UME6 is a key regulator of nitrogen repression and meiotic development

Randy Strich,1 Richard T. Surosky,2 Camille Steber,2 Evelyne Dubois,3 Francine Messenguy,3 and Rochelle Easton Esposito2

1Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 USA; 2Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois 60637 USA; 3Institut de Recherches du CERIA, Brussels B-1070, Belgium

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]

Received November 4, 1993; revised version accepted February 7, 1994.
has been studied extensively (for review, see Magasanik 1993). When confronted with limited environmental nitrogen, haploid cells arrest cell division in G1. 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) [Béchet et al. 1970; Deschamps et al. 1978; Dubois et al. 1978, 1991; Surosky and Esposito 1992]. To identify 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. 1989; 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 umel 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 umel-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 umel mutations. Therefore, these new mutations define an independent locus, designated umel. The recessive nature of the umel 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 umel 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 umel-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.

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, MATa and MATα 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 α-factor secretion [Fig. 2A, left]. However, the MATa ume6-2 mutant excretes significantly less α-factor compared with the wild-type [Fig. 2A, right]. This phenotype, as observed in nine4 mutants, is the result of BAR1 (encoding the α-factor protease) derepression in MATa cells (Vidal et al. 1991). Disruption of the BAR1 gene in a MATa ume6-2 strain restores α-factor production to wild-type levels [Fig. 2B], suggesting that UME6 [like UME4] is required for repression of the α-specific gene BAR1 in MATα 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.

**Figure 2.** Mating pheromone production in wild-type and ume6-2 mutants. (A) Halo assays using lawns of strains supersensitive to either α- or α-mating pheromone, and MATa and MATα 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 bar1Δ strains. BAR1 encodes the α-factor protease normally expressed only in MATa cells.
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 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-X2-Cys-X6-Cys-X<sub>α</sub>-Cys-X<sub>2</sub>-Cys-X<sub>α</sub>-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-Arg/Lys), 13 for protein kinase C (Ser/Thr-X-X-Asp/Glu), 4 for cAMP- and cGMP-dependent protein kinase (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).

Table 1. UME6 linkage analysis

| Marker          | PD<sup>a</sup> | NPD<sup>b</sup> | Tetratype | cM  |
|-----------------|---------------|---------------|-----------|-----|
| *ume6*­*hom2*   | 52            | 0             | 45        | 23.1|
| *ume6*­*aro1*   | 42            | 2             | 50        | 33.0|
| *ume6*­*pet14*  | 18            | 0             | 3         | 21.0|

<sup>a</sup>Parental ditotype.

<sup>b</sup>Nonparental ditotype.

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 ascis containing four viable spores segregated 2<sup>+</sup>:2<sup>-</sup> for the dis-
ruption allele, demonstrating that UME6 is dispensable for vegetative growth. Growth rates of umes-6-5 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 [sixfold for umes-6-5] than that of wild type [data not shown]. The overall spore viability of the heterozygous diploid, as measured by the ability to form macroscopic colonies, was significantly lower [sixfold for umes-6-5] than that of wild type [data not shown]. The overall spore viability of the heterozygous diploid, as measured by the ability to form macroscopic colonies, was significantly lower [sixfold for umes-6-5] than that of wild type [data not shown]. The overall spore viability of the heterozygous diploid, as measured by the ability to form macroscopic colonies, was significantly lower [sixfold for umes-6-5] than that of wild type [data not shown].

