A screen for genes required for meiosis and spore formation based on whole-genome expression

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Background: Meiosis is the process by which gametes are generated with half the ploidy of somatic cells. This reduction is achieved by three major differences in chromosome behavior during meiosis as compared to mitosis: the production of chiasmata by recombination, the protection of centromere-proximal sister chromatid cohesion, and the monoorientation of sister kinetochores during meiosis I. Mistakes in any of these processes lead to chromosome missegregation.

Results: To identify genes involved in meiotic chromosome behavior in *Saccharomyces cerevisiae*, we deleted 301 open reading frames (ORFs) which are preferentially expressed in meiotic cells according to microarray gene expression data. To facilitate the detection of chromosome missegregation mutants, chromosome V of the parental strain was marked by GFP. Thirty-three ORFs were required for the formation of wild-type asci, eight of which were needed for proper chromosome segregation. One of these (*MAM1*) is essential for the monoorientation of sister kinetochores during meiosis I. Two genes (*MND1* and *MND2*) are implicated in the recombination process and another two (*SMA1* and *SMA2*) in prospore membrane formation.

Conclusions: Reverse genetics using gene expression data is an effective method for identifying new genes involved in specific cellular processes.

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Background

The production of haploid gametes from diploid cells during meiosis relies on three aspects of chromosome behavior that are specific to the first meiotic division [1, 2]. The first is pairing and recombination between homologous chromosomes resulting in the production of chiasmata that hold maternal and paternal chromosomes together from pachytene until the onset of anaphase I. The second is the monoorientation of sister kinetochores, which ensures that homologs and not sister chromatids are pulled in opposite directions by the meiosis I spindle [3, 4]. The third is the retention of sister chromatid cohesion in the vicinity of centromeres until the onset of anaphase II [5, 6], which permits two rounds of chromosome segregation from a single round of DNA replication. Since none of these three properties have thus far been amenable to biochemical analysis, their study to date has been mainly by means of genetic analysis, namely by looking for mutants defective in meiosis I-specific chromosome properties. Such mutants missegregate chromosomes at high frequency specifically during meiosis.

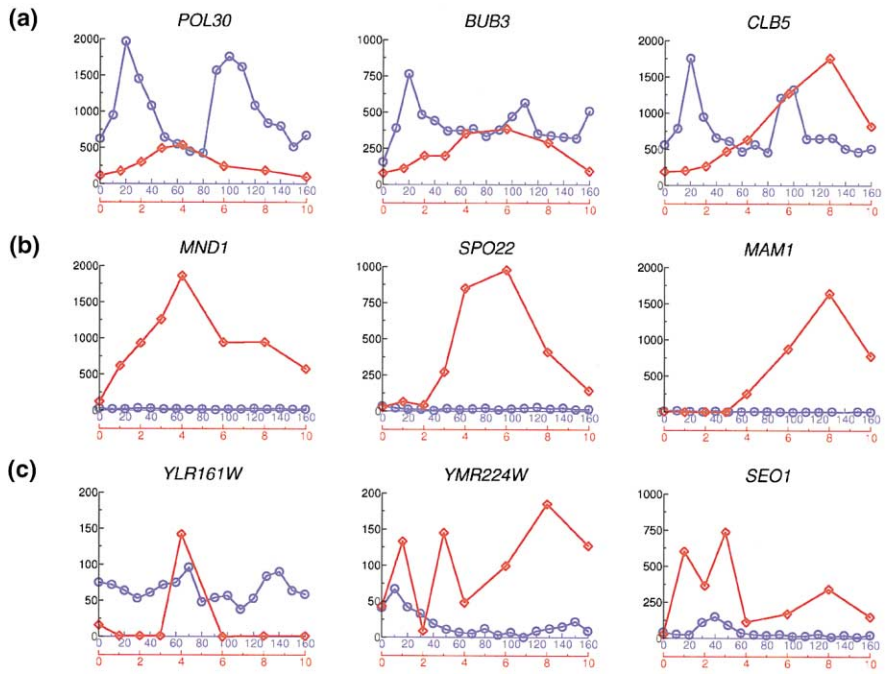
The completion of genome sequences and measurements of expression profiles of all genes within a genome during mitosis [7, 8] and meiosis [9, 10] has now made possible a new approach. On the assumption that many (albeit not all) of the genes required for meiosis I-type chromosome behavior will be expressed exclusively during meiosis I, we have made homozygous deletions in 301 genes preferentially expressed during meiotic divisions in yeast, and performed an initial characterization of their meiotic phenotypes. Thirty-three deletions had discernable phenotypes in meiosis and/or spore formation. Among these, eight were required for proper chromosome segregation.

