Supporting Text

Materials and Methods

Judging Morphological Mutants. Because statistical tests are not applicable to our data set, with only one datum for each deletion strain, we estimated the distribution of wild-type data and defined morphological normality of a deletion strain as the probability of wild-type data to take value outside of the deletion-strain value.

For each morphological parameter, the morphological abnormality of a deletion strain is measured in three steps.

Box–Cox Power Transformation. The wild-type data are divided by the mean and transformed by the function defined below (1).

$$F_{p,a}(x) := \begin{cases} \log(x+a) & \text{if } p = 0 \\ \dfrac{(x+a)^p - 1}{p} & \text{otherwise} \end{cases}$$

where p and a are transformation parameters.

Then the transformed data are standardized by $y = \dfrac{F_{p,a}(x) - Mean}{SD}$, where $Mean$ is the mean of the transformed data, and *SD* is the standard deviation of the transformed data. The transformation parameters are chosen to minimize the Anderson–Darling statistic,

$$D = \max_{-\infty < r < \infty} \frac{\left| S(r) - N(r) \right|}{\sqrt{N(r)(1 - N(r))}},$$

where S is the distribution function of the transformed wild-type data, and N is the distribution function of the fitted normal distribution.

Shapiro–Wilk Test for Normality. Then the Shapiro–Wilk test (2) is conducted to test how well the transformed data fit to a normal distribution. If the *P* value of the Shapiro–Wilk test is lower than a given threshold, this morphological parameter will not be used in judging abnormality of deletion strains because the estimated normal distribution is not reliable.

Thresholding *P* value with 0.3, 0.5, and 0.7, the number of parameters whose *P* value is greater than or equal to the threshold is 349, 254, and 161, respectively.

Abnormality of the Deletion Strain Morphology. On the assumption that the transformed wild-type data follow the estimated normal distribution, we defined the morphological normality of a deletion strain as $p \coloneqq min(N(F(x)),1-N(F(x)))$, where x is the deletion strain data, F is the transformation (including scaling and standardization described above) function that minimizes the Anderson–Darling statistic of the transformed wild-type data, and N is the distribution function of the estimated normal distribution. Given a threshold value, a deletion strain is judged to be a morphological mutant if the morphological normality *p* is less than or equal to the threshold.

Estimating the Number of Mutants. For a given *p* value threshold, denoted by P, deletion strains whose morphological normality *p* is less than or equal to the threshold in at least one parameter are counted as morphological mutants. The number of mutants expected by chance or the expected number of false positive mutants is calculated by $n = 2P \times n_p \times n_d (\approx (1-(1-2P)^{n_p}) \times n_d$ for $P \leq \mathrm{E}-03)$, where n_p is the number of parameters and n_d is the number of deletion strains. Independence of all parameters is assumed for $(1-(1-2P)^{n_p}) \times n_d$ and the worst-case dependency is assumed for $2P \times n_p \times n_d$.

Thresholding the Shapiro–Wilk test *P* value with 0.3, 0.5, 0.7, and changing the morphological normality threshold *P* from E-03 to E-06, the number of mutants

decreases as in Table 9. For further study, we chose thresholding the Shapiro–Wilk test *P* value with 0.5, resulting in statistically robust parameters of 254. Deletion strains whose morphological normality *p* is less than or equal to the threshold in at least one parameter are counted as mutants in Table 9; the number of mutants are 2,378 (the morphological normality threshold *P* of E-04) with a low false positive rate. Tables 2 and 3 describe the detail statistics of every parameter and ORF, respectively.

