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Novel Roles for Selected Genes in Meiotic DNA Processing

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High-throughput studies of the 6,200 genes of Saccharomyces cerevisiae have provided valuable data resources. However, these resources require a return to experimental analysis to test predictions. An in-silico screen, mining existing interaction, expression, localization, and phenotype datasets was developed with the aim of selecting minimally characterized genes involved in meiotic DNA processing. Based on our selection procedure, 81 deletion mutants were constructed and tested for phenotypic abnormalities. Eleven (13.6%) genes were identified to have novel roles in meiotic DNA processes including DNA replication, recombination, and chromosome segregation. In particular, this analysis showed that Def1, a protein that facilitates ubiquitination of RNA polymerase II as a response to DNA damage, is required for efficient synapsis between homologues and normal levels of crossover recombination during meiosis. These characteristics are shared by a group of proteins required for Zip1 loading (ZMM proteins). Additionally, Soh1/Med31, a subunit of the RNA pol II mediator complex, Bre5, a ubiquitin protease cofactor and an uncharacterized protein, Rmr1/Ygl250w, are required for normal levels of gene conversion events during meiosis. We show how existing datasets may be used to define gene sets enriched for specific roles and how these can be evaluated by experimental analysis.

Introduction

Meiotic DNA processing includes molecular functions such as DNA replication, repair, recombination, chromosome modification, and segregation. The fidelity of DNA processing events during meiosis is critically important as errors can give rise to mutations, genome rearrangements, and aneuploidies that are associated with genetic disorders.

A large number of high-throughput analyses have been performed to characterize the 6,200 genes of S. cerevisiae. These have included genomic screens for protein–protein [1–3] and protein complex interactions [4–7], high-throughput genetic interaction analyses [8–13], genome-wide measurements of gene expression under various environmental conditions [14–19], comprehensive measurements of subcellular localization of proteins [20,21], and assessments of deletion phenotypes of single genes [22–24]. Although these high-throughput datasets have proved to be useful, at the time of this work more than one third of the S. cerevisiae genes did not have a biological process and/or molecular function assigned on the Saccharomyces Genome Database (SGD) [25]. One major drawback of high-throughput studies is the difficulty in assessing the large amount of data that are produced, and to compound the problem further, spurious data are common [26,27]. However, it has been shown that problems with false information within datasets can be circumvented by combining data from different high-throughput experiments, as the data can either support or contradict one another [28,29].

In this report, a strategy of combining high-throughput data available for protein and genetic interactions, protein subcellular localization, and mRNA expression patterns, together with data from phenotype experiments, was used to identify minimally characterized genes potentially implicated in DNA processing. Homozygous deletion mutants were made for 81 genes selected with the data integration strategy and were assessed to detect roles in meiotic DNA processing. As a result, eleven (13.6%) genes were found to have novel roles in meiotic DNA processing.

Results

Integration of Datasets to Select Genes with Roles in DNA Processing

An in-silico selection strategy (Figure 1) was designed to combine high-throughput datasets, to identify mutants conferring DNA processing phenotypes. 81 genes (3.4% of the minimally characterized genes in the genome) were selected for further analysis. During primary selection, genes not annotated for a biological process and/or molecular function (minimally characterized genes) were selected if either a genetic or physical interaction partner involved in DNA processing could be identified. A gene was defined to be involved in DNA processing if its annotation was related to one or more of the following functions: DNA replication, repair, recombination, and related checkpoints, as well as chromosome segregation and chromatin structure/modification by the Comprehensive Yeast Genome Database (CYGD) or the Saccharomyces Genome Database (SGD). In this way a...
list of 752 DNA processing genes was created (Table S1). To increase stringency we required a minimum of two DNA processing interaction partners, which reduced the number of candidates from 718 to 316 genes. The interaction data were taken from the Yeast General Repository for Interaction Datasets (GRID) [30] and Database of Interacting Proteins (DIP) [31].

The secondary selection aimed to select against genes that had unfavourable characteristics. Of the initially chosen genes, 72 had well documented roles in DNA processing and were therefore removed (e.g., MAD1, well characterized for its role in the spindle checkpoint [32] and ZIP2, which has been shown to be an intrinsic component of the synaptonemal complex [33]). Of the genes essential for vegetative growth, 52 were not assessed. A further 61 genes were removed because of protein localization inconsistent with roles in DNA processing (e.g., mitochondria, endoplasmic reticulum, cell wall, bud neck, endosome, Golgi apparatus, vacuole, or lipid).

Also excluded were 37 genes annotated for roles in cell wall organization and biogenesis, bud site selection, vacuole transport, and nutrient metabolism. We excluded 13 genes because the fraction of their interaction partners involved in DNA processing was less than 1/5. The secondary selection resulted in the identification of 81 genes that were all subsequently analyzed experimentally (see Table S2 for the list of genes removed during the secondary selection).

It has been reported that genetic interaction data have a much higher confidence than physical interaction data [34], and it has been observed that mRNA expression patterns often correlate for proteins that interact physically [35–38]. To assess the 81 candidates further, the gene expression correlations of all physical interaction pairs were compiled. The method of assessing mRNA correlation used here has been described and assessed previously [29]. This method calculates a cosine correlation distance for a pair of proteins that is between zero for complete correlation, and two for anti-correlation. A correlation distance of below 0.9 was deemed sufficient expression correlation to support the interaction. This cut-off was decided for two reasons; firstly, yeast two-hybrid data generally have a weak relationship with gene expression correlation [38] and therefore a cut-off value too stringent would miss true interactions. Secondly, in general a correlation distance over 0.9 did not successfully

**Table S1.** List of 752 DNA Processing Genes

| Category | Description |
|----------|-------------|
| A        | Genes with two or more genetic interactions |
| B        | Genes with one genetic interaction and at least one physical interaction with co-expression |
| C        | Genes with one genetic interaction and no physical interaction with co-expression |
| D        | Genes with two or more physical interactions with co-expression |
| E        | Genes with one or no physical interactions with co-expression |

**Figure 1.** Integration of Datasets to Select Genes with Roles in DNA Processing

See text and Figure S1 and Tables S1, S2, and S3 for details of the selection strategy.

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Table 1. Summary of the Deletion Mutants for 16 of 81 Selected Genes That Had an Altered DNA Processing Phenotype

