Yeast Zinc Cluster Proteins Dal81 and Uga3 Cooperate by Targeting Common Coactivators for Transcriptional Activation of γ-Aminobutyrate Responsive Genes

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ABSTRACT In *Saccharomyces cerevisiae*, optimal utilization of various compounds as a nitrogen source is mediated by a complex transcriptional network. The zinc cluster protein Dal81 is a general activator of nitrogen metabolic genes, including those for γ-aminobutyrate (GABA). In contrast, Uga3 (another zinc cluster protein) is an activator restricted to the control of genes involved in utilization of GABA. Uga3 binds to DNA elements found in the promoters of target genes and increases their expression in the presence of GABA. Dal81 appears to act as a coactivator since the DNA-binding activity of this factor is dispensable but its mode of action is not known. In this study, we have mapped a regulatory, as well as an activating, region for Uga3. A LexA–Uga3 chimeric protein activates a lexA reporter in a GABA- and Dal81-dependent manner. Activation by Uga3 requires the SAGA complex as well as Gal11, a component of mediator. ChIP analysis revealed that Uga3 is weakly bound to target promoters. The presence of GABA enhances binding of Uga3 and allows recruitment of Dal81 and Gal11 to target genes. Recruitment of Gal11 is prevented in the absence of Dal81. Importantly, Dal81 by itself is a potent activator when tethered to DNA and its activity depends on SAGA and Gal11 but not Uga3. Overexpression of Uga3 bypasses the requirement for Dal81 but not for SAGA or Gal11. Thus, under artificial conditions, both Dal81 and Uga3 can activate transcription independently of each other. However, under physiological conditions, both factors cooperate by targeting common coactivators.

MANY unicellular organisms can use various nitrogen-containing compounds as a nitrogen source. In *Saccharomyces cerevisiae*, the favored sources of nitrogen are ammonium, glutamine, and asparagine. *S. cerevisiae* can also grow on nonpreferred nitrogen sources, including proline, urea, ornithine, allantoin, and γ-aminobutyrate (GABA). These nonpreferred nitrogen sources are converted into glutamate and glutamine. Nonpreferred nitrogen sources induce the derepression of many genes, which are involved in the utilization of these compounds and are unexpressed when a preferred nitrogen source is present (Godard *et al.* 2007). In *S. cerevisiae*, four GATA proteins are responsible for regulating the expression of these nitrogen catabolic genes. Gln3 and Gat1 are activators that induce the transcription of nitrogen catabolic genes in the presence of a non-favored nitrogen source, while Dal80 and Deh1 are transcriptional repressors (Wong *et al.* 2008).

The amino acid derivative GABA serves as a nonpreferred source of nitrogen in *S. cerevisiae* (Magasanik and Kaiser 2002). GABA can be degraded into succinate semialdehyde through the action of the GABA transaminase (encoded by the *UGA1* gene). This enzymatic reaction requires α-ketoglutarate or pyruvate, which is converted into glutamate or alanine, respectively. Succinate semialdehyde can be further degraded into succinate by the enzyme succinate semialdehyde dehydrogenase (encoded by *UGA2*), simultaneously catalyzing the production of NADPH from NADP (Coleman *et al.* 2001). Succinate can then be fed into the Krebs cycle for further energy production (Godard *et al.* 2007). Another important protein involved in the utilization of GABA is the permease Uga4, which mediates the specific

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uptake of GABA in *S. cerevisiae*. GABA is also able to enter *S. cerevisiae* nonspecifically through the proline permease Put4 and through the general amino acid permease Gap1 (André *et al.* 1993). Genome-wide studies have shown that expression of only five genes is induced by GABA, including the *UGA1* and *UGA4* genes (Godard *et al.* 2007). Expression of these two genes is regulated in part by GATA factors described above (Talibi *et al.* 1995; Marzluf 1997). In addition, the transcriptional activation of *UGA1* and *UGA4* depends on Dal81 (also called Uga35) and Uga3 (André 1990; Vissers *et al.* 1990; Coornaert *et al.* 1991; Talibi *et al.* 1995).

Dal81 and Uga3 are members of the family of zinc cluster proteins that form a major class of transcriptional regulators in *S. cerevisiae* (MacPherson *et al.* 2006). Zinc cluster proteins are typically composed of three domains: a DNA-binding domain containing a highly conserved cysteine-rich cluster, a regulatory domain, and an activation domain. The DNA-binding domain of most members of the zinc cluster protein family is situated in the N terminus of the proteins (MacPherson *et al.* 2006). The regulatory domain, also known as the middle homology region, is a region of approximately 80 amino acids that appears to play an important role in the regulation of the activity of zinc cluster proteins (Schjerling and Holmberg 1996). The activation domain of zinc cluster proteins is typically at the C terminus of the proteins and composed of acidic amino acid residues (Schjerling and Holmberg 1996). A number of zinc cluster proteins are activated by binding of small ligands and are the functional analogs of nuclear receptors found in metazoans (Naar and Thakur 2009).

Dal81 has been shown to be required for the transcriptional activation of a significantly large number of genes, whose functions include the catabolism of nitrogen sources such as urea, allantoin, arginine, and GABA. Dal81 is also required for the activation of *AGP1*, a wide-range specificity amino acid permease expressed in the presence of amino acids in the external environment of *S. cerevisiae* (Abdel-Sater *et al.* 2004). Dal81 has been shown to be necessary for the induction of *UGA1* and *UGA4* in the presence of GABA (André 1990; Talibi *et al.* 1995). GABA-dependent activation of *UGA1* and *UGA4* is also mediated by Uga3, which binds to upstream activating sequences (UAS_{GABA}) found in the promoters of these genes (André 1990; Vissers *et al.* 1990; Talibi *et al.* 1995).

Interestingly, Bricmont *et al.* (1991) have shown that a yeast strain expressing a mutant of Dal81 lacking the cysteine-rich zinc cluster motif did not have reduced levels of urea amidolyase, an enzyme encoded by the Dal81-regulated gene *DUR1,2*. Furthermore, this strain did not have detectable growth defects on medium containing GABA as the sole nitrogen source (Bricmont *et al.* 1991). It therefore appears that the zinc cluster motif of Dal81 is not required for the activation of at least some of its target genes. A similar observation was made for TamA, a protein found in the filamentous fungus *Aspergillus nidulans*, which is also required for the activation of genes involved in the catabolism of nitrogen sources such as GABA (Davis *et al.* 1996; Small *et al.* 2001).

Besides gene-specific activators and RNA polymerase, various complexes termed coactivators have been shown to play an important role in transcriptional activation. Mediator is a multiprotein complex that bridges gene regulators to general transcription factors and RNA polymerase II (Biddick and Young 2005; Casamassimi and Napoli 2007). The complex can be subdivided into three regions: the tail subcomplex that includes Gal11 and Sin4, the middle region, and the head region. Mediator produces its effect by directly interacting with the C-terminal domain of the largest subunit of RNA polymerase II. Another coactivator is the SAGA (Spt–Ada–Gcn5–acetyltranferase) complex, which possesses different enzymatic activities (Baker and Grant 2007). For example, the subunit Gcn5 is responsible for acetylation of various substrates including histone H3. Chromatin remodeling can be performed by the SWI/SNF complex, which can displace nucleosomes in an ATP-dependent manner (Mohrmann and Verrijzer 2005; Smith and Peterson 2005). *SWI2* encodes the catalytic subunit of this complex. RSC (remodel the structure of chromatin) is another example of an ATP-dependent chromatin remodeling complex (Mohrmann and Verrijzer 2005).