Verification of Candidate Strains. Replacement of the yeast genes by the kanamycin-resistant gene cassette (3) was verified by PCR using genomic DNA as a template. To confirm ploidy, DNA content of logarithmic-phase cells was determined by flow cytometry. Mating type was verified by crossing the candidate with either mating-type tester strain YOC1518 (*MAT***a** *leu1*) or YOC1811 (*MAT*α *leu1*) and by examining a leucine-auxotrophic phenotype of the resultant diploid. The *rad18* deletion strain was an α cell, and the *ctf8* deletion strain was a mixture of **a** and α cells, consistent with the notes of European *Saccharomyces cerevisiae* Archive for Functional Analysis (EUROSCARF). DNA content of the *scp160* deletion strain was 2*N*, which was the known phenotype (4). The *scp160* deletion strain was not further characterized. The rest of the strains used in Fig. 4 were all confirmed to be *MAT***a**, carrying a correct mutation with haploid-type DNA content.

To verify that the gene disruption in each strain results in the morphological phenotype, candidate strains were backcrossed with BY4742 (*MAT*α *leu2 his3 lys2 ura3*). Resultant diploids were sporulated, and a single ascus with tetrad spores was examined. Morphological phenotypes obtained from three sets of dissected tetrad (12 spores) were analyzed by CALMORPH, and linkage of the kanamycin-resistant phenotype to an average value of the morphological parameters was tested. In 29 mutants of a total of 30 candidate mutants, kanamycin resistance was linked to at least one parameter. We found that in the case of *rho4*, an average value of the morphological parameter used for the prediction was not linked with kanamycin cassette. Therefore, we omitted the *rho4* strain from further characterization.

Results and Discussion

Functional Prediction for the Category "Recombinational Repair." Based on the functional prediction for the category "recombinational repair," we identified 19 strains that were characterized by the parameters "ratio of cells in nuclear division" and "ratio of large-budded cells to budding cells before completion of nuclear division" (Fig. 4*A*). Among the deleted genes, seven genes are classified into two DNA repair epistasis groups, including recombination repair epistasis and postreplicational repair epistasis groups. Six genes (*RAD50*, *RAD51*, *RAD52*, *RAD54*, *MRE11*, and *XRS2*) are members of the recombination repair epistasis group, which are involved in the repair of double-stranded breaks (DSBs) in DNA (5). One gene (*RAD18*) belongs to the postreplicational repair epistasis group, whose product forms a stable complex with single-stranded DNA-binding, ATPase, and ubiquitin-conjugating activities (6). Mutants of these genes are defective in the repair of DNA damage caused by ionizing radiation and several damaging agents, such as methyl methanesulfonate (MMS) or hydroxyurea (HU).

In addition, we identified 12 less characterized genes (Fig. 4*A*). To know the functions of these genes, we referred to gene ontology. The vast majority belongs to gene ontology categories consisting of DNA metabolic processes. Three genes (*CTF4*, *CTF8*, and *CTF18*) are required for sister chromatid cohesion and faithful chromosome transmission (7, 8). Three genes (*RTT107*, *MMS1*/*RTT108*, and *RTT109*) were previously identified as genes involved in the regulation of Ty*1* transposition (9). Three genes (*HEX3*/*SLX5*, *ASF1*, and *EST1*) are involved in "DNA damage response," "chromatin assembly," and "telomere length regulation," respectively (10-12). The remaining three genes (*DIA2*, *SAC3*, and *YNL171C*) were not previously known to affect DNA metabolic processes.

To confirm whether deletion of each candidate affects DNA damage response, we examined the sensitivity of each strain to two DNA-damaging agents, MMS and HU. As anticipated from previous studies (13, 14), most of the mutants are sensitive to at least one agent (Table 8). We also found that *dia2*Δ or *sac3*Δ cells are also moderately

sensitive to MMS or HU (Table 8). These results suggested that all candidates have common functional defects related to DNA metabolism.