| Gene Deleted | Selection Category | HU Sensitive* | MMS Sensitive* | X-Ray Sensitive* | Sporulation Efficiency (%) | Low or No Meiotic Nuclear Divisions | Spore Viability (%) | Meiotic Gene Conversion Lys* Frequency (10^-4) | Meiotic Chromosome Missegregation Ade* Frequency (10^-4) |
|--------------|--------------------|---------------|----------------|------------------|----------------------------|-----------------------------------|-------------------|--------------------------------|---------------------------------|
| Wild type    | –                  | –             | –              | –                | 50.7                      | No                                | 94.59             | 3.868                          | 1.64                             |
| BRE1         | A                  | +             | –              | –                | <1                        | Yes                               | NA                | NA                             | NA                               |
| RAD61        | A                  | –             | –              | ++               | 47                        | No                                | 75                | 3.02                           | 1.95                             |
| VID21        | A                  | ++            | +               | +                 | <1                        | Yes                               | NA                | NA                             | NA                               |
| SGF73        | A                  | +             | –              | –                | 1.6                       | Yes                               | NA                | NA                             | NA                               |
| SOH1         | A                  | ++            | –              | –                | 22.6                      | No                                | 60                | 0.015                          | 6.08                             |
| PMR1 (HUR1)  | A                  | ++            | –              | –                | 10.3                      | Yes                               | 1.25              | NA                             | NA                               |
| YGL250W      | A                  | –             | –              | –                | 33.5                      | No                                | 85                | 0.663                          | 3.06                             |
| RTT101       | A                  | +             | ++             | –                | 37.9                      | Yes                               | NA                | NA                             | NA                               |
| DEF1         | A                  | +++           | +++            | ++               | <1                        | Yes                               | NA                | NA                             | NA                               |
| MMS22        | A                  | +++           | +++            | +++               | 13.3                      | No                                | 61.25             | 3.19                           | 13.6                             |
| BRES         | A                  | +             | –              | –                | 27.6                      | No                                | 82.5              | 0.013                          | 3.03                             |
| YPL017C      | A                  | –             | +               | –                | 17.2                      | Yes                               | NA                | NA                             | 50.3*                            |
| LGE1         | A                  | +             | –              | –                | <1                        | Yes                               | NA                | NA                             | NA                               |
| SWC5         | A                  | +             | –              | –                | 33.4                      | No                                | 91.25             | 3.17                           | 2.6                              |
| RMD11        | B                  | –             | –              | +                | 3.4                       | Yes                               | 90                | NA                             | NA                               |
| PSY3         | D                  | –             | +              | +                | 36.2                      | No                                | 85                | 3.55                           | 1.44                             |

The 16 mutants that had at least one altered DNA processing phenotype. See Table S3 for a summary of the results acquired for deletion mutants of all 81 genes. Numbers in bold signify the mutants that show phenotypes that are greatly different from the wild type for the particular assays referred to in the text.

a For HU or MMS sensitivity tests, overnight cultures were diluted by a factor of 10 from 10^-1 to 10^-7 and spot-plated onto YPD plates containing 100 mM HU and 0.03% MMS. For X-ray sensitivity, the same dilutions were plated onto a YPD plate that was then exposed to 120 kVp for 40 min.

b The meiotic chromosome missegregation phenotype was confirmed by assessing the mutant in a SK1 background. The SK1 strain has a locus, 35 kb away from the centromere of Chromosome V that has tandem arrays of the Tet operator that bind a Tet repressor-GFP fusion protein. This permits detection of Chromosome V segregation into the four meiotic products by fluorescence microscopy.

d, no hypersensitivity; ++, very high hypersensitivity; ++++, very high hypersensitivity; ++, high hypersensitivity; ++, moderate hypersensitivity; –, no hypersensitivity.

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predict interactions [29]. Using this information, the 81 selected genes were subdivided into five categories (Figures 1 and S1; Table S3 for all data and examples of this selection step). Category A consisted of 20 genes that possessed two or more genetic interactions with DNA processing genes. The 13 genes of Category B had a single genetic interaction and at least one physical interaction that showed correlated expression. The four genes of Category C had a single genetic interaction and at least one physical interaction without correlated expression. Category D consisted of 24 genes that had two or more physical interactions with correlated expression, and the remaining 20 genes of Category E had at least two physical interactions that do not have correlated expression.

Overview of the Screen

Deletion mutants for the 81 genes arising from our secondary selection were created in MAT-a and MAT-alpha W303 backgrounds. The experimental screen included testing for sensitivity to hydroxyurea (HU), methyl methanesulphonate (MMS), and X rays during vegetative growth, as well as assessing sporulation efficiency, meiotic nuclear division, spore viability, and levels of meiotic chromosome missegregation and gene conversion (Figure S3 and Table 1).

Twelve deletion mutants were shown to have increased sensitivity to HU, four of which were also sensitive to MMS, and three to X rays (Table 1). Additionally, two mutants, rad61 A and psy3 A, not sensitive to HU, were sensitive to X rays and MMS, respectively (Table 1). Results presented here are consistent with at least one previous genome-wide screen [39-43], with the exception of three mutants that show mild sensitivity to HU, sgf73 A, swc5 D, and rmd11 A. These phenotypes were shown to be the same in both MAT-a and MAT-alpha haploid strains.

Meiotic missegregation of Chromosome 1 was quantified by selecting spores which carry both ADE1 and ade1-ADE2 alleles indicating the presence of a second chromosomal copy (see Materials and Methods). Three mutants displayed increased levels of meiotic Chromosome 1 misse Segregation: soh1 A (5-fold), mms22 A (10-fold), and ypl017c A (5-fold) (Table 1). Meiotic gene conversion was measured by restoration of a functional LYS2 gene from lys2-5’nde1 and lys2-3’nde1 heteroalleles (Figure 2A). Spot tests and random spore analysis revealed a reduced level of gene conversion compared to the wild type (Figure 2B, Table 1) for ypl250w A (6-fold), soh1 A (>250-fold), and bre5 A (>250-fold). Further analysis of these mutants is discussed below. Six mutants (bre1 A, vid21 A, sgf73 A, rmd11 A, def1 A, and lge1 A) were found to have very low or no nuclear divisions in meiosis (Table 1). Further analyses of these genes’ roles in DNA processing are discussed below.

In summary, 11/81 (13.6%) of the selected genes were shown to have roles in meiotic DNA processing (Table 1). By far the highest proportion of meiotic DNA processing phenotypes 10/20 (50%) was found among mutants for genes with two or more genetic interactions (Category A). Only 1/13 (8%) from Category B, and none from Category C, D, or E conferred a meiotic DNA processing phenotype for any applied test.
Soh1, a Component of the Mediator Complex, Bre5, a Ubiquitin Protease Co-Factor, and an Uncharacterized Protein Ygl250w Are Required for Normal Levels of Gene Conversion during Meiosis

A reduced level of gene conversion was observed for ygl250wΔ, soh1Δ, and bre5Δ (Figure 2B, Table 1). To further characterize these mutants and ensure efficient and synchronous initiation of meiosis, deletions were made in a sporulation-proficient S. cerevisiae strain, SK1. In this background, bre5Δ had a sporulation efficiency of 80% after 24 h (unpublished data); however, in pre-sporulation conditions growth of bre5Δ was greatly inhibited and meiosis could not

Figure 2. Assessment of YGL250W, SOH1, and BRE5

(A) Schematic representation of Chromosome II from the diploid W303 background which consists of two LYS2 heteroalleles (lys2-5’nde1– and lys2–3’nde1–). These were used to measure meiotic gene conversion (see Materials and Methods).

(B) Spot test of wild type, ygl250wΔ, soh1Δ, and bre5Δ on haploid selection plates and haploid selection plates without lysine to measure meiotic gene conversion. The reduction in meiotic gene conversion of ygl250wΔ, soh1Δ, and bre5Δ was further assessed by random spore analysis (Table 1).

(C) Southern blot of DNA isolated from wild type, soh1Δ, and ygl250wΔ SK1 strains containing the ectopic URA3-ARG4 interval on Chromosome II. The DNA from the indicated times after initiation of sporulation were digested with Xhol then probed to detect COs and DSBs; mw1 represents the λ-HindIII molecular weight marker (Fermentas) and mw2 represents the 1-kb molecular weight marker (Fermentas). The full-sized Southern blots are presented in Figure S1.

For graphs (D–G), wild type, ygl250wΔ, and soh1Δ are represented by black diamonds, black circles, and white squares, respectively. The corresponding Xhol-digested Southern blots are presented in Figure 3C.

(D) Pre-meiotic DNA replication was assessed for synchronized meiotic cultures by fluorescence-activated cell sorting (FACS) and the change from 2c to 4c DNA content was plotted over time.