In this study, we show that GABA enhances DNA binding of Uga3 and recruitment of Dal81 and Gal11. Under artificial conditions, both Dal81 and Uga3 activate transcription independently of each other, a process mediated by SAGA and Gal11. Thus, under physiological conditions, Dal81 and Uga3 cooperate by targeting common coactivators for transcriptional activation.

Materials and Methods

Strains

Strains used in this study are listed in Table 1. The wild-type yeast strains used were BY4741 (*MAT***a** *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0), BY4742 (*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0) (Brachmann *et al.* 1998), and YPH499 (*MAT***a** *his3Δ200, leu2Δ1, lys2-801, trp1Δ1, ade2-101, ura3-52*) (Sikorski and Hieter 1989). Deletion strains were obtained from Invitrogen/Research Genetics (Huntsville, AL) (Winzeler *et al.* 1999). The open reading frame (ORF) of *UGA3* was tagged at its natural chromosomal location with a triple Myc epitope in strains BY4741 or BY4742 according to Schneider *et al.* (1995). Uga3 was tagged at the N terminus by transforming strain BY4741 with the PCR product obtained using plasmid pMPY-3XMYC as the template (Schneider *et al.* 1995) and the oligonucleotides CATGT ATGGATGCCAAGAAAACAAAGTTTTTTAAAGTGAGGT **ATG** AGGAACAAAAGCTGGAG and CCCATGCTTCGAATATTTCAA TTTCAGCTTCTCCACGCCATAATTTAGGGCGAATTGGGTACC. The nucleotides in boldface type correspond to the initiator-codon of the tagged ORF. After transformation, colonies were selected on plates lacking uracil, and homologous

Table 1 Partial list of strains used in this study

Strain	Genotype	Reference
BY4741	*MAT***a** *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0	Brachmann *et al.* (1998)
BY4742	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0	Brachmann *et al.* (1998)
YPH499	*MAT***a** *his3*Δ*200, leu2*Δ*1, lys2-801, trp1*Δ*1, ade2-101, ura3-52*	Sikorski and Hieter (1989)
BY4741 Δ*dal81*	*MAT***a** *his3*Δ1, *leu2*Δ0, *met15*Δ0 , *ura3*Δ0, *dal81*Δ::*kan*MX4	Research Genetics Winzeler *et al.* (1999)
BY4742 Δ*uga3*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0, *uga3*Δ::*kan*MX4	Research Genetics Winzeler *et al.* (1999)
BY4742 Δ*uga3*Δ*trp1* (parent BY4742 *uga3*)	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0, *trp1*Δ::*hisG, uga3*Δ::*kan*MX4	This work
BMASY1 Δ*dal81*Δ*uga3*Δ*trp1* (parent BY4741)	*MAT***a** *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0, *trp1*Δ::*hisG, dal81*Δ::*kan*MX4, *uga3*::*kan*MX4	This work
BY4742 HA-*UGA3*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0 [*UGA3* ORF tagged with 3 HA epitopes]	This work
BY4742 HA-*UGA3* Δ*dal81*	*MAT***a** *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0, Δ*dal81::LEU2* [*UGA3* ORF tagged with 3 HA epitopes]	This work
BY4742 HA-*DAL81*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0 [*DAL81* ORF tagged with 3 HA epitopes]	This work
HQY825 (parent BY4741)	MAT**a** *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0 *GAL11-myc*$_{13}$::*HIS3*	Qiu *et al.* (2005)
BMASY11 (parent HQY825)	*MAT***a** *his3*Δ1, *leu2*Δ0, *met15*Δ0, *ura3*Δ0 *GAL11-myc*$_{13}$::*HIS3, dal81*Δ::*LEU2*	This work
BY4742Δ*dal81*Δ*spt20*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0 *spt20*Δ::kanMX4, *dal81*Δ::*LEU2*	This work
BY4742Δ*dal81*Δ*spt3*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0 *spt3*Δ::kanMX4, *dal81*Δ::*LEU2*	This work
BY4742Δ*dal81*Δ*gal11*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0 *gal11*Δ::kanMX4, *dal81*Δ::*LEU2*	This work
BY4742Δ*dal81*Δ*med5*	*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0 *med5*Δ::kanMX4, *dal81*Δ::*LEU2*	This work

Strains not listed in this table are deletion strains that were obtained from Research Genetics (Winzeler *et al.* 1999).

recombination was verified by PCR. Cells were then grown overnight in YPD medium (Adams *et al.* 1997) to allow internal recombination between the two regions coding for the epitomes. *Ura3*$^-$ cells were selected on plates containing 5-fluoroorotic acid (Schneider *et al.* 1995). Tagging of *DAL81* was performed as described above using the oligos TGTTTA GACGAGCGGCAGAACGACAGGCAGCCATACTATCAA **ATG**A GGGAACAAAAGCTGGAG and CTTCGTAGGCGATGCGGCA TTATCAGCTGGTGATTGGTGAGGGTCTAGGGCGAATTGGG TACC and plasmid pMPY-3XHA as the template.

Strain BY4742 Δ*uga3*Δ*trp1* (*MAT*α *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0, *trp1*Δ::*hisG, uga3*Δ::*kan*MX4) was produced by gene disruption of the *TRP1* gene in BY4742 Δ*uga3*, as described by Alani *et al.* (1987). Specifically, strain BY4742 Δ*uga3* was transformed with a 4.7-kb fragment produced from the digestion of plasmid pNKY1009 (Alani *et al.* 1987) with the restriction enzymes *Eco*RI and *Bgl*II. The extremities of this 4.7-kb fragment were homologous to a portion of the *TRP1* gene and the middle section of the fragment was composed of the *URA3* gene flanked by two direct repeats of Salmonella *hisG* DNA. This fragment was inserted into the *TRP1* locus by homologous recombination and the *URA3* gene was eliminated by selecting for *Ura3*$^-$ strains on plates containing 5-fluoroorotic acid.

Strain BMASY1 Δ*dal81*Δ*uga3*Δ*trp1* (*MAT***a** *his3*Δ1, *leu2*Δ0, *lys2*Δ0, *ura3*Δ0, *trp1*Δ::*his, dal81*Δ::*kan*MX4, *uga3*Δ::*kan*MX4) was produced by first mating BY4742 Δ*uga3*Δ*trp1* with BY4741 Δ*dal81* followed by sporulation and tetrad dissection. Spore BMASY1 Δ*dal81*Δ*uga3*Δ*trp1* was confirmed as containing disruptions of *UGA3* and *DAL81* by Southern blot analysis (data not shown). Other strains carrying a deletion of *DAL81* were obtained by amplifying the *LEU2* marker using pRS405 (Sikorski and Hieter 1989) as a template and the oligonucleotides GCCTGTTTAGACGAGCGGCAGAA CGACAGGCAGCCATACTATCAAAGATTGTACTGAGAGTGCAC and TAGTTATTACCGTATTCCATTTTTACTTATGTGCTATTA TTTATACTGTGCGGTATTTCACACCG. The PCR product contains sequences homologous to the 5′ and 3′ regions of the *DAL81* gene.

Expression vectors

Expression vectors are described in Table 2. Plasmid p414MET25-3HA-*UGA3* was constructed in two steps. The *UGA3* ORF was first amplified by PCR, using genomic DNA from strain YPH499 as the template, with oligonucleotides CGGGATCCATGAATTATGGCGTGGAGAA and GGAATTCAC GCATAGTCAGGAACATCGTATGGGTATCAGGCAAAATTAAT ATTT. The resulting product was digested with the restriction enzymes *Eco*RI and *Bam*HI and subcloned into the low-copy plasmid p414MET25 (*TRP1* selective marker) (Mumberg *et al.* 1994) digested with the same enzymes, yielding the expression vector p414MET25-*UGA3*. The region coding for three HA epitopes was then amplified by PCR, using plasmid pMPY-3XHA (Schneider *et al.* 1995) as the template, with oligonucleotides GAAGATCTCTGCAGATGTACCCATACGATGT TCCT and GAAGATCTAGCAGCGTAATCTGGAACG. This PCR product was digested with the restriction enzyme *Bgl*II and subcloned into p414MET25-*UGA3* digested with *Bam*HI, thereby producing p414MET25-3HA-*UGA3*.

