Mol Gen Genet (1999) 262: 683–702

ORIGINAL PAPER

**K.-D. Entian · T. Schuster · J. H. Hegemann · D. Becher
H. Feldmann · U. Güldener · R. Götz · M. Hansen
C. P. Hollenberg · G. Jansen · W. Kramer · S. Klein
P. Kötter · J. Kricke · H. Launhardt · G. Mannhaupt
A. Maierl · P. Meyer · W. Mewes · T. Munder
R. K. Niedenthal · M. Ramezani Rad · A. Röhmer
A. Römer · M. Rose · B. Schäfer · M.-L. Siegler
J. Vetter · N. Wilhelm · K. Wolf · F. K. Zimmermann
A. Zollner · A. Hinnen**

Functional analysis of 150 deletion mutants in *Saccharomyces cerevisiae* by a systematic approach

Received: 11 March 1999 / Accepted: 9 June 1999

Abstract In a systematic approach to the study of *Saccharomyces cerevisiae* genes of unknown function, 150 deletion mutants were constructed (1 double, 149 single mutants) and phenotypically analysed. Twenty percent of all genes examined were essential. The viable deletion mutants were subjected to 20 different test systems, ranging from high throughput to highly specific test systems. Phenotypes were obtained for two-thirds of the mutants tested. During the course of this investigation, mutants for 26 of the genes were described by others. For 18 of these the reported data were in accordance with our results. Surprisingly, for seven genes, additional, unexpected phenotypes were found in our tests. This suggests that the type of analysis presented here provides a more complete description of gene function.

Key words *Saccharomyces cerevisiae* · Deletion mutants · Phenotypic analysis

Communicated by C.P. Hollenberg

K.-D. Entian (✉) · P. Kötter · A. Röhmer · M. Rose
Institut für Mikrobiologie,
Universität Frankfurt, Marie-Curie-Str. 9,
D-60439 Frankfurt/Main, Germany
e-mail: Entian@em.uni-frankfurt.de

T. Schuster · M.-L. Siegler
Institut für Medizinische Strahlenkunde und Zellforschung (MSZ), Universität Würzburg, Verbacher Str. 5,
D-97078 Würzburg, Germany

J. H. Hegemann · U. Güldener · S. Klein · R. K. Niedenthal
Institut für Mikrobiologie, Heinrich-Heine-Universität,
Universitätsstr. 1, Gebäude 26.12.01,
D-40225 Düsseldorf, Germany

H. Launhardt · T. Munder · A. Hinnen
Hans-Knöll-Institut für Naturstoff-Forschung e.V.,
Beutenbergstr. 11, D-07745 Jena, Germany

D. Becher · J. Kricke
Institut für Genetik und Biochemie,
Universität Greifswald, Jahnstr. 14,
D-17486 Greifswald, Germany

H. Feldmann · G. Mannhaupt · J. Vetter
Institut für Physiologische Chemie,
Physikalische Biochemie und Zellbiologie,
Universität München, Schillerstraße 44,
D-80336 München, Germany

C. P. Hollenberg · G. Jansen · M. Ramezani Rad
Institut für Mikrobiologie,
Universität Düsseldorf, Universitätsstr. 1,
Gebäude 26.12.01, D-40225 Düsseldorf, Germany

W. Kramer · P. Meyer · A. Römer
Institut für Mikrobiologie und Genetik,
Universität Göttingen, Grisebachstr. 8,
D-37077 Göttingen, Germany

A. Maierl · W. Mewes · A. Zollner
MIPS am Max-Planck-Institut für Biochemie,
Am Klopferspitz 18a,
D-82152 Martinsried, Germany

R. Götz · N. Wilhelm · F. K. Zimmermann
Institut für Mikrobiologie,
TH Darmstadt, Schnittspahnstr. 10,
D-64287 Darmstadt, Germany

M. Hansen · B. Schäfer · K. Wolf
Institut für Biologie IV,
RWTH Aachen, Worringer Weg,
D-52056 Aachen, Germany

K.-D. Entian, T. Schuster and J. H. Hegemann contributed equally to the preparation of the manuscript. The Project Coordinator was A. Hinnen

Supplementary material. Detailed descriptions of the methods used are available in electronic form on Springer Verlag's server at http://link.springer.de/link/service/journals/00438/index.htm.

Introduction

The yeast *Saccharomyces cerevisiae* represents a simple eukaryotic cell. It contains all the major structural elements characteristic of eukaryotes, such as a nucleus surrounded by a nuclear envelope, mitochondria, an endoplasmic reticulum, a Golgi apparatus for protein secretion and distribution, vacuoles which correspond to the lysosomes of higher eukaryotes, and ribosomes. *S. cerevisiae* cells are considerably smaller than those of higher eukaryotes, they proliferate by budding, and the nuclear envelope remains intact during mitosis. Differentiation processes – like those seen in tissues of multicellular organisms – do not appear in *S. cerevisiae*, but developmental processes that are comparable to differentiation do occur, such as mating of **a** and α cells, sporulation and pseudohyphal growth. In addition, carbohydrate and energy metabolism vary widely, depending on the carbon source provided.

Starting in 1988, the *S. cerevisiae* genome (with the exception of the mitochondrial genome and the highly repetitive rRNA-encoding region on chromosome XII) was sequenced in a worldwide project that was completed in April 1996 (Mewes et al. 1997). The genome comprises 12,000 kb, distributed on 16 chromosomes encoding approximately 6200 genes (Mortimer et al. 1989; Goffeau et al. 1996). Of these, about one-third are fairly well characterised with respect to their cell biological function, and for a further one-third a function is indicated by their homologies to genes previously described in other organisms. For about one-third of the genes (termed "orphans") there is no strong evidence concerning their possible cellular function. In silico analysis of all genes has suggested a function for 48% of the *S. cerevisiae* genes (Mewes et al. 1997).

Whereas in the past genetic and molecular analysis focused mainly on specific problems of cellular function, today, genome sequencing enables unknown genes to be analysed without any prior assumptions as to their possible function. Several genome-wide approaches have recently been initiated, including SAGE (Velculescu et al. 1997), high-density DNA arrays (Lashkari et al. 1997), genetic footprinting (Smith et al. 1996) and insertional mutagenesis (Burns et al. 1994; Ross-Macdonald et al. 1997). While the first two approaches allow genome-wide transcript analysis, the latter two require that mutants with specific properties be collected prior to analysis. This entails the risk that the selection system applied will not be sufficiently sensitive and that, in particular, mutants with reduced growth rates may be missed. Alternatively, functional analysis can start with a defined gene and employ a large number of individual tests (mutant scan analysis; Entian and Kötter 1998). Mutant scan analyses start with the directed deletion of a specific gene and search for any malfunction of the resulting deletion mutant. This requires the application of a large number of test systems, and overlaps between the test systems must be kept to a minimum. Furthermore, the test systems chosen must have a high throughput rate and should be inexpensive. The advantage of the mutant scan approach is that an extensive phenotypic database is generated that also includes negative test results, which in many cases can prove to be as informative as the positive findings.

