The Ume6 regulon coordinates metabolic and meiotic gene expression in yeast

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The Ume6 transcription factor in yeast is known to both repress and activate expression of diverse genes during growth and meiotic development. To obtain a more complete profile of the functions regulated by this protein, microarray analysis was used to examine transcription in wild-type and *ume6*Δ diploids during vegetative growth in glucose and acetate. Two different genetic backgrounds (W303 and SK1) were examined to identify a core set of strain-independent Ume6-regulated genes. Among genes whose expression is controlled by Ume6 in both backgrounds, 82 contain homologies to the Ume6-binding site (URS1) and are expected to be directly regulated by Ume6. The vast majority of those whose functions are known participate in carbon/nitrogen metabolism and/or meiosis. Approximately half of the Ume6 direct targets are induced during meiosis, with most falling into the early meiotic expression class (cluster 4), and a smaller subset in the middle and later classes (clusters 5–7). Based on these data, we propose that Ume6 serves a unique role in diploid cells, coupling metabolic responses to nutritional cues with the initiation and progression of meiosis. Finally, expression patterns in the two genetic backgrounds suggest that SK1 is better adapted to respiration and W303 to fermentation, which may in part account for the more efficient and synchronous sporulation of SK1.

Exposure of cells to changing nutritional conditions provides important cues that stimulate dramatic alterations in transcription, metabolism and cell division patterns. The *UME6* gene in *Saccharomyces cerevisiae* is a major regulator mediating some of these responses. *UME6* encodes a DNA-binding protein (1, 2) that associates with the upstream regulatory sequence $URS1_A$ (consensus 5′-TCGGCGGCT-3′) to regulate transcription of genes responding to metabolites such as glucose, nitrogen and inositol (3–8) and, in some cases, with a noncanonical URS1 ($URS1_B$; 5′-SGWGGMRRNANW-3′) to regulate genes involved in DNA repair (9). Although Ume6 is dispensable for growth, it controls efficient mating of haploids and is essential for the initiation and progression of meiosis (2, 10). Meiosis in yeast occurs when a/α diploid cells are starved for both nitrogen and a fermentable carbon source (e.g., glucose), culminating in the formation of four haploid spores (reviewed in ref. 11). Classic transcription studies (11) and more recent whole-genome analysis (12, 13) demonstrate that this process is accompanied by the ordered transcription of genes in multiple expression classes. Ume6/$URS1_A$ has been shown to be a central component of a key regulatory switch controlling both repression and activation of genes expressed early in the meiotic transcriptional program. In a *ume6*Δ mutant, these genes are derepressed during vegetative growth (exhibiting *u*nscheduled *m*eiotic *e*xpression; ref. 2). They also fail to be induced to full levels in meiosis, resulting in an inability to sporulate (10, 14).

Regulation of repression and activation by Ume6 occurs through physical association with other proteins that modulate its function. Repression involves recruitment of two independent corepressor complexes. One contains Sin3 and the histone deacetylase Rpd3 (15, 16), and the other contains the Isw2 chromatin-remodeling factor (17). Ume6 was, until recently, the only known DNA-binding partner for Sin3 in yeast (11, 18), and has provided a useful model for understanding regulation of transcription by histone deacetylation. It functions analogously to a variety of transcription factors in higher eukaryotes that interact with Sin3 homologs (e.g., nuclear hormone receptors, Mad-Max, etc.; refs. 19 and 20). The conversion of Ume6 from a repressor to an activator has been most extensively studied during meiosis. During this process, activation results from interaction of Ume6 with Ime1 (inducer of meiosis), which is up-regulated early in meiotic development (21, 22). Ime1 functions both to eliminate Ume6-dependent Sin3-mediated repression and to provide an activation domain for induction of the early genes (19, 23, 24). The Ume6/Ime1 complex also indirectly controls the transition from early to middle gene expression through its participation in the induction of Ndt80, a transcription factor that activates middle genes once recombination is completed (monitored by the pachytene checkpoint system; refs. 25–28). The observation from recent microarray analysis that URS1 occurs in the promoters of some of these genes (12, 13) suggests that Ume6 may also more directly regulate a subset of middle/late expression.

The following whole-genome analysis was done to gain a more complete picture of the diverse and complex genetic pathways coordinately regulated by Ume6. This study used high-density oligonucleotide arrays to define a minimal core set of genes directly regulated by Ume6/URS1 in diploid cells grown in glucose or acetate medium. The complete data sets can be found at our web sites (United States, http://re-esposito.bsd.uchicago.edu; Europe, www.bioz.unibas.ch/primig/ume6).

