Large-Scale Functional Genomic Analysis of Sporulation and Meiosis in *Saccharomyces cerevisiae*

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ABSTRACT

We have used a single-gene deletion mutant bank to identify the genes required for meiosis and sporulation among 4323 nonessential *Saccharomyces cerevisiae* annotated open reading frames (ORFs). Three hundred thirty-four sporulation-essential genes were identified, including 78 novel ORFs and 115 known genes without previously described sporulation defects in the comprehensive Saccharomyces Genome (SGD) or Yeast Proteome (YPD) phenotype databases. We have further divided the uncharacterized sporulation-essential genes into early, middle, and late stages of meiosis according to their requirement for *IME1* induction and nuclear division. We believe this represents a nearly complete identification of the genes uniquely required for this complex cellular pathway. The set of genes identified in this phenotypic screen shows only limited overlap with those identified by expression-based studies.

DURING meiosis cells exit the vegetative cell cycle and enter a linear divisional and differentiation pathway. The regulation of this process is probably best described in the budding yeast *Saccharomyces cerevisiae* (Pringle and Hartwell 1981). The decision to begin meiosis in this organism is controlled by two known major inductional mechanisms (Malone 1990). One is tuned to nutritional starvation, and the other is tuned to the presence of the a1/α2 heterodimer, found only in diploids formed by the joining of haploids of opposite mating type. Initiation of meiosis requires expression of many specialized genes. Transcriptional induction of these genes is controlled in large part by the early meiotic-specific transcription factor *IME1* (Kassir *et al.* 1988). Ime1p is required for the induction of most early meiotic genes except *IME4* (Shah and Clancy 1992) and is essential for all of the major downstream events of meiosis and sporulation.

Meiosis begins with a prolonged S phase during which a single replication of the genome occurs (Pringle and Hartwell 1981). S phase is followed by a very specialized meiotic prophase when homologous chromosomes pair and exchange DNA through recombination. Following recombination, meiotic cells undergo two rounds of division without further DNA replication, leading to the formation of four haploid nuclei. In *S. cerevisiae*, each of the nuclei is encapsulated in a hardened cell wall formed by growth of a double-membrane prospore wall adjacent to the outer plaque of the meiosis II spindle pole bodies (Moens and Rapport 1971). This capsule then differentiates into a multilayered spore wall by deposition of materials between the double layers of the prospore membrane (Lynn and Magee 1970). With normal division, four viable ascospores, each containing one haploid nucleus, become enclosed within the residual cell wall.

Several major genetic screens have been performed to identify the various contributors to the meiotic pathway in *S. cerevisiae*. These include screens for reduced sporulation (Esposito and Esposito 1969), for resistance to chemical agents (Dawes and Hardie 1974), for reduced spore viability (Rockmill and Roeder 1988), and for variations in spore wall components (Briza *et al.* 1990), as well as recent genomic-based screens (Rabitsch *et al.* 2001; Briza *et al.* 2002). The study of the genes identified in these screens has defined the pathway for meiotic activation and regulation in more detail in *S. cerevisiae* than in any other organism. More than 150 genes that have known roles in meiosis and sporulation have been identified by these traditional genetic screens (Kupiec *et al.* 1997). However, as shown here, many meiosis/sporulation genes remain to be identified and characterized.

MATERIALS AND METHODS

Strains and medium: All experiments were performed in the S288C background. Established protocols for media synthesis were used (Guthrie and Fink 1991). To test for growth on glycerol medium, the deletion mutants were resuspended from a nutrient-rich YEPD plate into water and transferred dropwise without dilution to a plate containing 2.5% glycerol as the primary carbon source.

Handling the deletion mutants and sporulation test: The mutant strains were received from Research Genetics (Birmingham, AL) in 96-well microtiter dishes on dry ice. The frozen cell pellets were thawed on ice and a portion was transferred to a YEPD plate and grown overnight at 26°. From the

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YEPD plate, a liquid culture was started in 300 μl of YEPD in 24-well microtiter plates (Becton Dickinson, Lincoln Park, NJ) and grown overnight in a 22° shaking water bath.

The sporulation test was performed by pelleting the cells in the 24-well microtiter plates by centrifugation in a Beckman 5810R centrifuge with an A-2-MTP rotor at 4000 rpm for 5 min at 22°. The supernatant was removed by aspiration and the cell pellet was washed twice by centrifugation with 300 μl of water, repelleted, and resuspended in sporulation medium (1 g/liter of yeast extract, 10 g/liter potassium acetate, 0.5 g/liter dextrose, 20 mg/liter leucine, 40 mg/liter uracil supplemented with 50 mg/ml uracil, and 250 mg/ml leucine). The 24-well microtiter plates were then shaken for 3 days at 22°. The cells were then examined for sporulation by DIC microscopy with an Olympus BX microscope with a ×100 oil immersion objective.