Here, we report such a phenotypic analysis of 150 deletion mutants for yeast genes whose function was not known when the project started. In a decentralised network 11 yeast laboratories generated deletion mutants and tested them for malfunctions in carbohydrate metabolism, mitochondrial function, reactions to stress, chromosome distribution, cytoskeleton and organelle morphology, pseudohyphal growth, mating efficiency, glycosylation, and cell division. In each case one of the two alleles was deleted in a diploid cell by replacing at least 80% of its coding region with a selection marker. Diploids were chosen for gene deletion so that the initial transformants were heterozygous for the gene under investigation. This enabled us to detect essential genes after haploid segregants were obtained from sporulated diploids (tetrad analysis). All data (positive and negative) were recorded and are available to the scientific community on the website (http://www.mips.biochem.mpg.de/proj/scdegen/index/html) created and maintained by MIPS (Martinsried Institute for Protein Sequences). Furthermore, a repository for the viable gene deletion mutants and complementing plasmids has been established (EUROSCARF, the European *Saccharomyces cerevisiae* Archive for Functional Analysis; see Entian and Kötter 1998), which makes the deletion mutants accessible to the scientific community (EUROSCARF website: http://wwwrz.uni-frankfurt.de/FB/fb16/mikro/EUROSCARF/).

Materials and methods

Yeast strains

The isogenic strain CEN.PK2 with different combinations of auxotrophies was used for gene deletion and as a reference strain. The list of wild-type strains and deletion mutants used is accessible under http://www.rz.uni-frankfurt.de/FB/fb16/mikro/euroscarf/. Strains and plasmids can be obtained from EUROSCARF, Institute for Microbiology, University of Frankfurt, Marie-Curie Str. 9, D-60439 Frankfurt, Germany. For mailing conditions and handling fees see EUROSCARF website.

The methods used are summarized in Table 1, and detailed descriptions of the methods are available as Electronic Supplementary Material on Springer Verlag's website (see Footnote).

Results

Growth and metabolism

Mutations which alter growth rates of yeast cells are easy to detect. But quantitation of such alterations requires as a standard an isogenic diploid wild-type strain in which all progeny segregants obtained after tetrad

Table 1 Summary of methods

Method	Brief description	References
Yeast strain	CEN.PK2	Entian and Kötter (1998)
Complete media	YPD (2% peptone, 1% yeast extract and carbon sources)	
Synthetic media	0.67% yeast nitrogene base	
Gene deletion strategy	Markers: *LEU2*, *HIS3*, *URA3*, *TRP1*, *loxP-kanMX-loxP*	Rothstein (1991); Güldener et al. (1996)
Tetrad analysis	Spore dissection was performed with a Singer micromanipulator MSM, De Fonbrune micromanipulator, or under the Zeiss Axioskop	
Carbon source utilization	Growth response on plates containing various carbon sources	This work
Oleic acid utilisation	Growth response on plates containing 0.1% oleic acid, 0.4% Tween 40, 0.1% yeast extract, 0.67% nitrogen base without amino acids	Erdmann et al. (1989)
Salt stress	Growth response on YPD plates containing increasing concentrations of NaCl or supplemented with 1.5 M KCl	This work
Temperature sensitivity	Growth response on YPD plates at 15° C, 30° C, and 37° C	This work
Oxidative stress	Growth response on YPD plates containing hydrogen peroxide	Schnell et al. (1992)
Rho^- formation	Growth response on YPG versus YPDG	This work
Maintenance of mitochondrial DNA	Staining of cells with DAPI	Moreno et al. (1991)
Respiratory chain enzymes	Photometric assays for succinate-ubiquinone oxidoreductase, ubiquinone cytochrome *c* reductase and cytochrome *c* oxidase	Rieske (1967); Tisdale (1967); Wharton and Tzagoloff (1967)
Oxygen consumption	Oxygen consumption of cells growing in YPD was determined with an oxygen electrode	This work
Copper toxicity	Growth response to increasing concentrations of $CuSO_4$	This work
Cell cycle profiling	FACS analysis of DAPI-stained cells	Hutter and Eipel (1978)
Minichromosome maintenance	FACS analysis of GFP expression from a CEN-based plasmid	Hegemann et al. (1999)
Thiabendazole sensitivity	Growth response on thiabendazole-containing complete synthetic media was determined by serial dilution drop tests	Matsuzaki et al. (1988)
Morphological characterisation	Nuclei, mitochondria, vacuoles, actin patches, microtubules and budding pattern were visualised by in situ fluorescence microscopy	Pringle et al. (1989); Rose et al. (1990); Adams and Pringle (1991)
Electron microscopy	Specimens for scanning electron microscopy were examined with a Zeiss DSM 940 A electron microscope. Ultrathin frozen sections and ultrathin resin-embedded sections were examined with a Zeiss 906 electron microscope	Reynolds (1963); Tokuyasu (1989); Zimmer et al. (1995); Hanschke and Schauer (1996)
Mating assays	Mating ability was determined by quantitative mating assays. Zygote formation was quantitated by microscopy.	Sprague (1991)
Invertase assays	Total invertase activity was measured in crude yeast cell extracts. Secreted invertase activity was determined using gel assays	Goldstein and Lampen (1975); Kaiser and Botstein (1986)
Invasive growth	Ability to grow invasively was determined by plating assays	Roberts and Fink (1994)
UV resistance and mutator phenotype analysis	UV resistance was scored in a semiquantitative plate assay. UV induced mutations, spontaneous (forward and reversion) and mitochondrial (Cm and Ery resistance) mutator phenotypes were scored in papillation plate assays using four clones each. Positive mutants were retested in quantitative assays	Lea and Coulson (1949); Grivell et al. (1973)
Heterologous signal transduction	Mutants were tested for their ability to transduce estrogen mediated signals by determining *lac*Z activity as reporter	Picard et al. (1990)

[a] A detailed description of the methods used is given in the Electronic Supplementary Material available on the Springer server (http://link.Springer.de/link/journals/00438/index.htm)

analysis have equal division rates. A strain which fulfils this condition is CEN.PK2. This homozygous diploid strain was obtained after mating type switching of a well characterised haploid strain (Entian and Kötter 1998). It generates spores which germinate equally well and grow out to form colonies of exactly the same size. Hence, this strain was chosen for the functional analysis of all deletion mutants.

We generated 150 diploid strains, each of which was heterozygous for a single-gene deletion (with one exception, which lacks two genes). The heterozygous diploids were sporulated and at least 10 tetrads per strain were dissected. The segregation of the deletion derivative was followed via the marker used to replace the deleted sequence. Among the 150 deletion mutants, 28 revealed a lethal phenotype as indicated by a 2:2 (viable:lethal) segregation pattern. Of the remaining 121 viable deletion mutants, 24 formed colonies of reduced size as compared to wild-type segregants (Table 2). Thus, simple tetrad analysis yields phenotypes (lethal or reduced growth) for one-third of the genes under investigation.