(E) Nuclear divisions (MI and MII) of the synchronized meiotic cultures in (E) were assessed with fluorescence microscopy using 4′,6-diamidino-2-phenylindole (DAPI) staining to visualize nuclear division.

(F) Molecular analysis for DSB (DSB1) signal/total lane signal from Southern blots of DNA extracted from synchronized meiotic cultures.

(G) Molecular analysis for CO (CO2) signal/total lane signal from Southern blots of DNA extracted from synchronized meiotic cultures.

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be synchronized. Therefore further analysis of bre5A was not performed. For wild type, ygl250wA and soh1A pre-meiotic DNA replication, meiotic nuclear divisions, as well as molecular analyses of meiotic double-strand breaks (DSBs) and crossovers (COs), was examined. The SK1 background used carries a 3.5-kb URA3-ARG4 fragment containing a recombination hotspot inserted at his4 on one copy of Chromosome III and at leu2 on the homologue [44]. DNA extracted from time courses of wild type, ygl250wA, and soh1A was digested with the XhoI restriction enzyme and used to assess both DSB and CO formation (Figures 2C–2E and S2).

For ygl250wA, pre-meiotic DNA replication initiates normally and progresses with similar kinetics to that of the wild type (Figure 2F), and although 15% fewer cells appear to have completed pre-meiotic DNA replication by 8 h, the level of meiotic nuclear divisions after 10 and 12 h is equivalent to the wild type (Figure 2G). DSB formation and repair during meiosis for ygl250wA also appears similar to wild type (Figure 2D), but strikingly, formation of COs was reduced by 4.5-fold (Figure 2E). For soh1A, initiation of pre-meiotic DNA replication appears to be delayed by 2 h and then proceeds with kinetics slightly below the wild type (Figure 2F). Meiotic nuclear divisions and formation of DSBs and COs are also delayed (Figure 2D, 2E, and 2G). Finally CO levels and meiotic nuclear divisions are mildly reduced compared to wild type (Figure 2E and 2G). In summary, for ygl250wA physical and genetic analysis suggests a parallel decrease in both gene conversion and CO formation, while the strong genetically determined decrease for meiotic gene conversion in soh1A was not matched by a similar lack of physical CO products in SK1.

Def1 Is Required for Efficient Synapsis between Homologues and Normal Levels of CO Recombination during Meiosis

Six mutants in the W303 background (bre1A, vid21A, sgf73A, rmd11A, def1A, and lge1A) were found to have very low or no nuclear divisions in meiosis (Table 1). These mutants were tested to determine whether IME1, the master regulator of entry into meiosis [45], was properly expressed (Figure 3A). They were also tested for changes in pre-meiotic DNA replication (Figures 3B and S3), meiotic DSB formation and repair at the THR4 hotspot (Figures 3C and S4) [46], and meiotic nuclear divisions (Figure 3D). Additionally, these six mutants showed differing levels of HU hypersensitivity (Figure S5A and Table 1); we therefore synchronized MAT-a cells by α-factor and monitored the progression of mitotic DNA replication by FACS (Figures 3E and S5B).

To ensure efficient and synchronous initiation of meiosis, deletions were made in the sporulation-proficient S. cerevisiae strain SK1. A synchronous culture of wild-type SK1 induces IME1 expression and pre-meiotic DNA replication almost immediately after transfer to complete starvation conditions, and 90% of the population completes DNA replication between 5–6 h (Figures 3B and S3). Meiotic DSB formation in the wild type peaks at 4 h, and all DSBs are repaired after 7 h (Figures 3C and S4). >90% of wild-type cells have completed the first meiotic division by between 9–10 h (Figure 3D). All six mutants showed differing degrees of aberrant progression of pre-meiotic DNA replication (see below and Figure 3A–3D). For wild-type MAT-a cells, >90% completed DNA replication 30 min after release from α-factor, while all six mutants were slower (see below and Figure 3E and 3F).

In the SK1 background, vid21A grew very slowly under pre-meiotic growth conditions and hardly showed any induction of IME1 expression (Figure 3A). Furthermore, pre-meiotic DNA replication and meiotic nuclear divisions were not detected (unpublished data).

In the rmd11A strain, the induction of IME1 was normal (Figure 3A); however DNA replication started with a delay of approximately 3 h, but proceeded with normal speed thereafter. Meiotic DSB formation and nuclear divisions occurred with a similar delay and also proceeded with fairly normal speed, but disappearance of DSBs was greatly delayed (Figure 3C and 3D), suggesting problems in DSB repair. Notably, in contrast to the SK1 background, a strong reduction in nuclear divisions had been observed for rmd11A in the W303 background (Figure 3D and Table 1). From α-factor synchronization a delay in G1 to S-phase transition was also observed for rmd11A (Figure 3E). Interestingly, according to the budding index, rmd11A does not affect the rate of bud formation (Figure 3F).

The remaining four mutants all were impaired for normal progression of pre-meiotic DNA replication. The bre1A and lge1A mutants showed normal induction of IME1 (Figure 3A). However, bre1A and lge1A started with a delay in pre-meiotic DNA replication, and 90% of the population completed DNA replication between 22–24 h (Figure 3B). Interestingly, the levels of DSBs formed in the sporulating bre1A and lge1A populations were reduced and the majority of them appeared to be repaired after 11–12 h (Figure 3C). Additionally, meiotic nuclear divisions were strongly reduced for bre1A and lge1A reaching only 57% and 52% after 48 h, respectively (Figure 3D). The sgf73A and def1A strains did not show a clear delay in entry into pre-meiotic DNA replication; however, they did show a lengthened time to complete pre-meiotic DNA replication (Figure 3B). Additionally, less than 80% of the population for both strains completed pre-meiotic DNA replication. The meiotic nuclear divisions for sgf73A and def1A were also strongly reduced reaching 27.5% and 35% after 48 h, respectively. The level of IME1 induction observed for sgf73A was reduced to 40% of wild-type levels (Figure 3A). The low level of IME1 induction could explain why sgf73A showed slow progression of pre-meiotic DNA replication and inefficient meiotic nuclear divisions. Additionally, def1A showed a mild reduction to roughly 70% of wild-type levels of IME1 induction (Figure 3A). However, this reduction does not explain the strength of the observed phenotypes in def1A. All four mutants also show a clear delay in initiation and progression of mitotic DNA replication (Figure 3E). In addition, FACS profiles show that 35% of def1A cells failed to enter G1 during α-factor synchronization (Figure 3E) and 11% of this population contained >2c (copies of the genome) DNA content. This may be partly due to defective cytokinesis after nuclear division, as 20% of def1A cells develop multiple large buds that sometimes contain more than a single nucleus.

The six mutations were also assessed in a spo11A, spo13A background to determine whether their sporulation phenotypes could be bypassed in the absence of meiotic recombination. Only def1A showed improvement of sporulation efficiency; however, spore viability was not improved in the absence of meiotic recombination (unpublished data).
To analyze sister chromatid cohesion and homologue synapsis in these mutants, meiotic nuclear spreads of each strain were immunostained for Rec8, the meiosis-specific cohesin subunit, and Zip1, a synapsis-specific component of the synaptonemal complex. As expected from the pre-meiotic DNA replication data, all strains showed a delay in the formation of Rec8 axes and were late in chromosome synapsis, with the exception of vid21Δ, which did not show

**Figure 3.** Further Characterization of VID21, BRE1, LGE1, RMD11, SGF73, and DEF1

Mutants for these genes were made in an SK1 background. The plots on each graph represent wild type (black diamonds), rmd11Δ (white diamonds), bre1Δ (black triangles), lge1Δ (white triangles), sgf73Δ (black circles), def1Δ (white circles), and vid21Δ (black squares). Where error bars are not shown, the time courses are of individual experiments. A total of three experiments were carried out in each case and the data shown are consistent with those obtained in the other experiments.