Test for ploidy of deletion strains: The mutant strains were taken from a YEPD plate, suspended in water, and transferred dropwise without dilution to a YEPD plate spread with a fresh layer of either the **a** or the α-mating-type tester strains. After overnight growth at 30°, velvet squares were used to transfer the yeast mixture to a minimal media plate to select for diploids (Guthrie and Fink 1991). Growth on the selective plate indicated a high level of haploids in the deletion parent strain, most likely due to incomplete selection when the diploid mutant strain was first created. In most of these cases, the diploid was further selected by streaking the original deletion mutant strain for single colonies on plates lacking methionine and lysine, which should allow growth of only the diploid. Single colonies of Met^+ Lys^+ cells were isolated and retested for sporulation and ploidy. If nonmating strains could be readily isolated, the sporulation results from these isolates are given. Otherwise, the strain was not evaluated.

Test for correct open reading frame deletion: The tested strains were deletions of YOL051w, YHR124w, YLL005c, YHL023c, YPL232w, YBR279w, YKL167c, YLR377c, YJR094c, and YDL226c. Two sets of conformational primers with the sequence chosen from the deletion project web site (http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html) were used. The first pair of primers, designated A and B, are unique to each open reading frame (ORF), where A is from regions 200–400 bases upstream of a particular ORF, and B is from regions within the particular ORF. This pair of primers gives a PCR product only if that ORF is intact. The second pair of primers, designated A and kanB, are unique to a deletion of the particular ORF, where A is as described above and kanB is specific for kanMX4 cassette. The second pair of primers gives a PCR product only if deletion of the specific ORF is present. In all 10 strains tested, a PCR product was observed only when primers A and kanB were used.

Nuclear division assay: Mutant strains were induced to sporulate by growing the cells in 1–2 ml of YEPD liquid medium in glass tubes, washing twice in water by centrifugation, and resuspending in sporulation medium in glass tubes. The cells were fixed at 24–96 hr by mixing equal volumes of culture medium and 3.7% formaldehyde in PBS. The mixture was immediately pelleted and resuspended in 3.7% formaldehyde in PBS for 2 hr at room temperature or ~15 hr at 4°. The cells were stained with 4′,6-diamidino-2-phenylindole (Sigma, St. Louis) at 1 μg/ml in PBS and examined by epifluorescence microscopy with an Olympus BX microscope and photographed with an Orca camera (Hamamatsu, Bridgewater, NJ).

RESULTS

An international consortium of laboratories have collaborated to individually delete most of the annotated ORFs in the *S. cerevisiae* genome as described previously (Giaever *et al.* 2002). Homozygous diploids were constructed by the consortium by mating independently mutated haploid strains. We have used this diploid deletion mutant collection to screen for genes required for meiotic division and sporulation.

Our primary screen was differential interference contrast microscopic inspection for the formation of visible spores following growth in liquid sporulation medium (materials and methods). Under these conditions, a majority of mutant strains were able to sporulate at levels of ~50% of all cells, with ~30% of the cells forming full tetrads. This is comparable to the sporulation levels for the S288C wild-type background from which the collection was derived and for auxotrophic mutants in the collection such as deletion of *ADE4* (not shown). The phenotypes for the mutant collection were divided into five categories of sporulation levels. The "normal" category was indistinguishable from wild type. A score of "low" was given if sporulated cells could be readily observed but at a lower frequency than that in wild type. "Very low" indicates that spores were only rarely seen, and "none" indicates the absence of spores. In addition, some mutants had high levels of sporulation but reduced numbers of spores per ascus. These mutants are listed as "low-4." No mutants had significant numbers of asci with more than four spores. Thirty-eight strains grew poorly or not at all and were not included in this analysis.

Each mutant with a sporulation defect was retested at least one additional time, and more if needed, until a consistent phenotype was observed. If normal sporulation was observed in any of these trials, that mutant was placed in the normal category. Three additional tests were performed to eliminate false identification of mutant phenotypes:

1. To confirm that the expected ORF was deleted and to verify that strain mixing did not occur during shipping and handling, the identity of the deleted ORF of 10 randomly chosen nonsporulating mutants was tested by PCR analysis (materials and methods). Five strains were chosen from each of the two releases and each strain was chosen from a different microtiter plate. All 10 of the chosen mutants contained a replacement of the expected ORF with the marker gene KanMX4 (not shown).
2. To distinguish the mutants unable to utilize nonfermentable carbon sources, all sporulation-defective mutants containing deletions of novel ORFs were tested for growth on plates containing glycerol as the sole carbon source (YEPG). Twenty-five novel mutant strains were unable to grow on YEPG and are assumed to be unable to sporulate due to respiratory defects. It should be noted that some of these may be defective for glycerol utilization rather than for respiration. After the phenotypes were confirmed,

