UME6 is a key regulator of nitrogen repression and meiotic development

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This report describes the identification, cloning, and molecular analysis of *UME6* (*CAR80/CARGRI*), a key transcriptional regulator of early meiotic gene expression. Loss of *UME6* function results in the accumulation of fully derepressed levels (70- to 100-fold increase above basal level) of early meiotic transcripts during vegetative growth. In contrast, mutations in five previously identified *UME* loci (*UME1* to *UME5*), result in low to moderate derepression (2- to 10-fold increase) of early meiotic genes. The behavior of insertion and deletion alleles indicates that *UME6* is dispensable for mitotic division but is required for meiosis and spore germination. Despite the high level of meiotic gene expression during vegetative growth, the generation times of *ume6* mutant haploid and diploid cells are only slightly reduced. However, both ascus formation and spore viability are affected more severely. The *UME6* gene encodes a 91-kD protein that contains a C6 zinc cluster motif similar to the DNA-binding domain of *GAL4*. The integrity of this domain is required for *UME6* function. It has been reported recently that a mutation in *CAR80* fails to complement an insertion allele of *UME6*. *CAR80* is a gene required for nitrogen repression of the arginine catabolic enzymes. Here, through sequence analysis, we demonstrate that *UME6* and *CAR80* are identical. Analyses of *UME6* mRNA during both nitrogen starvation and meiotic development indicate that its transcription is constitutive, suggesting that regulation of *UME6* activity occurs at a post-transcriptional level.

[*Key Words*: *CAR80*; *CARGRI*; transcription factor; yeast]

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The yeast *Saccharomyces cerevisiae* has evolved several mechanisms allowing survival under conditions of nutrient limitation. For example, diploids expressing both *MAT***a** and *MAT*α arrest cell division and initiate meiosis and spore development when starved for both a fermentable carbon source and nitrogen (for review, see Esposito and Klapholz 1981; Malone 1991; Honigberg et al. 1993), or switch to mycelial growth (foraging) when starved for nitrogen alone (Gimeno et al. 1992). These responses are mediated by a number of positive and negative regulators. The initiation of meiotic development is controlled by signal transduction pathways that monitor glucose and nitrogen levels and act in concert with an independent pathway responding to cell type. Together these pathways regulate transcription of a major inducer of meiosis, *IME1*, recently shown to function as a meiosis-specific transcriptional activator (Smith et al. 1993). The cell-type signal is transmitted by the **a**1–α2 negative regulator that inhibits transcription of *RME1*, a repressor of *IME1* (Mitchell and Herskowitz 1986; Kassir et al. 1988; Covitz et al. 1989). The glucose starvation cue is transduced, in part, through genes controlling cAMP-dependent protein kinase (cAPK) (Cameron et al. 1988; Matsumoto et al. 1988). The available evidence is consistent with the view that low cAPK activity, in response to limiting glucose, is also required for derepressed transcription of *IME1* (Smith and Mitchell 1989; Matsuura et al. 1990). After the induction of *IME1* transcription, a complex regulatory pathway appears to control both the onset and duration of the expression patterns observed for most meiotic genes. Previously, we have reported the identification of five genes (*UME1* to *UME5*) needed for the full repression of early meiosis-specific genes (*SPO11*, *SPO13*, and *SPO16*) during vegetative growth (Strich et al. 1989a). Mutations in any one of these *UME* genes relieve both nutritional and cell-type repression, allowing the unscheduled expression of early meiotic genes during vegetative growth of haploids and diploids, even in the absence of the *IME1* gene. In addition to these negative regulators, several positive regulators (*RIM* genes) have also been identified recently, which appear to act in conjunction with or downstream of *IME1* (Mitchell and Bowdish 1992; Su and Mitchell 1993).

At present, the components involved in transducing the nitrogen starvation signal during meiotic induction in diploid cells are poorly understood. On the other hand, the response of haploid yeast cells to nitrogen starvation

has been studied extensively (for review, see Magasanik 1993). When confronted with limited environmental nitrogen, haploid cells arrest cell division in G_1. Several biosynthetic pathways are repressed (e.g., arginine biosynthesis), whereas a number of catabolic pathways are induced (e.g., degradation and utilization of arginine). Catabolism of arginine requires the *CAR1* and *CAR2* genes, encoding arginase and ornithine transaminase, respectively. The *CAR1* gene is repressed by efficiently utilized nitrogen sources such as ammonia, glutamine, or asparagine (referred to as nitrogen catabolite repression). In the absence of nitrogen, both genes are strongly derepressed (Dubois et al. 1974). The presence of arginine as the sole nitrogen source induces *CAR1* and *CAR2* expression, which is dependent on four regulatory genes: *ARG80*, *ARG81*, and *ARG82* (previously designated *ARGRI*, *ARGRII*, and *ARGRIII*), acting in concert with *MCM1* (Thuriaux 1969; Messenguy et al. 1991; Messenguy and Dubois 1993). Interestingly, this same set of regulators is also required for the repression of arginine anabolism (Béchet et al. 1970; Dubois and Messenguy 1991). Negative regulation of the *CAR1* and *CAR2* genes is mediated by three genes *CAR80*, *CAR81*, and *CAR82* (formerly called *CARGRI*, *CARGRII*, and *CARGRIII*). Mutations in these genes allow growth of the *arg80* series of mutants on arginine or ornithine as a sole nitrogen source attributable to constitutive expression of *CAR1* and *CAR2* (Dubois et al. 1978; Deschamps et al. 1979), suggesting that induction takes place in part by inhibition or antagonism of the repressors. The effects of *car80*, *car81*, and *car82* mutations are additive, and a thorough analysis of the *CAR1* promoter shows that the targets of the negative and positive regulators are distinct, although located in close proximity to one another (Cunin et al. 1986; Sumadra and Cooper 1987; Kovari et al. 1990).

In addition to the recovery of the five *UME* genes described above, in two brief preliminary communications, we have reported the identification and cloning of a new regulator of early meiotic gene expression, designated *UME6* (Strich et al. 1989b; Steber et al. 1991). Recently, it was found that a mutation in the *CAR80* gene was unable to complement a disruption allele of *UME6*, suggesting that *UME6* and *CAR80* may be allelic (Park et al. 1992). Here, we describe in detail the initial identification, cloning, and characterization of *UME6* and demonstrate by DNA sequence analysis that *UME6* and *CAR80* are identical. This work indicates that the nitrogen-signaling systems that regulate early meiotic gene expression in diploid cells and nitrogen catabolic pathways in haploid cells are similar or at least share one common factor.

Results

Identification of the UME6 *gene by mutation analysis*

The *UME6* gene was identified in a screen designed to isolate mutants in the pathways involved in the degradation of meiosis-specific mRNAs. Previous studies have shown that the mRNA levels of genes induced early in meiosis decline rapidly when meiotic cells are returned to vegetative growth medium, because of a combination of rapid mRNA turnover and glucose repression of transcription (Surosky and Esposito 1992). To identify genes required for the degradation of these mRNAs, a search was initiated for mutants that would continue to express an early meiosis-specific mRNA (*SPO13*) when meiotic cells were returned to growth medium. Therefore, only isolates that exhibited meiotic levels of expression of a meiosis-specific reporter gene were chosen for further study. Four mutants were recovered that continued to express *SPO13* as assayed by elevated β-galactosidase (β-gal) activity of a *spo13–lacZ* fusion (see Materials and methods). Subsequent analysis revealed that these mutants express high constitutive levels of β-gal, even without prior meiotic induction. This finding suggested that the mutations caused altered transcriptional repression of *SPO13* during vegetative growth rather than altered mRNA turnover after meiotic induction. To verify that the mutations were *trans*-acting and chromosomal (rather than plasmid alterations), the plasmid containing the reporter gene was cured from the strains and a new plasmid containing the fusion gene was introduced. The four isolates continued to allow vegetative expression of the unmutagenized *spo13–lacZ* reporter gene, confirming that they contained chromosomal mutations. The phenotype exhibited by these four mutant strains is similar to, but significantly stronger than, the previously identified *ume* mutants mitotically derepressed for early meiotic genes (Strich et al. 1989a).