Plasmid p414MET25-3HA-*UGA3*(Δ124–300) was constructed by amplifying a region of the *UGA3* ORF by PCR, using p414MET25-3HA-*UGA3* as the template, with oligonucleotides GGGATTGCAGCTGTACAACTTCCCGATCGAACAATAC and TTACATGCGTACACGCGTTT. The resulting product was digested with the restriction enzyme *Bsr*G1 and subcloned into p414MET25-3HA-UGA3 digested with the same enzyme.

Table 2 Proteins used in the analysis of Uga3 and Dal81

HA-Uga3, Myc-Uga3	Full-length Uga3, tagged with 3 HA or Myc epitopes at the N terminus
HA-Uga3 (Δ124–300)	Internal deletion of Uga3 lacking aa 124–300, tagged with 3 HA epitopes at the N terminus
HA-Uga3 (Δ124–350)	Internal deletion of Uga3 lacking aa 124–350, tagged with 3 HA epitopes at the N terminus
HA-Uga3 (Δ518–528)	C-terminal deletion of Uga3 lacking aa 518–528, tagged with 3 HA epitopes at the N terminus
LexA-Uga3 (78–528)	Amino acids 78–528 of Uga3 fused to DNA-binding domain of LexA
HA-Dal81	Full-length Dal81, tagged with 3 HA epitopes at the N terminus
HA-Dal81ΔZn	Truncated Dal81 lacking aa 150–179 including all cysteines of the zinc cluster, tagged with 3 HA epitopes at the N terminus
LexA-Dal81	Full-length Dal81 fused to DNA-binding domain of LexA

Plasmid p414MET25-3HA-*UGA3*(Δ124–350) was constructed in two steps. A region of the *UGA3* ORF was first amplified by PCR, using p414MET25-*UGA3* as the template, with oligonucleotides ATCATTGTACAACGTCACAAATTTGA CGAG and TTACATGCGTACACGCGTTT. The resulting product was digested with *Bsr*G1 and subcloned into p414MET25-*UGA3*. The obtained plasmid was afterward digested with *Btg*I and *Nsi*I, and the smaller fragment was subcloned into p414MET25-3HA-*UGA3* digested with the same enzymes.

Plasmid p414MET25-3HA-*UGA3*(Δ518–528) was constructed by amplifying a region of the *UGA3* ORF by PCR, using p414MET25-3HA-*UGA3* as the template, with oligonucleotides GGAATTCATCAAATCATGTGGACCAGGTC and GACGATGCATGCTGAACTACAGATACTGAGA. The resulting product was digested with the restriction enzymes *Nsi*I and *Eco*RI, and subcloned into p414MET25-3HA-UGA3 digested with the same enzymes.

Plasmid p414MET25-lexA-*UGA3*(78-528) was constructed by amplifying the region coding for the DNA-binding domain of LexA by PCR, using plasmid pEG202 (Ausubel *et al.* 1997) as the template, with oligonucleotides CCGGGATCCATGAA AGCGTTAACGGCCA and GCTATGGCATGCGGCGGGAATTC CAGCCAGT. The resulting product was digested with *Bam*HI and *Sph*I and subcloned into p414MET25-*UGA3* digested with the same enzymes.

Plasmid p423*MET25*-lexA-HA-*DAL81* was constructed in two steps. The *DAL81* ORF was amplified by PCR using genomic DNA from strain BY4741 HA-*DAL81* (Akache *et al.* 2004) as the template and oligonucleotides AGCTAATCG TCGACTTACAGAGGGGTTTCCCTTG and CCATCGATGAGG GAACAAAAGCTGGA. The resulting product was digested with *Sal*I and *Cla*I and subcloned into p423MET25 (Mumberg *et al.* 1994) to yield p423MET25-3HA-*DAL81*. The region coding for the DNA-binding domain of LexA was amplified by PCR, using plasmid pEG202 (Ausubel *et al.* 1997) as the template, with oligonucleotides CCATCGATGAAAGCGTTAACGGCCAG and CCATCGATAACGGGAATTCCAGCCAGTCGC. The resulting product was digested with *Cla*I and subcloned into p423MET25-3HA-*DAL81* digested with the same enzyme.

Plasmid p423MET25-3HA-DAL81 was constructed by amplifying the *DAL81* ORF by PCR using genomic DNA from strain BY4742 HA-*DAL81* as template with oligonucleotides AGCTAATCGTCGACTTACAGAGGGGTTTCCCTTG and CCA TCGATGAGGGAACAAAAGCTGGA. The resulting product was digested with *Sal*I and *Cla*I and subcloned into the high-copy vector p423MET25 (Mumberg *et al.* 1994) cut with *Cla*I and *Xho*I.

Plasmid p423MET25-3HA-DAL81ΔZn was constructed by using plasmid p423MET25-3HA-DAL81 as a template for production of single-stranded DNA and oligonucleotide AGCAATACTGAAGGTAGATCCCATCAGATT for site-directed mutagenesis. This resulted in the deletion of *DAL81* sequences encoding aa 150–179.

Plasmid p413*MET25*-3HA-*UGA3* was constructed by subcloning a 1.5-kb *Spe*I–*Eco*RI fragment (containing the *UGA3* ORF) from plasmid p414*MET25*-3HA-*UGA3* into p413MET25 cut with the same enzymes (Mumberg *et al.* 1994).

Reporters

The reporter pUAS$_{GABA}$-lacZ is a high-copy plasmid containing a *URA3* marker that was constructed by inserting the double-stranded oligonucleotides TCGAAAAGCCGCGGGCG GGATTGTA and AATCCCGCCCGCGGCTTT in front of a minimal *CYC1* promoter driving lacZ transcription, as previously described ("UGA1-WT" in Noël and Turcotte 1998). The pSH18.34 reporter is a high-copy plasmid containing a *URA3* marker with eight lexA-binding sites in front of a minimal *GAL1* promoter driving lacZ transcription (Ausubel *et al.* 1997).

β-Galactosidase assays

Yeast strains were transformed with reporters, and expression vectors when appropriate, and were grown on selective medium lacking uracil and/or tryptophan and/or histidine, depending on the selective marker contained on the plasmids. Transformed colonies were grown overnight in YPD medium (Adams *et al.* 1997). Cells were then diluted in SC medium (Adams *et al.* 1997) (minus the amino acids used for selection) at 0.008% each, and 2% glucose. For assays shown in Figures 3 and 6, drop-out media were used. The activity of the reporters was assayed in the absence and in the presence of 0.1% GABA in the growth medium. The β-galactosidase assays were performed with permeabilized cells (Guarente 1983). Results were obtained from at least two independent experiments done with duplicate or triplicate samples.