Results

In silico selection of meiosis-specific genes

Genes to be deleted were selected on the basis of whole-genome expression profiles generated by PCR and oligonucleotide microarray technologies, and were initially examined cytologically for defects in chromosome segregation and spore formation. Our task of selecting genes expressed in a meiosis-specific fashion was complicated

Figure 1



Sample expression profiles. For each, gene expression data during mitosis (blue *x* axis [minutes] and blue line with circles) [7] and meiosis (red *x* axis [hours] and red line with diamonds) [10] is shown. The *y* axis shows the expression as signal intensity levels as in the original publications. Note that scaling is not identical for all genes shown. **(a)** Genes are shown that would have been selected by their meiotic expression but were excluded due to their mitotic expression. **(b)** Genes are shown that are expressed exclusively during meiosis and were disrupted in our screen. **(c)** Genes that were excluded due to their expression profiles are shown. Genes with only a single point of expression (e.g., *YLR161W*), erratic expression (e.g., *YMR224W*), or early expression (e.g., *SEO1*) were excluded.

by the fact that available data on gene regulation during mitosis [7, 8] and meiosis [9, 10] were obtained from independent experiments, and therefore signal intensity values were not directly comparable.

During our first attempt to identify meiosis-specific genes in silico, we used computational methods to select ORFs whose expressions were upregulated upon induction of meiosis. As a first step, we selected those ORFs that were upregulated according to PCR microarrays [9] and expressed at least 4-fold higher during meiosis according to oligonucleotide microarrays [10] than during the first 2 hr following the induction of meiosis. Using the above criteria, we selected 2116 ORFs, or about one third of all yeast genes. We next scrutinized (manually) the profiles of each of these ORFs, and eliminated all those clearly expressed in a cell cycle-specific manner during mitosis (for example, *CLB5* in Figure 1a) and all those lacking unambiguous induction during meiosis (such as shown in Figure 1c). We therefore eliminated all ORFs with single point peaks. In addition, those with multiple peaks were excluded to restrict our analysis to genes which are induced only during the meiosis I division. We excluded most ORFs whose expression peaked during the first 3 hr after shifting cells to sporulation conditions, because we presumed that most of these immediate-early genes would be involved in the nutritional response to sporulation medium and in early stages of commitment to the meiotic program [9, 10], but not in meiosis-specific chromosome behavior, our main interest. To fine tune our selection criteria, we used as a standard the meiotic and mitotic expression profiles of genes known to be required for meiosis-specific chromosome behavior such as *SPO11*, *SPO13*, *REC8*, *ZIP1*, and *HOP1*. Using the profiles of these genes as a guide, we selected ORFs whose peak expression during meiosis exceeded a signal intensity value of 150 [10], and whose peak during mitosis exceeded this value at not more than one time point (excluding the first three time points of the mitotic time course, because they showed unexpected expression levels for some genes, such as *ZIP1*) [7].

By this means, we selected 275 ORFs. We eliminated 112 out of these because their functions had already been characterized and deleted the remaining 163 ORFs (examples of expression profiles can be found in Figure 1b). Twenty-four out of the 163 deletions caused defects in meiosis and/or spore formation, and only five were essential for vegetative growth or germination.

To determine whether our initial selection criteria may have been too rigorous, we subsequently examined two additional classes of genes: those with peak values at more than one time point during mitosis greater than 150 (meiosis also greater than 150), and those with peak values during meiosis under 150 (mitosis also under 150). Manual inspection of these produced 316 new candidates, of which 178 were discarded because they had known roles. Of the 138 remaining genes, deletion of nine genes caused meiosis or spore formation defects and ten were essential

Table 1

Selection and disruption of genes expressed preferentially during meiosis.

	Known genes[a]					Deleted genes[b]				Known + deleted genes[c]			
		Vegetative function		Sporulation function									
	Total	Total	Essential	Total	Noticeable in our screen	Total	No apparent phenotype	Essential for mitotic growth	Meiosis/spore formation phenotype	Total	Essential for mitiotic growth	Meiosis/spore formation phenotype
First selection	112	78	16	41	32	163	134	5	24	275	21	56
Second selection	178	173	52	24	21	138	119	10	9	316	62	30
Total selection	290	251	68	65	53	301	253	15	33	591	83	86

Of the genes we selected in silico, we excluded those which already had known functions (a) because they either had a role during vegetative growth or during sporulation. However, with our screening scheme, we would not have identified all of these genes, but only those essential for vegetative growth and those showing clear defects in spore formation or chromosome segregation. Because some genes have roles during both vegetative growth and sporulation, numbers do not add up to the indicated total. Within the ORFs we did delete (b), we only identified genes which were essential for vegetative growth or showed clear defects in spore formation or chromosome segregation. If no function had been assigned to any of the genes selected by us in silico, we would have been able to identify the numbers indicated in (c).

for vegetative growth. Relaxation of our selection criteria therefore included a number of genes with meiotic functions missed by our initial choice but at the expense of less selectivity. Further rounds of selection with gradually reducing stringency might have produced even more genes required for meiosis and/or spore formation, but at the cost of progressively lower efficiency and cost effectiveness.