(A) The expression of IME1, a primary transcription factor required for entry into the meiotic cell cycle was assessed. SK1 strains carrying a plasmid that expresses the lacZ reporter gene under the control of the IME1 promoter were grown for synchronous meioses and assessed for lacZ expression via β-galactosidase activity [92]. W303 MAT-a mutant strains for the above genes were assessed for G1 to S phase transition in mitosis after release from α-factor arrest [87].

(B) Pre-meiotic DNA replication was assessed for synchronized meiotic cultures by FACS and the change from 2c to 4c DNA content was plotted over time. See Figure S2 for the raw data of the FACS analysis for meiotic DNA replication.

(C) DNA extractions from sporulation time courses were digested with BglII and meiotic DSB formation (DSBIII and IV) at the THR4 hotspot was assessed using Southern blotting and probing techniques [46]. See Figure S3 for the THR4 Southern blots.

(D) Nuclear divisions (MI and MII) of the synchronized meiotic cultures in (A) were assessed with fluorescence microscopy using DAPI staining to visualize nuclear division.

(E) DNA replication following release from α-factor arrest was assessed via FACS and the change from 1c to 2c DNA content was plotted against time. See Figure S4 for the raw data of the FACS analysis for mitotic DNA replication.

(F) The budding index of cells released from α-factor synchrony was assessed by phase contrast microscopy.

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axis formation or chromosome synopsis. The other five strains frequently contained Zip1 polycomplexes, an indication of a delay of synapsis relative to Zip1 expression (unpublished data). With the exception of the Zip1 polycomplexes, the majority of nuclei observed for the rmd11Δ, sgf74Δ, bre1Δ, and lge1Δ at later time points displayed normally synapsed chromosomes. However, nuclei containing long Rec8 axes and full synopsis were greatly reduced in sgf74Δ, bre1Δ, and lge1Δ mutants (unpublished data). The def1Δ strain showed interesting defects in chromosome morphology indicating uncoupling of axis formation from synopsis (Figure 4A and 4B). These events occur in parallel in wild type and also in most mutants with delayed synapsis. The def1Δ mutant synopsis phenotype represented in (A) was counted as “aligned” axes in the Rec8 analysis graph. At least 200 nuclei were counted per time point.

(B) Time course of the meiotic nuclei counted using immunocytochemistry for both wild type and def1Δ during meiosis. The def1Δ mutant synopsis phenotype represented in (A) was counted as “aligned” axes in the Rec8 analysis graph. At least 200 nuclei were counted per time point.
were formed and pairwise-aligned by one or more axial association sites, synopsis frequently did not commence, a situation not occurring in wild type (Figure 4A). Similar observations have been made for mutants of the ZMM class of meiotic genes (ZIP1, ZIP2, ZIP3, ZIP4/SPO22, MSH4, MSH5, and MER3) that are directly involved in initiation and progression of synopsis [33,47–50]. A hallmark of these ZMM mutants is the specific reduction in CO, without affecting noncrossover (NCO) recombination during meiosis. Therefore, molecular analysis of the level of CO and NCO recombination in def1 was assessed. For this, a mutation of DEF1 was created in a SK1 strain that carries a 3.5-kb URA3-ARG4 recombination interval inserted at his4 on one copy of Chromosome III and at leu2 on the homologue (Figure 4C) [44]. DNA extracted from synchronized sporulation time courses of wild type and def1 was digested with the XhoI and EcoRI restriction enzymes and used to assess DSB, NCO, and CO formation (Figures 4C–4G, S6A, and S6B). As observed for the THR4 hotspot (Figure 3C), DSB repair in def1 appears to take longer than in the wild type (Figure 4D and 4G). As a result, the appearance of CO and NCO recombination products are also delayed (Figure 4E–4G). Strikingly, the formation of COs is reduced in def1 to 35% of wild-type levels (Figure 4F), whereas NCO levels are largely unaffected (Figure 4E). Furthermore, as described in Figure 3C, DNA from the wild-type and def1 sporulation time courses were also digested with XhoI to assess meiotic DSBs and COs. Again DSB repair was delayed, and CO formation was reduced in def1 (Figure S6C–S6H). Thus, as predicted by the cytological phenotype, def1 is specifically defective in Zip1 assembly and CO control, identifying DEF1 as a ZMM gene.

**Deletion of PMR1 Causes HU Sensitivity and Formation of Multad in Meiosis**

Mutation of HUR1 has been reported to cause increased sensitivity to HU [51]. However, HUR1 partially overlaps with PMR1, a gene that encodes an ATPase required for Ca²⁺ and Mn²⁺ import into the Golgi apparatus (Mandal, et al. 2003). Therefore mutations that interrupt sections of the open reading frames of HUR1 and PMR1 separately were created (Figure S7A). This analysis revealed that deletion of PMR1 but not HUR1 affected resistance to HU (Figure S7B). FACS analysis showed that PMR1 is required for normal timing of initiation and progression of DNA replication during mitosis (Figure S7C). Additionally, pmr1Δ cells formed some abnormal “multad” asci containing more than four inviable spores (Figure S7D). The sporulation efficiency of pmr1Δ was 55%, and up to 52% of the asci contained >4 spores. Analysis of meiotic DNA replication in the pmr1Δ strain revealed that after 12 h, 30% of the cells had a DNA content greater than four copies of the genome suggesting re-replication or lack of cytokinesis prior to meiosis as the basis for multad formation (Figure S7E).

**Discussion**

**Systematic Integration of High-Throughput Data**

Although high-throughput experiments have provided insight into gene function, it has also become apparent that single datasets have limitations. False positive data are common. For yeast two-hybrid data it has been estimated that only 50% of the reported interactions are of biological relevance [27]. It is known that gene epitope tagging can result in incorrect protein localization data [20,21]. Additionally, 6.5% of the yeast genome deletion library is problematic with respect to background mutations [26]. Procedures used for high-throughput experiments can also give rise to limitations. For example, protein localization analyses have been performed in vegetative cells under normal growth conditions, whereas a number of proteins may only localize when exposed to a certain environmental condition. For yeast two-hybrid interaction experiments, the “bait” and “prey” proteins interact inside the nucleus, which in many cases is not their native cellular compartment. Protein complex purification experiments are biased towards proteins that are of high abundance [34].

Due to these limitations, a number of methods have been developed to combine datasets to determine whether the data support each other. Methods have been used to combine mRNA expression with protein interaction data [29,36,38] and from these studies it was found that proteins that interact often have a correlation in mRNA expression pattern. More recently, work combining mRNA expression, genetic interactions, and database annotations was used to validate protein interaction data [52].

Recently researchers have begun to develop a number of in-silico methods to predict gene function by integrating a number of high-throughput datasets [52–56]. However, to our knowledge only three integration methods include the high-throughput genetic interaction datasets for *S. cerevisiae* [52,55,57]. These studies either provide very little or no experimental analysis of their predictions [52,55,57]. Our data mining was based on the knowledge acquired from previous data integration techniques to set the selection criteria (Figure 1), and we have set out to test the predictions in experimental detail.