Haploid mutants were crossed to wild-type strains containing a *spo13–lacZ* fusion integrated at the *SPO13* locus. Wild-type levels of β-gal were observed in the resulting heterozygous diploids, indicating that the mutations are recessive (data not shown). Mutant segregants from this cross were backcrossed to generate diploids heterozygous for the new *ume*-like mutations and homozygous for the *spo13–lacZ* fusion gene. Tetrad analysis of these diploids yielded $2^+:2^-$ segregation of the mutant phenotype (60 tetrads analyzed), indicating that each of the mutations are in single nuclear genes. Subsequent intercrosses and complementation tests demonstrated that all four mutations are allelic and complement the five previously identified *ume* mutations. Therefore, these new mutations define an independent locus, designated *ume6*. The recessive nature of the *ume6* mutations, combined with the aberrant expression phenotype, indicate that the *UME6* gene product (Ume6) normally functions to repress *SPO13* during vegetative growth.

UME6 *is required for the mitotic repression of several early meiotic genes*

To verify that *ume6* mutations also cause derepression of the chromosomal *SPO13* gene, the abundance of *SPO13* mRNA was measured directly by S1 protection experiments. The level of vegetative *SPO13* mRNA in *ume6-2* mutants is increased ~70-fold relative to wild

type, similar to the induction observed during meiosis (Wang et al. 1987; cf. *ume6-2* with SK1 in Fig. 1). To determine whether this elevated accumulation of *SPO13* transcript is attributable to derepression and/or changes in mRNA turnover, the stability of the *SPO13* transcript was measured in wild-type and *ume6-2* mutant cells (Surosky and Esposito 1992). In both strains, the *SPO13* transcript was found to have a half-life of ~3 min, indicating that Ume6 is not involved in *SPO13* mRNA degradation (Surosky et al. 1994). Because the previously identified *UME* genes were found to regulate only meiotic genes expressed early in development, the vegetative levels of a number of other meiosis-specific transcripts were examined in a *ume6-2* mutant strain (Fig. 1). Two early meiotic genes, *SPO11* and *SPO16* (Atcheson et al. 1987; Malavasic and Elder 1990), are also fully derepressed, whereas no increase is seen in the expression of *SPO12* or *SPS2*, which are transcribed later in meiotic development (Percival-Smith and Segall 1984; Malavasic and Elder 1990). These data demonstrate that *UME6* is needed for the repression of the same subset of early meiotic genes controlled by *UME1* through *UME5*. However, in contrast to the previously identified *ume* mutants that exhibit a maximum 10-fold derepression, *ume6* mutants display a 70-fold induction of early meiosis-specific genes during vegetative division. Therefore, we conclude that *UME6* is a major component of the mitotic repression system governing early meiotic gene expression.

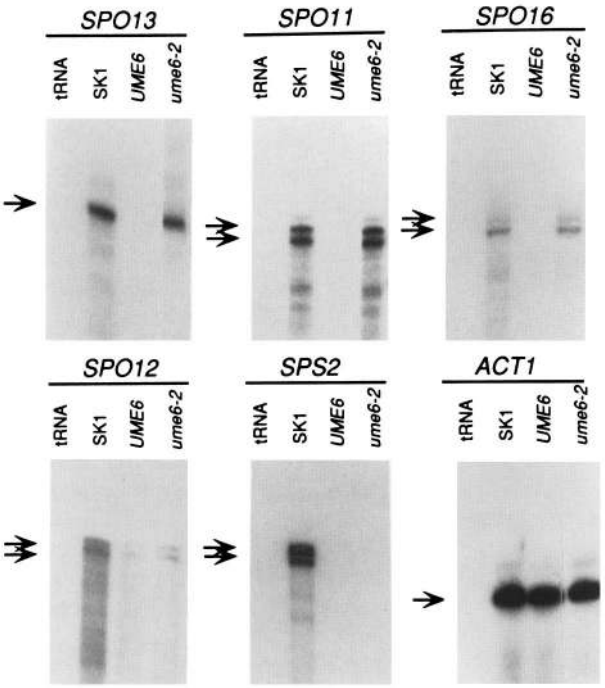

Figure 1. Nuclease S1 analysis of total RNA from vegetative cells in wild-type (*UME*) and *ume6*-2 mutants. Total RNA (20 μg) was prepared from late-logarithmic cultures. Arrows indicate probe sequences protected from S1 nuclease. (tRNA) Negative control for self-annealing of the probe; (SK1) RNA sample from the SK1 strain that illustrates the maximum derepression levels of the individual mRNAs during different points in meiosis; (*ACT1*) a control for mRNA fraction of the total RNA preparations.

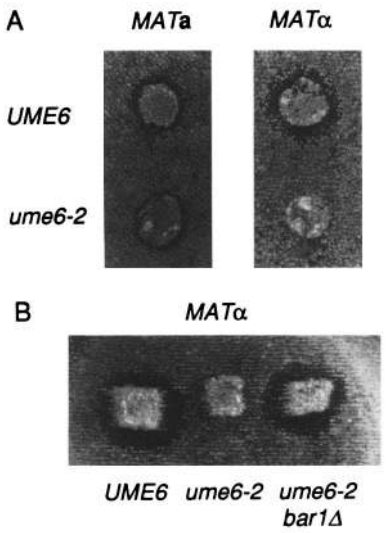

Figure 2. Mating pheromone production in wild-type and *ume6*-2 mutants. (*A*) Halo assays using lawns of strains supersensitive to either **a**- or α-mating pheromone, and *MAT***a** and *MAT*α wild-type and *ume6*-2 mutants. Zone of no growth reflects the relative amount of mating pheromone production. (*B*) Halo assay in wild-type, *ume6*-2 and *ume6*-2 *bar1*Δ strains. *BAR1* encodes the α-factor protease normally expressed only in *MAT***a** cells.

UME6 *and* UME4 *regulate an overlapping but not identical set of genes*

Among the previously characterized *UME* genes, *UME4* (*RPD1/SIN3*), was found to regulate a diverse set of nonmeiotic genes (Vidal et al. 1991). To determine whether *UME6* similarly plays a general role in regulating transcription, the expression of additional genes regulated by *UME4* was examined. First, *MAT***a** and *MAT*α wild-type and *ume6-2* haploid cells were tested for their ability to produce extracellular mating pheromones using strains supersensitive (*sst*) to pheromone growth arrest (MacKay et al. 1988). No decrease was observed in **a**-factor secretion (Fig. 2A, left). However, the *MAT*α *ume6-2* mutant excretes significantly less α-factor compared with the wild-type (Fig. 2A, right). This phenotype, as observed in *ume4* mutants, is the result of *BAR1* (encoding the α-factor protease) derepression in *MAT*α cells (Vidal et al. 1991). Disruption of the *BAR1* gene in a *MAT*α *ume6-2* strain restores α-factor production to wild-type levels (Fig. 2B), suggesting that *UME6* (like *UME4*) is required for repression of the **a**-specific gene *BAR1* in *MAT*α cells. It has also been shown recently that both Ume4 and Ume6 repress the *INO1* gene (Hudak et al. 1994). However, the regulation of several genes repressed by Ume4 (*FUS1, STE6, PHO5*) is not affected in *ume6* mutants

(data not shown). In addition, Ume4 appears to play no role in the control of *CAR1*, whereas Ume6 represents the major negative regulatory factor for this gene (Park et al. 1992; see below). These results indicate that although Ume4 and Ume6 coregulate some loci, the spectrum of genes that they control is not identical.

Cloning and mapping UME6

The *UME6* gene was isolated by complementation of the *ume6-2* mutant using the strategy described for cloning *UME4* (Vidal et al. 1991). The *ume6-2* complementing activity was subcloned and the minimal fragment required for *SPO13* repression during vegetative growth identified (Fig. 3). The putative *UME6* clone was shown by segregation analysis to integrate by way of homologous recombination at the *ume6* locus, confirming that the *UME6* gene had been recovered. The *UME6* gene was isolated independently by transposon tagging (see Materials and methods). Subcloning of the putative *UME6* gene indicated that the same region complementing the *ume6-2* allele also complements *car80* mutations (Fig. 3). Hybridization of a DNA fragment containing *UME6* sequences to a blot of separated yeast chromosomes and to a yeast contig library (see Materials and methods) revealed that *UME6* resides on the right arm of chromosome IV between the *pet14* and *hom2* loci (data not shown). More precise genetic mapping of the *UME6* gene was accomplished by segregation analysis using the *pet14*, *aro1*, and *hom2* markers (Table 1). These results indicate that *ume6* maps 21 cM proximal to *pet14*, 33 cM distal to *aro1*, and 23 cM distal to *hom2*.