Further analysis of the 24 mutants that exhibited a reduced colony size after tetrad analysis (strains 1, 12, 30, 32, 37, 43, 49, 51, 55, 56, 60, 62, 75, 85, 86, 87, 103, 105, 117, 119, 122, 130, 142, and 150) allowed classification into three major groups. The first was represented

Table 2 Growth phenotypes

Strain No.[a]	Systematic gene name	Gene designation	Reduced colony size in tetrads	Growth on glucose	Growth on other sugars[b]	Growth on ethanol	Growth on other C2/C3 carbon sources[c]	Respiration deficiency (%)[d]	Oxygen consumption (%)[e]	Complex II activity (%)[e]	Complex III activity (%)[e]	Complex IV activity (%)[e]	FACS[f]
1	*YBR163w*		+	+	+	+	+	100^{RD}	0	0	0	100	1n
9	*YBR171w*	*SEC66*											2n
12	*YNL264c*		+										
30	*YJR120w*		+	+	+	+	+	100^{RD}	20	23	63	45	1n
32	*YJR118c*		+	+	+	+	+	11/8	92	83	110	202	2n
37	*YNL252c*	*YML30*	+	+	+	+	+	100^{RD}	0	0	16	14	1n
41	*YBR095c*			+	+	+	+						2n
43	*YBR098w*		+	+	++	++	+						2n
49	*YJR106w*	*ECM27*	++	+	+	+	+	12/8	50	28	75	78	
51	*YKL135c*	*APL2*	+										2n
52	*YBR296c*												2n
55	*YJR144w*	*MGM101*	+	+	+	+	+	100^{RD}	0	23	19	72	1n
56	*YJR139c*	*HOM6*	++					10/10	85	126	166	128	
60	*YKL134c*		+	+	+	+	+	100^{RD}	0	0	0	20	1n
61	*YJR140c*	*HIR3*		+		+							
62	*YBR017c*	*KAP104*	++	+	+	+	+	100^{RD}	72	43	48	393	
65	*YJL094c*			+			+						S
68	*YIR001c*					+	+						
75	*YIR005w*		+	+	+								1n
80	*YJL096w*			+	+	+	+	100^{RD}	0	0	0	20	1n
85	*YJR101w*		+	+	+	+	+	100^{RD}	0	0	0	11	1n
86	*YJR102c*		+				+						
87	*YIR009w*		++			+	+						
96	*YGR056w*			+	+		+	7/8	90	66	57	91	
98	*YNR056c*	*BIO5*											2n
102	*YJR108w*			+									
103	*YKL137w*		+	+	+	+	+	100^{RD}	0	0	0	13	1n
105	*YBR106w*	*PHO88*	++										
114	*YBR242w*			+									1n, S
116	*YBR245c*					+							
117	*YBR152w*		++	+	+	+	+	100^{RD}	82	51	84	167	2n
119	*YJR132w*	*NMD5*	+	+	+	+	+						1n
121	*YJR134c*				+								
122	*YJL076w*		++	+	+	+	+						1n
124	*YGR045c*					+	+						
130	*YLR087c*		++	+	+	+	+						
137	*YOL060c*					+	+						
142	*YBR097w*	*VPS15*	++	+	+	+	+	100^{RD}	108	31	67	208	2n
150	*YGR057c*		+	+	+	+	+	3/5	48	70	53	90	2n

[a] Growth characteristics of the mutants on the different carbon sources are indicated as follows. +, mutants that show a detectable alteration compared to wild type; ++, mutants that show a drastic alteration relative to wild type. Deletion of the following 29 genes led to a lethal phenotype: *YBR087w*, *YBR088c*, *YBR091c*, *YBR102c*, *YBR121c*, *YBR155w*, *YBR156c*, *YBR159w*, *YBR167c*, *YBR168w*, *YBR170c*, *YGR046w*, *YGR048w*, *YGR060w*, *YIL003w*, *YIR010w*, *YIR012w*, *YJL074c*, *YJL091c*, *YJL097w*, *YJR112w*, *YJR141w*, *YKL125w*, *YKL144c*, *YNL251c*, *YNL256w*, *YNL258c*, *YNL260c*, *YNL263c*

[b] Other sugars tested were fructose, mannose and galactose

[c] C2/C3 carbon sources tested were acetate and glycerol

[d] Spontaneous respiration-deficiency was determined as the percentage of cells in a growing culture that lose the ability to grow on glycerol at 18° C and 37° C. The value for the wild type never exceeded 3%. Complete loss of respiratory activity at both temperatures is indicated by 100^{RD} (RD, respiration deficient)

[e] Oxygen consumption and the activity of complexes II, III and IV activity is expressed as a percentage relative to wild type

[f] The entries indicate whether the mutants show an increase, relative to wild type, in the proportion of cells in S phase or with a 1C or 2C DNA content

by 12 mutants (1, 30, 32, 37, 49, 55, 60, 62, 85, 103, 117, 142), which were either respiration deficient or had an increased tendency to lose mitochondrial DNA. The second group included three mutants (12, 51, 56) which showed normal growth on glucose, suggesting that spore germination is delayed. The third group consisted of 9 mutants (43, 75, 86, 87, 105, 119, 122, 130, 150) which were affected in central cell biological processes, such as chromosome segregation, DNA replication, mating, budding, microtubule stability, and stress responses.

Growth rates were estimated by cultivating the strains on solid media containing fermentable (glucose, fructose, mannose, galactose) and non-fermentable carbon sources (ethanol, acetate, glycerol). In addition, spontaneous petite (rho$^-$) formation was followed at 18° C and 37° C and mtDNA was stained with DAPI to differentiate rho$^+$ from rho$^-$ and rho° cells. Mutants showing phenotypes in one of these tests were further analysed by determining the activities of the respiratory chain complexes and their rates of oxygen consumption. In total, 32 out of the 121 viable mutants (1, 30, 32, 37, 41, 43, 49, 55, 60, 61, 62, 65, 68, 75, 80, 85, 86, 87, 96, 102, 103, 114, 116, 117, 119, 121, 122, 124, 130, 137, 142, 150) were affected in energy and carbohydrate metabolism (Table 2). Of these, 11 (1, 30, 37, 55, 60, 62, 80, 85, 103, 117, 142) failed to utilise non-fermentable carbon sources and were classified as totally respiration deficient (Table 2). Five mutants (32, 49, 56, 96, 150) exhibited high rates of spontaneous formation of respiration-deficient cells. DAPI staining of these mutants revealed a certain fraction of rho° cells in cultures of these mutant strains, which suggests instability of mtDNA. Typically, this coincided with a decrease in oxygen consumption. The activities of respiratory chain complexes II, III and IV were also changed. In two mutants (32, 56) the activity of complex IV was increased, whereas mutants 49, 96 and 150 had wild-type or decreased complex IV activities. Three of the respiration-deficient mutants (30, 62, 117) still showed residual oxygen consumption. Most deletion mutants with a complete loss of respiration lacked (1, 37, 60, 80, 85, 103) or had strongly reduced (30, 55, 62, 117, 142) complex II and complex III activities. Most of these mutants also displayed diminished complex IV activity.