Production of whole cell extracts for immunoblot analysis

Yeast cultures were grown overnight in YPD (Adams *et al.* 1997). Cells containing expression vectors and reporter plasmids were diluted in 100 ml of SD medium (Adams *et al.*

1997) lacking ammonium sulfate, supplemented with adenine, leucine, lysine, and histidine at 0.008%, as well as 2% glucose and 0.1% proline; cells expressing chromosomally tagged proteins were diluted in 300 ml SD medium (Adams *et al.* 1997) lacking ammonium sulfate, supplemented with adenine, leucine, lysine, histidine, tryptophan, and uridine at 0.004%, as well as 0.01% drop-out medium supplement, 2% glucose, and 0.1% proline. All cultures were grown to an OD_{600} of ~0.7. Cells were then pelleted, washed in ice-cold water, and resuspended in an equal volume of ice-cold IP-1 buffer (15 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% Triton X-100, 10 mM pyrophosphate, 2 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 μg/ml pepstatin, 1 μg/ml leupeptin), as modified from Mamnun *et al.* (2002). An equal volume of cold glass beads was then added and the cells were lysed by vortexing four times for 1 min at 4°C. The lysate was then separated from the debris by centrifugation and aliquots were boiled in 1.5× Laemmli buffer. The samples (containing 30 μg of proteins) were then run on a 7.5% SDS–polyacrylamide gel, transferred to a PVDF membrane, and analyzed by immunoblotting with 8 μg of anti-HA antibody (clone 12Ca5, Roche Applied Science).

Chromatin immunoprecipitation (ChIP) assay

Strains expressing chromosomally tagged proteins (Table 1) were grown as described in the previous section. ChIP assays were performed as described (Larochelle *et al.* 2006) except that quantitation was performed by qPCR. Enrichments were calculated over an untagged strain and normalized with the signal obtained with *ARN1* as an internal control using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen 2001). Oligonucleotides used for ChIP were ATTCGCGCTATCTCGA TTTC and CACCGCACCAATGGATAAAC for the *UGA1* promoter (−501 bp to −251 bp relative to the ATG) and TGC ACCCATAAAAGCAGGTGT and GAGAGCTATCGAATGTTTCCTC for the *ARN1* promoter (−260 bp to −86 bp relative to the ATG).

Results

Analysis of the functional domains of Uga3

Previous studies have shown that the DNA-binding domain of Uga3 is located at the N terminus (Noël and Turcotte 1998; Idicula *et al.* 2002). For example, a purified Uga3 polypeptide (aa 1–124) binds to UAS_{GABA} *in vitro* (Noël and Turcotte 1998). However, very little information about the other domains of Uga3 is available and sequence alignments of zinc cluster proteins failed to identify a middle homology region in Uga3 (Schjerling and Holmberg 1996). To map the functional domains of this regulator, the *UGA3* ORF was first amplified by PCR and was subcloned into the plasmid p414MET25, a low-copy yeast expression vector under the control of the repressible *MET25* promoter (Mumberg *et al.* 1994). The coding region of three

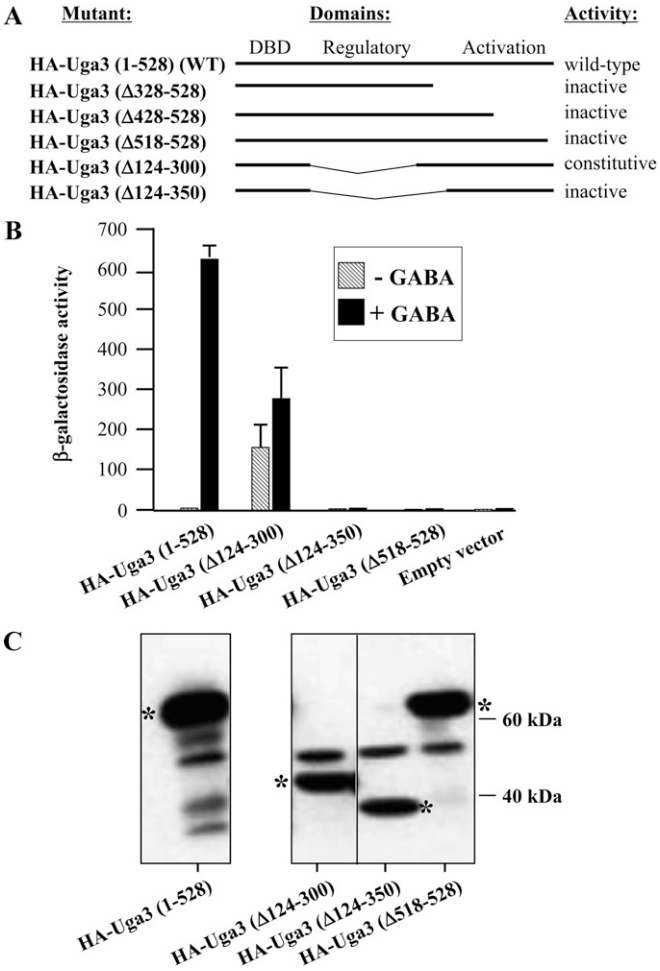

Figure 1 Functional domains of Uga3. (A) Scheme of the Uga3 mutants. Names of mutants are indicated on the left. Wild-type Uga3 is 528 amino acids long. Functional domains of Uga3 are given at the top and activity of mutants to the right. (B) Activity of Uga3 mutants. β-Galactosidase assays were performed with strain BY4742 Δ*uga3*Δ*trp1* (Table 1) cotransformed with the reporter plasmid $pUAS_{GABA}$-lacZ and with vector p414MET25-HA-UGA3, p414MET25-HA-UGA3(Δ124-300), p414MET25-HA-UGA3(Δ124-350), or p414MET25-HA-UGA3(Δ518-528). Refer to *Materials and Methods* for more details on the β-galactosidase assays. (C) Relative expression of Uga3 mutants. Strain BY4742 Δ*uga3*Δ*trp1* transformed with each respective expression vector was used to prepare whole-cell extracts. Western blot analysis was performed with an anti-HA primary antibody. Positions of wild-type and Uga3 mutants are indicated by an asterisk. Approximately 30 μg of whole-cell extracts were used for each sample.

HA epitopes was afterward inserted in-frame with the *UGA3* ORF, allowing the expression of Uga3 tagged at its N terminus with three HA epitopes. We also generated vectors expressing Uga3 derivatives with C-terminal or internal truncations and a summary of key mutants is presented in Figure 1A.

Uga3 and mutants (Table 2) were expressed in a Δ*uga3* strain and their activity assayed with the $pUAS_{GABA}$-lacZ reporter containing a single binding site for Uga3 (UAS_{GABA}) inserted upstream of a minimal *CYC1* promoter driving

expression of lacZ (Noël and Turcotte 1998). Since this reporter contains only a UAS_{GABA}, it allows specific monitoring of the activity due to Uga3 (or derivatives). Plasmid-expressed HA-Uga3 was able to induce high levels of reporter gene activity in a GABA-dependent manner (Figure 1B) while a reporter lacking the UAS_{GABA} gave background activity (data not shown and Noël and Turcotte 1998). Truncations of 100 or 200 aa at the C terminus of Uga3 completely abolished transcriptional activation (Figure 1A). Interestingly, an Uga3 mutant HA-Uga3(Δ518–528) carrying a very short (10 aa) deletion at the C terminus, also failed to activate the reporter even in the presence of GABA (Figure 1B). These results indicate that an activation domain is located at the C terminus of Uga3.

A mutant carrying an internal deletion [HA-Uga3(Δ124–300)] showed constitutive activity albeit at reduced levels when compared to wild-type Uga3. Addition of GABA slightly induced (less than twofold) reporter activity. A mutant with a larger internal deletion, HA-Uga3(Δ124–350), failed to activate the reporter. We assayed the relative expression of full-length HA-Uga3 and the three mutant forms of Uga3 described above, to test that the results of the β-galactosidase assays were not due to Uga3 derivatives not being expressed. Whole-cell extracts from BY4742 Δ*uga3*Δ*trp1* transformed with each of the four expression vectors were used in immunoblot analysis, using an anti-HA primary antibody (Figure 1C). Despite the fact that there is variation in protein levels, all of the Uga3 mutant proteins were clearly expressed. Furthermore, HA-Uga3(Δ124–300) induced levels of β-galactosidase activity lower than HA-Uga3 but the mutant protein was also expressed at lower levels than HA-Uga3. Our functional analysis of Uga3 shows that this factor has an activation domain located at its C terminus while a regulatory region is found in its "central" portion.