Our selection strategy identified 81 genes of which 16 (20%) caused at least one irregular DNA processing phenotype when mutated. Interestingly, all but one of these selected genes had at least one genetic interaction with a DNA processing gene. In an aim to avoid false candidates we saw fit to exclude 13 genes because the fraction of their interaction partners involved in DNA processing was less than 1/5. However, four of these genes have now been shown to have a role in DNA processing. Therefore this selection step was not beneficial.

**Genes Required for Normal Levels of Meiotic Gene Conversion and CO Formation during Meiosis**

Three genes were found to be required for normal levels of gene conversion during meiosis, SOH1, BRE5, and YGL250W.

(1) SOH1 was first discovered as a gene that suppressed the hyper-recombination phenotype of *hpr1Δ* [58]. Hpr1 is a component of the THO/TREX complex which couples transcription elongation with mitotic recombination [39]. Soh1 was later shown to be a component of the Mediator complex [60], which is required to stimulate gene transcription by transmission of regulatory signals from transcription activators to RNA polymerase II during stress responses [61]. SOH1 has a number of genetic interactions with genes required for DNA replication (e.g., RAD52, RAD50, RAD55, and RAD6), DNA repair (e.g., CDC45, MRC1, and ORC2) and chromatid cohesion (e.g., CTF4, CTF8, and CTF18), and the soh1Δ mutant...
was observed here to have sensitivity to HU in addition to reduced gene conversion levels, whilst not greatly affecting CO formation during meiosis. Our observations suggest that the Mediator complex also has a role in regulation of DNA replication and recombination.

(2) Bre5 is a conserved protein that has been shown to form a complex with ubiquitin protease Ubp3 that is required for the de-ubiquitination of subunits of coat protein complexes I and II that are involved in transport between the endoplasmic reticulum and Golgi apparatus [62,63]. BRE5 has been reported to have genetic interactions with genes involved in cell wall organization and biogenesis [13]. However, here the gene was selected for its genetic interactions with DNA processing genes [8,13]; therefore, it is conceivable that BRE5 functions in a number of cellular pathways, one of which is DNA processing. In this study, BRE5 was shown to be required for normal levels of sensitivity to HU and meiotic gene conversion. Perhaps Bre5 is required for de-ubiquitination of proteins required for DNA replication/recombination. Due to the slow growth phenotype of bre5A in pre-sporulation conditions, a meiotic-specific null allele would be required to assess its role in meiotic DNA processing more closely.

(3) YGL250W/RMR1 (named here Reduced Meiotic Recombination 1) was shown to be required for normal levels of gene conversion and CO formation during meiosis. This gene has been reported to have synthetic lethal interactions with both CDC7 and MCD1/SCC1 [13]. Interestingly, Cdc7-Dbf4 is required for recombination, synaptonemal complex formation, and chromosome segregation during meiosis [64,65]. Additionally, Mcd1/Scc1 is a subunit of the cohesin complex which is required for sister chromatid cohesion in mitosis and meiosis [66,67]. The meiotic DNA processing role of RMR1 remains to be determined. However Rmr1 appears to be sumoylated [68], which could be important for its function.

Genes Required for Normal Progression of Pre-meiotic DNA Replication

Six mutants sensitive to HU during vegetative proliferation also displayed reduced nuclear division in meiosis. These mutants were found to impair mitotic and meiotic DNA replication.

(1) The vid21A strain was found to be sensitive to X rays and MMS and the gene was found to be required for detectable expression of IME1 and initiation of pre-meiotic DNA replication. Since Ime1 is required for the initiation of meiotic events including pre-meiotic DNA replication [69], lack of Ime1 induction is sufficient to explain the phenotypes. Vid21 was recently identified as a novel component of the histone acetyltransferase NuA4 and is required for bulk H4 histone acetylation [70]. Other components of NuA4 are also required for maintenance of DNA integrity [70]. A mutant for another NuA4 subunit (Yng2) was found not to progress through meiosis [71], however expression of Ime1 and pre-meiotic DNA replication were not assessed. The chromatin remodelling Swi/Snf complex is required for high level expression of IME1 [72]. H4 histone acetylation is associated with transcriptional induction, and it is conceivable that NuA4 directly up-regulates IME1 and other early meiotic genes upon sporulation. However, a meiotic phenotype for other components of the histone acetyltransferase has not been reported.

(2) Recently, Sgf73 was found to be a component of two histone acetyltransferases, namely SAGA and SLIK [73], which are both required for gene expression. Here the Sgf73 mutant showed abnormal pre-meiotic DNA replication, and expression of IME1 was reduced. This reduced IME1 expression may account for the observed meiotic phenotypes. In addition to our observation that sgf73A is hypersensitive to HU, it has been shown that Sgf73 is required for the recruitment of the SAGA factor to upstream activating sequences that facilitates formation of the replication pre-initiation complex [74], thus confirming a direct role in DNA replication.

(3) Prior to this work, it was known that BRE1 and LGE1 are required for ubiquitination of histone H2B and K3 methyl- ation of H4 during vegetative proliferation [75]. However, their effects during meiosis had not been reported. Here we show that lge1A and bre1A strains are characterized by delayed initiation of meiotic DNA replication and lengthened time for completion. Recently, bre1A was shown to affect pre-meiotic DNA replication onset and progression, as well as DSB formation. That work found that Bre1 is an E3 ubiquitin ligase that exists as a complex with the E2 ubiquitin-conjugating enzyme Rad6 [76]. The Bre1-Rad6 complex was shown to ubiquitinate lysine 123 of histone H2B, which is required for normal levels and timing of DSB formation during meiosis. Here delay of onset and slowed progression of pre-meiotic DNA replication and reduced levels of meiotic DSBs for bre1A were also observed. Furthermore, the lge1A strain was observed to have the similar pre-meiotic S-phase pattern and reduction in meiotic DSB formation as the bre1A strain. As Lge1 co-purifies with Bre1 during vegetative proliferation [6,75], we predict that Lge1 may also function with Bre1 during meiosis. However, it should be noted that Lge1 does have a mitotic function that is independent to Bre1 and Rad6. In cells that have lost their mitochondrial genome, Lge1 is required for the induction of PDR3 and PDR5 expression which are both involved in multidrug resistance [77].

(4) Def1 forms a complex with Rad26 and recruits the E3 ubiquitin ligase Rsp5 to sites of DNA damage to ubiquitinate stalled RNA polymerase II to mark it for degradation [78–80]. However, only a minor fraction of the protein associates with Rad26 via immunoprecipitation [80], raising the possibility of other cellular roles. In fact, independent of its role with Rad26, Def1 was found to be required for telomere maintenance and shown to physically interact with Rrm3, a helicase required for replication of DNA at the telomeres [81]. Here we have shown that in addition to the sensitivity of def1A to HU, MMS, and X rays, both vegetative and pre-meiotic DNA replication are strongly affected. def1A also displays a prominent defect in the synapsis of homologous chromosomes during meiosis. Paired, but only loosely connected, chromosomes were observed, in which chromosome axes were fully formed, but synopsis had not commenced. This phenotype was also observed in mutants deficient for Zip1 loading (ZMM group) [33,47–50], some of which show similarity to components of the APC, a multi-subunit ubiquitin ligase [21]. Interestingly, def1A shows the specific reduction in COs, without affecting NCO recombination, which is another hallmark of ZMM mutants. From chromatin immunoprecipitation data, Def1 has been shown to bind to both telomeric and non-telomeric DNA [81].
Therefore it will be of interest to assess whether Def1 also binds to DNA during meiosis, and furthermore if its localization is correlated to its apparent requirement for efficient synopsis.