In summary, we selected 591 ORFs (275 + 316 based on the two sets of criteria) that appeared to be meiotically regulated. Two hundred and ninety had already been implicated either in meiosis and spore formation (65) and/or in other processes (251). Deletion analysis of the remaining 301 ORFs showed that 33 were required for meiosis/spore formation and 15 for vegetative growth. Our screen would have missed genes required for spore viability but not for chromosome segregation. Had nothing been known about any of the genes in our selection, our analysis would have identified 86 genes required for meiosis/spore formation and 83 genes essential for vegetative growth or germination. Table 1 summarizes the in silico selection and these results.

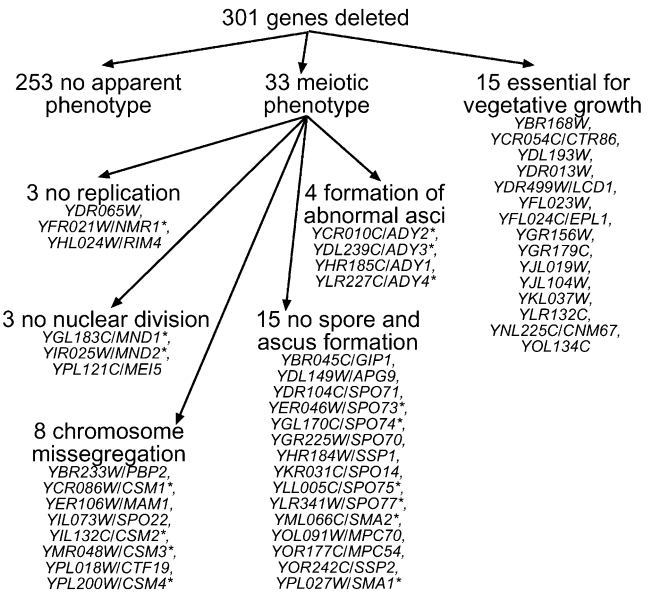
Overview of the screen

The descriptions of the phenotypes of all the gene deletions we constructed can be found on our web site together with the lists of genes we selected (http://mendel.imp.univie.ac.at/meiosis). A summary of genes whose deletion led to distinct phenotypes in meiosis and spore development is presented in Figure 2. Out of a total of 301 ORFs, deletion of 84% (253/301) had little or no effect on sporulation or chromosome V segregation and vegetative growth. On the other hand, deletion of 33 genes abolished the formation of wild-type asci. The phenotypes of these 33 mutants are described below. Despite our efforts to select preferentially meiosis-specific genes, approximately 5% of the ORFs (15/301) were essential for vegetative growth. All mutant strains created during this study have been deposited with ATCC.

Genes required for premeiotic S phase

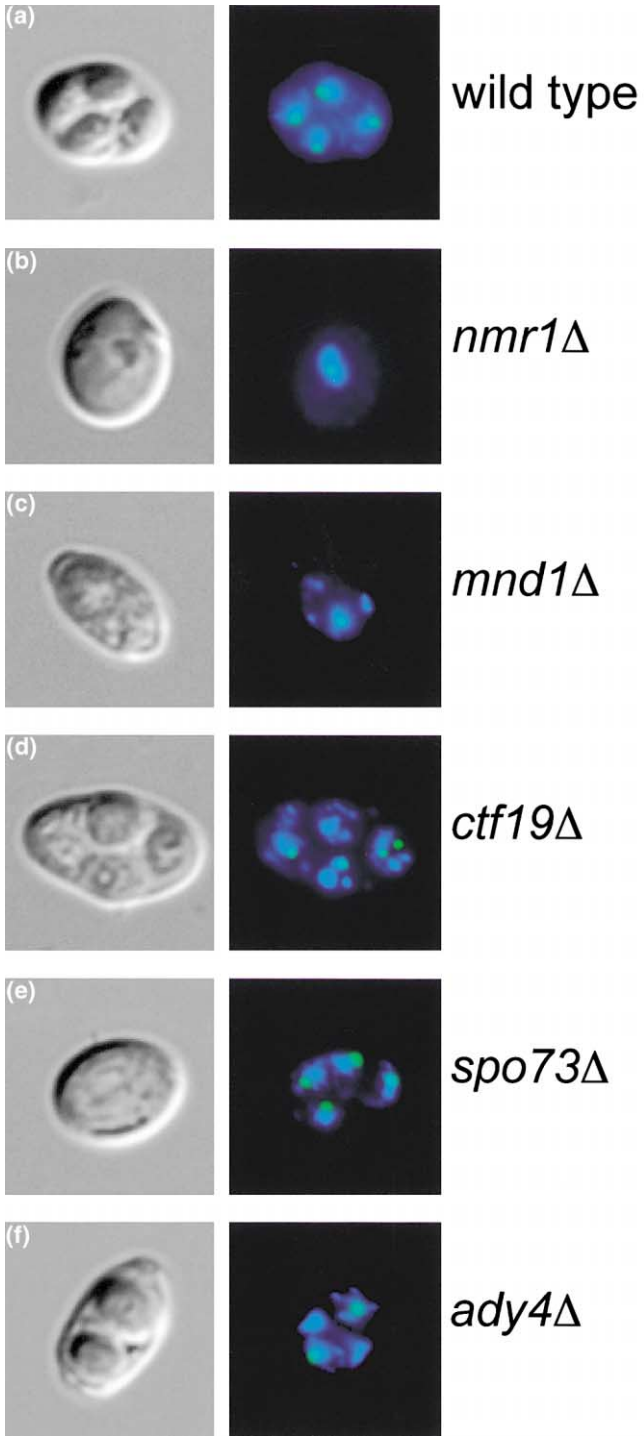
Flow cytometry was performed for all deletion mutants that did not form spores. This analysis showed that three mutants failed to undergo premeiotic DNA replication as well as nuclear divisions when shifted to sporulation medium. The failure of one of these (*YDR065W*) is presumably due to a metabolic defect because it also failed to grow on glycerol. A second gene (*YHL024W/RIM4*)