To further obtain the indications that *DIA2* and *SAC3* are involved in DNA metabolic processes, we examined whether they have a genetic interaction with the Rad18p- or Rad52p-dependent repair pathway. We decided not to analyze *YNL171C* further because this ORF overlaps with two other ORFs (*YNL170W* and *YNL172W*), and cells with only *YNL171C* deleted exhibited no phenotype with respect to cell morphology and damaging agent sensitivity (data not shown). The heterozygous diploids were constructed by crossing the *dia2*Δ or *sac3*Δ strains with *rad18* and *rad52*. After tetrad dissection, plates spotted with segregants were incubated for 3–5 days at 25°C. Interestingly, we found that coupling *dia2*Δ with *rad18*Δ or *rad52*Δ deletion causes extremely slow growth relative to any of the single mutant strains (Fig. 7*A*). Similarly, *sac3*Δ resulted in synthetic slow growth phenotype when coupled with each *rad* deletion (Fig. 7*A*). These synthetic genetic interactions may reflect either an enhanced delay in distinct repair from the Rad18p- or Rad52p-dependent pathway, or an enhanced frequency of spontaneous damages requiring the Rad18p- or Rad52p-dependent pathway for repair. In addition, the morphological phenotypes of the *sac3*Δ cells that are characteristic for DNA repair mutants were partially suppressed by deleting *RAD9* (Fig. 7 *B* and *C*). This result suggested that morphological phenotypes resulting from G_2/M accumulation seen in *sac3*Δ cells are dependent on *RAD9*. Our observation supports the hypothesis that unrepaired DNA damages monitored by Rad9p are accumulated in *sac3*Δ.

Dia2p is an F-box protein involved in invasive and pseudohyphal growth (15), although its molecular function remained unknown. Sac3p has been implicated in mRNA or protein export from the nucleus (16, 17) and has been shown to be required for normal mitotic progression and spindle morphology (18). This protein localized to the nuclear pore, where it interacts with nucleoporins (19). Therefore, current work suggests that nuclear pore and/or intranuclear transport somehow functions in DNA replication or DNA repair by preventing the accumulation of unrepaired DNA damage (20, 21).

Functional Prediction for the Category "Cell Wall Biosynthesis." We identified 10 mutants as candidates affecting cell wall integrity (Fig. 4*A*). To define the role of these gene products in cell wall assembly and remodeling, these mutants were further characterized. First, sensitivity of the candidate mutants to calcofluor white (CFW), echinocandin B (EB), caffeine, caffeine with sorbitol, and SDS were examined (Fig. 8*A*). It has been known that hypersensitivity or resistance to CFW, EB, caffeine, caffeine with sorbitol, or SDS is indicative of cell-surface defects (22-24). CFW is a negatively charged fluorescent dye that binds to nascent chains of chitin and, to a lesser extent, glucan through hydrogen bonding and dipole interactions. It is believed that CFW interferes directly with the molecular organization of the cell wall by preventing microfibril assembly (22, 23). EB is a glucan synthase inhibitor (25). Caffeine is an inhibitor of cAMP phosphodiesterases (26). Sorbitol and caffeine are used to analyze the mutants affecting the cell integrity *PKC1*-controlled MAP kinase pathway (27, 28). SDS is an anionic detergent that affects the growth of the cell wall mutants (29). Nine of 10 mutants showed hypersensitivity to one or multiple drugs, which indicate cell-surface defects (Fig. 8*A*). *cog1*, *mnn10*, *och1*, and *vps16* deletion strains exhibit hypersensitivity to all drugs. *arf1*, *cap1*, *end3*, *sac6*, and *she4* deletion strains showed hypersensitivity to multiple drugs. Sensitivity to these drugs by the *cap1* deletion strain was indistinguishable from the control strain.

Second, direct biochemical measurements of monosaccharide composition of alkali-soluble/insoluble fraction of cell extracts (30, 31) revealed that 9 of 10 candidates have some defects in the cell wall. The mass ratio of hexoses that are detected in each alkali-insoluble and alkali-soluble fraction was high in *arf1*, *cax4*, *cog1*, *end3*, *mnn10*, and *sac6* mutants, suggesting that glucan and/or mannoprotein (major components of the alkali-soluble fraction) contents were low in these mutants (Fig. 8*B*). The mass ratio of the proportions of chitin and hexose that was detected in the alkali-insoluble fractions was low in *arf1*, *cax4*, *mnn10*, and *och1* mutants, suggesting that chitin content was high in these mutants. Alternatively, alkali-insoluble glucan might be low in these mutants. The mass ratio of the proportions of glucose and mannose that was detected in the alkali-soluble fractions was low in *cax4*, *end3*, *mnn10*, *och1*, *sac6*, and *she4* mutants,

suggesting that mannose content was low in these mutants. Monosaccharide composition in the *cap1* mutant was indistinguishable from the control strain.