(5) **RMD11** was selected in this study due to a reported synthetic lethal interaction with *ade414-1A* [13] and a protein interaction with Dcc1 [2]. Prior to the completion of this work, **RMD11** was also shown to have synthetic sick interactions with **POL32** and **CSM3** [11]. Therefore, these interactions associate Rmd11 with the biological process of DNA replication. **RMD11** (Required for Meiotic Nuclear Divisions) was reported to be essential for sporulation but not to be required for **IME1** induction [22]. Here we confirmed these data for the W303 background, but showed that **RMD11** is not essential for meiosis in the efficiently sporulating SK1 strain background. In the SK1 background, pre-meiotic DNA replication was delayed, but eventually spores formed and were largely viable. Additionally, initiation of DNA replication during vegetative growth was delayed, suggesting that **RMD11** is required for the efficient initiation of DNA replication. Furthermore, *rmd11A* was found to have an increased sensitivity to HU, which slows or inhibits DNA replication. Interestingly, Rmd11 is a member of an uncharacterized protein family that includes members in many model organisms as well as **Homo sapiens**.

(6) In addition to HU hypersensitivity, *pmr1* was found by FACS analysis to affect pre-meiotic DNA replication and result in the formation of ascii with more than four inviable spores. Pmr1 is an ATPase required for Ca2+ and Mn2+ transport into the Golgi [82]. However, **PMR1** has genetic interactions with genes involved in DNA replication (e.g., **POL32** and **RRM3**), DNA repair (e.g., **MRE11**, **RAD55**, **RAD51**, **RAD18**, **MMS1**, and **RTT107**), and chromatid cohesion (e.g., **DCC1**, **CTF4**, and **CTF18**), and the mutant phenotypes observed in this study suggest that **PMR1** also plays a role in DNA processing.

Conclusions

A strategy of integrating high-throughput data can be successfully used to imply a role in DNA processing for minimally characterized genes. Genetic interaction data have proved to be extremely valuable in the success of our selection strategy. This feature encourages further genetic interaction analyses to be performed not only in yeast, but in all model organisms.

Among the 16 genes identified to be involved in DNA processing, 11 had a role in meiotic DNA processing, including **DEF1**, which was found to be required for efficient chromosome synopsis and specific reduction in CO, without affecting NCO recombination during meiosis. In addition, three genes (**SOH1**, **BRE5**, and **YGL250W/RMR1**) were found to be required for normal levels of meiotic gene conversion and three genes (**YPL017C**, **SOH1**, and **MMS22**) required for accurate chromosome segregation during meiosis.

Materials and Methods

Parent and deletion strains. All strains used in this work are presented in Table S4. Deletion strains were transformed with PCR-generated disruption cassettes containing the **KANMX4** marker gene [83-84]. Gene deletions were confirmed by PCR for three clones of each transformation.

Mitotic DNA processing screens. Spot plates were prepared on YPD (control) YPD + 100 mM HU (Sigma), YPD + 0.03% MMS (Sigma), YPD exposed to 40 min of X ray 120 kVp (Torrex cabinet X-ray system, Faxitron X-ray Corporation).

Molecular analyses. Meiotic DSBs and recombination products were detected by Southern blotting using 32P-ATP (GE Healthcare) labeled DNA probes. Signals were detected using the Storm Phosphoimager (GE Healthcare) and blots were quantified using ImagerVision 1.37 [85]. The methods used for the physical analysis of DSBs at the **THTR4** hotspot have been previously described [46]. The methods used for the physical analysis of DSBs, COs, and NCOs of the diploid strains that carry a 3.5-kb **URA3-ARG4** recombination interval inserted at his4 on one copy of Chromosome III and at les2 on the homolog have been previously described [44].

Cytology. Yeast meiotic spreads were performed as described [67,80]. Rec8-HA was detected using mouse anti-HA, 1:1,600 and CY3-conjugated goat anti-mouse antibody (1:200, Dianova). Rabbit anti-Zip1 antibody was raised against a purified Zip1-GST fusion protein and affinity purified against the same protein. The purified Zip1 antibody was used (1:50) and detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit serum (1:200, Sigma).

Cell synchrony and analysis of DNA replication. Haploid MAT-a cells were synchronized in G1 with α-factor using a method previously described [87]. SK1 diploids were synchronized for meiosis using a method previously described [88]. Cells were prepared for FACS analysis using a method previously described [89] and observed using a FACS Calibur (BD Biosciences) and CellQuant software, version 3.3 (BD Biosciences).

Random spore analysis for meiotic gene conversion and chromosome missegregation assays. Sporulation efficiency was determined for W303 diploids and the equivalent of 5 × 107 tetrads were digested with 100 µg/ml Zymolase (Zymo Research). Single spores were plated on YPE + 50 µg/ml CSM3 + Mn2+ or SC + CSM3 + 100 mM HU (Sigma) to select for haploid cells. To test for gene conversion, lysine was omitted, and to assess missegregation, adenine was omitted.

Supporting Information

Dataset S1. Gene Accession Numbers

Found at doi:10.1371/journal.pgen.0030222.s001 (21 KB XLS).

Figure S1. Examples of Selected Genes with an Implied Role in DNA Processing

Examples of genes having two interactions that have gene expression correlation distances with DNA processing genes below the cut-off value of 0.9.

Figure S2. Southern Blot of DNA Isolated from (A) Wild Type, (B) SK1 Strains Containing the Ectopic **URA3-ARG4** Interval on Chromosome III (D)

The DNA from the indicated times after initiation of sporulation
were digested with XhoI then probed to detect COs and DSBs; mw1 represents the λ-HindIII molecular weight marker (Fermentas) and mw2 represents the 1-kb molecular weight marker (Fermentas).

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Figure S3. FACS Analysis of Pre-Meiotic DNA Replication for VID21, BRE1, LGE1, RMD11, SGF73, and DEFI Mutants

Raw output from FACS analysis of each SK1 strain synchronized for entry into the meiotic cell cycle. Cells were counted at a rate between 250–280 cells per s. FACS Calibur apparatus and CellQuant Version 3.3 (BD Biosciences) were used for analysis.

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Figure S4. Southern Blot Analysis of DSB Formation during Meiosis for BRE1, LGE1, RMD11, and DEFI Mutants

(A) Schematic representation of the natural THR4 meiotic DSB hotspot on Chromosome III. DNA from synchronized sporulation time courses of each strain were cut with the BglIII restriction enzyme, and using a probe upstream to THR4, presence of DSBIII and DSBIV (indicated by the arrows) together with the parental were assessed [87]. Southern blots of wild type (B), mdr1Δ (C), del1Δ (D), bre1Δ (E), and dge1Δ (F). The DNA from the indicated times after initiation of sporulation were digested with BglIII then probed to detect DSBIII and DSBIV from the THR4 hotspot; mw represents the 1-kb molecular weight marker (Fermentas).

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Figure S5. Analysis of Mitotic DNA Replication for VID21, BRE1, LGE1, RMD11, and SGF73 Mutants

(A) Overnight cultures were diluted in series by a factor of 10 from 10−2 to 10−5 and plated on YPD and YPD + 100 mM HU. Mutants were defined as having increased sensitivity or decreased viability to HU in comparison to the wild type.

(B) FACS analysis of each MAT-a strain synchronized in G1 with 2-factor and then released into S phase. Cells were counted at a rate between 250–280 cells per s. FACS Calibur apparatus and CellQuant Version 3.3 (BD Biosciences) were used for analysis.