UME6 mediates CAR1 repression in response to nitrogen

As discussed earlier, expression of the catabolic genes CAR1 and CAR2, encoding arginase and ornithine aminotransaminase, 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 umes are derepressed for CAR1 and CAR2 in medium containing ammonia as a nitrogen source [Table 2]. The derepression in the umes 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 twofold increase in activity relative to ARG81; Table 2], suggesting that activation may occur in part by antagonism of repression [see Discussion]. [3] When the umes 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 umes 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].
Isolation and characterization of UME6

Ume6 interacts directly with URS1<sup>spo13</sup> 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<sup>spo13</sup>, electrophoretic mobility shift assays (EMSA) were performed using a 26-bp oligonucleotide containing URS1<sup>spo13</sup> 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<sup>spo13</sup> 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 lac1 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<sup>spo13</sup> 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<sup>spo13</sup> oligonucleotide, indicating that the interaction is specific. These results provide evidence that Ume6 is able to bind the URS1<sup>spo13</sup> 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

---

**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 do-

---

**Table A**

| Factor | Sequence | Codon Start |
|--------|----------|-------------|
| Ume6 | CWICIRLKXKCTRERPHCNCERLKLDC | 771 |
| Ga14 | CDICIRLKXKCTRERPHCNCERLKLDC | 10 |
| Hap1 | CTICRKLXKCTRERPHCNCERLKLDC | 63 |
| Ppr1 | CKICRKLXKCTRERPHCNCERLKLDC | 33 |
| Arg81 | CTWICRKLXKCTRERPHCNCERLKLDC | 20 |

---

**Figure 6.** 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 do-

---

**Figure 7.** 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 do-
on the following rationale. It has been reported that overexpression of either the \textit{IME1} or \textit{IME2} genes \cite{1} results in premature expression of early meiotic genes during vegetative growth, \cite{2} elevates levels of genetic recombination during mitotic division, and \cite{3} increases the kinetics and efficiency of ascus formation in sporulation medium \cite{Smith and Mitchell 1989}. Accordingly, if the functions of \textit{IME1} and/or \textit{IME2} in meiosis occur exclusively through negative regulation of \textit{UME6}, then \textit{ume6} mutants should behave in a manner similar to wild-type strains that overexpress the \textit{IME} genes. To test this hypothesis, wild-type and homozygous \textit{ume6-5} diploids were examined for recombination \cite{between leu1 heteroalleles}, for the appearance of binucleate or tetranucleate cells \cite{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 \textit{IME1} and \textit{IME2} overexpression, no significant increase in either recombination or multinucleate cells occurs in vegetative cultures of the \textit{ume6} mutant \cite{data not shown}. Moreover, sporulation is much less efficient in the mutant than in the wild type \cite{<5\% and 61\%, respectively}. Hence, although a disruption of the \textit{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 2. Effect of \textit{ume6} mutations on \textit{CAR1} and \textit{CAR2} expression**

| Strain number | Relevant genotype | Specific activities$^a$ arginase | ornithine transaminase |
|---------------|------------------|-------------------------------|------------------------|
| \textit{UME6} | \textit{ARG81}   | 8                             | 0.11                   |
| \textit{ume6} | \textit{arg81::CAR1} | 4                             | 0.05                   |
| \textit{ume6::URA3} | \textit{ARG81} | 41                           | 0.70                   |
| \textit{ume6} | \textit{arg81} | 51                           | 1.13                   |
| \textit{ume6::Ty} | \textit{arg81::CAR1} | 28                           | 0.93                   |
| \textit{ume6::URA3} | \textit{arg81} | 26                           | 0.84                   |

\textsuperscript{a}All of the strains are grown on minimal medium plus ammonium \textit{(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.

**Table 3. Effect of \textit{ume6} mutations on the response to nitrogen starvation**

| Strain | Genotype | Growth medium$^a$ | Specific activities$^b$ arginase | ornithine transaminase |
|--------|----------|-------------------|-------------------------------|------------------------|
| \textit{UME6} | + NH4 | 11                | 0.15                          |
| − NH4 (1 hr) | 82          | 0.50                    |
| − NH4 (2 hr) | 91          | 0.50                    |
| − NH4 + P | 28          | 0.07                    |
| \textit{ume6} | + NH4 | 39                | 1.20                          |
| − NH4 (1 hr) | 54          | 1.30                    |
| − NH4 + P | 75          | 0.70                    |
| \textit{ume6::URA3} | + NH4 | 99                  | 1.60                          |
| − NH4 (1 hr) | 121         | 1.90                    |
| − NH4 (2 hr) | 133         | 1.65                    |
| − NH4 + P | 192         | 1.20                    |

\textsuperscript{a}(+ N and − N) Growth with and without ammonium as a nitrogen source; (+ P) growth with proline as the sole nitrogen source.