Materials and Methods

Strains, Media, and Culture Conditions. The *UME6*$^+$ diploids (SK1: *MAT***a**/MATα *arg4-Nsp/arg4-Bgl his4x*::*LEU2-URA3/his4B*::*LEU2 ho*::*LYS2/ho*::*LYS2 leu2*::*hisG/leu2*::*hisG lys2/lys2 UME6/UME6 ura3/ura3* and W303: *MAT***a**/*MATα ade2/ade2 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 UME6/UME6 ura3-1/ura3-1*) were described in ref. 13. SK1 *ume6*Δ (*MAT***a**/*MATα gal80*::*LEU2/gal80*::*LEU2 ho*::*LYS2/ho*::*LYS2 leu2/leu2 lys2/lys2 trp1/trp1 ume6*Δ::*TRP1/ume6*Δ::*TRP1 ura3/ura3*) was provided by A. Mitchell and Y. Xiao. W303 *ume6*Δ (*MAT***a**/*MATα ade2/ade2 ade6/ADE6 can1-100/can1ADE2*:*CAN1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1*

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ume6D1/ume6D1 ura3-1/ura3-1) is a cross of YC105 and YC121 (19).

SK1 and W303 *UME6* and *ume6*Δ diploids were inoculated into yeast extract/peptone/dextrose (YPD) and yeast extract/peptone/acetate (YPA) (described in ref. 13) at 1×10^5 cells per ml and grown to 5×10^7 cells per ml (late log phase). Aliquots (400 ml) from each culture were collected by centrifugation and stored as pellets at −80°C. *UME6*$^+$ W303 YPA data are from a prior study (13); all other samples are from the current study.

Expression Data Analysis. RNA was prepared and hybridized to Affymetrix Ye6100 GeneChip arrays as described (13). Fluorescence intensities of hybridized arrays were measured with a Hewlett–Packard GeneArray Scanner. Primary data were collected by using GENECHIP 3.0 software, and the relative abundance of various mRNAs determined from the average difference for each gene (the mean of the differences between the hybridization intensity for the "Perfect Match" and "Mismatch" features), normalized for variation in hybridization intensity of individual arrays (by dividing each gene's average difference by the mean of all average difference values for the specific array). Normalized average differences at or below zero were adjusted to a small positive number (reflecting the average background value for that chip) to facilitate subsequent calculations. Differential fluorescence intensity signals in *UME6* and *ume6*Δ were statistically analyzed by using MATLAB 5.0 (MathWorks, Natick, MA) and GENESPRING 4.1 (Silicon Genetics, Redwood City, CA) to identify genes exhibiting a minimum 5-fold difference, a criterion based on the expression of genes known to be deregulated in *ume6*Δ (*ACS1*, *CAR1*, *CAR2*, *HOP1*, *IME2*, *INO1*, *REC104*, *SPO1*, *SPO11*, *SPO13*, *SPO16*). Genes with maximum normalized expression levels below 70 were excluded as being less reliable (too close to background levels).

A new software package (M.B., R. Koch, and M.P., unpublished data) was also used to identify statistically significant shifts in mRNA levels irrespective of the minimum 5-fold cutoff. Briefly, genes were ranked by normalized average difference, with all negative values brought to 1 (the lowest rank). Rank shift values for each gene were assigned by computing the rank differences in pair-wise combinations from each hybridization experiment, and normalized to compensate for rank shift dependence on signal strength (high in the weak signal range and low in the strong signal range). Background noise (random variation in rank shift) was empirically estimated by using independent samples of wild-type (*UME6*) diploids from our prior study of meiotic transcription (13). Genes were considered significantly deregulated in *ume6*Δ in either both strains and both media (strain and media-independent), or in both strains in at least one medium (strain-independent but not necessarily media-independent) (*i*) in the first case if the rank shifts between wild type and *ume6*Δ for each case had a predicted probability of $P < 0.25$ (resulting in a combined $P < 0.004$ for both strains and both media) and (*ii*) in the second case if the combined P value (derived by multiplying the rank-shift P values in each medium for a given strain) were below a threshold "false discovery rate" (29) of 10%. Finally, the media-dependent and media-independent lists described above were combined to obtain a more comprehensive set of Ume6-regulated genes.

Search for Regulatory DNA Motifs. Regulatory motifs were detected using the "Find-Regulatory-Sequences" analysis subroutine in GENESPRING 2.3.4, which iteratively scans a region of specified length (in this case, 4–10 nucleotides) upstream of each ORF.

Results and Discussion

Genes regulated by *UME6* were identified by comparing microarray expression profiles from wild-type and *ume6*Δ strains. A set of marker genes known to be deregulated in *ume6*Δ was used to establish initial criteria for inclusion. Based on the behavior of these markers, loci that showed at least a 5-fold increase or decrease in expression in the mutant were considered to be Ume6-regulated. A statistical approach was also used to compare multiple data sets from different strains and media (see *Materials and Methods*). Ume6-regulated genes were expected to fall into two major classes: (*i*) direct target genes, whose promoters are bound by Ume6, and (*ii*) indirect targets showing altered expression as a result of downstream effects caused by loss of Ume6. To identify the subset of genes that are most likely direct targets of Ume6, the promoters of differentially expressed genes (−600 to +200 with respect to the ATG start codon) were screened for the presence of the core hexamer $URS1_A$ sequence (5′-GGCGGC-3′ or its reverse complement) bound by Ume6 *in vivo* and *in vitro* (2, 17).