The Dal81 zinc cluster domain is not required for activation of a UAS_{GABA} reporter

In line with studies on a native *UGA1* promoter (Bricmont *et al.* 1991), results show that removal of *DAL81* greatly reduced activity of the UAS_{GABA} reporter (Figure 2, left part). Introduction of a high copy expression vector for HA-Dal81 into a Δ*dal81* strain increased promoter activity at levels higher than a wild-type strain, presumably because *DAL81* is overexpressed under these conditions. We also expressed a truncated version of Dal81 lacking amino acids 150 to 179 encompassing all 6 cysteines residues of the zinc cluster domain. Many studies on zinc cluster proteins predict that this truncated protein would be defective for binding to DNA (MacPherson *et al.* 2006). However, the truncated Dal81 protein was as efficient as wild-type Dal81 for activation of the UAS_{GABA} reporter suggesting it does not need to directly contact DNA for activation of a Uga3-responsive promoter (Figure 2, right part).

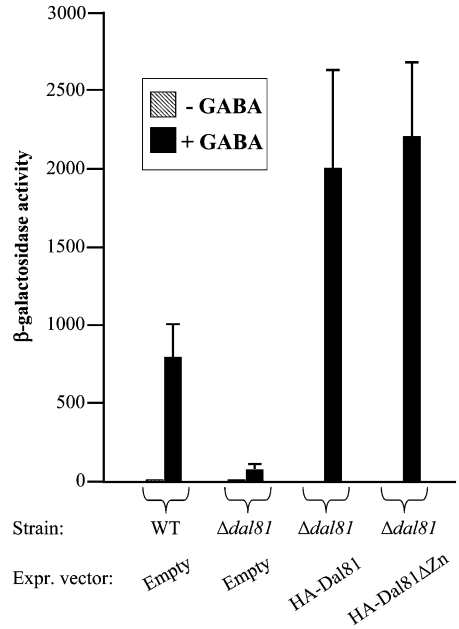

Figure 2 The zinc cluster region of Dal81 is not required for transcriptional activation. Wild-type strain BY4742 (WT) or Δ*dal81* was transformed with the lacZ reporter $pUAS_{GABA}$-lacZ and plasmids p423MET25 (Empty), or p423MET25-3HA-DAL81 (HA-Dal81), or p423MET25-3HA-DAL81ΔZn (HA-Dal81ΔZn), for β-galactosidase assays. Refer to *Materials and Methods* for more details on the β-galactosidase assays.

Activity of LexA-Uga3 chimeric protein is dependent on the presence of GABA and Dal81

We were then interested in determining if the GABA-induced activation of Uga3 requires a specific DNA-binding domain or can be transferred to a heterologous DNA-binding domain. To this end, we expressed a chimeric protein consisting of the LexA DNA binding protein fused to amino acids 78 to 528 of Uga3. Interestingly, this chimeric protein activated expression of a lacZ reporter bearing lexA sites in a GABA-dependent manner (Figure 3A) while expression of LexA by itself gave background reporter activity (data not shown). A LexA-Uga3 fusion protein lacking N-terminus of Uga3 [LexA-Uga3(300-528)] was constitutively active, in analogy to the Uga3 Δ124-300 mutant described previously. Taken together, these results suggest that a GABA-responsive domain could be present between aa 78 and 300 of Uga3.

As observed with wild-type Uga3, activation by LexA-Uga3(78-528) was abolished in the absence of *DAL81* (Figure 3A). Thus, Dal81 dependence of Uga3 for transcriptional activation by Uga3 can be transferred to a chimeric protein bearing a heterologous DNA-binding domain. Moreover, activation was as efficient as wild-type Dal81 when a truncated Dal81 lacking the zinc cluster region was co-expressed along with LexA-Uga3(78-528) (Figure 3B). Taken together, these results raises the possibility that the role of Dal81 is not (only) to facilitate DNA-binding of regulators such as Stp1 (Boban and Ljungdahl 2007), but also to directly contribute to transcriptional activation (see below).

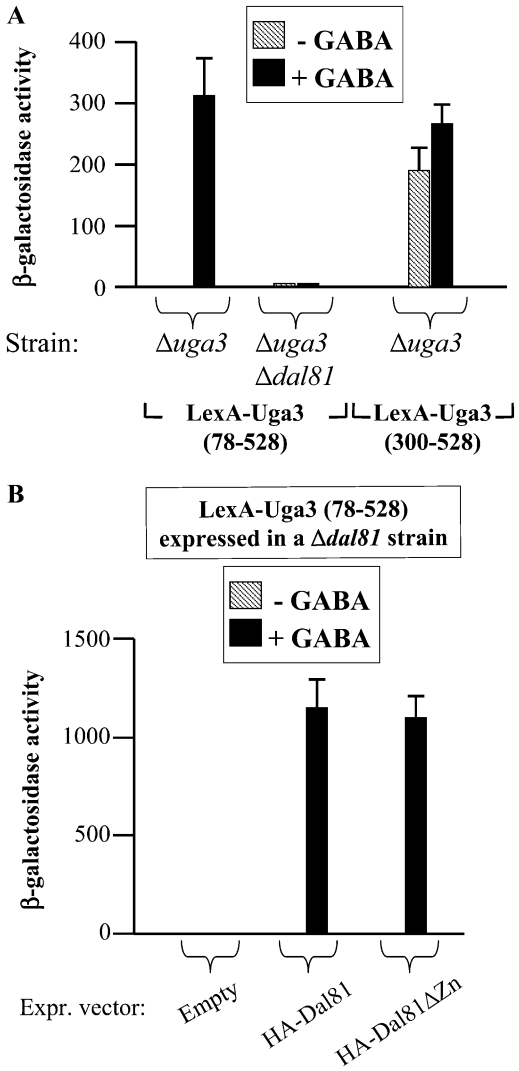

Figure 3 A LexA-Uga3 chimeric protein is responsive to GABA and requires Dal81 for transcriptional activation. (A) Strain BY4742 Δ*uga3*Δ*trp1* (Δ*uga3*) or BMASY1 Δ*dal81*Δ*uga3*Δ*trp1* (Δ*uga3* Δ*dal81*) were cotransformed with the lexA reporter pSH18-34 (Ausubel *et al.* 1997) and expression vectors p414MET25-lexA-*UGA3*(78–528), or p414MET25-lexA-*UGA3*(300–528), for β-galactosidase assays. Refer to *Materials and Methods* for more details on the β-galactosidase assays. (B) Strain BMASY1 Δ*dal81*Δ*uga3*Δ*trp1* (Δ*uga3* Δ*dal81*) was transformed with the lexA reporter pSH18-34 (Ausubel *et al.* 1997), the expression vector p414MET25-LexA-*UGA3*(78–528) along with p423MET25 (Empty), or p423MET25-3HA-DAL81 (HA-Dal81), or p423MET25-3HA-DAL81ΔZn (HA-Dal81ΔZn), for β-galactosidase assays. Refer to *Materials and Methods* for more details on the β-galactosidase assays.