\textsuperscript{b}Specific activities calculated as in Table 2.
Isolation and characterization of UME6

EMTs 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 complex formation. Competition experiments were performed with 33-, 100-, and 300-fold excess of unlabeled URS1 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/CARG1). 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 (CARG1) 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
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 HOPI [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 [Messengu 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., T4C 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

---

**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(r-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 URS1SPO13 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
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 formation and arrest phenotype as observed with ume6 mutants [Strich et al. 1989a]. Thus, the failure to down-regulate 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 de-repress 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 de-repress 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 (e.g., 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 de-repressed during nitrogen starvation. We have shown here that in the ume6 mutant, arginase is still de-repressed 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 pSPO13/28 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 TaqI restriction fragment of URA3 (+4 to +212) into the AccI site of pUC19 (Yannisch-Perron et al. 1985). The BamHI-BglII fragment of SPO13 containing the transcriptional control region and first 15 codons [Buckingham et al. 1990] was introduced in-frame into the BamHI site immediately upstream of the URA3 fragment, and the BamHI–Ncol fragment of the resulting construct [Ncol = +200 in URA3] was then used to replace the BamHI–Ncol fragment of YCp19 [Stinchcomb et al. 1982]. Plasmid pEF1271 [a gift from J. Boeke, The Johns Hopkins University, Baltimore, MD] contains a galactose-inducible Ty element used to generate and mark ura40 mutations. Plasmid p3, containing ampicillin and tetracycline resistance genes crippled by amber mutations, was used to rescue transposon tagged derivatives in E. coli. Plasmids YTPK26-106 [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 bart::LEU2 mutation in RSY237. The ume6-5 disruption allele was made by inserting the 2.25-kb BglII fragment of LEU2 into the BamHI site of UME6 (at codon 140) in plasmid p5914, and then transposing the chromosomal UME6 gene in strain JX150 with a 6.5-kb fragment containing the ume6-5 allele [Rothstein 1991]. The ume6-61 allele was constructed by inserting a 4.2-kbp SaI–ClaI fragment containing UME6 into SaI–ClaI-digested Ytp5. An internal 2.4-kbp BstXI–PacI fragment containing the entire coding sequence of UME6 was liberated and the vector recoupled using XbaI linkers. This plasmid (pCS4) was digested with BstEI 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 BglII 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 Shimazuichi 1986] and the halo assay for detection of mating pheromone excretion [Her- skowitz 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    | MATα 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      | MATα his3-11,13 leu2 lys1 lys2 trp1 Ume6-2 ura3                            | this study                         |
| NKY278   | MATa/MATa lys2/ lys2 ura3/ura3 hco::LYS2                                  | N. Kleckner [Yale University, New Haven, CT] |
| JX192    | MATα/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    | MATα his6 lys2ΔA201 sst1-3 trp1Δ1 ura3-52                                  | R. Gaber [Northwestern University, Evanston, IL] |
| R1924    | MATα can1 cyh2 his6 met1 sst2-1                                           | this study                         |
| 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 Ume6-5 ura3                         | this study                         |
| RSY270   | MATα can1 his4-519 leu2-3,112 lys2 trp1 Ume6-5 ura3                         | this study                         |
| RSY271   | MATα can1 his4-519 leu2-3,112 lys2 trp1 Ume6-5 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| MATα arg81::CAR1 ura3                                                     | Qiu et al. [1991]                 |
| Σ1278b   | MATα                                                           | Béchet et al. [1970]              |
| 02296b   | MATα car80 ura3                                                           | this study                         |
| 02459c   | MATα arg81 car80 ura3                                                     | this study                         |
| 02536c   | MATα arg81::CAR1 ura3                                                     | this study                         |
| 02532d   | MATα ura3                                                                | this study                         |
| 02446b   | MATα ura3                                                                | this study                         |
| yC105    | MATα ade2 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1               | this study                         |