Two genetically distinct strain backgrounds (W303 and SK1) and two growth media containing either fermentable or nonfermentable carbon sources (glucose or acetate) were examined. W303 is commonly used for genetic and biochemical analysis in yeast, whereas SK1 is often used in meiotic studies because of its highly efficient and synchronous sporulation. These diploids permitted identification of a core set of strain-independent Ume6 targets and direct comparison to a core set of genes previously found to be induced in meiosis in these same strains (13). The use of both glucose (typically used for growth) and acetate (generally used to culture cells before sporulation) facilitated identification of Ume6-regulated genes that are glucose-repressed, and allowed examination of Ume6-dependent transcription associated with respiring cells. Analysis of the two strains in these growth media also further defined transcriptional differences that potentially contribute to their distinct phenotypes during growth and sporulation.

Ume6-Regulated Genes in SK1 and W303. A comparison of SK1 wild-type and *ume6*Δ strains grown in glucose revealed 152 genes differentially expressed at least 5-fold in the mutant (Fig. 1). This set is highly enriched for genes that contain the URS1 hexamer (41% vs. 13% with a URS1 hexamer in the genome as a whole). These URS1-containing genes are expected to be directly regulated by Ume6 binding to their promoters, and include most genes previously shown to be under Ume6 control (e.g., *HOP1*, *IME2*, *INO1*, *REC104*, *SPO1*, *SPO11*, *SPO13*, *SPO16*). For simplicity, all Ume6-regulated genes containing the URS1

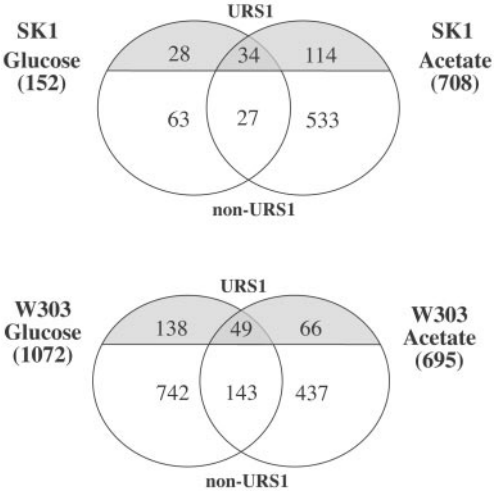

Fig. 1. Ume6-regulated genes in SK1. Venn diagram showing the number of genes deregulated during growth of SK1 *ume6*Δ and W303 *ume6*Δ in glucose and acetate media. Shaded regions indicate genes containing promoter sequences corresponding to the URS1 hexamer.

hexamer will be referred to as "direct targets," and genes lacking this sequence will be referred to as "indirect targets." It should be noted that although genes containing the $URS1_B$ sequence (5′-SGWGGMRRNANW-3′) may be classified as "indirect" by this approach, none were detected in our study (see below). The following analysis will focus primarily on the direct targets.

Detection of differentially expressed genes in SK1 *ume6*Δ differs significantly when cells are assayed in glucose vs. acetate medium (62 direct targets in glucose vs. 148 in acetate; Fig. 1). The smaller number of deregulated genes seen in SK1 glucose may reflect decreased expression of loci that are also subject to glucose repression (and/or acetate activation). This explanation is consistent with results from W303 (initially selected for high cytochrome *c* expression), which is known to exhibit less stringent glucose repression than other commonly used strains (30). In W303, the numbers of direct targets deregulated in glucose (187 genes) and acetate (115 genes) are both more similar to SK1 acetate.

A subset of the Ume6 direct targets in each strain is deregulated in both media (34 genes in SK1 and 49 genes in W303; Fig. 1). These represent a unique class that responds to loss of Ume6 independently of any effects of carbon source on metabolism and transcription (e.g., respiration, glucose repression, etc.). Among the genes lacking the URS1 hexamer, a much smaller proportion are media-independent, suggesting that most of the indirect effects are related to growth on specific carbon sources (see web sites for lists of indirectly regulated genes).