Cofactors involved in Uga3 transcriptional activation

We were interested in determining the possible requirement of Uga3 for coactivators other than Dal81. To this end, the UAS_{GABA} reporter was introduced into various strains carrying deletions of genes encoding components of SAGA (*ADA1*, *GCN5*, *SPT3*, *SPT20*), mediator (*GAL11*, *MED5*, *SIN4*), ATP-dependent chromatin remodeling complexes (*RSC2*, *SWI2*), and β-galactosidase activity assayed. Deletion of genes encoding various components of the SAGA complex (*ADA1*, *GCN5*, *SPT3*, *SPT20*) resulted in significantly decreased activation by Uga3 (Figure 4). A strain lacking *GAL11* showed 30% β-galactosidase activity relative to a wild-type strain but no marked effect was observed when deleting *SIN4* or *MED5* encoding other components of mediator. Finally, deletion of *RSC2* or *SWI2* had little effect on activation by Uga3. In summary, the SAGA complex as well as Gal11 modulate the transcriptional activity of Uga3.

Dal81 and Gal11 are recruited to the UGA1 *promoter in the presence of GABA*

Dal81 was shown to enhance activation of the factor Stp1 by facilitating its binding to target promoters (Boban and Ljungdahl 2007). To test if a similar mechanism is responsible for the requirement of Dal81 for Uga3 activation, we performed ChIP experiments using Uga3 tagged at its natural chromosomal location with three Myc epitopes. Results show that Myc-Uga3 is very weakly bound (1.6-fold enrichment) to the promoter of the target gene *UGA1*, as assayed with proline as the nitrogen source (Figure 5, column 1). In the presence of GABA, binding of Myc-Uga3 is enhanced approximately fourfold. Deletion of *DAL81* decreased but, importantly, did not abolish binding of Uga3 to the *UGA1* promoter. We also assayed the recruitment of Dal81 at the *UGA1* promoter by ChIP. No enrichment of HA-Dal81 at *UGA1* was detected in the absence of GABA while addition of the inducer allowed recruitment of this factor (Figure 5, column 3), in agreement with another study performed with the *UGA4* promoter (Cardillo *et al.* 2010). As shown above, Gal11, a component of the mediator complex, is required for full activation by Uga3. In agreement with

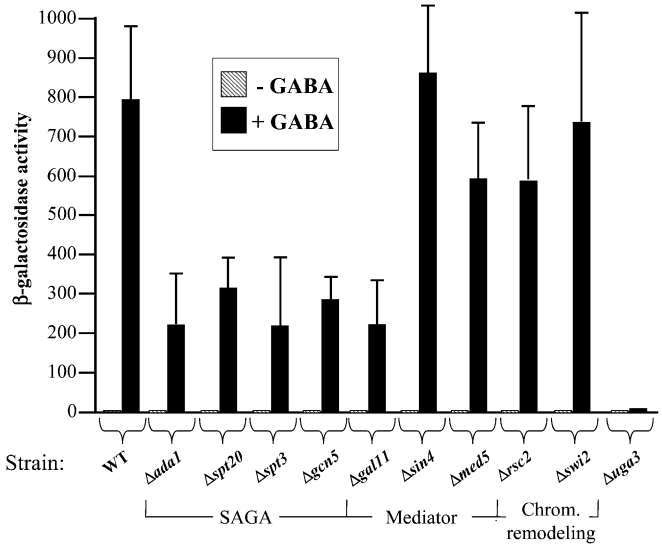

Figure 4 Cofactor requirements for activation by Uga3. Wild-type strain BY4742 (WT) and strains carrying deletion of genes encoding various cofactors (as indicated at the bottom) were transformed with the reporter $pUAS_{GABA}$-lacZ for β-galactosidase assays. Refer to *Materials and Methods* for more details on the β-galactosidase assays.

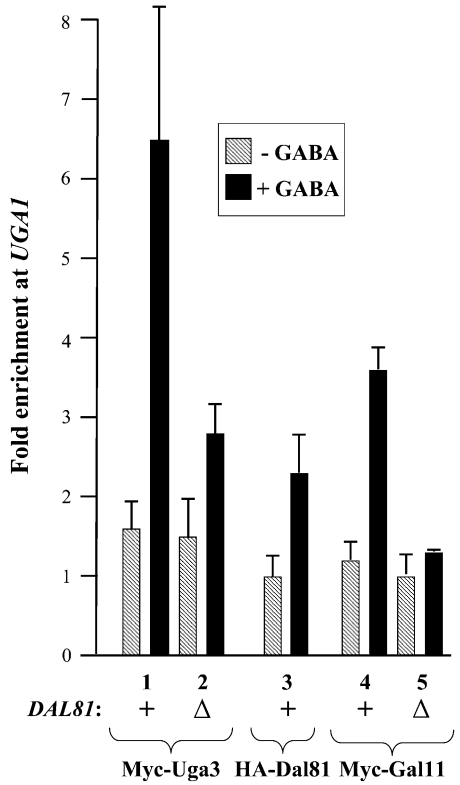

Figure 5 GABA-dependent recruitment of Dal81 and Gal11 to the *UGA1* promoter. Chip assays were performed with strains BY4742 Myc-*UGA3* or BY4742 Myc-*UGA3* Δ*dal81* (Myc-Uga3 + or Δ *DAL81*, respectively), strain BY4742 HA-*DAL81* (HA-Dal81) or strains HQY825 and BMASY11 (HA-Gal11 + or Δ *DAL81*, respectively). Data are given as fold enrichment calculated by normalizing signals to a control gene (*ARN1*) and to an untagged control strain as measured in the presence (solid bars) or in the absence (stippled bars) of GABA.

these observations, ChIP analysis showed that Gal11 is recruited to the *UGA1* promoter in the presence of GABA (Figure 5, column 4). Interestingly, removal of *DAL81* prevented recruitment of Gal11 (Figure 5, column 5). Similar results were obtained with *UGA4*, another target gene of Uga3 (data not shown). Taken together, these results suggest that Dal81 facilitates binding of Uga3 and recruitment of Gal11 at the *UGA1* and *UGA4* promoters.

Dal81 acts as a transcriptional activator when tethered to DNA

We wanted to test if the effect of Dal81 is strictly restricted to helping Uga3 bind to DNA or if the effect of Dal81 is mediated via recruitment of coactivators. We first tested to see if Dal81 by itself has transcriptional activation properties; to this end, we fused the DNA-binding domain of LexA to Dal81 and measured the activity of a reporter containing lexA binding sites. Strong activity of the reporter was observed when expressing LexA-Dal81 in the absence of GABA but not LexA alone (Figure 6). Addition of GABA did not increase activity of LexA-Dal81, in agreement with the fact that Dal81 is a general coactivator of nitrogen-regulated genes. Activation by LexA-Dal81 was approximately 2.5-fold stronger when performing the assay in a strain lacking *UGA3* while addition of GABA had no effect. Reduced activity of LexA-Dal81 in the presence of wild-type Uga3 may be explained by the fact that, under these artificial conditions, Uga3 may compete with Lex-Dal81 for cofactors. These results provide additional evidence that the mechanism of action of Dal81 is not only exerted by favoring binding of transcription factors to target sites but that Dal81 also contributes directly to transcriptional activation. Activation by LexA-Dal81 was measured in various strains carrying deletions of genes encoding coactivators, as described for Uga3. As observed with Uga3, deleting *SPT20, SPT3*, and *GAL11* (but not *GCN5*) significantly reduced activation by LexA-Dal81 (Figure 6). In contrast to Uga3, removal of *SIN4* or *RSC2* did reduce (approximately 2-fold) the activation by LexA-Dal81. These differences could be explained by the fact that the assay was performed in different promoter contexts. Taken together, our data clearly show that Dal81 can directly activate transcription regardless of the presence of Uga3 and that *SPT20, SPT3*, and *GAL11* are important for transcriptional activation by native Uga3/Dal81 and LexA-Dal81.