Ume6 *contains a C6 zinc- cluster domain required for its function*

We have determined independently the complete nucleotide sequence of the *ume6* (p5905) and *car80* (pED30) complementing fragments (Fig. 3). The sequences are identical and contain a single large open reading frame encoding a predicted 91-kD protein of 836 amino acids (Fig. 4). The Ume6 protein has a calculated isoelectric point of 10.4 and is rich in acidic and basic residues, with

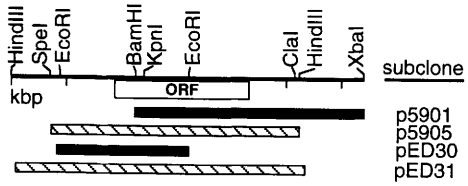

Figure 3. Restriction map of the *UME6* locus. The *top* line represents a partial restriction map of the *UME6* region. The *UME6* ORF is depicted in the open box below the line with the 5′ → 3′ orientation running *left* to *right*. Solid bars represent noncomplementing fragments; hatched bars represent fragments able to complement *ume6* and *car80* mutations. Insertion mutations were constructed by placing the coding sequences of *LEU2* or *URA3* in the *Bam*HI and *Kpn*I sites, respectively to generate *ume6-5* and *ume6::URA3* alleles.

Table 1. UME6 *linkage analysis*

Marker	PD[a]	NPD[b]	Tetratype	cM
ume6–hom2	52	0	45	23.1
ume6–aro1	42	2	50	33.0
ume6–pet14	18	0	3	21.0

[a]Parental ditype.
[b]Nonparental ditype.

basic residues clustered at the carboxyl terminus (21/60 arginine or lysine). It contains relatively high levels of proline (7.2%), asparagine (10.5%), serine (14.7%), and threonine (8.1%) residues.

FASTA searches (Pearson and Lipman 1988) of the GenBank, EMBL, and SwissProt protein data bases reveal homology of Ume6 to the C6 zinc cluster DNA-binding domain of the form Cys-X_2-Cys-X_6-Cys-X_{6-9}-Cys-X_2-Cys-X_6-Cys (Evans and Hollenberg 1988; Vallee et al. 1991), found in a number of regulatory proteins including Gal4, Hap1, and Arg81 (Fig. 5). To determine whether the C6 motif in Ume6 is required for its function, amino acid residues known to be important for Gal4 DNA binding were altered by site-directed mutagenesis (see Materials and methods). Crystallographic analysis of Gal4 has shown that the Lys-18 residue forms multiple sequence-specific bonds with the Gal4-binding site, whereas Cys-14 participates in zinc binding (Marmorstein et al. 1992). In Ume6, the Cys-774 corresponds to Gal4 Cys-14, and Lys-778 to Gal4 Lys-18. Mutations in either of these residues (Fig. 5) fail to complement a *ume6-D1* deletion allele as determined by vegetative *spo13–lacZ* expression (elevated 55- and 64-fold over the isogenic wild-type levels for the Cys-774 > Ser and Lys-778 > Leu mutations, respectively). This is comparable with the 54-fold increase seen in the *ume6-D1* deletion strain. A search of the PROSITE data base using MacPattern indicates that Ume6 contains a consensus Kex2 cleavage site (Julius et al. 1983) and a number of consensus kinase target sites, including 16 for casein kinase II (Ser/Thr-X-X-Asp/Glu), 13 for protein kinase C (Ser/Thr-X-Arg/Lys), and 4 for cAMP- and cGMP-dependent protein kinase (Arg/Lys-Arg/Lys-X-Ser/Thr) (Edelman and Krebs 1987).

The *UME6* mRNA was detected by Northern blot analysis and found to be a relatively nonabundant transcript of 2.7 kb (data not shown). This is in good agreement with the size of the predicted *UME6* open reading frame (2.5 kb). Three distinct doublets of 5′ mRNA start sites (indicated by arrowheads in Fig. 4) were detected by primer extension studies (data not shown).

UME6 *is dispensable for vegetative growth but is required for efficient spore germination*

To determine whether *UME6* is essential for mitotic division, a diploid was constructed that was heterozygous for *ume6-5*, a *ume6::LEU2* disruption allele (see Materials and methods). This diploid was sporulated and 60 tetrads dissected on rich medium. All of the asci containing four viable spores segregated $2^+:2^-$ for the dis-

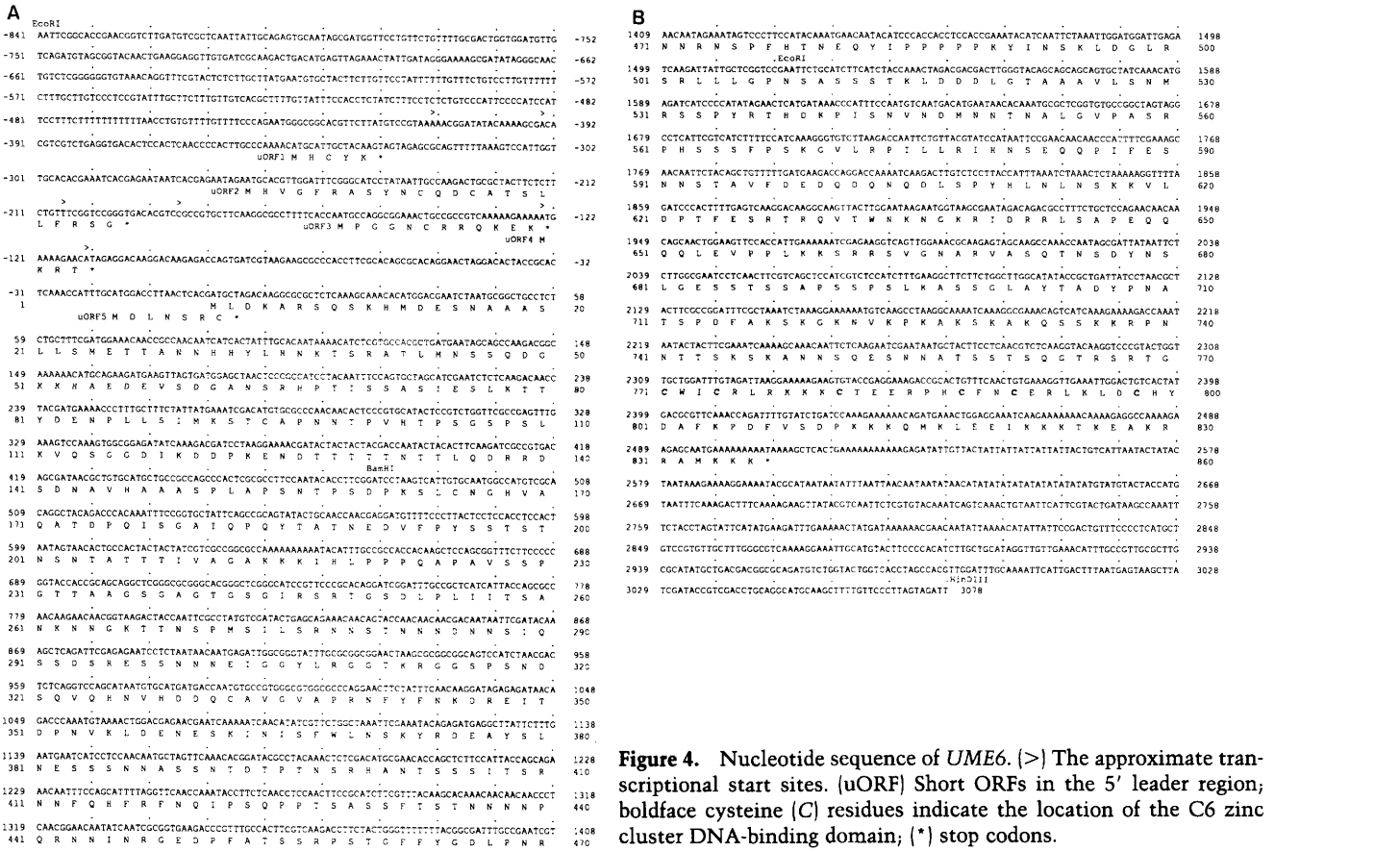

Figure 4. Nucleotide sequence of *UME6*. (>) The approximate transcriptional start sites. (uORF) Short ORFs in the 5′ leader region; boldface cysteine (*C*) residues indicate the location of the C6 zinc cluster DNA-binding domain; (*) stop codons.

ruption allele, demonstrating that *UME6* is dispensable for vegetative growth. Growth rates of *ume6-5* strains were reduced 10–60%, depending on the strain background (data not shown). The overall spore viability of the heterozygous diploid, as measured by the ability to form macroscopic colonies, was significantly lower (72%) than an isogenic wild type (95%). Further examination revealed that although the *UME6* spores resulting from the heterozygous *UME6/ume6-5* diploids produced viable colonies at the wild-type rate, only 46% of *ume6-5* spores were able to form macroscopic colonies. These results indicate that although *UME6* is not required for mitotic growth, it plays a role in efficient spore germination.