Defining a Core Set of Strain-Independent Ume6-Regulated Genes. The results from the two strain backgrounds were examined further to derive a core set of genes regulated in common in both W303 and SK1. The requirement for deregulation in both backgrounds due to loss of *UME6* eliminates genes whose deregulation depends on strain-specific differences (e.g., known genetic markers and numerous strain-specific polymorphisms; ref. 13). It also excludes genes whose expression changes as a result of marker differences between the wild-type and *ume6*Δ derivative (SK1 wild-type and *ume6*Δ strains differ at *arg4*, *his4*, *trp1*, and *gal80*, whereas the W303 wild-type and *ume6*Δ strains differ at *ade2* and *can1*; see *Materials and Methods*). Finally, to exclude strain-specific responses to carbon source (e.g., glucose repression), we included genes that were deregulated in either glucose or acetate, or both media.

The resulting core set (Fig. 2*A*) contains 74 genes that have the URS1 hexamer and are therefore expected to be directly regulated by Ume6. The levels of deregulation of this subclass are shown in Fig. 2*B* (the 143 indirect targets in the core can be found at our web sites). Several genes for which Ume6 is known to play an important regulatory role (e.g., *ACS1* and *CAR1*) are not present in the core because they were not sufficiently deregulated in both strains to meet the initial criteria for inclusion. The resulting core therefore represents those genes whose expression is most clearly dependent on Ume6, independent of strain background. Among these, 20 genes are media- as well as strain-independent (deregulated in both glucose and acetate in each strain). This smaller subset is expected to exclude many genes that are regulated by both carbon source and Ume6.

In addition to applying the empirically derived 5-fold criteria described above, as noted earlier, we also used a more sophisticated statistical analysis. This method employed a new software package designed to detect significant shifts in expression patterns irrespective of simple fold-deregulation criteria. The strength of the algorithm is the identification of genes that display a weak but reproducible change in their signal intensities (see *Materials and Methods*). It applies more stringent criteria for inclusion of low expression genes (which may be subject to more variation in signal fluctuation and lead to potential false positives; ref. 31). It also allows more sensitive detection of deregulated genes with high expression levels, which may be undetected by the 5-fold criteria. This statistical approach independently defined a more restricted core set of 45 genes. As expected, the vast majority of these (37 of 45) are present among