Figure 2



Overview of the results of our screen. The flow chart categorizes the 301 genes we deleted in our screen. Systematic open reading frame names are indicated. In addition, standard three letter names are given. Names indicated with an asterisk (*) were assigned during this study.

Figure 3



Sample pictures of the major phenotypes. On the left, differential interference contrast micrographs are shown, as are fluorescence micrographs of DNA stained with DAPI (blue) and CenV-GFP (green) on the right. **(a)** Wild-type (strain K8409). **(b)** Cells arrested before premeiotic DNA replication (*nmr1*Δ). **(c)** Cells arrested after replication but before the first nuclear division (*mnd1*Δ). **(d)** Chromosome missegregation observed in *ctf19*Δ (one nucleus lacks a chromosome V, and another one inherited two and the nuclei are fragmented). **(e)** A sporulation-deficient mutant that undergoes both nuclear divisions without chromosome missegregation (*spo73*Δ). **(f)** A mutant that shows a high frequency of dyads (*ady4*Δ). Note that before spore formation a GFP signal was visible in all four nuclei, indicating that this mutant does not missegregate chromosomes.

has meanwhile been shown to be a regulator of Ime2 expression [11]. We named the third ORF (*YFR021W*) *NMR1* (*n*eeded for pre*m*eiotic *r*eplication). The *nmr1*Δ phenotype is shown in Figure 3b.

Genes required for meiotic nuclear divisions

Deletion mutants in three genes (*YGL183C*, *YIR025W*, and *YPL121C*) failed to undergo efficient nuclear divisions despite an apparently normal premeiotic S phase as assessed by flow cytometry. They also failed to form spores efficiently. *YPL121C* corresponds to *MEI5*. We called *YGL183C* and *YIR025W MND1* and *MND2* (needed for *m*eiotic *n*uclear *d*ivisions), respectively. A typical example of an *mnd1*Δ cell is shown in Figure 3c.

If the lack of normal levels of nuclear divisions in *mnd1*Δ and *mnd2*Δ mutants were due to defects during recombination, then deletion of *SPO11* (which encodes the double-strand endonuclease that initiates recombination) [12] should suppress their nuclear division defects. Because deletion of *SPO11* itself causes random segregation of homologous chromosomes during meiosis I [13], we analyzed the effects of deleting *MND1* and *MND2* in *spo11*Δ *spo13*Δ double mutants (deletion of *SPO13* allows mutants defective in the initiation of recombination to undergo a single equational division and to form dyads containing two diploid viable spores [14, 15]; in this experiment, 34 of 60 spores derived from dyads were viable). Indeed, both *mnd1*Δ *spo11*Δ *spo13*Δ and *mnd2*Δ *spo11*Δ *spo13*Δ triple mutants formed dyads with high efficiency. Dissection of these dyads showed that 25 of 60 *mnd1*Δ *spo11*Δ *spo13*Δ but only 1 of 40 *mnd2*Δ *spo11*Δ *spo13*Δ spores were viable. Deletion of *SPO11* alone in *mnd2*Δ cells also rescued spore formation efficiently but produced inviable spores due to random chromosome segregation, whereas the deletion of *SPO13* alone had a much weaker effect. These data suggest that the lack of meiotic nuclear divisions in *mnd1*Δ and *mnd2*Δ mutants may be caused by defects after initiation of recombination. The inviability of spores from *mnd2*Δ *spo11*Δ *spo13*Δ mutants further indicates that *MND2* must have a second function that is independent of the meiotic recombination process.

We also analyzed the ability of *mnd1*Δ and *mnd2*Δ cells to form chromosomal axes and to synapse homologous chromosomes using a polyclonal antibody against the Zip1 synaptonemal complex (SC) component [16] and a tagged version of the meiotic cohesin subunit Rec8 [17]. On chromosome spreads from *mnd2*Δ nuclei, no axial cores could be observed at any stage after transfer to sporulation

medium. In wild-type cells, Rec8 and Zip1 line the axes of bivalents during pachytene. In contrast, in *mnd2*Δ mutants, Rec8 and Zip1 were found associated with chromatin in numerous largely nonoverlapping foci (Figure 4a–d). This suggests that *MND2* has an essential function in the formation of chromosomal axes, which precedes the initiation of recombination.