UME6 *mediates* CAR1 *repression in response to nitrogen*

As discussed earlier, expression of the catabolic genes *CAR1* and *CAR2*, encoding arginase and ornithine transaminase, respectively, is repressed by nitrogen. Three lines of evidence indicate that the *UME6* gene is involved in mediating this repression. (1) Mutants containing either disruption or point mutations of *ume6* are derepressed for *CAR1* and *CAR2* in medium containing ammonia as a nitrogen source (Table 2). The derepression in the *ume6* disruption mutant (sixfold for *CAR1*), is comparable with wild-type expression levels under nitrogen starvation (presumably the point mutant is derepressed to a lower extent because of leakiness of the mutation). (2) Derepression occurs even in the absence of the transcriptional activator *ARG81* (the *arg81* mutant exhibits a threefold increase in activity relative to *ARG81*; Table 2), suggesting that activation may occur in part by antagonism of repression (see Discussion). (3) When the *ume6* disruption strain is starved for nitrogen, only a slight additional increase is seen in *CAR1* and *CAR2* gene expression (Table 3). *CAR1* shows a twofold greater repression when grown in efficiently utilized nitrogen sources (e.g., ammonia) compared with poorly utilized sources (e.g., proline). This difference is thought to result from the functioning of at least two independent pathways of nitrogen repression: One pathway (referred to as nitrogen catabolite repression) is derepressed in the presence of poor nitrogen sources, and the other in the complete absence of environmental nitrogen. Interestingly, the *UME6* gene does not appear to participate in the pathway that responds to the quality of the nitrogen source, as mutations in *ume6* have no effect on the normal (twofold) derepression seen when cells are grown in the presence of proline (Table 3). These results suggest that the *UME6* gene is essential for only one of the pathways of nitrogen regulation and functions to transduce a general nitrogen repression signal (see Discussion).

A

Factor	Sequence	Codon Start
	S L	
Ume6	CWICRLRKKKCTEERPHCFNCERLKLDC	771
Gal4	CDICRIKKLKCSKEKPKCAKCLKNNWEC	10
Hap1	CTICRKRKVKCDKLRPHCQQCTKTGVAH	63
Ppr1	CKRCRLKKIKCDQEFPSCKRCAKLEVPC	33
Arg81	CWTCRGRKVKCDLRHPHCQRCEKSNLPC	20

B

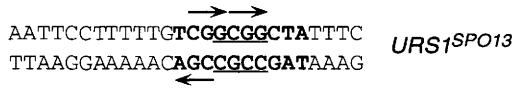

Figure 5. Sequence comparisons of C6 zinc cluster family members. (*A*) Zinc cluster homologies of *UME6* with other yeast transcription regulators. (Vertical bars) Identity; (colon) similarity; (*) deviation from consensus. The codon start refers to the residue number of the first cysteine in the motif. The arrows indicates the C → S and K → L substitutions in the domain. (*B*) Putative Ume6 DNA-binding sites. The $URS1^{SPO13}$ region is shown depicting the consensus *URS1* homology (**boldface type**; Luche et al. 1990) with the core element required for *SPO13* repression as defined by in vivo experiments underlined (L. Buckingham, R. Strich, and R.E. Esposito, in prep.). Half-sites of the consensus C6 zinc cluster protein-binding sites (CGPu) are depicted with arrows.

Ume6 interacts directly with $URS1^{SPO13}$ *in vitro*

As discussed earlier, the *CAR1* and *SPO13* promoters share a similar *cis* regulatory element (*URS1*) that is required for repression of both genes (Sumrada and Cooper 1985; Buckingham et al. 1990). This element, in conjunction with adjacent sequences, also functions in the activation of *SPO13* (L. Buckingham, R. Strich, and R.E. Esposito, in prep.). Epistasis analysis of strains carrying mutations in both the *URS1* element and the *UME6* gene indicate they have a similar (i.e., nonadditive) level of *SPO13* derepression during vegetative growth as strains carrying each single mutation, strongly suggesting that Ume6 acts through the *URS1* control region (L. Buckingham, R. Strich, and R.E. Esposito, in prep.). To further examine the interaction between Ume6 and $URS1^{SPO13}$, electrophoretic mobility shift assays (EMSAs) were performed using a 26-bp oligonucleotide containing $URS1^{SPO13}$ and extracts from vegetative cells carrying either the *UME6* wild-type or the *ume6-5* disruption allele (for details, see Materials and methods). As shown in Figure 6, six DNA–protein complexes are observed in the wild-type extracts. These complexes are specifically competed by the addition of unlabeled $URS1^{SPO13}$ oligonucleotide and are therefore *URS1* specific. The relative intensities of the six complexes are reproducible using different strain backgrounds, extract preparation procedures, and binding conditions. Significantly, in extracts prepared from *ume6-5* mutants, two new complexes are observed (C7 and C8), whereas C1 and C2 are absent. The C8 complex appears to be composed of several distinct members. Although the specific nature of the various complexes is not presently known, the EMSAs do suggest that Ume6 regulates *SPO13* expression through regulating protein interactions (either directly or indirectly) at the *URS1* element.

To investigate further whether Ume6 binds the *URS1* element directly, the carboxy-terminal third of Ume6 containing the zinc cluster domain (amino acids 560–836) was fused to the amino portion of the *Escherichia coli* maltose-binding protein (MBP). This fusion gene (pMAL–Ume6) is under the control of the *lacI* promoter and is therefore, inducible by IPTG. The fusion protein (MBP-Ume6) and MBP were affinity purified from extracts prepared from *E. coli* transformants containing either the pMAL–Ume6 fusion gene or the MBP vector alone (pMAL; for details, see Materials and methods). PAGE analysis of resulting protein preparations revealed species of the predicted size of MBP and MBP–Ume6 proteins (data not shown). Approximately 0.4 μg of protein from each sample was incubated with labeled $URS1^{SPO13}$ in a standard EMSA as described above. The MBP–Ume6 fusion protein produced a significant shift (arrow in Fig. 7) in probe migration, whereas the control MBP alone did not. This complex is competed by unlabeled $URS1^{SPO13}$ oligonucleotide, indicating that the interaction is specific. These results provide evidence that Ume6 is able to bind the $URS1^{SPO13}$ directly without the assistance of additional yeast proteins or yeast-specific modifications.

UME6 *represses meiotic gene expression independently of* IME1 *and* IME2

Previous studies have shown that normal early meiotic gene induction is dependent on the function of two positive effectors of meiotic development, *IME1* and *IME2*. A recent report (Bowdish and Mitchell 1993) has shown that Ume6 represses the transcription of *IME2* (another early meiosis-specific gene that, like *SPO13*, contains a *URS1* element). To test whether *UME6*-dependent repression of early meiotic genes occurs by effects on either of the *IME* genes during vegetative growth, we examined *SPO13* mRNA levels in *ume6-2* strains containing gene disruptions of either *IME1* or *IME2*. These studies revealed that the derepressed vegetative levels of *SPO13* mRNA in the *ume6-2* mutant do not require the presence of either *IME1* or *IME2*, similar to results reported previously for *ume1* to *ume5* (Fig. 8). Thus, like all other *UME* genes, *UME6*-mediated repression of *SPO13* must occur either downstream or independently of the *IME1* and *IME2* functions.