Overexpression of Uga3 bypasses the requirement for Dal81

Two models could explain the results described above. GABA-bound Uga3 allows recruitment of Dal81 that, in turn, would bring coactivators such as SAGA and Gal11 to target promoters. Alternatively, both Uga3 and Dal81 would be responsible for recruitment of SAGA and Gal11. To distinguish between these two possibilities, we took advantage of our observation that overexpression of Uga3 bypasses the requirement for Dal81 (Figure 7). As expected, activation by Uga3 expressed from its native promoter is strongly dependent on Dal81 (Figure 7, columns 1 and 2). However, introduction of an expression vector for Uga3 in wild-type yeast cells results in a *DAL81*-independent activation of the reporter (Figure 7, columns 3 and 4). Western blot analysis showed that, under these conditions, Uga3 was overexpressed by a factor of at least 20 (data not shown). We then performed β-galactosidase assays with Uga3 overexpressed in strains lacking both *DAL81* and genes encoding other coactivators. β-Galactosidase activity was greatly reduced in strains Δ*dal81*Δ*spt20* and Δ*dal81*Δ*spt3* and approximately 3-fold in strain Δ*dal81*Δ*gal11* (Figure 7, columns 5–7). However, with *MED5*, a slightly stronger effect was observed with overexpressed Uga3 when compared with native Uga3/Dal81 or LexA-Dal81 (Figure 7, column 8). Taken together, these results show that the role of Uga3 is not restricted to DNA binding and the recruitment of Dal81 but that it also directly participates in transcriptional activation via SAGA and Gal11.

Discussion

In this study, we characterized the zinc cluster proteins Dal81 and Uga3, a general positive regulator of nitrogen

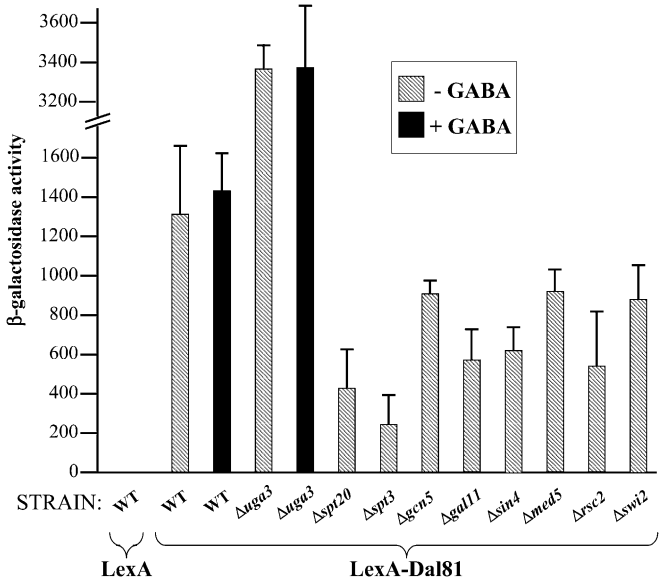

Figure 6 Dal81 acts as a transcriptional activator when tethered to DNA. Wild-type strain BY4742 (WT) and strains carrying deletions of genes encoding Uga3 or various cofactors (as indicated at the bottom) were transformed with an expression vector for LexA alone or LexA-Dal81 (p423*MET25*-lexA-HA-*DAL81*) along with the lexA reporter pSH18.34 for β-galactosidase assays. Refer to *Materials and Methods* for more details on the β-galactosidase assays.

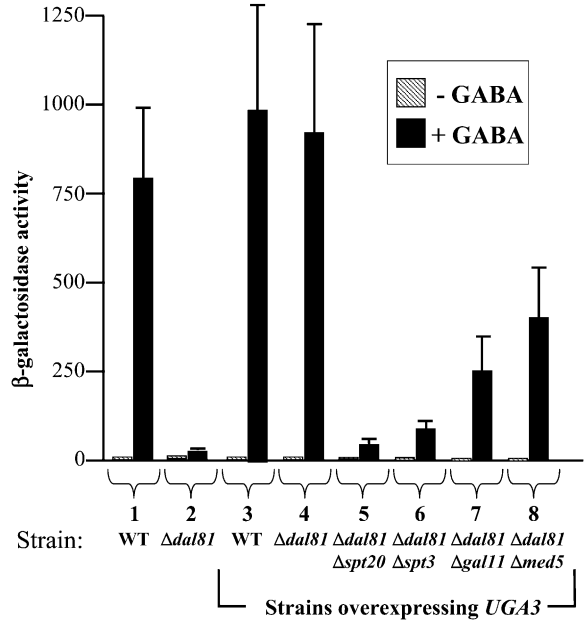

Figure 7 Overexpression of Uga3 bypasses the requirement for Dal81. Columns 1 and 2: wild-type strain BY4742 (WT) and an isogenic Δ*dal81* strain were transformed with the reporter pUAS$_{GABA}$-lacZ for β-galactosidase assays. Columns 3–8: wild-type strain BY4742 (WT) and various deletion strains (as indicated at the bottom) were transformed with plasmids p423MET25-3HA-Uga3 and pUAS$_{GABA}$-*lacZ* for β-galactosidase assays. Refer to *Materials and Methods* for more details on the β-galactosidase assays and Table 1 for strains.

utilization genes and an activator of genes for GABA catabolism, respectively. The mutational analysis done on Uga3 provided new information on the functional domains of this zinc cluster protein (Figure 1). Previous comparative genome analysis did not identify a regulatory domain (also named the middle homology region) in Uga3 (Schjerling and Holmberg 1996). However, our experiments showed that a mutant lacking the region corresponding to amino acids 124–300 was constitutively active. These results were further supported by the chimeric protein LexA-Uga3(300–528), which functioned in a similar constitutive manner (Figure 3A). Analogous observations were made with the zinc cluster proteins Leu3 and Hap1, which become constitutively active when their regulatory domains are deleted (Pfeifer *et al.* 1989; Zhou *et al.* 1990). It therefore appears that Uga3 possesses such a regulatory domain, located between the DNA-binding domain and the activation domain. The reason why comparative genome analysis failed to identify a regulatory domain in Uga3 may be due to the fact that this domain is not actually highly conserved among zinc cluster proteins. It is possible that the regulatory domain of Uga3 diverged from the other zinc cluster proteins to accommodate for its function in the response to GABA.

Our mutational analysis also demonstrated that the region of Uga3 encompassing amino acid residues 300–350 is necessary for the activity of Uga3 as a transcriptional activator. The reason this domain is necessary for the function of Uga3 is, however, not clear. It is possible that an important part of the activation domain of Uga3 could be between amino acids 300 and 350. The Uga3 truncation mutant lacking the last 10 amino acid residues of the full-length protein was also unable to induce the activity of the pUAS$_{GABA}$-lacZ reporter in the presence of GABA. The C terminus of Uga3 is required for the activity of this transcription factor but does not show homology to known domains involved in transcription.

Besides Dal81, Uga3 requires the SAGA complex as well as the Gal11 component of mediator for transcriptional activation (Figure 4). For example, removal of *GAL11* resulted in reduced (3.3-fold) reporter activity. Similarly, activation of transcription by the zinc cluster protein Pdr1 was shown to be mediated by direct interaction with the component Gal11 of the mediator complex (Thakur *et al.* 2008). Although we observed reduced binding of Uga3 at the *UGA1* (and *UGA4*) promoter in cells lacking *DAL81*, there was still significant binding of this factor to target genes, as assayed by ChIP (Figure 5). In contrast to our observations, removal of Dal81 almost completely abolished binding of the transcription factor Stp1 to target promoters (Boban and Ljungdahl 2007).