Extensive synapsis and SC formation occurred in *mnd1*Δ mutants. However, we rarely observed nuclei containing 16 fully synapsed bivalents. Dissolution of the SC was defective in *mnd1*Δ mutant cells, with the result that nuclei accumulated in a state with extensive but not complete synapsis during the first 10 hr after transfer to sporulation medium (Figure 4e–h). The accumulation of incompletely synapsed nuclei in *mnd1*Δ mutant cells and the recovery of spore viability in the *spo11*Δ *spo13*Δ background suggests that they may be defective in a late phase of recombination, synapsis, and/or in SC dissolution.

Genes needed for chromosome segregation

Eight deletion mutants underwent both nuclear divisions and formed spores but missegregated chromosome V. One of these (*YER106W*, which we called *MAM1*) has been analyzed in detail and been shown to be necessary for monopolar attachment of sister kinetochores during meiosis I [4]. Deletion of two genes (*YPL018W*/*CTF19* and *YCR086W*) caused massive meiotic chromosome missegregation, which was associated with spore viabilities below 1%. Both of these deletions also reduced the vegetative growth rate in glucose. Deletion of a third gene (*YBR233W*/*PBP2*) also led to a decreased growth rate and caused a more modest meiotic chromosome missegregation, resulting in a spore viability of 5%. The three mutants showing a reduced growth rate were examined for chromosome V (marked by GFP at the *URA3* locus) segregation in binucleate vegetative cells. No mitotic chromosome missegregation could be observed with this method (data not shown). The remaining four deletion mutants in this category (*YIL073C*/*SPO22*, *YIL132C*, *YMR048W*, and *YPL200W*) showed mild chromosome missegregation, resulting in spore viabilities between 20% and 60%. We called the four unnamed genes in this category *CSM1–4* (*c*hromosome *s*egregation in *m*eiosis). The missegregation of chromosomes in *ctf19*Δ cells is shown in Figure 3d.

Genes needed for spore and ascus formation

Fifteen deletion mutants (*YBR045C*/*GIP1*, *YDL149W*/*APG9*, *YDR104C*/*SPO71*, *YER046W*, *YGL170C*, *YGR225W*/*SPO70*, *YHR184W*/*SSP1*/*SPO3*, *YKR031C*/*SPO14*, *YLL005C*, *YLR341W*, *YML066C*, *YOL091W*/*MPC70*, *YOR177C*/*MPC54*, *YOR242C*/*SSP2*, and *YPL027W*) underwent both meiotic nuclear divisions without any apparent chromosome missegregation but failed to form spores or asci when shifted to sporulation medium. In accordance with previous nomenclatures, we called unnamed genes in this category *SPO* (apart from *YML066C* and *YPL027W*; see below). The *spo73*Δ (*yer046w*Δ) phenotype is shown in Figure 3e. We suggest that the corresponding proteins might be essential components of the prospore membrane, prospore wall or ascus, or regulators of the formation of either. In fact, during the course of our work, two of the ORFs mentioned above (*YOL091W*/*MPC70* and *YOR177C*/*MPC54*) were shown to be regulators of prospore membrane assembly localizing to meiosis II spindle pole bodies [18].

To further characterize these mutants, we investigated them for their ability to form wild-type-like prospore membranes. Localization of Don1, a specific marker for this membrane (see Figure 5a and corresponding legend) by immunofluorescence revealed that among those mutants investigated (deletions of *YBR045C*/*GIP1*, *YER046W*, *YGR225W*/*SPO70*, *YLL005C*, *YLR341W*, *YML066C*, and *YPL027W*), two showed aberrant prospore membrane structures. We named these genes (*YPL027W* and *YML066C*) *SMA1* and *SMA2* (*s*pore *m*embrane *a*ssembly). The other mutants did not show any obvious differences in the Don1 localization compared to wild-type.