To determine whether the reverse is true, that the *IME1* or *IME2* genes exert their positive control on *SPO13* expression through negative regulation of *UME6*, we examined other phenotypes of *ume6* mutants based

Table 2. *Effect of* ume6 *mutations on* CAR1 *and* CAR2 *expression*

Strain number	Relevant genotype		Specific activities[a]	
	UME6	*ARG81*	arginase	ornithine transaminase
Σ1278b	*UME6*	*ARG81*	8	0.11
10R34d-II	*UME6*	*arg81::CAR1*	4	0.05
02296b	*ume6*	*ARG81*	41	0.70
02654a	*ume6::URA3*	*ARG81*	51	1.13
02459c	*ume6*	*arg81*	28	0.93
02446b	*ume6::Ty*	*arg81*	26	0.84
02536c	*ume6::URA3*	*arg81::CAR1*	34	0.72

[a]All of the strains are grown on minimal medium plus ammonium (M.am) as a nitrogen source and containing the appropriate amino acid supplements. Arginase specific activity is expressed in micromoles of urea per hour per milligram of protein. Ornithine transaminase specific activity is expressed in micromoles of D-5-pyrroline carboxylic acid per hour per milligram of protein.

on the following rationale. It has been reported that overexpression of either the *IME1* or *IME2* genes (1) results in premature expression of early meiotic genes during vegetative growth, (2) elevates levels of genetic recombination during mitotic division, and (3) increases the kinetics and efficiency of ascus formation in sporulation medium (Smith and Mitchell 1989). Accordingly, if the functions of *IME1* and/or *IME2* in meiosis occur exclusively through negative regulation of *UME6*, then *ume6* mutants should behave in a manner similar to wild-type strains that overexpress the *IME* genes. To test this hypothesis, wild-type and homozygous *ume6-5* diploids were examined for recombination (between *leu1* heteroalleles), for the appearance of binucleate or tetranucleate cells (reflecting the occurrence of one or both meiotic divisions) during vegetative growth, and for the efficiency of ascus production in sporulation medium. In contrast to *IME1* and *IME2* overexpression, no significant increase in either recombination or multinucleate cells occurs in vegetative cultures of the *ume6* mutant (data not shown). Moreover, sporulation is much less efficient in the mutant than in the wild type (<5% and 61%, respectively). Hence, although a disruption of the *UME6* gene results in unscheduled expression of early meiotic genes, this phenotype does not promote meiotic events during vegetative growth and, furthermore, is del-

Table 3. *Effect of* ume6 *mutations on the response to nitrogen starvation*

Strain	Genotype	Growth medium[a]	Specific activities[b]	
			arginase	ornithine transaminase
Σ1278b	*UME6*	+NH4	11	0.15
		−NH4 (1 hr)	82	0.50
		−NH4 (2 hr)	91	0.50
		−NH4 + P	28	0.07
02296b	*ume6*	+NH4	39	1.20
		−NH4 (1 hr)	54	1.30
		−NH4 + P	75	0.70
02532d	*ume6::URA3*	+NH4	99	1.60
		−NH4 (1 hr)	121	1.90
		−NH4 (2 hr)	133	1.65
		−NH4 + P	192	1.20

[a](+N and −N) Growth with and without ammonium as a nitrogen source; (+P) growth with proline as the sole nitrogen source.
[b]Specific activities calculated as in Table 2.

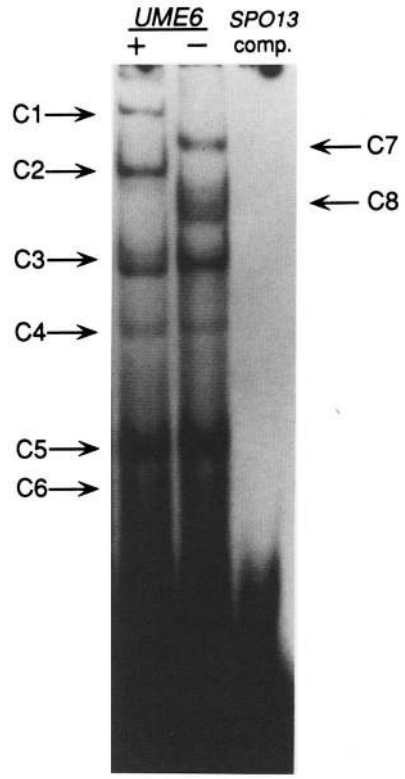

Figure 6. EMSAs using crude extracts. Wild-type (+) and *ume6-5* mutant (−) extracts were incubated with a 26-bp *URS1*^{SPO13} oligonucleotide, and DNA–protein complexes separated on nondenaturing polyacrylamide gels (see Materials and methods). Complexes associated with wild-type extracts are designated C1–C6. Complexes C7 and C8 are observed only in *ume6* mutant extracts. (*SPO13* comp.) The wild-type extract with a 100-fold excess of unlabeled *URS1*^{SPO13} oligonucleotide.

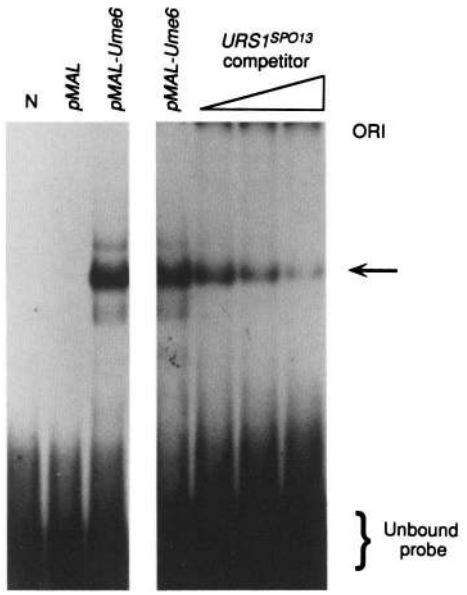

Figure 7. EMSAs with MBP–Ume6 fusion protein. EMSAs were conducted as described in the legend to Fig. 6. (N) Naked DNA. Extracts were prepared from *E. coli* transformed with MBP plasmid alone (pMAL) or the fusion gene construct (pMAL–Ume6). The arrow indicates specific MBP–Ume6/$URS1^{SPO13}$ complex formation. Competition experiments were performed with 33-, 100-, and 300-fold excess of unlabeled $URS1^{SPO13}$ oligonucleotide, respectively.

eterious to the sporulation process. These results suggest that induction of early meiotic gene expression by *IME1* and *IME2* does not occur simply by down-regulating *UME6* and/or that *UME6* may play a positive role in regulating meiosis (see Discussion).

UME6 *mRNA is constitutively expressed during meiosis and nitrogen deprivation*

Because *UME6* negatively regulates pathways of early meiotic transcription and arginine degradation, we examined whether the *UME6* gene itself is repressed transcriptionally when these genes are induced. The steady-state levels of *UME6* transcript in total RNA from a wild-type strain (NKY278) during vegetative growth and meiosis were determined using primer extension analysis. Primer extension was used because of the multiple transcriptional start sites observed for *UME6* and the presence of small open reading frames (ORFs) upstream of the authentic initiator codon (see Fig. 4). If these small ORFs play a role in regulation, transcriptional start usage might be an important consideration. These experiments revealed that *UME6* transcript levels from all transcriptional start sites remain constant throughout meiosis (1.5–9 hr) and subsequent spore formation (12–48 hr), including the period when *SPO13* mRNA is expressed transiently (Fig. 9A). A similar result was obtained when *UME6* mRNA levels were determined by Northern blot analysis of cultures grown on a rich nitrogen source or starved for nitrogen (Fig. 9B). We conclude from these results that *UME6* itself is not subject to transcriptional regulation, indicating that other mechanisms are used to inactivate Ume6 repressor activity and allow the expression of *CAR1* and *SPO13*.

Discussion

This report describes the identification, cloning, and characterization of *UME6* (*CAR80/CARGRI*). Evidence is provided that (1) *UME6* represses a unique but diverse set of genes responding to environmental conditions (*CAR1* and *CAR2*), cell-type control (*BAR1*), or both types of regulatory signals (*SPO11,13,16*), (2) *UME6* and *CAR80* (*CARGRI*) are identical and encode a 91-kD protein containing a C6 zinc cluster required for its function, (3) *UME6* is dispensable for mitosis but plays a role in maintaining optimum growth rates in haploids and diploids, (4) the gene is essential for nitrogen regulation of degradative enzymes during vegetative growth and for proper meiotic development, and (5) *UME6* transcription is constitutive during nitrogen starvation and sporulation.

How does *UME6* act to repress a diverse set of genes? The upstream regulatory regions of *CAR1*, *CAR2*, and several early meiotic genes contain a *cis*-acting element (*URS1*) that is required for transcriptional repression (Sumrada and Cooper 1987; Park and Craig 1989; Buckingham et al. 1990; Vershon et al. 1992). Three sets of results argue that Ume6 repression is mediated through *URS1*. First, independent genetic studies demonstrate that the derepressed levels of *CAR1* and *SPO13* in *ume6* mutants are not additive when combined with point mutations in $URS1^{CAR1}$ or $URS1^{SPO13}$, respectively (Park et al. 1992; L. Buckingham, R. Strich, and R.E. Esposito, in prep.). Second, most C6 zinc cluster family members have been shown to recognize the nucleotide triplets

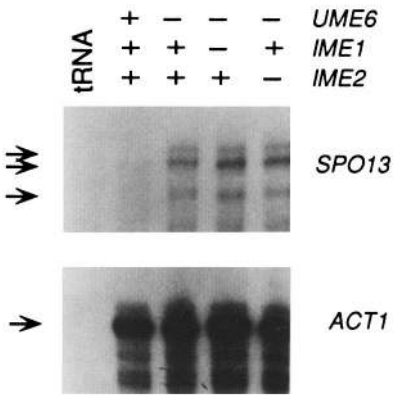

Figure 8. The *ume6* mutant phenotype does not require *IME1* or *IME2*. The *UME6*, *IME1*, or *IME2* genotypes are presented above the gel. Arrows illustrate the bands corresponding to the protected *SPO13* probe using S1 protection experiments. *ACT1* mRNA levels are used to normalize the poly(A)$^+$ fraction in the total RNA preparations.