Nitrogen starvation was achieved by filtering cells grown on minimal medium with ammonia (referred to as + NH₄) and culti

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

A **MATα** haploid strain [S29] containing two single-copy (CEN) plasmids, one bearing a **MATα** gene (pSG2228) and the other, a **SPO13-lacZ** protein fusion gene (pSspo1330), was subjected to ethylmethane sulfonate (EMS) mutagenesis. The presence of the **MATα** plasmid enables haploid cells to form the a1–a2 repressor, which allows the initiation of meiotic development when cells are transferred to sporulation medium [Wagstaff 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 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. 1989a].

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 restested 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 S905 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 S905 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 pI7. 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 GALI 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-
cells containing the plasmid p3, which contains the gene for kanamycin resistance. E. coli were grown in 1 mg/ml of l-ornithine as the sole nitrogen source. Because of its requirement for ornithine for growth, three candidates were obtained that harbored derepressed levels of arginine 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 [BJ110]. 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 EcoRI, 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 (2x10^6 to 4x10^6 total cells) or S1 analysis (≈1x10^6 total cells) were prepared from late log cultures (6x10^6 to 8x10^6 cells/ml) of YPD- or YPA-grown cells and from sporulation cultures (5x10^6 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 [Acheson 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 HindIII 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 [Arcangiolli and Lessure 1985] from RSY269 and RSY270 cultures harvested in late logarithmic growth. A 26-bp oligonucleotide GAAATAGGACGGCGACAAAAAGGAATT containing UR51 produced by the Fox Chase Cancer Center DNA synthesis facility was labeled at one end with [γ-32P]ATP using polynucleotide kinase and hybridized to a threelfold molar excess of unlabeled complementary DNA to drive most of the labeled probe into a duplex type. The probe was either used directly or gel purified. Reactions containing 10 μg of crude extract, 2.5 μg of pol[dI-dC] and 1.0 μg of pol[dA-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 UR51pspo13, pMAL and pML-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'-GCTGATTGTACATTAG-3' (Cys-774 > Ser, creates an XhoI site) and 5'-TCTAGATGCGCGCTTAA-3' (lys-778 > Leu, creates an AffII 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 yme6 deletion strain yC105 and into the isogenic wild-type strain RSY10. These strains had been transformed previously with p spo1346, a YCp50-based plasmid containing the 5' spo13--lacZ fusion (Buckingham et al. 1990). Two independent transformants of each strain [with p spo1346 alone, or with p spo1346 and pPL5905 or one of its derivatives] were grown at 30°C to 1x10^7 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].

Acknowledgments
We are grateful to M. Rose and J. Boecke for the gift of a genomic DNA library and plasmids. We thank E. Joris for his skillful technical assistance and J.P. Ten Have for the illustration. This work was supported in part by the Research Council for the Université Libre de Bruxelles to F.M. and E.D., grant CA-06927
from the National Cancer Institute and by an appropriation from the Commonwealth of Pennsylvania to R.S., and grant GM29182 from the National Institutes of Health to R.E.E.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

Arcangioli, B. and B. Lescure. 1985. Identification of proteins involved in the regulation of yeast iso-1-cytochrome C expression by oxygen. EMBO J. 4: 2627–2633.

Atcheson, C.L., B. DiDomenico, S. Frackman, R.E. Esposito, and R.T. Elder. 1987. Isolation, DNA sequence, and regulation of a meiosis-specific eukaryotic recombination gene. Proc. Natl. Acad. Sci. 84: 8035–8039.