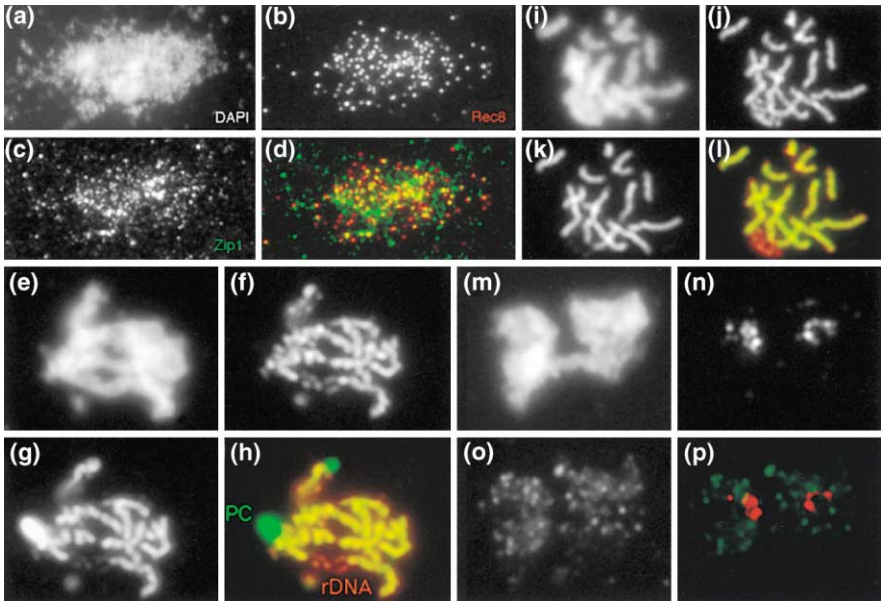
In the *sma1*Δ mutant, Don1 localized to clustered structures adjacent to the spindle poles in meiosis II, with some structures dispersed throughout the cytoplasm (Figure 5d). The efficient assembly of the precursors of the prospore membrane to a continuous prospore membrane seems to be defective. So far, only two other genes, *MPC54* and *MPC70*, have been implicated in this process. In the *sma1*Δ mutant, however, most of the Don1 containing precursors of the prospore membrane can be found adjacent to the spindle poles, which contrasts with the situation in the *mpc54*Δ or *mpc70*Δ mutants, where only small amounts of the precursors localize to the spindle poles [18].

In *sma2*Δ cells, Don1 localized to four larger-than-normal rings inside the cells during meiosis II (compare Figure 5c with 5b). This suggests that these cells are able to form continuous prospore membranes. However, the correct shaping of the membranes into domed, nuclear lobes engulfing pouches seems to be impaired. This is reminiscent of the phenotype of a mutant that lacks Spo20 [19], a meiosis-specific homolog to the mammalian-soluble NSF attachment protein 25 (SNAP-25), which is involved in membrane fusion during exocytosis.

Genes needed for ascus formation

Four deletion mutants (*YCR010C*, *YDL239C*, *YHR185C*/*ADY1*, and *YLR227C*) formed asci with an aberrant morphology when shifted to sporulation medium. All mutants underwent both nuclear divisions without observable chromosome missegregation, but the asci formed contained a high frequency of dyads, that is, asci that contain only two spores. We therefore named these genes using

Figure 4



*mnd1*Δ and *mnd2*Δ mutants arrest with different subnuclear morphologies. (a–d) Spread nucleus of *mnd2*Δ mutant (6 hr). **(a)** Chromatin, **(b)** Rec8-HA3, **(c)** Zip1, and **(d)** Rec8-HA3 (red) and Zip1 (green) merged. Signals are independently distributed. (e–h) Spread nucleus of *mnd1*Δ mutant (10 hr). **(e)** Chromatin, **(f)** Rec8-HA3, **(g)** Zip1, and **(h)** Rec8-HA3 (red) and Zip1 (green) merged. Extensively synapsed nuclei accumulate at later time points. A polycomplex (PC) is present in most nuclei, even in the ones exhibiting almost complete synapsis. The rDNA axes remain unsynapsed and devoid of any Zip1, as has been shown for wild-type. (i–l) Spread wild-type nucleus at 6 hr. **(i)** Chromatin, **(j)** Rec8-HA3, **(k)** Zip1, and **(l)** Rec8-HA3 (red) and Zip1 (green) merged. All 16 chromosomes are fully synapsed, except for the rDNA region on chromosome XII. (m–p) Spread wild-type nucleus at 10 hr. **(m)** Chromatin, **(n)** Rec8-HA3, **(o)** Zip1, and **(p)** Rec8-HA3 (red) and Zip1 (green) merged. Most nuclei have undergone anaphase I and removed Zip1 and most of Rec8 from chromatin.

the nomenclature already used for *YHR185C/ADY1* — *ADY* (*a*ccumulation of *dy*ads). Having completed both nuclear divisions, *ady* mutants proceed to package only two of the four meiotic products. The *ady4*Δ (*ylr227c*Δ) phenotype is shown in Figure 3f.

