy.** 1991. Dissection of the bifunctional ARGRII protein involved in the regulation of arginine anabolic and catabolic pathways. Mol. Cell. Biol. **11:**2169–2179.
53. **Rai, R., F. S. Genbauffe, and T. G. Cooper.** 1987. Transcriptional regulation of the *DAL5* gene in *Saccharomyces cerevisiae*. J. Bacteriol. **169:**3521–3524.
54. **Rai, R., F. S. Genbauffe, R. A. Sumrada, and T. G. Cooper.** 1989. Identification of sequences responsible for transcriptional activation of the allantoate permease gene in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**602–608.
55. **Richardson, J. S., and D. C. Richardson.** 1988. Amino acid preferences for specific locations at the ends of α-helices. Science **240:**1648–1652.
55a. **Rost, B., and C. Sander.** 1993. Prediction of protein structure at better than 70% accuracy. J. Mol. Biol. **232:**584–599.
55b. **Rost, B., and C. Sander.** 1994. Combining evolutionary information and neural networks to predict protein secondary structure. Proteins **19:**55–72.
56. **Rowen, D. W., E. Nwadiuto, and B. Magasanik.** 1997. Role of GATA factor Nil2p in nitrogen regulation of gene expression in *Saccharomyces cerevisiae*. J. Bacteriol. **179:**3761–3766.
57. **Schmitz, M. L., M. A. dos Santos Silva, H. Altmann, M. Czisch, T. A. Holak, and P. A. Baeurle.** 1994. Structural analysis of the NF-κB p65 C terminus. J. Biol. Chem. **269:**25613–25620.
58. **Seipel, K., O. Georgiev, and W. Schaffner.** 1994. A minimal transcription activation domain consisting of a specific array of aspartic acid and leucine residues. Biol. Chem. Hoppe-Seyler **375:**463–470.
59. **Shen, F., S. J. Triezenberg, P. Hensley, D. Porter, and J. R. Knutson.** 1996. Transcription activation domain of the herpesvirus protein VP16 becomes conformationally constrained upon interaction with basal transcription factors. J. Biol. Chem. **271:**4827–4837.
60. **Shen, F., S. J. Triezenberg, P. Hensley, D. Porter, and J. R. Knutson.** 1996. Critical amino acids in the transcriptional activation domain of the herpesvirus protein VP16 are solvent-exposed in highly mobile protein segments. J. Biol. Chem. **271:**4818–4826.
61. **Sigler, P. B.** 1988. Acid blobs and negative noodles. Nature **333:**210–212.
62. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
63. **Smith, V., K. N. Chou, D. Lashkari, D. Botstein, and P. O. Brown.** 1996. Functional analysis of the genes of yeast chromosome V by genetic footprinting. Science **274:**2069–2074.
64. **Soussi-Boudekou, S. Vissers, A. Urrestarazu, J.-C. Jauniaux, and B. Andre.** 1997. Gzf3p, a fourth GATA factor involved in nitrogen-regulated transcription in *Saccharomyces cerevisiae*. Mol. Microbiol. **23:**1157–1168.
65. **Stanbrough, M., and B. Magasanik.** 1996. Two transcription factors, Gln3p and Nil1p, use the same GATAAG sites to activate the expression of *GAP1* of *Saccharomyces cerevisiae*. J. Bacteriol. **178:**2465–2468.
66. **Svetlov, V., and T. G. Cooper.** 1996. Review: compilation and characteristics of dedicated transcription factors in *Saccharomyces cerevisiae*. Yeast **11:** 1439–1484.
67. **Svetlov, V., and T. G. Cooper.** Efficient PCR-based random mutagenesis of sub-genic (100 bp) DNA fragments. Yeast, in press.
68. **Tanaka, M., W. M. Clouston, and W. Herr.** 1994. The Oct-2 glutamine-rich and proline-rich activation domains can synergize with each other or duplicates of themselves to activate transcription. Mol. Cell. Biol. **14:**6046–6055.
69. **Van Hoy, M., K. K. Leuther, T. Kodadek, and S. A. Johnston.** 1993. The acidic activation domains of the GCN4 and GAL4 proteins are not α helical but form β sheets. Cell **72:**587–594.
70. **Vissers, S., B. Andre, F. Muyldermans, and M. Grenson.** 1989. Positive and negative regulatory elements control the expression of the *UGA* gene coding for the inducible 4-aminobutyric-acid-specific-permease in *Saccharomyces cerevisiae*. Eur. J. Biochem. **181:**357–361.
71. **Wiame, J.-M., M. Grenson, and H. Arst.** 1985. Nitrogen catabolite repression in yeasts and filamentous fungi. Adv. Microb. Physiol. **26:**1–87.
72. **Wickner, R. B.** 1994. [*URE3*] as an altered Ure2 protein: evidence for a prion analog in *Saccharomyces cerevisiae*. Science **264:**566–569.
73. **Wu, Y., R. J. Reece, and M. Ptashne.** 1996. Quantitation of putative activator-target affinities predicts transcriptional activating potentials. EMBO J. **15:**3951–3963.
74. **Xu, S., D. A. Falvey, and M. C. Brandriss.** 1995. Roles of *URE2* and *GLN3* in the proline utilization pathway in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:**2321–2330.
75. **Zhu, X., J. Garrett, J. Schreve, and T. Michaeli.** 1996. GNP1, the high affinity glutamine permease of *S. cerevisiae*. Curr. Genet. **30:**107–114.