Béchet, J., M. Grenson, and J.M. Wiame. 1970. Mutations affecting the repressibility of arginine biosynthetic enzymes in Saccharomyces cerevisiae. Eur. J. Biochem. 12: 31–39.

Boeke, J.D., D.J. Garfinkel, C.A. Styles, and G.R. Fink. 1985. Ty elements transpose through an RNA intermediate. Cell 40: 491–500.

Boonchird, C., P. Messenguy, and E. Dubois. 1991. Characterization of the yeast ARGS6 gene: Determination of the nucleotide sequence, analysis of the control region and of ARGS6 transcript. Mol. Gen. Genet. 226: 154–166.

Bowditch, K. and A.P. Mitchell. 1993. Bipartite structure of an early meiotic upstream activation sequence from Saccharomyces cerevisiae. Mol. Cell. Biol. 13: 2172–2181.

Brill, S.J. and B. Stillman. 1991. Replication factor-A from Saccharomyces cerevisiae is encoded by three essential genes coordinately expressed at S phase. Genes & Dev. 5: 1589–1600.

Buckingham, L.E., H.-T. Wang, R.T. Elder, R.M. McCarroll, M.R. Slater, and R.E. Esposito. 1990. Nucleotide sequence and promoter analysis of SPO13, a meiosis-specific gene of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. 87: 9406–9410.

Cameron, S., L. Levin, M. Zoller, and M. Wigler. 1988. cAMP-dependent control of sporulation, Glycogen metabolism, and heat shock resistance in S. cerevisiae. Cell 53: 555–566.

Covitz, P.A., I. Herskowitz, and A.P.Mitchell. 1989. The yeast RME1 gene encodes a putative zinc finger protein that is directly repressed by al-1a. Genes & Dev. 5: 1982–1989.

Cunin, R., E. Dubois, G. Vanthienen, K. Tinel, A. Jacobs, and M. Crabbeel. 1986. Positive and negative regulation of CAR1 expression in S. cerevisiae. Mol. Gen. Genet. 205: 170–175.

Deschamps, J., E. Dubois, and J.M. Wiame. 1979. L-ornithine transaminase synthesis in S. cerevisiae: Regulation by inducer exclusion. Mol. Gen. Genet. 174: 225–232.

Dubois, E. and F. Messenguy. 1991. In vitro binding studies of the ARGR proteins to the ARGS6 promoter. Mol. Cell. Biol. 11: 2162–2168.

Dubois, E., M. Grenson, and J.M. Wiame. 1974. The participation of the anabolic glutamate dehydrogenase in the nitrogen catabolite repression of arginase in S. cerevisiae. Eur. J. Biochem. 48: 603–616.

Dubois, E., M. Hierneux, M. Grenson, and J.M. Wiame. 1978. Specific induction of catabolism and its relation to repression of biosynthesis in arginine metabolism in S. cerevisiae. J. Mol. Biol. 122: 383–406.

Edelman, A.M. and E.G. Krebs. 1987. Protein serine/threonine kinases. Ann. Rev. Biochem. 56: 567–613.

Elder, R.T., E.Y. Loh, and R.W. Davis. 1983. RNA from yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. Proc. Natl. Acad. Sci. 80: 2432–2436.

Esposito, R.E. and S. Klapholz. 1981. Meiosis and ascospore development (ed. J.N. Strathern, E.W. Jones, and J.R. Broach), pp. 211-287. In The molecular biology of the yeast Saccharomyces: Life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. Science 240: 889–895.

Evans, R.M. and S.M. Hollenberg. 1988. Zinc fingers: Guilt by association. Cell 52: 1–3.

Gardiner, K., W. Laas, and D. Patterson. 1986. Fractionation of large mammalian DNA restriction fragments using vertical pulsed-field gradient gel electrophoresis. Som. Cell Mol. Genet. 12: 185–195.