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Figure S6. Assessment of del1Δ in Meiosis

Southern blot of DNA isolated from SK1 wild-type (A) and del1Δ (B) strains containing the ectopic URA3-ARG4 interval on Chromosome III (Figure S2). The DNA from the indicated times after initiation of sporulation were digested with XhoI and EcoRI then probed to detect NCOs, COs, and DSBs; mw1 represents the λ-HindIII molecular weight marker (Fermentas) and mw2 represents the 1-kb molecular weight marker (Fermentas).

Southern blot of DNA isolated from SK1 wild-type (C) and del1Δ (D) strains containing the ectopic URA3-ARG4 interval on Chromosome III described in Figure 3. The DNA from the indicated times after initiation of sporulation were digested with XhoI then probed to detect COs and DSBs; mw1 represents the λ-HindIII molecular weight marker (Fermentas) and mw2 represents the 1-kb molecular weight marker (Fermentas).

(E) Molecular analysis for DSB (DSB1 + DSB2) signal/total lane signal from Southern blots of DNA extracted from synchronized meiotic cultures.

(F) Molecular analysis for CO1 signal/total lane signal from Southern blots of DNA extracted from synchronized meiotic cultures.

(G) Molecular analysis for CO2 signal/total lane signal from Southern blots of DNA extracted from synchronized meiotic cultures.

(H) Molecular analysis for CO (CO1 + CO2) signal/total lane signal from Southern blots of DNA extracted from synchronized meiotic cultures.

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Figure S7. Assessment of pmr1Δ and hur1Δ in Mitosis and Meiosis

(A) Diagram showing the 181-bp overlap between the open reading frames for HUR1 and PMR1 on Chromosome VII; two KANMX4 gene deletion mutants were constructed using cassettes that only interfere with either HUR1 (1–81 bp of HUR1 ORF, hur1Δ–81, represented by black region) or PMR1 (1–2,233 bp of PMR1 ORF, pmr1Δ–2,233, represented by grey region). The arrows on each strand represent direction of transcription.

(B) HU sensitivity assay using the hur1Δ–81 and pmr1Δ–2,233 strains shows that PMR1 and not HUR1 is required for resistance to HU.

(C) FACS analysis of cells released from α-factor synchomy shows that the progression of mitotic DNA replication is slowed in pmr1Δ–2,233.

(D) Microscopy of sporulation sample of SK1 wild type and pmr1Δ–2,233 using differential interference contrast (DIC) and fluorescence microscopy to view segregation of Chromosome V (tagged with green fluorescent protein). The SK1 pmr1Δ–2,233 mutant not only gives rise to tetrads, but also “multids” that contain greater than four spores.

(E) DNA replication during meiosis was assessed via FACS. 35% of the population of the pmr1Δ–2,233 cells have a DNA content that is greater than a single round of diploid DNA replication (>4c) observed in the wild type. The pmr1Δ–2,233 strain does not grow well during pre-meiotic conditions; therefore the FACS analysis experiment was not optimal.

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Table S1. List of DNA Processing Genes Used for Selection

Found at doi:10.1371/journal.pgen.0030222st001 (124 KB XLS).

Table S2. List of Strains Used in This Work

Found at doi:10.1371/journal.pgen.0030222st002 (474 KB XLS).

Table S3. List of Five Subdivided Categories of the 81 Genes Selected for Analysis

Found at doi:10.1371/journal.pgen.0030222st003 (175 KB XLS).

Table S4. List of Twenty Categories of the 81 Genes Selected for Analysis

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References

1. Hazbun TR, Malstrom L, Anderson S, Graczyk BJ, Fox B, et al. (2003). Assignment function to yeast proteins by integration of technologies. Mol Cell 12: 1353.

2. Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, et al. (2001). A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc Natl Acad Sci U S A 98: 4569–4574.

3. Uetz P, Giot L, Cagney G, Mansfield TA, Judson A, et al. (2000). A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature 403: 623.

4. Gavin AC, Aloy P, Grandi P, Krause R, Boesche M, et al. (2006). Proteome survey reveals modularity of the yeast cell machinery. Nature 440: 631.

5. Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415: 141.

6. Ho Y, Gruhler A, Heilbut A, Bader GD, Moore L, et al. (2002). Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. Nature 415: 180.

7. Krokan NJ, Cagney G, Yu H, Zhong G, Guo X, et al. (2006). Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. Nature 440: 637.

8. Bellouai M, Chang M, Ou J, Xu H, Boone C, et al. (2003). Egl1 forms an alternative RFC complex important for DNA replication and genome integrity. EMBO J 2: 4304–4313.

9. Davierwala AP, Haynes J, Li Z, Brost RL, Robinson MD, et al. (2005). The synthetic genetic interaction spectrum of essential genes. Nat Genet 37: 1147–1152.
10. Meadlay V, Baetz K, Guazzo J, Yuen K, Kwok T, et al. (2005). Systematic yeast synthetic lethal and synthetic dosage lethal screens identify genes required for chromosome segregation. Proc Natl Acad Sci U S A 102: 13956–13961.

11. Pan X, Ye P, Yuan DS, Wang X, Badger JS, et al. (2006). A DNA integrity network in the yeast Saccharomyces cerevisiae. Cell 124: 1069.

12. Tong AHY, Evangelista M, Parsons AB, Xu H, Badger GD, et al. (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294: 2364–2380.

13. Tong AHY, Lesage G, Badger GD, Ding H, Xu H, et al. (2004). Global mapping of the yeast genetic interaction network. Science 303: 808–813.

14. Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, et al. (1998). The transcriptional program of sporulation in budding yeast. Science 282: 699–705.

15. Gach AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, et al. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11: 4241–4257.

16. Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, et al. (2000). Functional discovery via a compendium of expression profiles. Cell 102: 109.

17. Primig M, Williams RM, Winzeler EA, Tevzadze GG, Conway AR, et al. (2000). The core mitotic transcriptionic in budding yeast. Nat Genet 26: 185.

18. Roberts CJ, Nelson B, Marton MJ, Stoughton R, Meyer MR, et al. (2000). Signaling and circuitry of multiple MAPK pathways revealed by a matrix of global gene expression profiles. Science 287: 889–895.

19. Tong AHY, Patel CJ, Widicka L, Lockhart DJ, Weissman JS, et al. (2000). Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101: 249.

20. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, et al. (2003). Global gene expression analysis of yeast cells grown in shaking culture. Proceedings of the National Academy of Sciences of the United States of America 100: 1102–1107.

21. Turner EA, Batt WD, Fioretti G, Capra MD, Smith JL, et al. (2003). A set of ordered yeast deletion mutants and its application to comparative genome analysis. Nat Genet 31: 449–454.

22. Enyenihi AH, Saunders W (2003). Large-scale functional genomic analysis of the human genome. Curr Opin Biotechnol 14: 459–464.

23. Kassir Y, Granot D, Simchen G (1988). IME1, a positive regulator gene of meiosis in Saccharomyces cerevisiae. Cell 52: 853.

24. Hannich TJ, Lewis A, Kroetz MB, Li SJ, Heide H, et al. (2005). Defining the central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. Cell 124: 1069.

25. Balakrishnan R, Christie KR, Costanzo MC, Dolinski K, Dwight SS, et al. (2001). Yeast General Repository DIP: Database of Interacting Proteins. Nucleic Acids Res. 28: 289–291.

26. Krogan NJ, Baetz K, Keogh MC, Datta N, Sawa C, et al. (2004). Regulation of the yeast Golgi to endoplasmic reticulum retrograde transport. J Biol Chem 278: 27507–27512.