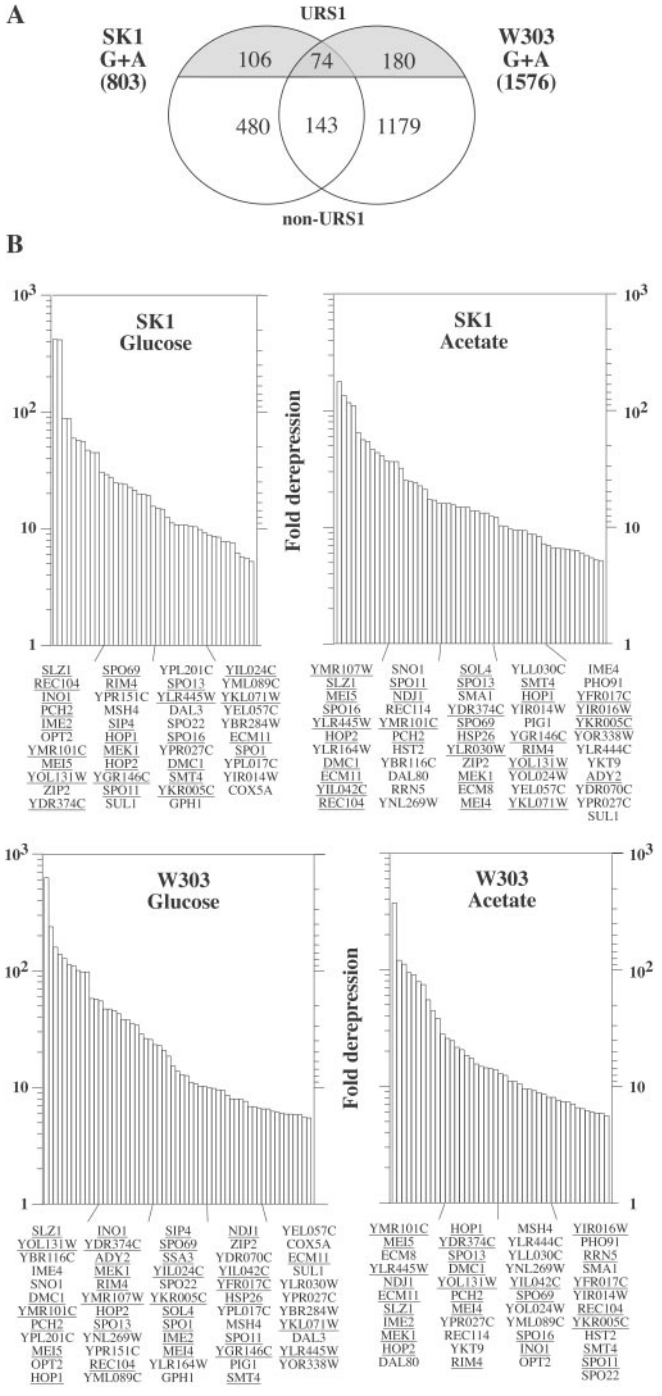

Fig. 2. Strain-independent Ume6-regulated genes. (*A*) Venn diagram showing the genes deregulated in each strain during growth in glucose and/or acetate by the 5-fold criteria. This "core" set of Ume6-regulated genes consists of 74 "direct" (URS1-containing) and 143 "indirect" targets deregulated in common in both strains. (*B*) Histograms indicating the levels of derepression of the core Ume6 direct target genes in glucose and acetate (fluorescence intensities for *ume6*Δ/*UME6*). Only one gene, *REV1*, exhibited increased expression in the *ume6* mutant (25-fold in SK1 glucose and 14-fold in W303 glucose; not shown), suggesting it is likely activated rather than repressed by Ume6 during vegetative growth.

the 74 loci found to be deregulated by the 5-fold criteria (Fig. 2*B*). Significantly, the remaining set of core genes detected by the 5-fold criteria alone contains a number of known early meiotic genes (e.g., *MSH4*, *REC114*, *SPO22*, and *ZIP2*). These are unlikely to be false positives, because all early $URS1_A$ genes studied thus far are regulated by the Ume6-Ime1 activator complex. The statistical approach above identified two additional known Ume6 targets (*ACS1* and *CAR1*), as well as six other genes (*AUT7*, *MPC54*, *MTC2*, *PRE10*, *RNP1*, and *YLR414C*) not detected by the 5-fold criteria. Given that both approaches each detected additional expected Ume6 targets, the data from the two methods was combined in the discussion below to generate a more inclusive core list of 82 likely targets (with the 37 genes identified in common by the two methods representing the most conservative estimate) in the Ume6 regulon.

Functional Classification of Ume6 Direct Targets. Among the 82 core Ume6-regulated genes defined above, 52 encode proteins with known or proposed functions (32), and fall into several classes (Fig. 3*A*). Strikingly, half of them (26 of 52 genes) play important roles in meiosis. Most of these act during chromosome pairing, recombination or cohesion (*ADY2*, *DMC1*, *HOP1*, *HOP2*, *MEI4*, *MEI5*, *MEK1*, *MSH4*, *NDJ1*, *REC104*, *REC114*, *SPO11*, *SPO13*, *SPO69*, *ZIP2*) or at other early steps in the process (*IME2*, *IME4*, *PCH2*, *RIM4*, *SPO1*, *SPO22*). Two (*MPC54*, *SMA1*) act late, during prospore development. The stages in meiosis at which the remaining ones act are not yet known (*AUT7*, *SPO16*, *ULP2*). A second large class of Ume6/URS1-regulated genes participates in metabolic pathways. These 15 genes encode proteins involved in metabolism of carbohydrates (*GPH1*, *PIG1*, *SOL4*), nitrogen (*CAR1*, *DAL3*, *DAL80*), oxidizable carbon sources (*ACS1*, *COX5A*), or inositol (*INO1*). Others in this group are involved in nutrient transport (*OPT2*, *PHO91*, *SUL1*), or encode undefined putative metabolic functions (*SNO1*, *YIL042C*, *YKL071W*). A third group encodes transcription regulators (*HST2*, *RRN5*, *SIP4*). Significantly, altered expression of this latter class may be one mechanism by which loss of Ume6 causes many of the "indirect" effects on transcription of non-URS1 genes discussed earlier. Finally, the few other known genes outside these categories include three cell wall maintenance genes (*YMR101C*, *ECM8*, *ECM11*), two heat shock genes (*HSP26*, *SSA3*), a proteasome subunit (*PRE10*), a ribonucleoprotein (*RNP1*), and a DNA repair gene (*REV1*). *REV1* is the only gene in the core set that exhibits reduced rather than increased expression in a *ume6*Δ mutant, and may represent a class of loci for which Ume6 functions as a transcriptional activator during log-phase growth. The remaining 30 Ume6 core target genes encode proteins of unknown functions (*MTC2*, *SLZ1*, *YKT9*, *YBR116C*, *YBR284W*, *YDR070C*, *YDR374C*, *YEL057C*, *YFR017C*, *YGR146C*, *YIL024C*, *YIR014W*, *YIR016W*, *YKR005C*, *YLL030C*, *YLR030W*, *YLR164W*, *YLR414C*, *YLR444C*, *YLR445W*, *YML089C*, *YMR107W*, *YNL269W*, *YOL024W*, *YOL131W*, *YOR338W*, *YPL017C*, *YPL201C*, *YPR027C*, *YPR151C*). Many of these might be expected to act in meiosis or metabolism, based on the identity of the known genes studied above. Indeed, two of these (*YDR374C* and *SLZ1*) have recently been shown in genome-wide deletion analysis to be required for efficient sporulation (A. Deutschbauer and R.W.D., unpublished data).