Gimeno, C.J., P.O. Ljungdahl, C.A. Styles, and G.R. Fink. 1992. Unipolar cell divisions in yeast S. cerevisiae lead to filamentous growth: Regulation by starvation and RAS. Cell 68: 1077–1090.

Hashimoto, H., H. Morikawa, Y. Yamada, and A. Kimura. 1985. A novel method for transformation of intact yeast cells by electroinjection of plasmid DNA. Appl. Microbiol. Biotechnol. 21: 236–244.

Herskowitz, I. 1988. Life style of the budding yeast Saccharomyces cerevisiae. Microbiol. Rev. 52: 536–553.

Heyer, W.D., R.S. Rao, L.F. Erdile, T.J. Kelly, and R.D. Kolodner. 1991. An essential Saccharomyces cerevisiae single-stranded binding protein is homologous to the large subunit of human RP-A. EMBO J. 9: 2321–2329.

Honigberg, S.M., R.M. McCarroll, and R.E. Esposito. 1993. Regulatory mechanisms in meiosis. Curr. Opin. Cell Biol. 5: 219–225.

Hudak, K.A., J.M. Lopes, and S.A. Henry. 1994. A pleotropic phospholipid biosynthetic regulatory mutation in Saccharomyces cerevisiae is allelic to sin3 [sd1, ume4, rpd1]. Genetics 136: 475–483.

Julius, D., L. Blair, A. Brake, G. Sprague, and I. Thorner. 1983. Yeast α factor is processed from a larger precursor polypeptide: The essential role of a membrane-bound dipetidyl aminopeptidase. Cell 32: 838–852.

Kassir, Y., D. Granot, and G. Simchen. 1988. IMEl1: a positive regulator of meiosis in S. cerevisiae. Cell 52: 853–862.

Klapholz, S. and R.E. Esposito. 1980. Recombination and chromosome segregation during the single division meiosis in spo12-1 and spo13-1 diploids. Genetics 96: 567–572.

Kovari, L., R. Sumrada, G. Kovari, and T.G. Cooper. 1990. Multiple positive and negative elements mediate induced arginase [CAR1] gene expression in S. cerevisiae. Mol. Cell. Biol. 10: 5087–5097.

Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Method Enzymol. 154: 367–382.

Luche, R.M., R. Sumrada, and T.G. Cooper. 1990. A cis-acting element present in multiple genes serves as a suppressor protein binding site for the yeast CAR1 gene. Mol. Cell. Biol. 10: 3884–3895.

Luche, R., W.C. Smart, and T.G. Cooper. 1992. Purification of the heteromeric protein binding to the URS1 transcriptional repression site in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. 89: 7412–7416.

Luche, R., W.C. Smart, R.A. Sumrada, and T.G. Cooper. 1993. Saccharomyces cerevisiae BUF protein binds to sequences participating in DNA replication in addition to those mediating transcriptional repression [URS1] and activation. Mol. Cell. Biol. 13: 5749–5761.
MacKay, V.L., S.K. Welch, M.Y. Insley, T.R. Manney, J. Holly, G.C. Saari, and M.L. Parker. 1988. The *Saccharomyces cerevisiae* BAR1 gene encodes an exported protein with homology to pepsin. *Proc. Natl. Acad. Sci.* 85: 55–59.

Madden, S.L., D.M. Cook, J.F. Morris, A. Gasher, V.P. Suchatne, and F.J. Rauscher III. 1991. Transcriptional repression mediated by the WT1 Wilms Tumor gene product. *Science* 253: 1550–1553.

Magasanik, B. 1993. Regulation of nitrogen utilization (ed. E.W. Jones, J.R. Pringle, and J.R. Broach), pp. 283–313. *In The molecular biology of the yeast Saccharomyces: Gene expression*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Malavasic, M.J. and R.T. Elder. 1990. Complementary transcripts from two genes necessary for normal meiosis in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 10: 2809–2819.