Fifteen of the mutants not involved in mitochondrial metabolism were affected in the utilisation of other carbon sources (41, 43, 61, 65, 68, 75, 86, 87, 116, 119, 121, 122, 124, 130, 137). Mutants 41, 43, 61, 65, 75, 119, 122, and 130 showed lower growth rates on glucose as well, suggesting secondary effects. Mutant 87 grew slowly on all non-fermentable carbon sources, and mutant 116 grew more slowly on ethanol than the wild-type. Remarkably, mutant 124 showed an increased colony size after tetrad analysis but also revealed reduced growth on ethanol. Two mutants (65, 86) showed reduced growth with acetate but were not affected on other carbon sources. Number 121 utilised galactose and mannose poorly, and mutant 68 used glycerol and ethanol less efficiently.

A comparison of the set of 24 deletion mutants that formed small segregant colonies after tetrad analysis with the 25 mutants that grew slowly on glucose reveals that 18 mutants are members of both groups (Table 2). Further analysis of those mutants which showed an effect in only one test system revealed that most of them (tetrad analysis: 51, 87, 105; growth on glucose: 41, 61, 65, 102, 114) had rather specific phenotypes. The data therefore demonstrate that these rapid and convenient test systems are very powerful tools for the identification of mutants with quite specific physiological effects.

DNA profile analysis of logarithmically growing cells

Logarithmically growing yeast cell cultures are characterised by a typical distribution of cells in the different stages of the cell cycle, which can be determined by quantification of the cellular DNA by fluorescence-activated cell sorting (FACS) (Hutter and Eipel 1978). Alterations in this distribution are indicative of mutations which influence cell cycle progression. About 20% of the mutants tested (23 mutants: 1, 9, 30, 32, 37, 41, 43, 51, 52, 55, 60, 65, 75, 80, 85, 98, 103, 114, 117, 119, 122, 142, 150) displayed notable changes in this respect (Table 2). Almost equal numbers of strains showed an increase in the relative size of the 1n cell population (11 strains: 1, 30, 37, 55, 60, 75, 80, 85, 103, 119, 122) or the 2n cell population (10 strains: 9, 32, 41, 43, 51, 52, 98, 117, 142, 150). In addition, mutant 65 showed an increase in the proportion of cells present in the S-phase of the cell cycle, while in cultures of mutant 114 increased numbers of 1n and S-phase cells were observed. All strains except mutant 98 showed additional phenotypes and about 80% of them were pleiotropic (1, 9, 30, 32, 37, 41, 43, 55, 60, 65, 75, 80, 85, 103, 117, 119, 122, 142). Comparison of the FACS data with the other phenotypes revealed a good correlation between reduced growth and a change in DNA profile.

More specific effects were found for four mutants (9, 51, 52, 98) which grew normally on glucose: the proportion of 2n cells was increased, suggesting that the corresponding proteins are involved in cell cycle progression. In mutant 98 this was the only phenotype observed. The corresponding ORF has been named BIO5 and shows homology to a transmembrane regulator of KAPA/DAPA transport which is involved in biotin synthesis (Phalip et al. 1999). The increase in the proportion of 2n cells in rich media may result from a biotin deficiency in the medium.

For three mutants (41, 51, 52) the additional phenotypes (reduced growth or delayed germination, increased or reduced mating efficiency) may be directly associated with the FACS phenotype. The protein deleted in mutant 114 contains a domain with similarities to purine nucleotide binding domains, suggesting a possible regulatory function for this protein, which would be compatible with the other phenotypes observed for this strain (increased number of 1n/S-phase cells, slightly

reduced growth on glucose, resistance to $CuSO_4$, reduced signal transduction activity).

Responses to stress and toxic agents

Yeast cells respond to environmental stress conditions or toxic agents, such as high or low temperatures, starvation, changes in osmotic pressure, and heavy metals, by activating specific protection mechanisms. For example, yeast cells respond to hypertonic shock by activating a MAP kinase cascade called the high osmolarity glycerol (HOG) response pathway (Brewster et al. 1993, Brewster and Gustin 1994; Schüller et al. 1994), which leads to an accumulation of glycerol that permits the cell to withstand the increased osmotic pressure. To examine whether any of the mutants considered here fail to respond to stress, we tested their growth behaviour on plates containing hydrogen peroxide or high concentrations of salt.

Of the 121 viable mutants, 18 (1, 30, 37, 42, 43, 44, 55, 58, 60, 75, 80, 85, 86, 103, 117, 122, 130, 142) were hypersensitive to hydrogen peroxide (Table 3). Of these, 10 mutants (1, 30, 37, 55, 60, 80, 85, 103, 117, 142) also exhibited respiration deficiency, suggesting that oxidoreductase reactions are important for the oxidative stress reaction. The strongest oxidative stress phenotype seen among the 8 mutants which were not respiration deficient was found for mutants 42, 58, 75, 86 and 130. Twenty-three mutants (1, 12, 30, 37, 42, 43, 49, 55, 60,

Table 3 Stress phenotypes

Strain number[a]	Systematic gene name	Gene designation	$NaCl^s$	$NaCl^r$	KCl^s	$H_2O_2^s$	Cu^s	Cu^r	ts	cs	Respiratory deficiency
1^S	*YBR163w*		+		+	+			+	+	+
8	*YGL236c*				++						
9	*YBR171w*	*SEC66*							+		
11	*YNL265c*			+							
12	*YNL264c*		+								
16	*YJR117w*	*STE24*						+			
30^S	*YJR120w*		+		+	+					+
37^S	*YNL252c*	*YML30*	+		+	+					+
39	*YBR086c*			+							
42	*YBR096w*		+			+					
43^S	*YBR098w*		+			+	++				
44	*YBR101c*					+					
49^S	*YJR106w*	*ECM27*	++							+	
55^S	*YJR144w*	*MGM101*	+		+	+					+
58	*YKL129c*	*MYO3*		+		+					
60^S	*YL134c*		+			+					+
61	*YJR140c*	*HIR3*	+								
62^S	*YBR017c*	*KAP104*	+		+			+	+		+
65	*YJL094c*			+							
71	*YIR004w*						++				
75^S	*YIR005w*			+		+					
80	*YJL096w*		+		+	+					+
85^S	*YJR101w*		+		++	+					+
86	*YJR102c*		++		++	+					
87	*YIR009w*			+				+			
89	*YLR098c*	*CHA4*	++								
92	*YLR099c*							+			
95	*YCR008w*		++								
96	*YGR056w*		++				+			+	
103^S	*YKL137w*		+			+					+
105	*YBR106w*	*PHO88*					+				
106	*YBR111c*						+				
114	*YBR242w*							+			
117^S	*YBR152w*		+			+					+
118	*YBR151w*						+				
119^S	*YJR132w*	*NMD5*		++	++		+				
122^S	*YJL076w*			++	++	+				+	
130^S	*YLR087c*		++			+				+	
140	*YOL054w*		++								
142^S	*YBR097w*	*VPS15*	+			+					+
146	*YBR157c*						+				
147	*YJL077c*						+				
148	*YBR158w*						+				
150	*YGR057c*		+					+			

[a] Sensitivity (s) and resistance (r) of mutant cells to different stress conditions is indicated as follows: + indicates that mutants show a detectable phenotypic alteration compared to wild type; ++ indicates a drastic phenotypic alteration relative to wild type. Strains numbered with the suffix S show a slow-growth phenotype

61, 62, 80, 85, 86, 89, 95, 96, 103, 117, 130, 140, 142, 150), including all the respiration mutants, showed a moderate or strong increase in sensitivity to NaCl. Of these, 14 (1, 30, 37, 42, 43, 55, 60, 80, 85, 86, 103, 117, 130, 142) were also sensitive to H_2O_2. Only eight mutants (12, 49, 61, 89, 95, 96, 140, 150) were sensitive to NaCl alone.