In addition to the 82 core genes detected in diploids grown in either glucose or acetate, two recent independent microarray studies also identified a number of Ume6 targets, using haploid glucose-grown cells lacking components of histone deacetylase or chromatin remodeling complexes. These identified 62% (deregulated >2-fold; ref. 33) and 41% (deregulated >3-fold; ref. 34) of the genes in our core. Some of the 28 genes found only in our core may be specific to diploids and/or growth in acetate (i.e., glucose-repressed and/or acetate-activated). Interestingly, at least two of these genes (*IME4*, *SMA1*) are known to be important for meiosis.

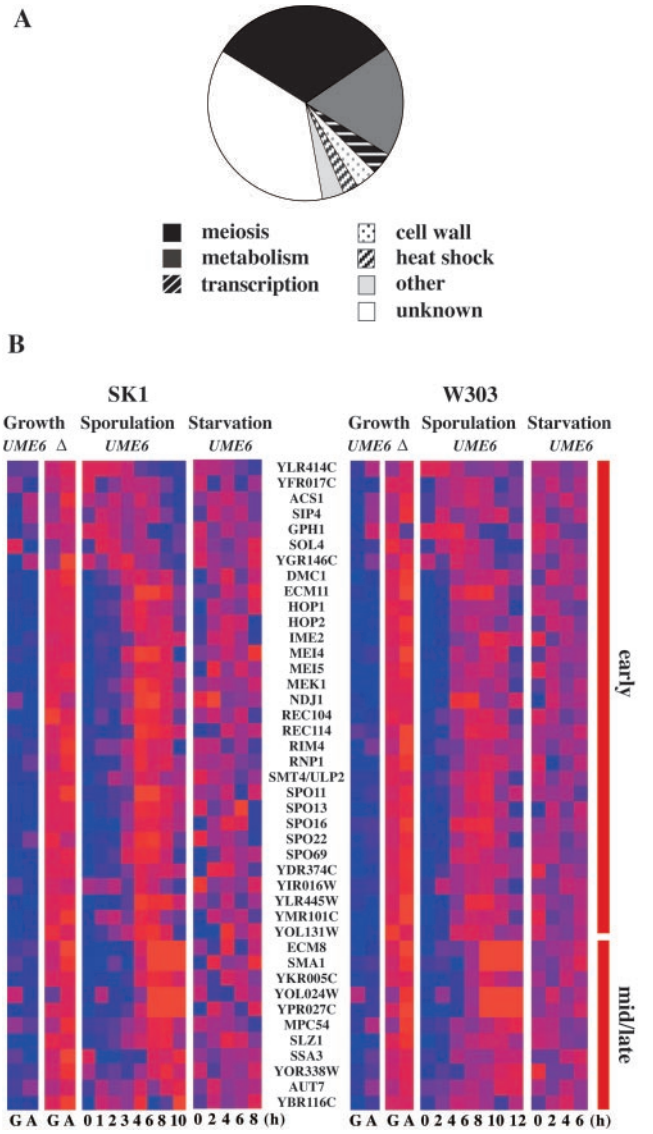

Fig. 3. Function and expression of Ume6 targets. (*A*) Functional classifications of the 82 direct Ume6 targets in the core. The indirect core of 143 genes (from the 5-fold criteria) with known functions fall into the following Munich Information Center for Protein Sequences (MIPS) functional classes: energy (19%), metabolism (16%), cell growth/division/DNA synthesis (12%), intracellular transport (9.5%), cell rescue/defense/aging (8.1%), transcription (8.1%), cellular biogenesis (6.8%), protein destination (6.8%), protein synthesis (6.8%), transport facilitation (4%), ionic homeostasis (2.7%) (40). (*B*) Expression of the 42 meiotically induced core Ume6-regulated genes during growth and meiosis. Red and blue indicate high and low expression levels, respectively. For each strain, columns represent vegetative growth of *UME6* and *ume6*Δ in acetate and glucose (*Left*) or timepoints during meiosis (*Right*; ref. 13). The meiotic expression classes of the genes are indicated on the far right. Note that Ime2 exhibits a biphasic induction and therefore has been categorized as both an early and middle gene (13).