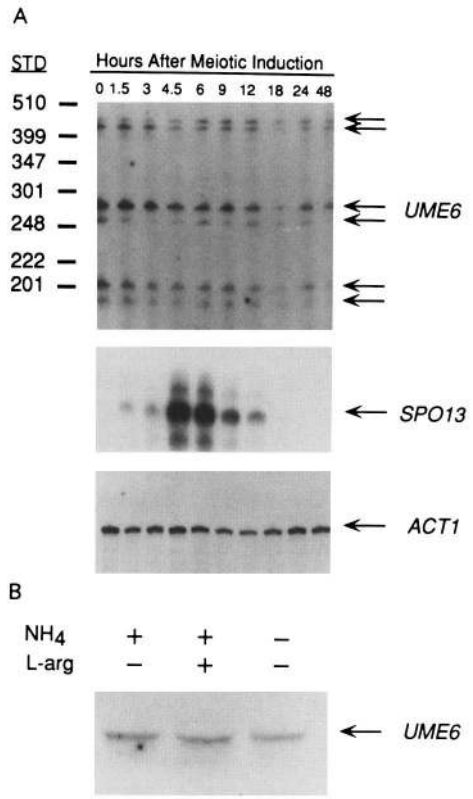

Figure 9. *UME6* mRNA levels during meiotic development and nitrogen starvation. (*A*) Primer extension analysis of 30 μg of total RNA extracted from vegetative cells (time point = 0 hr) and at subsequent times after the shift to sporulation medium. The arrows indicate the extension products from a primer at +13 to +27 with respect to the initiator ATG. S1 nuclease protection assays were performed with probes for *SPO13* and *ACT1* using the same RNA samples. The *SPO13* samples illustrate the typical induction pattern of this gene, and *ACT1* is used to normalize the poly(A)$^+$ fraction in the total RNA preparations. (*B*) Northern blot analysis of 50 μg of total mRNA probed with a fragment of pED30. Nitrogen sources are given above each lane. The sample lacking a nitrogen source was grown on ammonia and shifted to a nitrogen-free medium for 1 hr before harvesting the cells.

CGG-N$_{(8-11)}$-CCG or an asymmetric site containing GCA (Reese and Ptashne 1993). The *URS1* element also contains the canonical C6 zinc cluster DNA-binding sequence (GCPu) within the region shown to be required for *SPO13* repression in vivo (underlined in Fig. 5B; Buckingham et al. 1990; L. Buckingham, R. Strich, and R.E. Esposito, in prep.). Finally, we provide direct biochemical evidence that Ume6 is able to bind directly to the *URS1*^{SPO13} region in vitro. Taken together, these results argue strongly that Ume6 regulates gene expression through the *URS1* element. Interestingly, it is clear that Ume6 does not regulate all genes that contain a *URS1* element (e.g., *SSA1*; E. Craig, pers. comm.; *TRK2*, R. Gaber, pers. comm.; *ARG4*, E. Dubois and F. Messenguy, unpubl.). Hence, it is possible that the specificity of Ume6/*URS1* repression occurs through association with additional proteins. Two proteins (Buf1 and Buf2), neither of which is the product of the *UME6* gene, have been purified that bind the *URS1*^{CAR1} (Luche et al. 1992). The genes encoding these proteins are allelic to *RPA1* and *RPA2* (Luche et al. 1993), two factors isolated by their ability to bind single-stranded DNA derived from origins of chromosomal replication (Heyer et al. 1990; Brill and Stillman 1991). Although the roles *RPA1* and *RPA2* play in transcriptional control have not been determined, they may represent general DNA-binding factors that, in combination with Ume6, direct the specific expression pattern observed for *CAR1*.

The carboxy-terminal location of the C6 zinc cluster domain in the Ume6 negative regulator is unusual compared with its position in other *trans*-regulators such as Gal4, Ppr1, Put3, and Arg81. All of these other factors function as transcriptional activators and contain the C6 domain in the amino-terminal 25% of the protein. In addition, the activators of the steroid and thyroid hormone receptor superfamily also contain zinc cluster domains in the amino-terminal end of the protein (Evans 1988). Interestingly, there is at least one example of a zinc finger protein that acts as a negative regulator, which provides a possible paradigm for the mode of Ume6 action. The *wt1* gene is expressed in the developing kidney and is believed to function as a tumor suppressor gene, as mutations in *wt1* are associated with some Wilms tumors (Madden et al. 1991). After mitogen stimulation, the zinc finger protein EGR-1 accumulates in the nucleus and is thought to stimulate transcription by displacement of the WT1 repressor. Although the zinc finger domain of WT1 is found at the carboxyl end of the protein, it is not yet clear whether this location has functional significance in repression. It is tempting to speculate that zinc finger transcriptional activators in vegetative growth or meiosis may antagonize the *UME6* repressor in a similar fashion. For example, in the case of *SPO13* transcription, the *URS1* element, together with adjacent sequences, is required for activation as well as repression (L. Buckingham, R. Strich, and R.E. Esposito, in prep.). The binding of an activator that also contains a C6 zinc cluster may occur at or adjacent to *URS1* and thus prevent the binding of the *UME6* negative regulator. Significantly, the *URS1* element has been shown to be required for both activation and repression of other meiotic genes, including *HOP1* (Vershon et al. 1992), *IME2* (Bowdish and Mitchell 1993), and *SPO11* (C. Atcheson and R.E. Esposito, unpubl.). A similar argument could also be made for regulation of *CAR1* transcription where the targets of the *ARG81* C6 zinc cluster activator, the "arginine boxes," have been localized to sequences immediately adjacent to the *URS1*^{CAR1} (Messenguy et al. 1991). Alternatively, meiotic induction may occur through formation of a heterodimer between Ume6 and another protein that is either unable to bind DNA or binds to another site (e.g., T_4C in *IME2*). In these cases, only half of the zinc finger recognition site (i.e., an asymmetric site) may be required for function.

Why are *ume6* diploids unable to complete normal meiotic development? One explanation proposed by

Bowdish and Mitchell (1993) is based on their finding that *UME6* appears to be required both for repression of *IME2* as well as for the *IME1*-dependent activation of *IME2*. Like *ume6* mutants, loss of *IME2* activity results in an early arrest of meiotic development. They have suggested that *ume6* mutants may terminate development as a result of the absence of *UME6*-dependent transcriptional activation. An alternative explanation for the *ume6* sporulation defect is suggested by the finding that *ume4* diploids accumulate *SPO13* mRNA to nearly normal levels after meiotic induction but that they remain high throughout development (i.e., *ume4* mutants fail to re-establish transcriptional repression; R. Strich, unpubl.). Loss of *UME4* (*RPD1/SIN3*) activity, which is reported to have no effect on *IME2* activation (Bowdish and Mitchell 1993), results in a similar reduction in asci production and arrest phenotype as observed with *ume6* mutants (Strich et al. 1989a). Thus, the failure to downregulate some early meiotic genes (such as *SPO13*) later in meiosis may inhibit the progression of meiotic development. In a similar manner, the loss of Ume6 activity may also result in a failure to re-establish repression of the early meiotic genes during meiosis, thereby causing an arrest in development similar to *ume4* mutants.