Finally, to visualize the cell wall of the strains, cells were cultured in yeast extract/peptone/dextrose (YPD) and the cell wall was observed with transmission electron microscopy (TEM). Eight mutants possessed abnormal cell wall morphology compared with the wild type (Fig. 8*C*). Whereas cell wall layers of the control cells (*his3* deletion strain) were 50–100 nm in thickness, the cell-wall thickness of seven mutants (*cax4*, *cog1*, *end3*, *mnn10*, *och1*, *sac6*, and *she4*) was more than 150 nm. The *end3* and *sac6* deletion strains are composed of sparse materials. A characteristic property of the *she4* deletion strain was that in this strain, the cell wall in mother cells was thicker than that in bud. In addition, thick septa at the bud neck between the mother and daughter cells were formed in *vps16*, *cax4*, *cog1*, *mnn10*, and *och1* deletion strains. These mutants appeared similar to the *chs2* and *myo1* strains in that the lateral walls thicken gradually and finally meet over an extended region, giving rise to a thick septum lacking normal trilaminar structure (32). Apparent structural abnormalities of the cell wall were not observed in *arf1* and *cap1* deletion strains.

Previous studies indicated that 3 genes of 10 candidate genes are involved in cell wall biosynthesis. *MNN10*, *OCH1*, and *CAX4* were annotated to N-linked glycosylation, according to GO terms. *MNN10* and *OCH1* were identified as the α-1,6-mannosyltransferase in the biosynthesis of N-glycoproteins (33). *CAX4* encodes the dolichyl-pyrophosphate phosphatase, which is required for dolichyl pyrophosphate-linked oligosaccharide intermediate synthesis and protein N-glycosylation (34). Although the other seven genes have not been annotated to the process of cell wall biosynthesis, our analyses on the deleted strains suggest that most of the identified mutations result in defects of cell wall remodeling. To maintain cell wall integrity, two biological processes seem to be required: secretory transport and actin organization (35). Our results are consistent with the idea that cell wall remodeling links to secretory transport pathway and actin organization.

1. Box, G. E. P. & Cox, D. R. (1964) *J. R. Stat. Soc.* **26,** 211–252.

2. Shapiro, S. S. & Wilk, M. B. (1965) *Biometrika* **52,** 591–611.

3. Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P. & Boeke, J. D. (1998) *Yeast* **14,** 115–132.

4. Wintersberger, U., Kuhne, C. & Karwan, A. (1995) *Yeast* **11,** 929–944.

5. Symington, L. S. (2002) *Microbiol. Mol. Biol. Rev.* **66,** 630–670.

6. Broomfield, S., Hryciw, T. & Xiao, W. (2001) *Mutat. Res.* **486,** 167–184.

7. Mayer, M. L., Gygi, S. P., Aebersold, R. & Hieter, P. (2001) *Mol. Cell* **7,** 959–970.

8. Hanna, J. S., Kroll, E. S., Lundblad, V. & Spencer, F. A. (2001) *Mol. Cell. Biol.* **21,** 3144–3158.

9. Scholes, D. T., Banerjee, M., Bowen, B. & Curcio, M. J. (2001) *Genetics* **159,** 1449–1465.

10. Mullen, J. R., Kaliraman, V., Ibrahim, S. S. & Brill, S. J. (2001) *Genetics* **157,** 103–118.

11. Ramey, C. J., Howar, S., Adkins, M., Linger, J., Spicer, J. & Tyler, J. K. (2004) *Mol. Cell. Biol.* **24,** 10313–10327.