Ume6 May Play a Broader Role in Meiosis and Spore Formation than Previously Thought. Prior studies in our lab and others showed that Ume6 is a major regulator of early meiotic expression (2, 10, 22, 35). Indeed, the present analysis shows that a significant fraction of Ume6 activity is devoted to meiotic functions, because more than half of the known direct Ume6 targets play important roles

%	C	T	**T**	**C**	**G**	**G**	**C**	**G**	**G**	**C**	**T**	A	A
A:	18	26	18	9	0	0	0	0	0	0	24	68	47
T:	23	39	63	18	0	0	0	0	0	0	58	17	31
G:	23	19	10	24	*	*	0	*	*	0	11	9	12
C:	35	16	8	48	0	0	*	0	0	*	6	5	10

Fig. 4. URS1 consensus sequence derived from alignment of GGCGGC hexamer sequences in the promoters (+200 to −600 upstream) of the 82 direct core genes. The previously described consensus (3) is in bold, and has been extended to include additional flanking nucleotides present at a frequency (indicated below the consensus) of at least 50% higher than the average frequency for that nucleotide in the genome (≈20% for G and C, ≈30% for A and T). The most common nucleotide in each position is shaded.

in meiosis. To more broadly examine the overlap between Ume6 targets and meiotically regulated genes, we compared the entire core of direct Ume6 targets to the meiotic transcriptome. Our previous study of genome-wide transcription during meiosis identified a set of ≈900 core genes meiotically regulated in SK1 and W303 (13). A substantial fraction of the core Ume6 transcriptome (42 of 82) is also present among these, supporting the view that one of the primary functions of Ume6 is the regulation of meiotic gene expression (Fig. 3*B*). As expected, most of them (31 of 42) are induced early in the process as part of expression clusters 1–4. However, several Ume6-regulated genes (mostly of unknown function) appear to be induced later in meiosis (in expression clusters 5–7; *AUT7*, *ECM8*, *MPC54*, *SMA1*, *SLZ1*, *SSA3*, *YBR116C*, *YKR005C*, *YOL024W*, *YOR338W*, *YPR027C*). This is the first indication that Ume6 may be required for some middle and late gene regulation (prior analyses of the URS1-containing middle genes *SMK1* and *NDT80* showed that the absence of Ume6 has little effect on their vegetative expression; refs. 27 and 36). Although the present study shows that Ume6 represses a subset of middle genes during growth, it remains to be determined whether it participates in their activation in meiosis. Nevertheless, the identification of meiotic genes repressed by Ume6 beyond the early classes suggests it plays a broader role in meiotic regulation than previously thought.

Promoter Analysis of Ume6-Regulated Genes. To identify elements that control expression of Ume6-regulated genes, the promoters (+200 to −600 bp upstream of the ATG) of the entire core sets of 82 direct and 143 indirect targets were scanned for over-represented sequences ranging from 4–10 bp in length. As expected, the consensus sequence for URS1 found in the direct targets matches the previously published sequence (5′-TCGGCGGCT-3′) derived from independent studies of common laboratory strains (3). The present survey of 82 Ume6-regulated genes indicates additional flanking nucleotides are also significantly conserved (Fig. 4). It is important to emphasize that this analysis is based on the published sequence of *Saccharomyces cerevisiae*, derived from strain S288C (32). Although S288C is more closely related to W303 than SK1 (13), the actual nucleotide frequencies in the two strains used in our studies may thus differ from that shown in Fig. 4. These and other promoter polymorphisms may in some cases result in strain-specific differences in the regulation of genes controlled by Ume6.

The $URS1_B$ sequence (5′-SGWGGMRRNANW-3′) previously linked to Ume6 regulation of DNA repair genes (9) was not detected in either the direct or indirect sets, suggesting it may be less common among Ume6-regulated genes, not active under the conditions tested (log phase growth), or too degenerate for recognition by the statistical techniques used. This analysis also failed to detect over-representation of sequences corresponding to UAS_H or T_4C (in either the direct or indirect sets), two other elements found in some Ume6-regulated early meiotic genes (11). Intriguingly, one new sequence associated with URS1 at higher than random frequencies was identified. This sequence, TTCGTTAW, was enriched 2.6-fold among direct Ume6 targets in the strain-independent core, and 3.3-fold among direct targets in SK1. However, deletion of this sequence in four of these genes (*COS7*, *GAL1*, *SNO1*, *SPO16*) had no effect on their expression in wild-type or *ume6*Δ strains during growth or meiosis (data not shown). Therefore the role, if any, of this sequence in transcriptional regulation remains unclear.

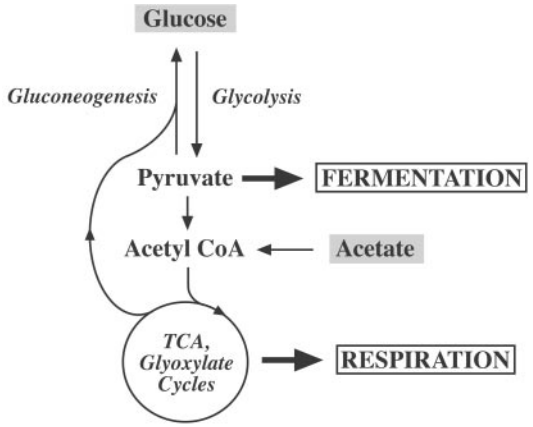

Fig. 5. Schematic of acetate and glucose utilization pathways in yeast. Glucose is metabolized by fermentation or respiration [via tricarboxylic acid (TCA) cycle]. Acetate is converted to acetyl-CoA and metabolized via the TCA cycle (respiration) and/or the glyoxylate cycle (which provides substrates for gluconeogenesis) (41, 42).