How is *UME6* itself regulated? To derepress *CAR1* and the early meiotic genes, the repressor function of *UME6* must be deactivated. We report here that this deactivation is likely to be post-transcriptional, as *UME6* mRNA levels are unaltered during nitrogen deprivation and meiosis. This finding is not surprising considering the wide range of genes controlled by *UME6*. Removal of the protein by inhibiting transcription would derepress several genes that normally remain repressed. Moreover, the probable need for Ume6 in *IME2* activation and in re-establishing transcriptional repression of the early meiotic genes after only a brief expression window (~2 hr; see Fig. 9) suggests that the *UME6* protein is not simply destroyed by degradation or translational control and then synthesized de novo. As noted in Results, expression of *CAR1* but not *CAR2* is regulated in response to the quality of the nitrogen source, whereas both enzymes are derepressed during nitrogen starvation. We have shown here that in the *ume6* mutant, arginase is still derepressed in the presence of proline, a poor nitrogen source. However, no additional increase is observed when the *ume6* mutant is starved completely for exogenous nitrogen. Thus, the regulation of arginine catabolism by *UME6* may represent the endpoint of the transduced environmental nitrogen signal. Such a role fits well with the control of meiosis and sporulation, as this process is initiated only with nitrogen starvation. *UME6* activity could then be modulated by translational control through the upstream ORFs (uORF; Fig. 4), interactions with a specific effector, or by post-translational modifications. Translational control may be less likely for the same reasons described for transcriptional control. Because the primary sequence of Ume6 did not reveal striking homology to a known motif that mediates protein–protein interactions (e.g., amphipathic helix) but does contain several possible targets for a variety of protein kinases, including cAMP-dependent protein kinase, we favor the latter possibility. The analysis of the phosphorylation state of Ume6 during different periods in cell growth and development may help to resolve this question.

Materials and methods

Strains and plasmids

The genotypes of strains used in these studies are listed in Table 4. Construction of the *SPO13–lacZ* reporter gene fusion in p(*SPO13*)28 is described elsewhere (Wang et al. 1987). The *spo13–URA3* fusion gene in plasmid pMS49 (constructed by M. Slater in our laboratory) was made by inserting the 208-bp *Taq*I restriction fragment of *URA3* (+4 to +212) into the *Acc*I site of pUC19 (Yannisch-Perron et al. 1985). The *Bam*HI–*Bgl*II fragment of *SPO13* containing the transcriptional control region and first 15 codons (Buckingham et al. 1990) was introduced in-frame into the *Bam*HI site immediately upstream of the *URA3* fragment, and the *Bam*HI–*Nco*I fragment of the resulting construct (*Nco*I = +200 in *URA3*) was then used to replace the *Bam*HI–*Nco*I fragment of YCp19 (Stinchcomb et al. 1982). Plasmid pJEF1271 (a gift from J. Boeke, The Johns Hopkins University, Baltimore, MD) contains a galactose-inducible Ty element used to generate and mark *car80* mutations. Plasmid p3, containing ampicillin and tetracycline resistance genes crippled by amber mutations, was used to rescue transposon tagged derivatives in *E. coli*. Plasmids YIpK26-106 (Kassir et al. 1988) and pAM412-2 (Smith and Mitchell 1989), kindly provided by Y. Kassir (Tel Aviv University, Israel) and A. Mitchell (Columbia College of Physicians and Surgeons, NY), respectively, were used to disrupt *IME1* and *IME2* in strain D15. Plasmid pUZ77, containing the *BAR1*-coding sequence disrupted by the insertion of the *LEU2* gene (provided by G. Sprague, University of Oregon, Eugene), was used to construct the *bar1::LEU2* mutation in RSY237. The *ume6-5* disruption allele was made by inserting the 2.25-kb *Bgl*II fragment of *LEU2* into the *Bam*HI site of *UME6* (at codon 140) in plasmid p5914, and then transplacing the chromosomal *UME6* gene in strain JX150 with a 6.5-kb fragment containing the *ume6-5* allele (Rothstein 1991). The *ume6-D1* allele was constructed by inserting a 4.2-kbp *Sal*I–*Cla*I fragment containing *UME6* into *Sal*I–*Cla*I-digested YIp5. An internal 2.4-kbp *Bst*XI–*Pac*I fragment containing the entire coding sequence of *UME6* was liberated and the vector reclosed using *Xho*I linkers. This plasmid (pCS4) was digested with *Bst*EII and used to delete *UME6* from the genome using the pop-in/pop-out method (Rothstein 1991). The *ume6::URA3* allele was constructed by inserting a 1.1-kb *Bgl*II fragment containing the *URA3* gene at codon 230. DNA fragments and plasmids were transformed into yeast by either the lithium acetate procedure (Schiestl and Gietz 1989) or by electroporation (Hashimoto et al. 1985) using a Bio-Rad Gene Pulser TM set at 2.5 kV and 25 μF as per manufacturer's instructions.

Media and plate assays

Growth, sporulation, and media conditions have been described previously (Klapholz and Esposito 1980), as has the plate assay for β-gal activity (Strich et al. 1989). The overlay plate assay for acid phosphatase activity (Toh-e and Shimauchi 1986) and the halo assay for detection of mating pheromone excretion (Herskowitz 1988) were performed as described previously. All yeast strains used in *CAR80* analyses were grown on minimal me-

Table 4. *Strains*

Strain	Genotype	Source
RSY10	*MAT***a** *ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	Strich et al. (1989a)
D15	*MATα his4 leu2 lys1 trp1 ume6-2 ura3*	this study
D16	*MAT***a** *his3-11,13 leu2 lys1 lys2 trp1 ume6-2 ura3*	this study
NKY278	*MAT***a**/*MATα lys2/lys2 ura3/ura3 ho::LYS2*	N. Kleckner (Yale University, New Haven, CT)
JX192	*MAT***a**/*MATα can1/can1 his4-519/his4-519 leu2-3,112/leu2-3,112 lys2/lys2 trp1/trp1 UME6/ume6-5 ura3/ura3*	this study
R1887	*MAT***a** *his6 lys2Δ201 sst1-3 trp1Δ1 ura3-52*	R. Gaber (Northwestern University, Evanston, IL)
R1924	*MATα can1 cyh2 his6 met1 sst2-1*	R. Gaber
RSY219	*MATα his4 ime2Δ leu2 lys1 trp1 ume6-2 ura3 ime2::LEU2*	this study
RSY260	*MATα his4 ime1Δ leu2 lys1 trp1 ume6-2 ura3 ime1::URA3*	this study
RSY269	*MATα can1 his4-519 leu2-3,112 lys2 trp1 ura3*	this study
RSY270	*MAT***a** *can1 his4-519 leu2-3,112 lys2 trp1 ume6-5 ura3*	this study
RSY271	*MAT***a** *can1 his4-519 leu2-3,112 lys2 trp1 ura3*	this study
RSY280	*MATα can1 his4-519 leu2-3,112 lys2 trp1 ume6-5 ura3*	this study
RSY300	*MATα bar1Δ his4 leu2 lys1 trp1 ume6-2 ura3 bar1::LEU2*	this study
10R34d-II	*MAT***a** *arg81::CAR1 ura3*	Qiu et al. (1991)
Σ1278b	*MAT***a**	Béchet et al. (1970)
02296b	*MAT***a** *car80 ura3*	this study
02459c	*MAT***a** *arg81 car80 ura3*	this study
02536c	*MAT***a** *arg81::CAR1 ume6::URA3*	this study
02532d	*MATα ume6::URA3*	this study
02446b	*MAT***a** *arg81 ume6::Ty ura3*	this study
yC105	*MAT***a** *ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ume6-D1 ura3-1*	this study

dium containing 3% glucose, vitamins, and mineral traces as described (Messenguy 1976). Nitrogen sources were 0.02 M $(NH_4)_2SO4$, or 1 mg/ml of L-arginine, L-ornithine, or L-proline. Nitrogen starvation was achieved by filtering cells grown on minimal medium with ammonia (referred to as $+NH_4$) and cultivating them for 1 or 2 hr on fresh minimal medium without nitrogen ($-NH_4$).

Isolation, cloning, and mapping the ume6 *complementing activity*

A *MAT***a** haploid strain (S29) containing two single-copy (CEN) plasmids, one bearing a *MATα* gene (pSG228) and the other, a *SPO13–lacZ* protein fusion gene (p[spo13]30), was subjected to ethylmethane sulfonate (EMS) mutagenesis. The presence of the *MATα* plasmid enables haploid cells to form the a1–α2 repressor, which allows the initiation of meiotic development when cells are transferred to sporulation medium (Wagstaff et al. 1982). The second plasmid provided a convenient indirect assay of *SPO13* expression by monitoring β-gal activity of the fusion protein. The *spo13–lacZ* fusion was placed on a single-copy *CEN* vector (rather than a high-copy plasmid used in the detection of *ume1* to *ume5*) to recover mutants exhibiting a higher level of derepression. Survivors of mutagenesis were lifted with filter paper discs from YPD growth medium, placed on sporulation medium (to induce *SPO13* expression), and returned to rich growth medium. β-Gal activity of the *spo13–lacZ* fusion was assayed on filters after the shift as described previously (Strich et al. 1989).