12. Viscardi, V., Baroni, E., Romano, M., Lucchini, G. & Longhese, M. P. (2003) *Mol. Biol. Cell* **14,** 3126–3143.

13. Chang, M., Bellaoui, M., Boone, C. & Brown, G. W. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 16934–16939.

14. Hartman, J. L., IV, & Tippery, N. P. (2004) *Genome Biol.* **5,** R49.

15. Palecek, S. P., Parikh, A. S. & Kron, S. J. (2000) *Genetics* **156,** 1005–1023.

16. Lei, E. P., Stern, C. A., Fahrenkrog, B., Krebber, H., Moy, T. I., Aebi, U. & Silver, P. A. (2003) *Mol. Biol. Cell* **14,** 836–847.

17. Jones, A. L., Quimby, B. B., Hood, J. K., Ferrigno, P., Keshava, P. H., Silver, P. A. & Corbett, A. H. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 3224–3229.

18. Bauer, A. & Kolling, R. (1996) *J. Cell Sci.* **109 (Pt 6),** 1575–1583.

19. Fischer, T., Strasser, K., Racz, A., Rodriguez-Navarro, S., Oppizzi, M., Ihrig, P., Lechner, J. & Hurt, E. (2002) *EMBO J.* **21,** 5843–5852.

20. Hieronymus, H., Yu, M. C. & Silver, P. A. (2004) *Genes Dev.* **18,** 2652–2662.

21. Loeillet, S., Palancade, B., Cartron, M., Thierry, A., Richard, G. F., Dujon, B., Doye, V. & Nicolas, A. (2005) *DNA Repair (Amsterdam)* **4,** 459–468.
22. Ram, A. F., Wolters, A., Ten Hoopen, R. & Klis, F. M. (1994) *Yeast* **10,** 1019–1030.
23. Lussier, M., White, A. M., Sheraton, J., di Paolo, T., Treadwell, J., Southard, S. B., Horenstein, C. I., Chen-Weiner, J., Ram, A. F., Kapteyn, J. C., *et al.* (1997) *Genetics* **147,** 435–450.
24. Page, N., Gerard-Vincent, M., Menard, P., Beaulieu, M., Azuma, M., Dijkgraaf, G. J., Li, H., Marcoux, J., Nguyen, T., Dowse, T., *et al.*. (2003) *Genetics* **163,** 875–894.
25. Douglas, C. M., Marrinan, J. A., Li, W. & Kurtz, M. B. (1994) *J. Bacteriol.* **176,** 5686–5696.
26. Parsons, W. J., Ramkumar, V. & Stiles, G. L. (1988) *Mol. Pharmacol.* **34,** 37–41.
27. Costigan, C., Gehrung, S. & Snyder, M. (1992) *Mol. Cell. Biol.* **12,** 1162–1178.
28. Martin, H., Arroyo, J., Sanchez, M., Molina, M. & Nombela, C. (1993) *Mol. Gen. Genet.* **241,** 177–184.
29. Bickle, M., Delley, P. A., Schmidt, A. & Hall, M. N. (1998) *EMBO J.* **17,** 2235–2245.
30. Johnson, A. R. (1971) *Anal. Biochem.* **44,** 628–635.
31. York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T. & Abersheim, P. (1985) *Methods Enzymol.* **118,** 3–40.
32. Schmidt, M., Bowers, B., Varma, A., Roh, D. H. & Cabib, E. (2002) *J. Cell Sci.* **115,** 293–302.
33. Orlean, P. (1997) in *Molecular and Cellular Biology of the Yeast Saccharomyces. Vol. 3: Cell Cycle and Cell Biology*, eds. Pringle, J. R., Broach, J. R. & Jones, E. W. (Cold Spring Harbor Lab. Press, Woodbury, New York), pp. 229–362.
34. Fernandez, F., Rush, J. S., Toke, D. A., Han, G. S., Quinn, J. E., Carman, G. M., Choi, J. Y., Voelker, D. R., Aebi, M. & Waechter, C. J. (2001) *J. Biol. Chem.* **276,** 41455–41464.
35. Pruyne, D. & Bretscher, A. (2000) *J. Cell Sci.* **113,** 365–375.