Eleven mutants (1, 8, 30, 37, 55, 62, 80, 85, 86, 119, 122) were sensitive to high levels of KCl; eight of these also showed H_2O_2 sensitivity (1, 30, 37, 55, 80, 85, 86, 122) (Table 3). Comparison of the groups showing sensitivity to NaCl or KCl revealed a high degree of overlap (1, 30, 37, 55, 62, 80, 85, and 86 fall into both groups), which suggests that in this case both salts invoke the same osmotic stress response. Only mutant 8 was specifically sensitive to KCl – the only phenotype which may indicate a defect in potassium ion transport.

A surprisingly large number (8) of deletion mutants (11, 39, 58, 65, 75, 87, 119, 122) showed increased resistance to NaCl. Although such mutants are easily obtained by classical genetic selection, the molecular characterisation of many of them has been hampered by the fact that the corresponding genes were difficult to isolate because of the dominant nature of the phenotype. Six of our mutants (11, 39, 58, 65, 75, 87) were only slightly more resistant than wild-type, whereas NaCl resistance was strongly increased in two mutants (119, 122).

Temperature-sensitive (ts) and cold-sensitive (cs) mutants

In some cases deletion of certain genes results in growth arrest at high temperatures. Such an effect has been observed for the *MPK1/SLT2* gene, which encodes a MAP kinase that regulates cytoskeleton dynamics as well as cell wall assembly (Mazzoni et al. 1993; Zarzov et al. 1996), *SIN4*, which codes for a subunit of the RNA polymerase II holoenzyme-mediator complex (Jiang and Stillman 1992), and *SWP73*, which is important for Swi/Snf-dependent chromatin remodelling (Cairns et al. 1996).

Our functional analysis uncovered two viable deletion mutants (9, 62) which showed a temperature-sensitive (ts) phenotype, and four mutants (49, 96, 122, 130) which were cold-sensitive (cs) (Table 3). One additional mutant (1), however, was sensitive to high as well as low temperatures. With the exception of mutant 9, all these mutants also exhibited a strongly reduced growth rate at 30° C and were sensitive to either NaCl or KCl, suggesting that they are generally stress sensitive. The gene deleted in mutant 9 has recently been described as *SEC66* and the ts phenotype of the *sec66* deletion has been confirmed (Feldheim et al. 1993).

Copper toxicity

The heavy metal copper is an essential trace nutrient for yeast since it is required for a variety of enzymatic reactions. However, copper is also toxic if it is present in higher concentrations. Yeast cells have evolved a variety of regulatory mechanisms to control levels of intracellular copper (Butt et al. 1984; Hamer 1986; Fürst et al. 1988; Munder and Fürst 1992; Jungmann et al. 1993; Dancis et al. 1994). Damage to one of these mechanisms may lead to increased sensitivity to copper. But mutations that result in copper resistance are also conceivable. To test the 121 viable mutants for changes in growth behavior we plated them on media containing increasing amounts of $CuSO_4$. This test allowed us to detect 10 mutants with an increased sensitivity to copper (43, 71, 96, 105, 106, 118, 119, 146, 147, 148) and six which were more resistant (16, 62, 87, 92, 114, 150) than the wild type (Table 3). The fact that there was little overlap between sets of mutants that showed salt and copper sensitivity/resistance suggests that the effect of copper is not associated with defects in the response to osmotic shock or other stresses.

Cell morphology, cytoskeleton and organelles

Morphological changes, cytoskeletal defects or alterations in organelles can cause a wide variety of phenotypes. For example, defective mitochondria can lead to respiration deficiency. Also, aberrant distributions of microtubules and actin patches can result in morphological modifications and genetic instability. Therefore, we asked whether any of the deletion mutants exhibit such changes. All mutants were investigated by fluorescence microscopy, using specific stains, for morphological changes in mitochondria, vacuoles, nuclei and the patterns of microtubules, actin and bud scars. Those in which organelle structure was changed were then analysed by transmission and scanning electron microscopy. Attention was mainly focused on (i) mitochondrial integrity as evaluated by the presence of cristae and double membranes, (ii) the localisation of the mitochondria within the cytoplasm, and (iii) their presence as individual organelles. In all but two cases (32, 49) the light microscopy data were confirmed by electron microscopic observations (see comments in Table 4).

Fluorescence microscopy identified one mutant (1) which appeared to have a smaller nucleus (Fig. 1A) than wild-type cells (Fig. 1B), which was often fragmented. This was confirmed by our electron microscopic observations (Fig. 1C). Many cells of this strain even lacked a nucleus, a phenotype which was also observed in a second mutant (88). Three mutants contained altered vacuoles, one of which was barely detectable by light microscopy (88). The other two contained dispersed (117; Fig. 2A) or enlarged vacuoles (142; Fig. 3A). Three additional deletion mutants showed fewer actin patches or changes in their distribution (101, 103, 118). This defect should lead to changes in the cytoskeleton; and indeed, approximately 10% of the cells of mutant 118 gave rise to elongated daughter cells. In these cells the actin patches were mainly found distal to the mother