Discussion

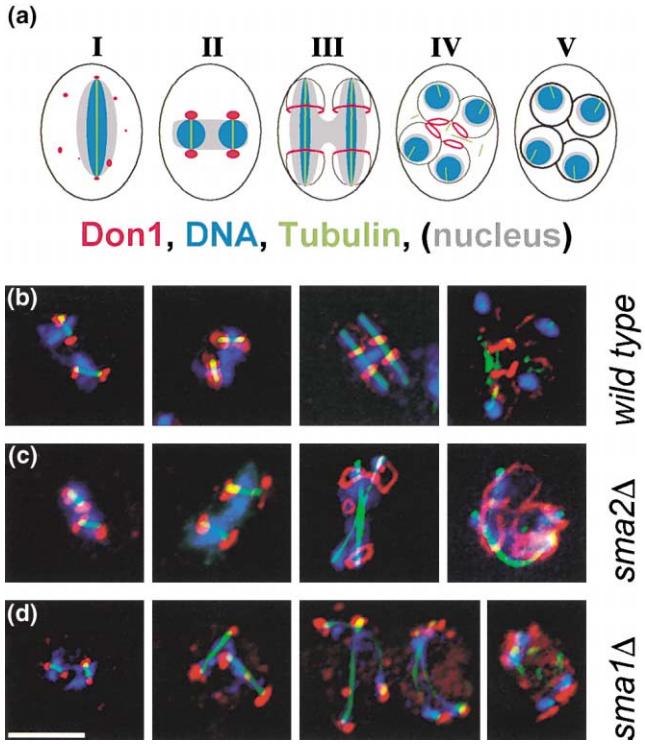
Functional genomic approaches

The determination of genome sequences has identified large numbers of genes with no known function. It is generally thought that one of the key challenges for the future is to identify by more systematic means than has hitherto been possible the functions of those genes whose functions have supposedly been recalcitrant to conventional forward genetic or biochemical analyses. This goal can now be formulated for any organism with a fully sequenced genome and methods to inactivate gene function. There have already been several attempts to meet this challenge. More than 2,000 yeast gene deletions have been examined [20], whereas the functions of 4,600 genes have been investigated by RNA interference in the nematode *C. elegans* [21, 22]. However, limited insights into specific biological processes have resulted from the initial characterization of systematic gene knockouts in either yeast or *C. elegans*.

The work described here was founded on a specific premise: that genomic sequences can provide extremely valuable resources to understand the mechanistic basis of biological processes when the investigator already has some insight into the process being investigated and has tools to measure its activity inside the cell or organism. We chose to use genomic information to investigate chromosome segregation during meiosis I. For this purpose, we used a yeast strain that sporulates synchronously and with high efficiency, and which contains markers that greatly facilitated the analysis of chromosome segregation. Most if not all of the genes disrupted in this study have already been disrupted by other consortia, but this was performed in strains that sporulate too poorly for detailed physiological analysis of meiosis. To reduce the number of genes to be deleted, we chose only those genes known to be expressed preferentially during meiosis. For this purpose, we used expression profiles of all 6,000 yeast genes [7–10]. Our study identified eight genes whose deletion causes a significant frequency of chromosome missegregation.

In the course of our work, two other studies adopted a similar, albeit less extensive, approach to ours. In one, 2 out of 18 ORFs whose mRNAs were upregulated during meiosis and were predicted to code for coiled-coil proteins were shown by GFP tagging to encode novel meiosis-specific spindle pole body proteins needed for the formation of prospore membranes [18]. In another study, ORFs predicted to encode transmembrane domain-containing proteins whose mRNAs were upregulated during α pheromone arrest were investigated for roles during conjugation. This identified a novel gene required for cell fusion [23]. These three examples demonstrate that novel insights into gene function can readily be obtained by

Figure 5



Prosporewall formation defect in *sma1*Δ and *sma2*Δ. Genes *SMA1* and *SMA2* are required for specific steps in the assembly of the prospore membrane. **(a)** Schematic illustration of the assembly pathway of the prospore membrane. Precursors of the prospore membrane appear during meiosis I in the cytoplasm. Toward the end of meiosis I, some of the precursors bind to the spindle poles. At the beginning of meiosis II, all precursors are found at the spindle poles where they assemble into a continuous prospore membrane [18]. During meiosis II, the prospore membranes, one per spindle pole body, extend like pouches around the lobes of the nucleus. After meiosis II and completion of the nuclear division, the prospore membranes eventually fuse with themselves to form prospore walls that enclose haploid nuclei [28–30]. The prospore walls then become subsequently matured to the spore walls. Don1 (red) is a specific marker (I) for the precursor membranes of the prospore membrane (II-IV) and for the leading edge of the prospore membrane during meiosis II. Detection of Don1 in **(b)** wild-type, **(c)** *sma*2Δ, or **(d)** *sma1*Δ cells from different stages of meiosis. Cells that are representative for specific steps in meiosis, as judged from the number and length of microtubule bundles, are shown. Don1 (red), tubulin (green), and DNA (blue) are labeled. The scale bar represents 5 μm.

knocking out genes with specific expression profiles targeted at understanding a specific biological process. The use of specialized screening schemes to study specific cellular processes is the paradigm of forward genetics. This approach is equally valid for the reverse genetics-dominated postgenomic era. The main difference between forward and reverse genetic screens is the method of mutagenesis. Several recent studies have demonstrated the potential for using RNA interference for similar purposes in *C. elegans* [21, 22, 24, 25].

How informative are expression profiles?

Our study follows up recent expression profiling data by gene inactivation on a large scale. In two rounds, we selected 591 genes as potentially biologically important loci for meiosis and/or spore formation on the basis of their expression profiles alone. We excluded 290 genes from our selection because they already had known functions. Among the 301 ORFs we deleted, we found 33 new genes (11%) whose absence leads to striking meiotic or sporulation phenotypes and 15 genes (5%) whose deletion was lethal during vegetative growth. The identification of functions for the 253 genes whose deletion had no discernible phenotype in our assay may require new ways of analyzing meiotic cells or analysis of multiple mutant combinations.