Malone, R.E. 1991. Dual regulation of meiosis in yeast. *Cell* 61: 375–378.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Marmorstein, R., M. Carey, M. Ptashne, and S.C. Harrison. 1992. DNA recognition by GAL4: Structure of a protein–DNA complex. *Nature* 356: 408–414.

Matsumoto, K., I. Uno, and T. Ishikawa. 1988. Initiation of meiosis in yeast mutants defective in adenylate cyclase and cAMP dependent protein kinase. *Cell* 32: 417–423.

Matsuura, A., M. Treinin, H. Mitsuzawa, Y. Kassir, I. Uno, and G. Simchen. 1990. The adenylate cyclase/protein kinase cascade regulates entry into meiosis in *Saccharomyces cerevisiae* through the gene *IME1*. *EMBO J.* 9: 3225–3232.

McKnight, S.L. and R. Kingsbury. 1982. Transcription control signals of a eukaryotic protein-coding gene. *Science* 217: 316–324.

Messenguy, F. 1976. Regulation of arginine biosynthesis in *S. cerevisiae*: Isolation of a cis-dominant constitutive mutant for ornithine carbamoyltransferase synthesis. *J. Bacteriol.* 128: 49–55.

Messenguy, F. and E. Dubois. 1983. Participation of transcription and post-transcriptional regulatory mechanisms in the control of arginine metabolism in yeast. *Mol. Gen. Genet.* 189: 148–156.

——. 1993. Genetic evidence for a role of *MCM1* in the regulation of arginine metabolism in *S. cerevisiae*. *Mol. Cell. Biol.* 13: 2586–2592.

Messenguy, F., E. Dubois, and C. Boonchird. 1991. Determination of the DNA binding sequences of ARGR proteins to arginine anabolic and catabolic promoters. *Mol. Cell. Biol.* 11: 2852–2863.

Messenguy, F., M. Penninckx, and J.M. Wiame. 1971. Interaction between arginase and ornithine carbamoyltransferase in *S. cerevisiae*. *Eur. J. Biochem.* 22: 277–286.

Mitchell, A.P. and K.S. Bowdish. 1992. Selection for early meiotic mutants in yeast. *Genetics* 131: 65–72.

Mitchell, A.P. and I. Herskowitz. 1986. Activation of meiosis and sporulation by repression of the RME1 product in yeast. *Nature* 319: 738–742.

Park, H.-D., R.M. Luche, and T.G. Cooper. 1992. The yeast *UME6* gene product is required for transcriptional repression mediated by the *CARI URS1* repressor binding site. *Nucleic Acids Res.* 20: 1909–1915.

Park, H.-O. and E.A. Craig. 1989. Positive and negative regulation of basal expression of a yeast *HSP70* gene. *Mol. Cell. Biol.* 9: 2025–2033.

Pearson, W.R. and D.J. Lipman. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci.* 85: 2444–2448.

Percival-Smith, A. and J. Segall. 1984. Isolation of DNA sequences preferentially expressed during sporulation in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 4: 142–150.

Qui, H.F., E. Dubois, and F. Messenguy. 1991. Dissection of the bifunctional ARGR1 protein involved in the regulation of arginine anabolic and catabolic pathways. *Mol. Cell. Biol.* 11: 2169–2179.

Reece, R.J. and M. Ptashne. 1993. Determinants of binding-site specificity among yeast C6 zinc cluster proteins. *Science* 261: 909–911.

Rigby, P.W.J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling desoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* 113: 237–251.

Rothstein, R. 1991. Targeting, disruption, replacement, and allelic rescue: Integrative DNA transformation in yeast. *Methods Enzymol.* 194: 281–301.

Sanger, R., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* 74: 5463–5467.

Schiestl, R.H. and R.D. Gietz. 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.* 16: 339–346.

Sikorski, R.S. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122: 19–27.

Smith, H.E. and A.P. Mitchell. 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 9: 2142–2152.