27. Krogan NJ, Baetz K, Keogh MC, Datta N, Sawa C, et al. (2004). Regulation of the yeast Golgi to endoplasmic reticulum retrograde transport. J Biol Chem 278: 27507–27512.

28. Hwang D, Rust AG, Ramsey S, Smith JJ, Leslie DM, et al. (2005). A high-resolution protein interaction map of the yeast mediator complex. Nucleic Acids Res 33: 5367–5374.

29. Game JC, Williamson MS, Baccari C (2005). X-ray survival characteristics and genetic analysis for nine Saccharomyces deletion mutants that show altered radiation sensitivity. Genetics 169: 51–63.

30. Measday V, Baetz K, Zeniv D, Arguiñano M, Simchen G (1993). A short distance from the centromere to cellular target pathways. Nat Biotechnol 22: 62.

31. Allers T, Lichten M (2001). Synthetic lethal and synthetic dosage lethal screens identify genes required for meiotic chromosome synopsis. Cell 102: 245.

32. Mandel S, Robybr K, Kassir Y (1994). MEI1 gene encodes a transcription factor which is required to induce meiosis in Saccharomyces cerevisiae. Dev Genet 15: 139–147.

33. Tong AHY, Lesage G, Badger GD, Ding H, Xu H, et al. (2004). Global mapping of the yeast genetic interaction network. Science 303: 808–813.

34. Game JC, Williamson MS, Baccari C (2005). X-ray survival characteristics and genetic analysis for nine Saccharomyces deletion mutants that show altered radiation sensitivity. Genetics 169: 51–63.

35. Parsons AB, Brost RL, Ding H, Li Z, Zhang C, et al. (2004). Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways. Nat Biotechnol 22: 62.

36. Begley TJ, Rosenbach AS, Ideker T, Samson LD (2004). Hot spots for chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways. Nat Biotechnol 22: 62.

37. Allers T, Lichten M (2001). Synthetic lethal and synthetic dosage lethal screens identify genes required for meiotic chromosome synopsis. Cell 102: 245.

38. Mandel S, Robybr K, Kassir Y (1994). MEI1 gene encodes a transcription factor which is required to induce meiosis in Saccharomyces cerevisiae. Dev Genet 15: 139–147.

39. Goldway M, Sherman A, Zeniv D, Arguiñano M, Simchen G (1993). A short distance from the centromere to cellular target pathways. Nat Biotechnol 22: 62.

40. Measday V, Baetz K, Zeniv D, Arguiñano M, Simchen G (1993). A short distance from the centromere to cellular target pathways. Nat Biotechnol 22: 62.

41. Allers T, Lichten M (2001). Synthetic lethal and synthetic dosage lethal screens identify genes required for meiotic chromosome synopsis. Cell 102: 245.

42. Mandel S, Robybr K, Kassir Y (1994). MEI1 gene encodes a transcription factor which is required to induce meiosis in Saccharomyces cerevisiae. Dev Genet 15: 139–147.

43. Goldway M, Sherman A, Zeniv D, Arguiñano M, Simchen G (1993). A short distance from the centromere to cellular target pathways. Nat Biotechnol 22: 62.

44. Measday V, Baetz K, Zeniv D, Arguiñano M, Simchen G (1993). A short distance from the centromere to cellular target pathways. Nat Biotechnol 22: 62.

45. Allers T, Lichten M (2001). Synthetic lethal and synthetic dosage lethal screens identify genes required for meiotic chromosome synopsis. Cell 102: 245.

46. Mandel S, Robybr K, Kassir Y (1994). MEI1 gene encodes a transcription factor which is required to induce meiosis in Saccharomyces cerevisiae. Dev Genet 15: 139–147.

47. Goldway M, Sherman A, Zeniv D, Arguiñano M, Simchen G (1993). A short distance from the centromere to cellular target pathways. Nat Biotechnol 22: 62.
histone H4 acetylation activity is required for mitotic and meiotic progression. J Biol Chem 276: 43653–43662.

72. Yoshimoto H, Wada H, Yamashita K (1995). Transcriptional regulation of meiosis-inducing IME1 and IME2 genes by GAM gene products in Saccharomyces cerevisiae. Biosci Biotech Biochem 57: 1784–1787.

73. Pray-Grant MG, Schlietz D, McMahon SJ, Wood JM, Kennedy EL, et al. (2002). The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. Mol Cell Biol 22: 8774–8780.

74. Shukla A, Bajwa P, Bhaumik SR (2006). SAGA-associated Sgf73p facilitates formation of the preinitiation complex assembly at the promoters either in a HAT-dependent or independent manner in vivo. Nucleic Acids Res 34: 6225–6252.

75. Hwang WW, Venkatasubrahmanyan S, Lanculescu AG, Tong A, Boone C, et al. (2003). A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. Mol Cell 11: 261.

76. Yamashita K, Shinohara M, Shinohara A (2004). Rad6-Bre1-mediated histone H2B ubiquitylation modulates the formation of double-strand breaks during meiosis. Proc Natl Acad Sci U S A 101: 11380–11385.

77. Zhang X, Kolaczkowska A, Devaux F, Panwar SL, Hallstrom TC, et al. (2005). Transcriptional regulation by Lge1p requires a function independent of its role in histone H2B ubiquitination. J Biol Chem 280: 2759–2770.

78. Reid J, Svejstrup JQ (2004). DNA damage-induced Def1-RNA polymerase II interaction and Def1 requirement for polymerase ubiquitylation in vitro. J Biol Chem 279: 29875–29878.

79. Somesh BP, Reid J, Liu WF, Erdjument-Bromage H, Tempst P, et al. (2005). Multiple mechanisms confining RNA polymerase II ubiquitylation to polymerases undergoing transcriptional arrest. Cell 121: 913.

80. Woudstra EC, Gilbert C, Fellows J, Jansen L, Brouwer J, et al. (2002). A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. Nature 415: 929.

81. Chen YB, Yang CP, Li RX, Zhou JQ (2003). Def1p is involved in telomere maintenance in budding yeast. J Biol Chem 280: 24784–24791.

82. Mandal D, Rulli SJ, Rao R (2003). Packing interactions between transmembrane helices alter ion selectivity of the yeast Golgi Ca2+/Mn2+-ATPase PMR1. J Biol Chem 278: 35292–35298.

83. Gietz DR, Woods RA (2002). Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. Methods Enzymol 350: 87–96.

84. Wach A, Brachat A, Pohlmann R, Philipsen P (1994). New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast 10: 1793–1808.

85. Abramoff MD, Magelhaes PJ, Ram SJ (2004). Image processing with ImageJ. Biophotonics International 11: 36–42.

86. Loidl J, Klein F, Engebrecht J (1998). Genetic and morphological approaches for the analysis of meiotic chromosomes in yeast. Methods Cell Biol. 53: 257–285.

87. Breeden LL (1997). a-factor synchronization of budding yeast. Methods Enzymol 283: 332–342.

88. Amon A (2002). Synchronization procedures. Methods Enzymol 351: 457–467.

89. Haase SB, Lew DJ (1997). Flow cytometric analysis of DNA content in budding yeast. Methods Enzymol 283: 322–332.

90. Rockmill B, Lambie EJ, Roeder GS (1991). Spore enrichment. Methods Enzymol 194: 146–149.

91. Breitkreutz B, Stark C, Tyers M (2003). Osprey: A network visualization system. Genome Biol 4: R22.

92. Rose M, Botstein D (1983). Construction and use of gene fusions to lacZ (β-galactosidase) that are expressed in yeast. Methods Enzymol 101: 167–180.