It is possible that Dal81 has two, nonexclusive, modes of action: (1) one operating by facilitating binding of transcription factors to DNA and (2) another by contacting coactivators for a more efficient formation of an initiation complex at target promoters. The observation that Dal81, when tethered to DNA, acts as a strong and Uga3-independent transcriptional activator (Figure 6) supports (at least in part) the

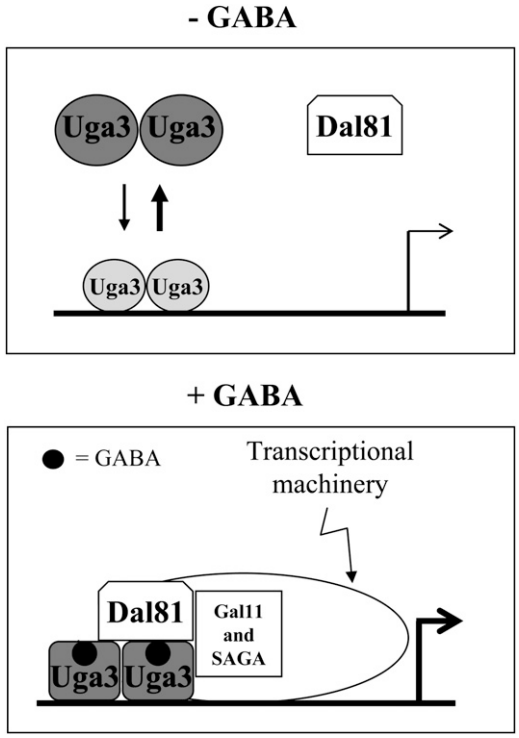

Figure 8 Model for the transcriptional activation by Uga3 and Dal81. A Uga3 homodimer is weakly bound to target sites under noninducing conditions (top). GABA (shown as solid circles) induces a conformational change of Uga3 allowing interaction with Dal81 and recruitment of components of the transcriptional machinery such as Gal11 (bottom). Dal81 can also interact with the transcriptional machinery, thus facilitating formation of a Uga3–Dal81-coactivator complex at target promoters. DNA binding activity of Dal81 is not required for its function. It is not known if Dal81 interacts directly with Uga3 or if Gal11 interacts directly with Uga3 and Dal81.

second mechanism of action of Dal81. A Dal81 mutant lacking the cysteine-rich region and, as a result, most likely defective in DNA binding, was fully functional as a coactivator for Uga3 as well as LexA-Uga3(78–528) (Figure 3). In the latter case, both factors act in concert in spite of the fact that the truncated Dal81 (Dal81ΔZn) cannot bind DNA and that LexA-Uga3 lacks the natural Uga3 DNA binding domain. These results, as well as previous observations (Bricmont *et al.* 1991), strongly suggest that Dal81 does not need to directly contact DNA but may associate with Uga3 to form a stable complex at target promoters, such as *UGA1*. We failed to observe such an interaction using co-immunoprecipitation assays perhaps because this interaction is weak (M.-A. Sylvain and B. Turcotte, unpublished results). However, in support of a Dal81–Uga3 interaction, Dal81 interacts with Dal82, an activator of allantoin catabolic genes, in a two-hybrid assay (Scott *et al.* 2000). A parallel can also be made between Dal81 and its *A. nidulans* homolog TamA. In analogy to Dal81, TamA activates transcription when tethered to DNA (Small *et al.* 1999). As shown in a two-hybrid assay, TamA interacts with the transcriptional activator LeuB (Polotnianka *et al.* 2004). LeuB, a homolog of *S. cerevisiae* Leu3, and TamA are both involved in controlling the expression of the *gdhA* gene encoding NADP-linked glutamate dehydrogenase. Moreover, mutating a critical cysteine residue in the zinc cluster domain of TamA has no effect on its functionality (Davis *et al.* 1996). Thus, results suggest that the DNA-binding domain of Dal81 and TamA are dispensable in spite of the fact that the primary amino acid sequence of the cysteine-rich region of these factors corresponds to a *bona fide* zinc cluster. Dal81 and TamA DNA-binding activity may be required for activation at a subset of the target genes, yet to be identified.

Importantly, our results show that the role of Uga3 is not restricted to recruiting Dal81. This was demonstrated by showing that overexpressed Uga3 can activate transcription at wild-type levels even in the absence of Dal81 (Figure 7). Moreover, both LexA-Dal81 and overexpressed Uga3 mainly rely on the same set of coactivators (SAGA and Gal11) for transcriptional activation, suggesting a cooperative mode of action for these factors (Figures 6 and 7). As stated above, binding of Stp1 to DNA is more dependent on Dal81 than Uga3. However, it is also possible that a mechanism similar to Uga3 operates for Stp1. Recruitment of Dal81 would increase formation of a transcription complex at target promoters of Stp1, the only difference being that Stp1 DNA affinity would be lower than Uga3. The yeast transcriptional activator Gcn4 also requires SAGA and mediator for activity. Recruitment of these factors is mediated by Gcn4 itself (Qiu *et al.* 2005). In contrast, our genetic studies and ChIP assays suggest that both Uga3 and Dal81 can independently recruit Gal11 and SAGA. It will be interesting to determine if Dal81 also relies on SAGA and Gal11 at other nitrogen metabolic genes.

What is the mechanism of activation of Uga3 by GABA? Activity of a number of zinc cluster proteins is controlled by intermediary metabolites (for a review, see Sellick and Reece 2005). For example, the activity of the zinc cluster proteins Leu3 and Lys14 is controlled by intermediary metabolites of leucine and lysine biosynthesis, respectively (Feller *et al.* 1994; Feller *et al.* 1999; Sze *et al.* 1992). In contrast, our unpublished results showed that activation by Uga3 is not dependent on the presence of GABA metabolites since no reduction of reporter activity is observed in strains Δ*uga1* and Δ*uga2*, which are unable to catabolize GABA. We hypothesize that Uga3 is directly activated by GABA in a manner analogous to the zinc cluster protein Put3, an activator of proline utilization genes that is directly activated by binding to its ligand proline (Sellick and Reece 2003). Similarly, the zinc cluster protein Pdr1, an activator of drug resistance genes, is activated by direct binding of diverse drugs and xenobiotics (Thakur *et al.* 2008). Attempts to show direct binding of GABA to purified Uga3 failed since overexpression of this factor from yeast or bacterial cells did not yield sufficient purified Uga3 for binding studies.

Our unpublished results suggest that Uga3 is found in the form of a homodimer, even in the absence of GABA. In addition, a large-scale localization study showed that, under noninducing conditions, Uga3 is found in the nucleus (Huh

et al. 2003). Thus, Uga3 is most probably found as a homodimer in the nucleus regardless of the presence of GABA (see proposed model in Figure 8, top). However, our ChIP assays (Figure 5) demonstrated that Uga3 is only weakly bound to the *UGA1* promoter. As stated above, we hypothesize that GABA binds directly to Uga3 resulting in a conformational change that would favor binding of Uga3 to *UGA1* (Figure 8, bottom). This conformational change would also permit Uga3 to interact with Dal81. Both Uga3 and Dal81 can then interact with coactivators such as Gal11 and SAGA to allow the formation of a pre-initiation complex. The absence of Dal81 results in reduced surface for interaction with coactivators leading to greatly diminished transcriptional activation by Uga3. Conversely, overexpression of Uga3 bypasses the requirement of Dal81 because higher levels of Uga3 would increase its binding to target promoters for recruitment of Gal11 and SAGA. In conclusion, our studies show that Dal81 and Uga3 act in concert by targeting common components of the transcriptional machinery.

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