Previously identified genes

ACN9	low	*ATP10*	none	*CVT17*	v. low	*GCS1*	none	*LEO1*	low	*NEM1*	v. low	*RAD6*	none	*RPL35A*	low-4	*SNF3*	low	*SPR3*	low	*TOP1*	low-4	*VPS4*	low
ADA2	v. low	*AUT1*	none	*CVT9*	low	*GIM5*	low	*LRS4*	low	*NGG1*	none	*RAD50*	low-4	*RPL40A*	low	*SNF4*	v. low	*SPS1*	low	*TPS1*	v. low	*VPS5*	low-4
ADY1	low-4	*AUT10*	none	*CYC8*	none	*GIP1*	none	*LUV1*	low	*NPR2*	none	*RAD51*	low	*RPN10*	low-4	*SNF6*	none	*SPT3*	v. low	*TPS2*	v. low	*VPS13*	none
ADY2	low-4	*AUT7*	none	*DEF1*	v. low	*GSF2*	low-4	*MAF1*	v. low	*NUP120*	low	*RAD55*	low	*RTF1*	low	*SNF7*	low	*SPT4*	v. low	*TUP1*	none	*VPS20*	low
AKR1	low	*BIM1*	none	*DEP1*	v. low	*GSG1*	low-4	*MAM1*	low	*NUP170*	low	*RAD57*	low	*SAE2*	low	*SOD2*	none	*SSN8*	low	*UBA3*	none	*VPS24*	low
AMA1	none	*BPH1*	low	*DOA4*	none	*HEX3*	low	*MCK1*	v. low	*NUP84*	low	*RAI1*	low	*SAP190*	low	*SPF1*	low	*SSO1*	none	*UBC11*	low-4	*VPS30*	none
APG1	none	*BRE1*	none	*DRS2*	low	*HMG2*	low	*MEI5*	v. low	*PAF1*	none	*RAM1*	none	*SCS2*	low	*SPO1*	none	*SPT10*	low	*UBI4*	none	*VPS41*	none
APG10	none	*BST1*	low	*DFG5*	low-4	*HMT1*	low	*MET13*	low	*PCK1*	none	*RCY1*	v. low	*SEC22*	v. low	*SPO7*	low-4	*SPT20*	none	*UBP14*	low	*VPS53*	low
APG12	none	*BTN2*	low-4	*DHH1*	none	*HOP2*	none	*MET22*	low	*PCL8*	low-4	*REF2*	low	*SED4*	low	*SPO11*	low-4	*SRT1*	low	*UFD2*	low	*XRS2*	low-4
APG13	none	*BTS1*	low	*DID4*	low	*HPR1*	none	*MLS1*	none	*PEP12*	none	*RIM4*	none	*SEP7*	low-4	*SPO12*	low-4	*SRV2*	low	*UME1*	low	*YPT7*	none
APG16	v. low	*BUB3*	none	*DMC1*	low	*HSL7*	low	*MMS4*	low	*PEP3*	none	*RIM11*	none	*SHE9*	v. low	*SPO13*	low-4	*SSN2*	low-4	*UME6*	none	*SDS3*	low-4
APG2	none	*BUD28*	low-4	*DOA1*	v. low	*IDS2*	low	*MND2*	v. low	*PEP7*	none	*RIM15*	v. low	*SHP1*	v. low	*SPO14*	none	*SSP1*	none	*UMP1*	low-4	*YVH1*	low
APG5	v. low	*CBC2*	none	*ECM8*	low	*INO2*	low-4	*MUM2*	none	*PFK26*	low	*RIM101*	low	*SIC1*	low-4	*SPO16*	low-4	*SSP2*	v. low	*VAC8*	none	*ZIP1*	v. low
APG7	none	*CDC10*	none	*EMP70*	low	*IME1*	none	*NAM8*	low-4	*PGD1*	low	*RPA49*	low	*SIN3*	none	*SPO19*	v. low	*SSZ1*	low	*VAM3*	none	*ZUO1*	low
APG9	none	*CDC40*	low	*ERG4*	low	*IME2*	none	*MON1*	none	*PHO91*	low-4	*RPL7A*	low	*SLG1*	none	*SPO20*	none	*STP4*	low	*VAM6*	none		
APS3	low	*CIS1*	low	*ERV14*	none	*IMP2'*	none	*MPC54*	none	*PKH2*	v. low	*RPL13A*	low-4	*SLK19*	low-4	*SPO21*	none	*SWI3*	none	*VAM7*	none		
APT1	low	*CKB1*	low	*FAT1*	low	*IRA2*	low	*MRE11*	low-4	*POP2*	v. low	*RPL19A*	low	*SLX8*	none	*SPO69*	v. low	*SWM1*	low	*VID28*	low		
ARC1	low	*CLB5*	low	*FEN1*	low-4	*ISC1*	none	*MRK1*	low	*PPA1*	none	*RPL27A*	low	*SMA1*	low	*SPO71*	none	*TFP1*	v. low	*VMA2*	none		
ARD1	none	*CSM1*	low-4	*FUS1*	low-4	*ISC10*	low	*MSO1*	none	*PRB1*	v. low	*RPL31A*	low	*SMA2*	none	*SPO73*	none	*THI12*	low-4	*VMA13*	none		
ARG82	low	*CST9*	low	*GAL11*	low	*KAR4*	low-4	*NAT1*	none	*PRE9*	low-4	*SAC2*	low	*SMK1*	v. low	*SPO74*	none	*THR4*	low	*VMA6*	none		
ARO2	low	*CTK1*	none	*GCN5*	none	*KCS1*	low	*NBP2*	low	*PRS3*	low	*SAC7*	v. low	*SNF1*	none	*SPO75*	none	*TIF4631*	low	*VPH1*	low		
ATF1	low-4	*CTK3*	none	*GPB2*	v. low	*LAS21*	low	*NDT80*	none	*PTC1*	low	*RPL34B*	low	*SNF2*	none	*SPO77*	none	*TOM1*	low	*VPS3*	v. low		