The Eurofan consortium recently characterized meiotic phenotypes of systematic knockouts irrespective of their expression profiles. In this study, 31 genes with strong meiotic defects were identified by visual screening among 483 mutants analyzed (6.4%; F. Klein and A. Nicolas, personal communication). Comparing this number to our 86/591 (15.6%) meiotic mutants yields a 2.3-fold enrichment by using meiotically expressed ORFs.

Had we stopped our screen after the first round of selection (see Results) and only disrupted 163 of the 275 genes selected, we would have identified 24 new genes with distinct roles during sporulation (14.7%) and five genes essential for vegetative growth (3.1%). Our first round of selection therefore yielded approximately 4.8 times more genes with roles during meiosis and/or spore formation than essential genes. The inclusion of genes chosen by less stringent criteria during our second round of selection lowered that figure to 2.2 times. Had we not excluded genes due to known roles, we would have been able to identify 56 genes (20.4% of 275 ORFs) with a role during meiosis/sporulation and 21 genes (7.6% of 275 ORFs) needed for vegetative growth with our first round of selection, resulting in a factor of 2.7 (see Table 1). The corresponding figures after including genes from our second round of selection would have been 86 genes needed for meiosis/sporulation and 83 for vegetative growth, with a factor of almost 1. Thus, while reducing the stringency of selection did indeed identify new meiosis-specific genes, it also greatly reduced their enrichment. We based our selection criteria on the expression profiles of genes already known to have roles in meiosis (such as *SPO11*, *SPO13*, *REC8*, *ZIP1*, and *HOP1*), which appears to be a sensible approach also for future studies of different biological processes. However, several of the players in meiosis-specific chromosome behavior that we set out to study may be expressed at significant levels also in mitotic cells (for example, *SPO12*) and might therefore have been eliminated as candidates.

Conclusions

Two major conclusions emerge from this analysis. First, using mRNA expression profiles to select genes to be deleted is clearly an effective alternative to conventional untargeted mutagenesis for the genetic study of meiosis. Meiosis-specific genes are uniquely suitable for functional analysis through gene disruption because they are not essential for vegetative growth. For example, it would have been impossible to apply a similar approach to genes required for mitosis. Furthermore, this approach should be applicable to other developmental processes in any organism where genes can be reliably inactivated, whether by deletion or RNA interference. Second, a large fraction (approximately 50%) of the yeast genes selected by us as potentially important for meiosis based on expression profiles had already been identified by conventional mutagenesis at the onset of our analysis.

Among new meiotic genes identified by our approach was *MAM1*, required for the monoorientation of sister kinetochores during meiosis I [4], a process unique to meiosis which forms the basis of segregation of alleles to different gametes. We also identified two novel genes (*SMA2* and *SMA1*) which are necessary for the correct formation of prospore membranes, and two genes (*MND1* and *MND2*) that seem to act downstream of DSB formation during recombination. In addition, we identified 28 further genes that are essential for the formation of wild-type asci.

Materials and methods

Creating deletions

We used a sporulation-proficient SK1 strain harboring two markers that facilitated detection and characterization of chromosome missegregation. The *URA3* locus, 35 kb away from the centromere of chromosome V, contained tandem arrays of the Tet operator which were bound by a Tet repressor-GFP fusion protein [26]. This permitted detection of chromosome V segregation into the four meiotic products by fluorescence microscopy (see Figure 3). In addition, the scissile Rec8 subunit of meiotic cohesin was tagged with multiple HA epitopes, which enabled us to characterize the state of sister chromatid cohesion by cytological means.

Our parental strain (K8409; *Mat***a**/*Mat*α, *HO*, P_{URA3}*-tetR-GFP, URA3:tetO*$_{224}$*, REC8-HA3, his3::hisG*) was transformed with PCR-generated disruption cassettes containing the *HIS3MX6* marker gene [27]. Primers were synthesized on ABI 394 and PerSeptive Expedite DNA synthesizers according to an optimized protocol. Correct heterozygous deletions were identified by PCR, sporulated, and tetrads of three independent clones were dissected. Due to the presence of the *HO* gene, tetrads gave rise to four diploid clones, two of which were homozygous for the gene deletion. Gene deletions were confirmed by PCR, and homozygous diploid deletion mutants were then transferred to sporulation medium (8.2 g NaOAc, 1.9 g KCl, 0.35 g $MgSO_4$, and 1.2 g NaCl per liter) for 36 hr and checked for spore formation and chromosome V segregation.

Immunofluorescence

Immunofluorescence, and collection and rendering of the images were performed as described [18]. The pictures in Figure 5b–d show planar projections of 3D-rendered cells. Don1p was detected using affinity-purified polyclonal antibodies made in rabbit; tubulin was detected using the Wa3 mouse monoclonal antibody. Secondary antibodies were goat anti-mouse $ALEXA_{488}$ and goat anti-rabbit $ALEXA_{546}$ (Molecular Probes). DNA was detected using DAPI.

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