Table 4 Morphological phenotypes

Strain No.[a]	Systematic gene name	Gene designation	Mitochondria		Nucleus	Vacuoles	Microtubules	Thiabendazole	Actin	Budding pattern	Comments
			LM	EM							
1[S]	*YBR163w*		+	+[RD]	+					+	Mitochondria poorly stainable by DiOC6(3), unbranched and shorter than WT. Nuclei smaller, many daughter cells lack a nucleus. Increase in number of cells lacking bud scars. Mitochondria visible as vesicle-like structures by EM, often clotted, with interior structures missing
9	*YBR171w*	*SEC66*						+ (37° C)		+	Cells tend to form clumps (*SEC66*):
18	*YBR150c*							+			
30[S]	*YJR120w*		+	+[RD]				+			Mitochondria clumped
32	*YJR118c*			+							Mitochondria appear shorter under LM but seem normal in the EM
37[S]	*YNL252c*	*YML30*	+	+[RD]				+			Mitochondia not stainable by DiOC6(3), but appear clumped in the EM
41	*YBR095c*									+	Increase in the number of cells lacking bud scars
43[S]	*YBR098w*						+	+ + +		+	Mitochondria barely detectable by LM; cell size is heterogeneous and cell morphology abnormal
44	*YBR101c*							+			
48	*YJR092w*	*BUD4*								+	Bipolar budding (*BUD4*)
49[S]	*YJR106w*	*ECM27*		+			+	+ + +			Mitochondria appear shorter or barely detectable by LM but seem normal in the EM
55[S]	*YJR144w*	*MGM101*	+	+[RD]				+			Mitochondria not stainable with DiOC6(3), but detectable by EM
56	*YJR139c*	*HOM6*						+ +			
60[S]	*YKL134c*		+	+[RD]				+ +		+	Mitochondria not stainable with DiOC6(3), but detectable with DAPI. The size of the lumen appears larger in the EM. Increase in the number of cells lacking bud scars
62[S]	*YBR017c*	*KAP104*	ND	+[RD]						+	Cells tend to clump (*KAP104*). Mitochondria shorter; Elongated cells have elongated mitochondria
80	*YJL096w*							+			
85[S]	*YJR101w*							+			
87	*YIR009w*							+ +			
88	*YLR092w*	*SUL2*	+	+	+	+					Mitochondria not associated with plasma membrane. Vesicles barely stainable (*SUL2*). In the EM many cells appear anucleate
101	*YBR294w*	*SUL1*							+		Fewer actin patches than WT (*SUL1*)
102	*YJR108w*						+				Mitochondria shortened
103[S]	*YKL137w*							+	+		Fewer, but larger actin patches than WT
105	*YBR106w*	*PHO88*					+	+ +			Mitochondria shortened
115	*YBR244w*		ND	+							Mitochondria short, rounded
117[S]	*YBR152w*		+	+[RD]		+				+	Mitochondria not stainable with DiOC6(3); DAPI staining reveals round mitochondria. Vesicles enlarged; chitin patches; unipolar budding. In the EM mitochondria show less structure, with cristae rarely visible; unipolar budding, tendency to form pseudohyphae
118	*YBR151w*								+		In 10% of daughter cells, actin patches are found distal to the birth scar. In the LH, cells appear clumped
122[S]	*YJL076w*		+	+							Mitochondria barely stainable with DiOC6(3). In the H, mitochondria appear clumped; cells club-shaped
132	*YLR093c*		+	+							Mitochondria barely stainable with DiOC6(3). In the EM, cristae appear irregular
137	*YOL060c*		ND	+							Mitochondria round and short
142[S]	*YBR097w*	*VPS15*				+		+ + +			Vesicles occupy up to 70% of the cell; cells aggregated (*VPS15*)

[a] Presence and degree of morphological alterations are indicated by +, + + or + + + (see Table 2). Details are given in the rightmost column. Strains numbered with the suffix S show a slow-growth phenotype

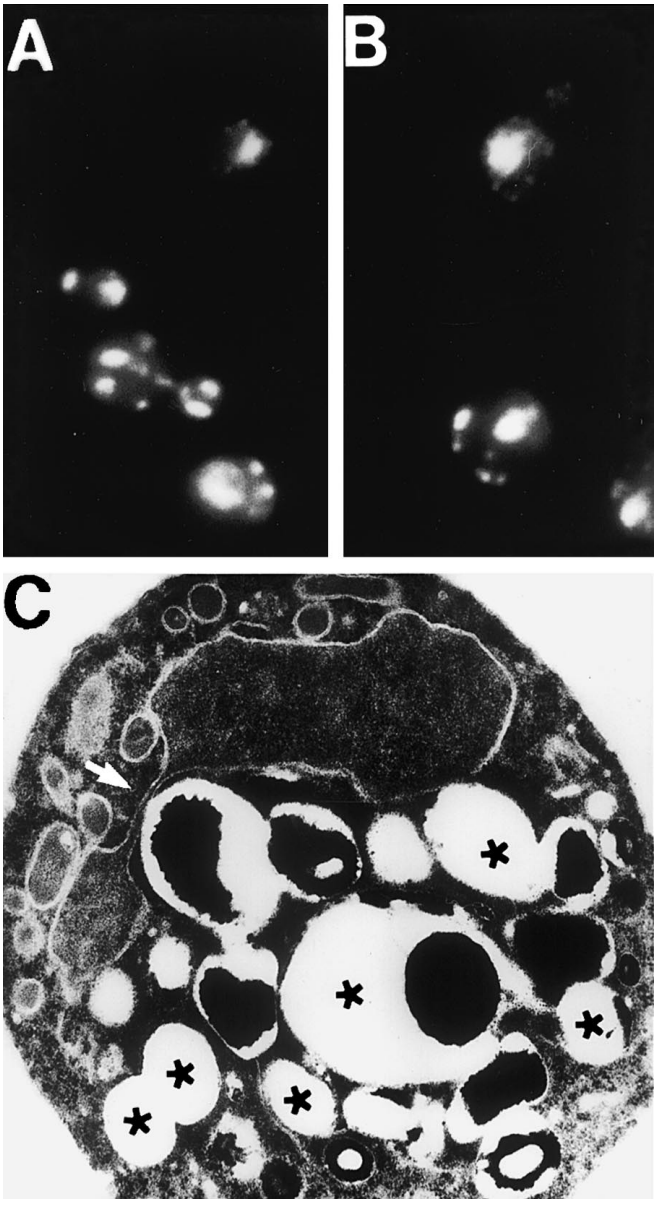

Fig. 1 **A** DAPI staining of cells of mutant strain 1 (*YBR163w*). Mutant cells exhibit more than one stained body which indicates fragmentation of the nucleus. **B** DAPI-stained wild type cells. **C** Electron micrograph of a typical mutant strain 1 cell. The nucleus is divided into two masses linked by a bridge (*arrow*). Mutant 1 also produced a large number of vacuolar structures (*asterisks*)

cell during budding. Alterations in the microtubule structures should also influence the cytoskeleton. In four of our mutants (43, 49, 102, 105), microtubules were shorter than normal or scarcely detectable, leading in one case to heterogeneity in cell size and abnormal cell morphology (43). It is interesting to note that three of these mutants were highly sensitive to the microtubule-destabilising agent thiabendazole. Only one mutant with short microtubules did not exhibit a thiabendazole-sensitive phenotype (102).