Novel genes

YAL035W/*FUN12*	none	YDR048C/YDR049W	v. low	YEL072W/*RMD6*	v. low	YHR116W	low	YLR358C	low	YOR199W	low
YAL068C	low	YDR070C	low-4	YER083C/*RMD7*	none	YHR132W-A	low	YLR370C	low	YOR255W	low
YBL100C	none	YDR117C	low	YER119C-A	low	YIL017C/*VID28*	low	YML009W-B	low	YOR296W	low-4
YBR090C	low	YDR126W/*SWF1*	v. low	YFR048W/*RMD8*	none	YIL157C	low	YMR010W	low-4	YOR298W	low
YBR159W	low	YDR255C/*RMD5*	low	YGL020C/*MDM39*	v. low	YJL160C	low	YMR158W-A	none	YOR333C/*SWF5*	none
YBR174C	low	YDR326C	low	YGL066W/*SGF73*	none	YJL175W	low	YMR306C-A	low-4	YOR338W	low-4
YCL010C/*SGF29*	none	YDR359C/*VID21*	low	YGL107C/*RMD9*	none	YJR003C	low-4	YNL170W	v. low	YPL055C/*LGE1*	v. low
YCR105W	low	YDR417C	low	YGL218W	none	YKL118W	none	YNL196C	low	YPL144W	low-4
YDL001W/*RMD1*	v. low	YDR433W/*KRE22*	v. low	YGL246C	low	YKR089C	low	YNL296W	none	YPL157W	low
YDL033C	v. low	YDR442W	low	YGR004W	low	YLR021W	low-4	YNL332W	low-4	YPL166W	low
YDL041W	v. low	YDR455C	low	YGR226C	none	YLR054C	low-4	YOL071W/*EMI5*	none	YPL183W-A	none
YDL072C	low	YDR512C/*EMI1*	none	YHL023C/*RMD11*	none	YLR235C	v. low	YOR008C-A	low	YPL205C	low
YDL118W/YDL119C	low	YDR516C/*EMI2*	none	YHR067W/*RMD12*	none	YLR269C	low	YOR135C	none	YPR053C	v. low

FIGURE 1.—Genes found to be required for sporulation. Genes are listed alphabetically followed by the ORF names for unpublished or uncharacterized loci. The level of sporulation observed in the mutant is shown to the right of the name. Included are genes previously shown to be required for sporulation or meiosis (red) and genes previously characterized but without a meiosis or sporulation phenotype listed in the SGD or YPD (green). All of the strains with deletions of these genes and ORFs were found to be able to grow on YEPG except deletions of the known sporulation/meiosis genes (red), which were not tested. When two ORFs are listed, they overlap in position and the functional gene is not known.

the identity of each of the ORFs was determined and a comparison to known phenotypes was made by reference to the SGD and YPD (Table S1, supplemental material at http://www.genetics.org/supplemental/). One hundred eighteen genes that were known or suspected to be required for respiratory growth are shown in yellow in Table S1 and were not studied further. Deletion mutants for the genes previously characterized, but not shown to be involved in respiration in the phenotypic description of the SGD and YPD, were tested for growth on YEPG. Any of the mutants that failed to grow on YEPG plates were not included in subsequent analyses.

3. All of the sporulation mutants were tested for ploidy by crossing the presumptive diploid strains to haploid mating-type tester strains (MATERIALS AND METHODS). Fifteen mutants were apparently haploid and were not studied further.