Smith, H.E., S.E. Driscoll, R.A.L. Sia, H.E. Yuan, and A.P. Mitchell. 1993. Genetic evidence for transcriptional activation by the yeast *IME1* gene product. *Genetics* 133: 775–784.

Steber, C., R. Surosky, R. Strich, and R.E. Esposito. 1991. Identification of *UME6*, a negative regulator of early meiotic gene expression. "Proceedings from yeast genetics and molecular biology," p. 102. San Francisco, CA. Genetics Society of America.

Stinchcomb, D., C. Mann, and R. Davis. 1982. Centromeric DNA from *Saccharomyces cerevisiae*. *J. Mol. Biol.* 158: 157–179.

Strich, R., M.R. Slater, and R.E. Esposito. 1989a. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. *Proc. Natl. Acad. Sci.* 86: 10081–10022.

Strich, R., M.R. Slater, R.T. Surosky, and R.E. Esposito. 1989b. Regulatory mutants that allow unscheduled expression of two genes that function in separable pathways in meiotic development. "Proceedings from yeast genetics and molecular biology," p. 36. Atlanta, GA. Genetics Society of America.

Su, S.S.Y. and A.P. Mitchell. 1993. Identification of functionally related genes that stimulate early meiotic gene expression in yeast. *Genetics* 133: 67–77.

Sumrada, R.A. and T.G. Cooper. 1985. Point mutation generates constitutive expression of an inducible eukaryotic gene. *Proc. Natl. Acad. Sci.* 82: 643–647.

——. 1987. Ubiquitous upstream repression sequences control activation of the inducible arginine gene in yeast. *Proc. Natl. Acad. Sci.* 84: 3997–4001.

Suroskey, R.T. and R.E. Esposito. 1992. Early meiotic transcripts are highly unstable in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12: 3948–3958.

Suroskey, R.T., R. Strich, and R.E. Esposito. 1994. The yeast *UME5* gene regulates the stability of meiotic mRNAs in...
response to glucose. *Mol. Cell. Biol.* [in press].

Thuriaux, P. 1969. “Existence de gènes régulateurs couplant la répression de la biosynthèse et l’induction du catabolisme de l’arginine dans *S. cerevisiae*.” Ph.D. thesis. Brussels University, Brussels, Belgium.

Toh-e, A. and T. Shimauchi. 1986. Cloning and sequencing of the PH080 gene and CEN15 of *Saccharomyces cerevisiae*. *Yeast* 2: 129–139.

Vallee, B.L., J.E. Coleman, and D.S. Auld. 1991. Zinc fingers, zinc clusters, and zinc twists in DNA-binding protein domains. *Proc. Natl. Acad. Sci.* 88: 999–1003.

Vershon, A.K., N.M. Hollingsworth, and A.D. Johnson. 1992. Meiotic induction of the yeast HOPI gene is controlled by positive and negative regulatory sites. *Mol. Cell. Biol.* 12: 3706–3714.

Vidal, M., R. Strich, R.E. Esposito, and R.F. Gaber. 1991. RPD1 [SIN3/UME4] is required for maximal activation and repression of diverse yeast genes. *Mol. Cell. Biol.* 11: 6306–6316.

Wagstaff, J.E., S. Klapholz, and R.E. Esposito. 1982. Meiosis in haploid yeast. *Proc. Natl. Acad. Sci.* 79: 2986–2990.

Wang, H.-T., S. Frackman, J. Kowalisyn, R.E. Esposito, and R. Elder. 1987. Developmental regulation of SPO13: A gene required for separation of homologous chromosomes at meiosis I. *Mol. Cell. Biol.* 7: 1425–1435.

Yannisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 33: 175–185.
UME6 is a key regulator of nitrogen repression and meiotic development.

R Strich, R T Surosky, C Steber, et al.

*Genes Dev.* 1994, 8:
Access the most recent version at doi:10.1101/gad.8.7.796