Fluorescence microscopy revealed that 14 deletion mutants had morphologically aberrant mitochondria, which could not be stained or were abnormally shaped (Table 4). Eleven of these strains (1, 30, 32, 37, 49, 55, 60, 62, 117, 122, 137) were respiration deficient or unable to grow on non-fermentable carbon sources, while three others (88, 115, 132) did not show such defects. Mutant 30 exhibited fused mitochondria (see Fig. 3B), whereas in mutant 117 the distribution of mitochondria and their morphology was altered. In the latter case, the mitochondria were distributed throughout the cytoplasm, sometimes dislodged by dispersed vacuoles, while in wild-type cells mitochondria are found close to the plasma membrane (Fig. 2B–E). In addition, the nucleus appeared to be vacuolated in many cases. In five mutants (1, 32, 49, 115, 137) we observed short mitochondria by light microscopy. Four other mutants (37, 55, 122, 132) possessed mitochondria which could be stained only weakly with DiOC6(3) or not at all. Although not respiration deficient, mutant 122 showed very heterogeneous mitochondrial structures. They possess regular cristae and vary in size and shape. However, rings of fused mitochondria were frequently formed (Fig. 3C). In addition, this mutant showed an abnormal morphology with most cells being club-shaped (Fig. 3D).

Eight other deletion mutants showed an altered cell morphology (1, 9, 41, 43, 48, 60, 62, 117). Generally, haploid wild-type cells show an axial budding pattern. The mutant strain 117, however, often showed an unipolar budding pattern (Fig. 4A–C). In addition, these cells not only had typical bud scars but also chitin spots randomly distributed over the whole cell surface (Fig. 4D–G). The haploid mutant strain 48 displayed a bipolar budding pattern. The gene which is deleted in this mutant has previously been described by Sanders and Herskowitz (1996) as *BUD4*, which is required for axial budding pattern. The mutant strains 9 and 62 often formed cell clusters. The gene deleted in mutant 9 has already been described as SEC66 (Feldheim et al. 1993), which encodes an integral membrane protein of the endoplasmic reticulum required for translocation of presecretory proteins in *S. cerevisiae*. In mutant 62, the gene *KAP104* has been deleted; its product is thought to be functionally involved in recycling mRNA-binding proteins to the nucleus after mRNA export (Aitchison et al. 1996). Why these deletion mutants also show an altered budding pattern in combination with cell clustering is not known.

The daughter cells of mutant strains 1, 41 and 60 showed a defect in budding. About 30% (10% in wild type) of the cells were unbudded and no bud scars were detectable. In addition, cells of strains 1 and 60 tended to accumulate in G1, while strain 41 was characterised by an increased proportion of G2 cells in our FACS profiles. Moreover, the cells without bud scars did retain methylene blue, indicating that they are not viable. This phenotype suggests a defect in cell cycle progression.

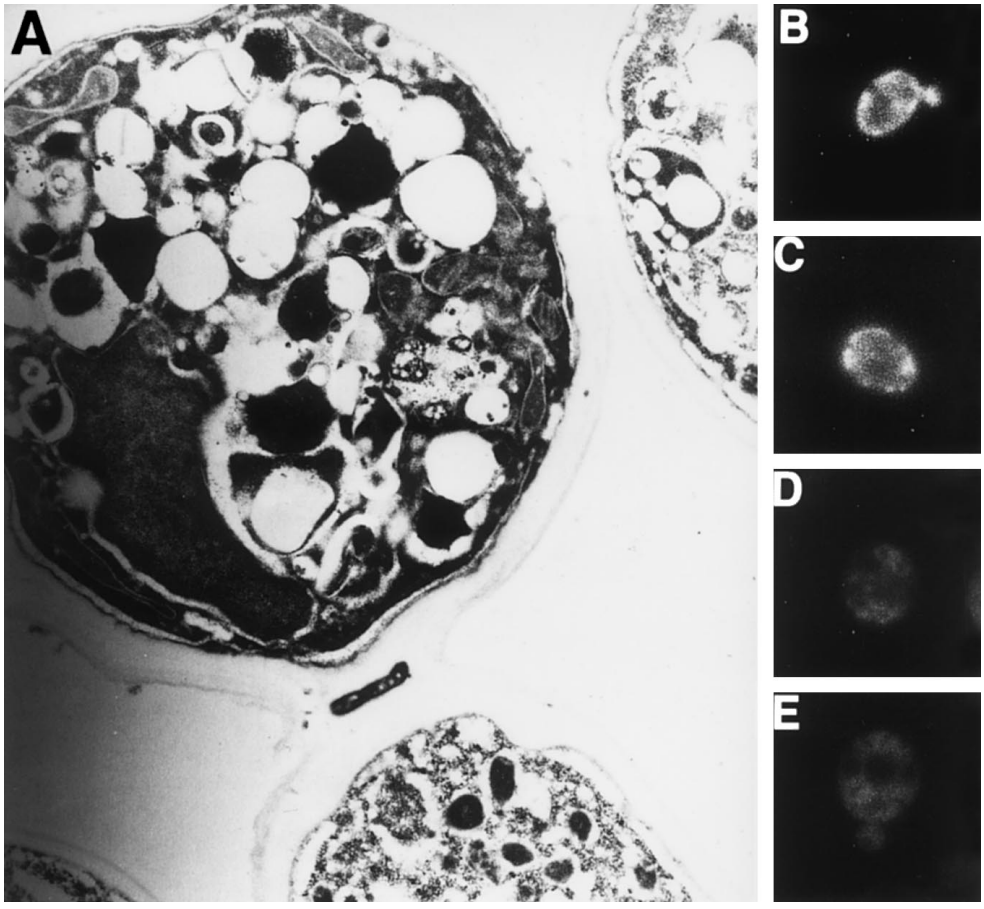

Fig. 2A–E Dispersed vacuoles and dislodged mitochondria in mutant strain 117 (*YBR152w*). **A** Electron micrograph of a typical mutant cell with dispersed vacuolar inclusions. **B–D** Immunofluorescence microscopy of DiOC6(3)-stained cells demonstrates that this leads to a random distribution of mitochondria in many cases (**D** and **E**), while in wild-type cells mitochondria are usually observed close to the plasma membrane (**B** and **C**)

Differentiation

Although yeast does not undergo major developmental changes, it can differentiate under certain growth conditions. For instance, when starved completely for nitrogen, diploid cells enter meiosis and sporulate (Fowell 1952), or they grow out as pseudohyphae when nitrogen levels in the medium are low (Gimeno et al. 1992). Starved haploid cells, however, can enter the quiescent G0 stage (Hartwell 1974) or start to grow invasively into solid medium (Liu et al. 1993; Roberts and Fink 1994). They are also able to conjugate with partners of the opposite mating type to generate zygotes which grow out as diploid cells (Byers 1981). Pseudohyphal and invasive growth, as well as mating, are controlled by MAP kinase cascades, some members of which are common to all three processes (Liu et al. 1993; Ammerer 1994; Roberts and Fink 1994). While these pathways have already been extensively studied, there are still many open questions with respect to how the signals are processed downstream of the kinase cascades.

To examine the mutant strains for defects in invasive growth we used the assays described by Roberts and Fink (1994). In addition, we tested the strains for their ability to conjugate, using mating assays (Sprague 1991). Indeed, eight out of 121 viable mutant strains showed diminished invasive growth (Table 5), and 17 mutants tested showed variations in mating behavior (Table 5). Eleven of these mutants exhibited reduced or poor mating efficiency. In four of them only the mating efficiency of MAT**a** cells was impaired, in four others only MATα cells were affected. Three strains showed reduced mating ability in both mating types. Mating efficiency was enhanced in six deletion mutants; one of these mutations affected both mating types (Table 5).