The mutants with a consistent sporulation phenotype and normal growth on YEPG and YEPD plates are interpreted to be the genes important for sporulation in *S. cerevisiae* and are shown in Figure 1. By this analysis, 154 (3.6% of total) of the tested genes are essential for sporulation, with very few, if any, spores visible. One hundred eighty-one (4.2%) play an important but nonessential role, including 48 (1.1%) that are required to efficiently produce all four spores.

To further define the role of the novel genes in the meiotic/sporulation pathway we tested mutants of 56 novel and unpublished ORFs, including most of those with severe sporulation defects, for induction of the early meiotic inducer *IME1* (MITCHELL *et al.* 1990) and for meiotic nuclear division (Figure 2; Table 1). The selected strains were transformed with a plasmid containing a β-galactosidase reporter gene under the control of the *IME1* promoter. Thirteen mutants were un-

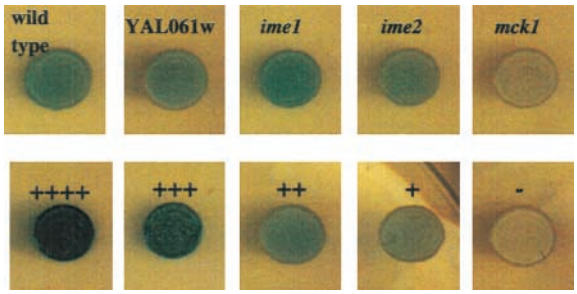

FIGURE 2.—Examples of *IME1* induction. Top, selected mutants transformed with an *IME1*-lacZ reporter. *MCK1* is required for *IME1* induction and shows the phenotype expected if *IME1* expression is absent. *IME1* and *IME2* are required for later steps of meiosis, after *IME1* induction. These mutants show the expression expected of meiotic mutants that are able to induce *IME1* to wild-type levels. *YAL061W* is a representative essential sporulation gene and the deletion mutant shows an *IME1* expression phenotype consistent with an arrest after *IME1* induction. Bottom, various examples of *IME1* expression phenotypes found among the tested strains.

able to induce β-galactosidase expression, showing that these ORFs are required for full activation of this early meiotic inducer. The bulk of the novel mutants (27) were able to induce *IME1*, but were unable to undergo nuclear division. Thus, these genes were not required for initiation of meiosis, but were required for essential steps in the middle stages of meiosis. Twelve mutants were able to induce *IME1* and to divide meiotically, but were blocked for spore wall formation. Note that three of the mutants (deletions of YOR338W, YAL068C, and YNL296W) failed to induce detectable expression of *IME1* yet still showed high levels of nuclear division. Since *IME1* expression is essential for all but the earliest stages of meiosis (MITCHELL *et al.* 1990), some low level of *IME1* induction may still be occurring in these strains. Alternatively, mutations in these genes may bypass the requirement for *IME1* induction. Further analysis will be required to determine what role the gene products play in activation of *IME1* expression. However, it is important to note that a "0" in our assay may not represent the complete absence of *IME1* expression. Higher than normal *IME1* expression levels could be due to lack of *IME2* induction, which is known to downregulate *IME1* (SMITH and MITCHELL 1989). Four of the mutants, deletions of YAL035W, YNL170W, YPL183W-A, and YBL100C, did not give clear and reproducible *IME1* expression profiles.

On the basis of these phenotypes, we have named 14 of the most essential sporulation loci. The genes required for *IME1* induction were named *EMI1-5* (*E*arly *M*eiotic *I*nduction). Those that were not required for entry into meiosis, but were required for meiotic nuclear division, were named *RMD1-12* (*R*equired for *M*eiotic nuclear *D*ivision). Genes essential for spore wall formation were named *SWF1* (*S*pore *W*all *F*ormation) and *SWF5*.

TABLE 1

Further evaluation of mutants in 56 novel or unpublished ORFs

ORF	*IME1* induction	Nuc. div.	ORF	*IME1* induction	Nuc. div.
YAL035W	ND	No	YAL056W	0	No
YAL068C	0	Yes	YBL100C	ND	No
YBR174C	+	No	YCL010C	++	Yes
YCR105W	+	Yes	YDL001W	++	No
YDL041W	+++	No	YDL118W	++	No
YDL151C	0	No	YDR048C	++++	No
YDR070C	+	Yes	YDR117C	++	Yes
YDR126W	+++	Yes	YDR255C	+++	No
YDR359C	+	No	YDR433W	++	Yes
YDR512C	0	No	YDR516C	0	No
YEL072W	+	No	YER083C	++	No
YFR048W	+	No	YGL020C	+++	Yes
YGL066W	+	No	YGL107C	+	No
YGL218W	0	No	YGR226C	++	No
YHL023C	++++	No	YHR067W	++	No
YHR116W	+	No	YIL017C	0	No
YKL054C	+++	No	YKL118W	+	No
YKR089C	+	Yes	YLR021W	+++	Yes
YLR235C	+++	No	YLR269C	0	No
YML009W-B	+++	No	YMR010W	+	Yes
YMR158W-A	++	Yes	YMR306C-A	+	No
YNL170W	ND	No	YNL296W	0	Yes
YOL071W	0	No	YOR135C	0	No
YOR298W	++	No	YOR333C	++	Yes
YOR338W	0	Yes	YPL055C	++++	No
YPL144W	++	No	YPL157W	+++	No
YPL166W	+	No	YPL183W-A	ND	No
YPL205C	0	No	YPR053C	++	No