The *UME6* gene was cloned using the same procedure that was used to clone *UME4* (Vidal et al. 1991). Briefly, a yeast *CEN* library containing *LEU2* as a selectable yeast marker (obtained from P. Hieter, Johns Hopkins University, Baltimore, MD), was introduced into a *ume6-2* mutant strain bearing a *spo13–URA3* gene fusion on a *CEN* plasmid (pMS49). This strain exhibited a Ura^+ phenotype. Selection for Ura^- colonies on medium containing 5-fluoro-orotic acid yielded one *ume6* complementing clone (per 5400 transformants), which was retested and subcloned into pRS vectors (Sikorski and Hieter 1989). To determine the minimum complementing fragment, the subclones were examined for their ability to repress expression of a *spo13–lacZ* reporter gene fusion in a *ume6-2* strain during mitosis. The *UME6* gene was subsequently localized to chromosome IV by hybridization of *UME6* subclone 5905 to yeast chromosomes separated by transverse alternating field electrophoresis (TAFE; Gardiner et al. 1986), except that switch times were 60 sec for 15 hr and 90 sec for 8 hr. Further localization of *UME6* was accomplished by hybridizing 5905 to filters containing subclones of chromosome IV provided by M. Olsen (L. Riles and M. Olson, pers. comm.). Standard segregation analysis with markers on the same chromosomal fragment was conducted to determine the precise map location.

Isolation of car80/cargRI *complementing clones*

The complementing activity for *car80* mutations was cloned by Ty insertional mutagenesis. The Ty element used contains the *supF*-marked *E. coli* plasmid pian7. To select Ty-marked *car80* mutations, strain 10R34d-II (*arg81/argRII, ura3*) was transformed with pJEF1271, which contains a Ty element whose expression is dependent on the *GAL1* promoter (Boeke et al. 1985). The transformed strain was grown for 5 days at 22°C on minimal medium containing 2% galactose to induce high levels of transposition and plated on solid minimal medium contain-

ing 1 mg/ml of L-ornithine as the sole nitrogen source. Because the strain was mutant for *arg81*, the activator of *CAR2*, only cells containing the *car80* mutation were able to express *CAR2* and use ornithine for growth. Three candidates were obtained that exhibited derepressed levels of arginase and ornithine transaminase and were unable to complement a *car80* point mutation. Southern blot analysis revealed that each isolate shared one Ty-tagged band among the multiple Ty insertions. One candidate was crossed with an *arg81* mutant (BJ210). The diploid was sporulated and meiotic products assayed by Southern blot analysis and tested for arginase activity to obtain a *car80* segregant containing a single Ty insertion at the *car80* locus (02446b; Table 2). To recover this allele, total DNA from 02446b was digested by *Eco*RI, ligated, and used to transform an *E. coli* host by selecting pian7 (*supF*) on plates containing ampicillin and tetracycline. This *E. coli* strain already contained the plasmid p3, which contains the gene for kanamycin resistance as well as the tetracycline and ampicillin resistance genes made nonfunctional by amber mutations. Using the *car80*::Ty fragment as a probe, the complete complementing activity was recovered by colony hybridization to the yeast genomic library constructed by M. Rose (Princeton University, NJ). Among 5000 colonies, one positive candidate was obtained. To verify that this clone contained the complete gene, it was recovered and retested for its ability to complement a *car80* mutation (strain 02459c; see Fig. 3).

DNA sequencing

Subclones of 5905 and pED30 were inserted into a Bluescript vector (Stratagene) or a derivative (pVZ1) for use as sequencing templates. For *UME6*, nested deletions were generated in each subclone using exonuclease III as prescribed by the Erase-A-Base kit supplied by Promega (Madison, WI). *UME6*-specific oligonucleotides (synthesized by P. Gardner, University of Chicago, IL) were used to prime sequencing reactions across subclone junctions. For the *car80* complementing fragment, universal T3 and sequence-specific primers were used to perform dideoxy sequencing (Sanger et al. 1977) of double-stranded DNA template using Sequenase DNA polymerase (U.S. Biochemical) as directed by the manufacturer. Searches for homology to protein sequences in the GenBank, EMBL, and SwissProt data bases were performed with the FASTA program in the UWGCG analysis package. The GenBank accession number of the sequence reported here is L24539.

Nuclease S1 analyses and primer extension assays

RNA samples for primer extension (2×10^8 to 4×10^8 total cells) or S1 analysis ($\sim1\times10^8$ total cells) were prepared from late log cultures (6×10^6 to 8×10^6 cells/ml) of YPD- or YPA-grown cells and from sporulation cultures (5×10^7 cells/ml) as described previously (Elder et al. 1983). The S1 protection probes for the following genes are described in previous reports: *SPO13* (Wang et al. 1987), *SPO11* (Atcheson et al. 1987), *SPO12* (Malavasic and Elder 1990), *SPS2* (Strich et al. 1989a), *SPO16* (Malavasic and Elder 1990), and *ACT1* (Strich et al. 1989a). Primer extension experiments were performed with the oligonucleotide 5′-GCTTTGAGAGCGCGC-3′ (+27 to +13 with respect to the initiator ATG) according to McKnight and Kingsbury (1982). Northern blots of total RNA were prepared using glyoxyl denaturing conditions (Maniatis et al. 1982; Messenguy and Dubois 1983; Boonchird et al. 1991). The 5-kb *Hin*dIII fragment containing the *CAR80* gene was labeled by nick translation (Rigby et al. 1977) and used as a probe.

Electrophoretic mobility shift assays

Electrophoretic mobility shift assays (EMSAs) with crude extracts were conducted as described elsewhere (Arcangioli and Lescure 1985) from RSY269 and RSY270 cultures harvested in late logarithmic growth. A 26-bp oligonucleotide GAAATAGCCGCCGACAAAAAGGAATT containing *URS1* (produced by the Fox Chase Cancer Center DNA synthesis facility) was labeled at one end with [γ-^{32}P]ATP using polynucleotide kinase and hybridized to a threefold molar excess of unlabeled complementary DNA to drive most of the labeled probe into a duplex state. The probe was either used directly or gel purified. Reactions containing 10 μg of crude extract, 2.5 μg of poly[d(I-C)] and 1.0 μg of poly[d(A-T)] as nonspecific competitors, and 0.1 ng probe were incubated at 16°C for 20 min, loaded directly onto a 6% nondenaturing polyacrylamide gel, and electrophoresed at 10 V/cm for 2 hr. For in vitro binding of the MAL–Ume6 fusion protein to $URS1^{SPO13}$, pMAL and pMAL–Ume6 *E. coli* transformants were induced with IPTG during log phase growth. Soluble MBP and MBP-Ume6 were affinity purified using maltose-coated beads as per the manufacturer's instructions (New England Biolabs). The proteins were eluted from the beads using 10 mM maltose and the eluate concentrated using Centricon units (Amicon). The same assay conditions were used as just described except ~0.4 μg of purified protein was substituted for the crude extract.

Oligonucleotide-directed mutagenesis

Oligonucleotide-directed mutagenesis of p5905 was performed as described previously (Kunkel et al. 1987). Oligomers used were synthesized by P. Gardner and were of the sequence 5′-GCTGGATTTCTAGATTAAGG-3′ (Cys-774 > Ser, creates an *Xba*I site) and 5′-TGTAGATTAAGGCTTAAGAAGTGTACC-3′ (Lys-778 > Leu, creates an *Afl*II site). The altered nucleotides are underlined. Plasmid isolates were first screened for the engineered restriction site and sequenced to confirm the nature of the mutation.

β-Gal assays

The *UME6* clone pPL5905 and derivatives were transformed into the *ume6* deletion strain yC105 and into the isogenic wild-type strain RSY10. These strains had been transformed previously with p(spo13)46, a YCp50-based plasmid containing the 5′ *spo13–lacZ* fusion (Buckingham et al. 1990). Two independent transformants of each strain [with p(spo13)46 alone, or with p(spo13)46 and pPL5905 or one of its derivatives] were grown at 30°C to 1×10^7 cells/ml in 3 ml of medium selective for the plasmids. Cells were harvested, and the level of β-gal expression resulting from *spo13–lacZ* expression was assayed as described previously (Buckingham et al. 1990).

Enzyme assays

Arginase (E.C.3.5.3.1) and ornithine transaminase (E.C.2.6.1.13) were assayed as described previously (Messenguy et al. 1971; Dubois et al. 1978).

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