Transcription Profiles Suggest That SK1 Is Better Adapted to Respiration and W303 Is Better Adapted to Fermentation. In addition to providing a genome-wide view of the Ume6 regulon, this study provides potential insights into the metabolic states of SK1 and W303 that may affect their growth and sporulation properties. In particular, several lines of evidence support the view that SK1 is better adapted to a respiratory lifestyle than W303. First, in comparison to W303 in glucose, SK1 has elevated expression of genes involved in the tricarboxylic acid (TCA) cycle and respiration. These include genes encoding subunits of succinate dehydrogenase (*SDH1* and *SDH2*, required for electron transfer to ubiquinone in the respiratory chain), components of the respiratory apparatus (*BIO2*, *COX4*, *COX8*, *CYB2*, *CYC1*, and *NDI1)*, and proteins with well-characterized roles in ubiquinone metabolism (*QCR6*, *QCR9*, *QCR10*, and *RIP1*). Mutations in many of these genes profoundly effect growth on oxidizable carbon sources, including acetate, glycerol and ethanol (37). Second, in contrast to SK1, W303 in acetate exhibits lower expression of many genes important for respiration. For example, several mitochondrial genes exhibiting very large acetate-dependent induction in SK1 fail to be induced in W303, including *PET123*, *ISM1*, *MSY1*, *MRP7*, *COX10*, and *MSM1*. Conversely, W303 has elevated expression of genes required for gluconeogenesis (e.g., glucose synthesis from nonfermentable carbon sources such as acetate; see Fig. 5). This pathway is effectively repressed in both strains growing in glucose, but is induced by W303 in acetate to levels that are 3- to 4-fold higher than in SK1. Significantly, the gene encoding the Cat8 transcription factor (required for derepression of gluconeogenic genes) is induced significantly higher in W303 than SK1, as are the gluconeogenic genes *NTH2*, *ATH1*, *GLC3*, *GLG2*, *GSY1*, *GCG1*, *TPS1*, and *TPS2*. These strain-specific differences in gene expression may result in lowered respiratory capacity and/or increased gluconeogenesis in W303. Because sporulation is generally dependent on respiration and inhibited by glucose (11, 38, 39), these properties

could account for W303's less efficient and less synchronous sporulation.

Several additional differences in transcription between the strains may explain other aspects of their growth. For example, many protein synthesis genes are specifically repressed in the W303 *ume6*Δ mutant growing in acetate, in agreement with the observed slow growth rate and accumulation of enlarged unbudded cells (compared with SK1 *ume6*Δ) in this medium. Also, the *MUC1* flocculin is significantly more highly expressed in SK1 than W303, which may account for the unusually high degree of flocculation observed in SK1 (limiting its use in growth studies). The complete list of genes exhibiting at least a 5-fold difference in expression between wild-type SK1 and W303 in glucose (120 genes) and acetate (480 genes) can be found at our web sites.

Summary and Conclusions

The present study identifies a set of genes that exhibit strain-independent regulation by Ume6 in diploid budding yeast. Among these, 82 contain homologies to the Ume6-binding site and are expected to be directly regulated by Ume6. The identities of known genes in this set indicate that in diploid cells Ume6 functions primarily as a regulator of meiosis and nitrogen/carbon metabolism. It ensures that cells respond appropriately to their nutritional environment to turn catabolic/anabolic genes on/off and turn on the genes required for initiation and progression of meiosis. How is repression by Ume6 relieved at the different promoters? For the early meiotic genes, Ime1 converts Ume6 into an activator, whereas for some nitrogen-regulated genes Ume6 interacts with the ArgR-Mcm1 activator complex. It remains to be determined whether additional activators bind Ume6 to regulate genes responding to different metabolites, or meiotic genes induced after the early class (in mid/late meiosis). Moreover, it is also not yet clear how binding of these various factors to Ume6 is coordinated to achieve specificity at different promoters. This study provides a minimal set of Ume6-regulated genes to investigate these questions. In addition, a comparison of transcription in SK1 vs. W303 revealed significant differences in expression of genes required for respiration and gluconeogenesis, which may in part account for their different growth and sporulation phenotypes. Finally, in addition to the many direct targets described above, the large number of indirect targets detected in both strains underscores the downstream effects and far-reaching consequences of Ume6 regulation in the cell.

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