The ability of each of the listed deletion mutants to express an *IME1*-lacz reporter gene (on a scale of 0 to ++++) and to undergo nuclear division (Nuc. div.) is shown. ND means the mutant could not be reliably assayed for *IME1* induction.

DISCUSSION

The sporulation genes of *S. cerevisiae*: We have used a collection of 4323 single-gene deletion mutants in nonessential genes to identify 334 sporulation-essential genes. Included within this group of genes are 140 previously identified meiosis/sporulation genes and 78 novel or unpublished ORFs. We also found that 115 genes that had been previously characterized, but did not show a sporulation phenotype on the SGD or YPD, were actually essential for full sporulation. For some of these genes the sporulation defect will be a novel finding, and in others it may be known but not included as a phenotype in these databases. Fifty-three genes were not screened because of weak growth or apparent haploidy. Most of the remaining ~1944 genes and predicted ORFs in the *S. cerevisiae* genome are essential for life and by definition are required for steps other than the meiotic sporulation pathway. Thus, we believe the data presented here define a nearly complete collection of sporulation genes in this pathway. This represents one

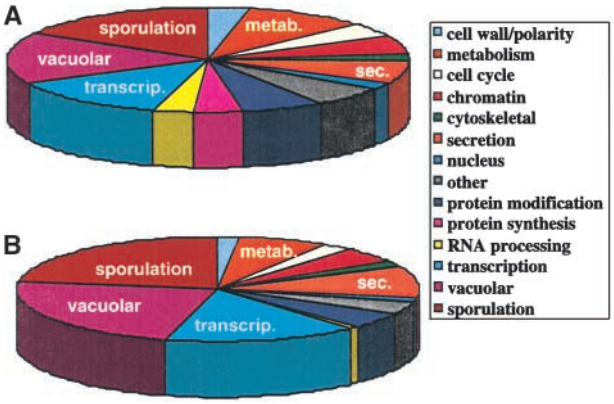

FIGURE 3.—Pie charts showing functional groupings of the genes required for sporulation in *S. cerevisiae*. Data include both known sporulation genes and previously characterized genes not described as essential for sporulation in the SGD and YPD phenotypic descriptions. (A) Genes that are required for full sporulation but not for life or growth on nonfermentable carbon sources. (B) Genes that are essential for even low levels of sporulation.

of the first large-scale genetic characterizations of a complex cellular pathway in a eukaryotic organism.

That being said, many questions concerning the function of these genes obviously remain, including where in the sporulation pathway they exert their influence and in what order the gene products are active. We and others are in the process of applying functional genomic techniques to further define the role of these gene products in the sporulation/meiotic pathway. It is also important to emphasize that many genes essential for a normal meiotic sporulation pathway, including inhibition of sporulation in rich medium, recombination and accurate chromosome segregation, complete spore wall synthesis, and formation of viable spores, may not be included in this screen since these defects often do not block the formation of visible spores.

Many of the novel ORFs overlap with the sequences of known genes or other ORFs in the genome. Since overlapping functional genes are very rare, or nonexistent, in *S. cerevisiae*, we assume that the phenotypes may represent the loss of function of the other gene or ORF found on the opposite DNA strand. In some cases, the overlapping gene is known or suspected to be required for sporulation. Thus, we suggest that the phenotypes due to loss of the ORFs YBL100C, YGR226C, YJL175W, YKL118W, YMR158W-A, YOR135C, YOR199W, YOR333C, YLR235C, YPL205C, and YPR053C are most likely due to loss of portions of the known meiotic genes on the opposite strand of DNA, which overlap with these hypothetical ORFs. In other cases, the overlapping gene has not been shown to be required for sporulation, and in some cases the deletion of the overlapping ORF in our hands does not cause sporulation defects. Thus, while deletions of the ORFs YBR174C, YDL041W, YGL218W, YLR358C, YML009W-B, YMR306C-A, YNL170W, YNL-296W, YDR417C, YDR442W, and YDR455C all give a sporulation phenotype, further analysis will be required to determine if these sequences represent functional genes. In addition, a few of the deleted ORFs overlap with larger, uncharacterized ORFs. We suggest that the phenotypes from deletion of YDL118W, YLR269C, and YDR048C may be due to the loss of the larger overlapping ORFs on the opposite DNA strand, YDL119C, YLR270W, and YDR049W, respectively. In each case, further analysis will be required to define the functional transcriptional unit in the case of these overlapping ORFs.

Functional classes for the sporulation genes: Figure 3 shows how the previously characterized genes (red and green in Figure 1) fit into functional groups. Classification was made by analysis of mutant phenotype at the SGD and YPD and selective review of the literature. Figure 3A shows 252 known genes, which we found to be required for full sporulation. Surprisingly, only 17% of the identified genes appear to be primarily specialized for meiosis/sporulation, having little or no known function during vegetative growth. Thus, while meiosis and sporulation have many specialized features, only a small minority of the genes required for this transformation have functions unique to meiosis. Nearly equally well represented are genes primarily involved in vacuolar function (15%) and transcription (13%). The significance of both of these categories is expected. The vacuole is important for survival under the starvation conditions required to induce sporulation (VAN DEN HAZEL *et al.* 1996). During sporulation, ~1000 genes show increased or decreased expression (CHU *et al.* 1998; PRIMIG *et al.* 2000), most likely explaining the large number of transcription-related proteins we find important for sporulation.

Figure 3B shows the 124 genes whose absence produces a severe sporulation defect (mutants with none or very low phenotypes in Figure 1). Again, a majority are involved in vacuolar function (23%) or transcription (15%) or are specialized for meiosis/sporulation (23%). Genes primarily functioning in the secretory pathway (10%) also become prominent. This may reflect the essential role of this pathway in prospore membrane synthesis when membrane vesicles are targeted to the growing tips. A notable difference between these groups is that genes involved primarily in protein synthesis or RNA processing, while making up 8.0% of the genes required for full sporulation, are rarely essential for sporulation (0.88%). We can add 78 ORFs to the list of genes essential for full sporulation. A majority of the novel ORFs are required between the beginning of meiotic induction and nuclear division. Others play roles in the initiation of meiosis and spore wall formation. Additional analysis will be required to narrow the mutant phenotypes further.

Efficiency and accuracy of this screen: There are two

general issues to consider regarding the usefulness of this type of large-scale functional genomic screen: the efficiency and the accuracy. The screens were performed blind with the identity of the deleted ORFs initially distinguished only by their position on the microtiter plate. Only after the phenotypes were established was the identity of the locus known by the investigator. By determining our success at finding known genes required for sporulation we can estimate our efficiency at identifying unknown sporulation genes. To make this comparison we searched the SGD for genes previously shown to be required for sporulation and verified the phenotypes in the appropriate literature references. Seventy-seven sporulation-essential phenotypic citations were found. Of these 77 mutants, we found 68 to be sporulation defective in our double-blind screen. For the remaining 9 mutants, a sporulation deficiency was reported previously that was inconsistent with the data presented here. Null mutations in two genes (*RIM9* and *LIF1*) are reported to reduce sporulation to between 47 and 67% of wild-type levels (Klapholz *et al.* 1985; Li and Mitchell 1997; Herrmann *et al.* 1998; Jiao *et al.* 1999) but had no effect on sporulation in our hands. This relatively low reduction in sporulation may not be readily detected in our large-scale, nonquantitative assay. Also, some differences between these previous studies and ours in regard to time in sporulation medium or temperature could contribute to the differences in sporulation levels. Loss of any of seven genes (*UMP1*, *FEN1*, *INO2*, *SDS3*, *GSG1*, *PHO2*, and *SNF8*) was previously shown to produce severe or complete loss of sporulation (Berben *et al.* 1988; Hammond *et al.* 1993; el-Sherbeini and Clemas 1995; Yeghiayan *et al.* 1995; Vannier *et al.* 1996; Byrd *et al.* 1998; Ramos *et al.* 1998) but sporulated at ≥69% of wild-type levels in our hands. Furthermore, *ubr1* mutants were reported previously to have a deficiency of four-spore tetrads (Bartel *et al.* 1990) but were normal in our hands. Reasons for the discrepancy between our data and the established literature remain to be determined, but could include differences in strain backgrounds or experimental error. Thus, we believe our efficiency at detecting sporulation genes is ~88%, including both severe and mild phenotypes.

To ensure the accuracy of our screen, we eliminated several sources of false positives within the data, including respiratory-deficient strains, poor growing strains, and haploid contaminants. We also showed that in all of the strains examined the expected locus was deleted. However, we have not shown that the phenotype was due to the deleted locus. To estimate the frequency of random false-positive errors we can ask how often we picked a known nonessential gene by mistake. For comparison we checked auxotrophic mutants in biosynthetic pathways known to be dispensable for sporulation. Twenty-six mutants with deletion of genes in the adenine, histidine, leucine, lysine, tryptophan, and uracil pathways are included in the tested collection (Table S1). None of these were found to be required for sporulation in this blind study. Thus the frequency of random false-positives is apparently <4% (1/26).

Comparison with other genomic-based screens: The Eurofan set of 624 mutants was screened for dityrosine spore wall components and, secondarily, for sporulation and nuclear division (Briza *et al.* 2002). Of the 16 sporulation mutants they found that were also in our collection, we identified 12 and failed to identify 4. Deletions of genes *SEP7/SHS1*, *PHO91*, *YDL121C*, and *YJR003C* had little or no effect on sporulation in our hands. As with the examples above, further evaluations will be required to resolve these discrepancies.

Rabitsch *et al.* (2001) have taken a slightly different approach to identify genes primarily involved in meiotic chromosome segregation. They deleted 301 loci in genes that showed increased expression during meiosis I (Chu *et al.* 1998; Primig *et al.* 2000). Eighteen were required for sporulation. Of these, 14 were in the collection we used, and all 14 were also found by us to be required for sporulation.

Comparing phenotypic and expression-based genetic screens: As functional genomic assays like this one are completed, it will be useful to compare the genes identified as essential with the genes showing the greatest transcriptional regulation. We can compare our phenotypic analysis to the sporulation expression data of Chu *et al.* (1998). These investigators have shown that ~500 ORFs increase expression levels during sporulation. Do those genes that show an increase in expression represent the genes most likely to be essential? Of 78 YEPG+ mutants in our study that were blocked for sporulation, 39% of the deleted ORFs showed increased expression during sporulation in wild-type strains, 21% showed a decrease in expression, and 40% showed no change (or both an increase and a decrease) during sporulation. For comparison we checked a random sample of 50 novel ORFs whose deletion we show did not produce a sporulation defect. Fourteen percent had increased expression during meiosis, 38% had decreased expression, and 48% had no change of expression. Thus, we see 39% of essential sporulation genes, but only 14% of random sporulation-positive genes, show increased expression during sporulation. Analysis of the Eurofan collection produced a similar result, showing 35% of the sporulation-essential genes to have a sporulation-specific induction (Briza *et al.* 2002). So there appears to be a correlation between requirement and increased expression for sporulation.

However, there was surprisingly little total overlap between the genes essential for sporulation and those showing the most increase in expression. Of the 84 genes in our study that showed a >7-fold induction at any time during sporulation, only 21% gave a sporulation phenotype when deleted (Chu *et al.* 1998). Of the genes with a >15-fold induction, only 24% produced

any sporulation phenotype when deleted. Thus, loss of highly expressing sporulation genes in most cases produced no sporulation phenotype. Perhaps most significantly, only 10.5% of the genes required for normal sporulation were found in the collection of ~200 genes with >7-fold induction of expression at any time during sporulation. This comparison indicates that expression-based profiling and phenotypic analysis are yielding different sets of sporulation genes. Similarly, previous studies have shown that there is little correlation between the expression of different genes and phenotypic requirement for those same genes for growth in selected media (Winzeler *et al.* 1999; Giaever *et al.* 2002).

Meiosis and sporulation are complicated pathways and expression profiles may be expected to vary depending on exactly when the protein is needed. Therefore, considering expression at specific time points may produce greater overlap with the phenotypic data. Rabitsch *et al.* (2001) used this approach by first identifying genes upregulated at least fourfold higher during meiosis than during the first 2 hr of sporulation conditions. Additional analysis, including comparison with the expression profiles of known meiotic genes, yielded 301 candidates, but even in this case only ~11% showed defects in meiosis or spore formation when deleted. This is higher than our recovery of 7.6% (327 sporulation mutants among 4323 candidates). In comparison, it seems that expression profiling will increase efficiency at a cost of reduced total yield. Most recently, the use of clustering algorithms has had the greatest success predicting function by combining data from different expression arrays. In 23 of 42 functional sets, transcriptional coregulation accurately predicted function in ≥30% of the test cases (Wu *et al.* 2002).

In summary, data from this and other studies suggest that the phenotypic and expression-based studies will yield different sets of genes involved in sporulation and other pathways in budding yeast. Given the unique cellular processes occurring during sporulation and the well-known transcriptional activation pathways, this is an unexpected result. There are many possible explanations. For example, small subtle changes in expression may be all that are needed for regulation of some of the meiotic genes. Another possibility, suggested by the data presented here, is that most of the sporulation-essential genes function during vegetative growth and therefore are not expected to be preferentially transcribed during sporulation. Whatever the explanation, it appears that these two types of screens will yield different sets of genes involved in sporulation and other pathways.

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