Eleven mutants with reduced mating efficiency or with defects in invasive growth also show abnormal responses to osmotic stress (see Tables 3 and 5). Since yeast cells respond to hypertonic and hypotonic shock by activating MAP kinase pathways (Brewster et al. 1993; Brewster and Gustin 1994; Schüller et al. 1994; Davenport et al. 1995), it is likely that crosstalk may influence the osmotic shock pathway, the pheromone signal pathway and the invasive growth signalling pathway. Such crosstalk has been shown for the mating pheromone pathway, which is negatively influenced by the HOG pathway (Hall et al. 1996).

Glycosylation and secretion: the invertase assay

Yeast invertase encoded by the *SUC* genes is expressed as an unglycosylated cytoplasmic and a glycosylated extracellular enzyme. Thus, the enzyme provides an

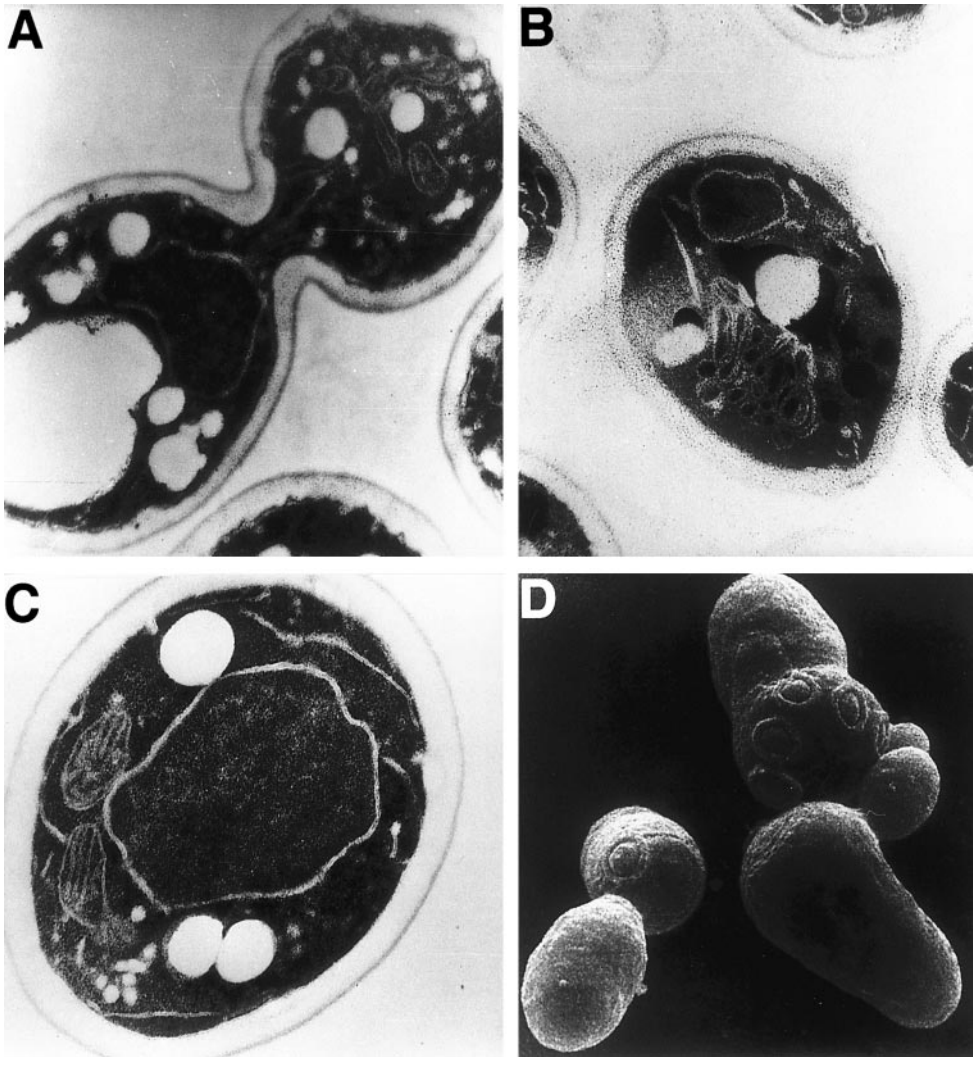

Fig. 3A–D Morphology of mutant strains 142 (*YBR017w = VPS15*), 30(*YJR120w*) and 122(*YJL076w*). Mutant 142 (**A**) is characterized by a large number of vacuoles, while mutant strain 30 (**B**) showed clumping of mitochondria. Mutant 122 (**C**) also exhibited fused mitochondria which were larger than wild type. This mutant also frequently formed club-shaped cells as shown by scanning electron microscopy (**D**)

excellent model for studying protein glycosylation and secretion. Therefore, we tested our viable mutants for defects in these pathways. None of them failed to secrete invertase, as revealed by the invertase-overlay assay, whereas seven mutants showed an altered electrophoretic mobility of invertase (9, 65, 86, 117, 125, 130, 135), which indicates a change in the glycosylation pattern of the enzyme (see Table 7). Four mutants (9, 65, 86, 135) secreted invertase with short mannose outer chains (see Table 7); of these only mutant 9 showed a reduction in specific activity in comparison to homozygous wild-type strains. In mutant 86 only 40% of the invertase proteins were glycosylated – a considerably lower proportion than found in wild-type cells, in which more than 65% is glycosylated. In the other mutants the mobility of the extracellular invertase on native gels was heterogeneous, with both short and full-length mannose outer chains. However, in MATα, but not MAT**a**, cells of mutant 117, invertase exhibited reduced specific activity but had a wild-type molecular weight. The glycosylation defect in five of the above mutants (9, 65, 86, 117, 130) was associated with other phenotypes, e.g. defects in the response to stress or in utilization of carbon sources.

Chromosome segregation and genetic stability

The fidelity of chromosome segregation is a prerequisite for the correct distribution of genetic material during cell growth. Therefore, it is essential for every living organism. The processes involved in achieving this goal are collectively grouped together in the so-called chromosome cycle, which describes the duplication and segregation of the genetic material during the cell cycle. Many proteins are involved in the fundamental processes of DNA replication and chromosome segregation (Hegemann and Fleig 1993; Page and Snyder 1993; Bell 1995). Six of the 121 viable deletion strains exhibited an increase in the rate of mitotic loss of an artificial minichromosome (Hegemann et al. 1999) (Table 6). Three of these strains (49, 62 and 119) displayed pleiotropic phenotypes, suggesting that the reduced segregation efficiency might not be the primary phenotype of the deletion (e.g. for strain 49: see Lussier et al. 1997). The other three strains (53, 63 and 133) exhibited increased plasmid loss as the only phenotype detected by the various assays. Interestingly, none of these strains showed sensitivity to the microtubule (MT)-destabilising

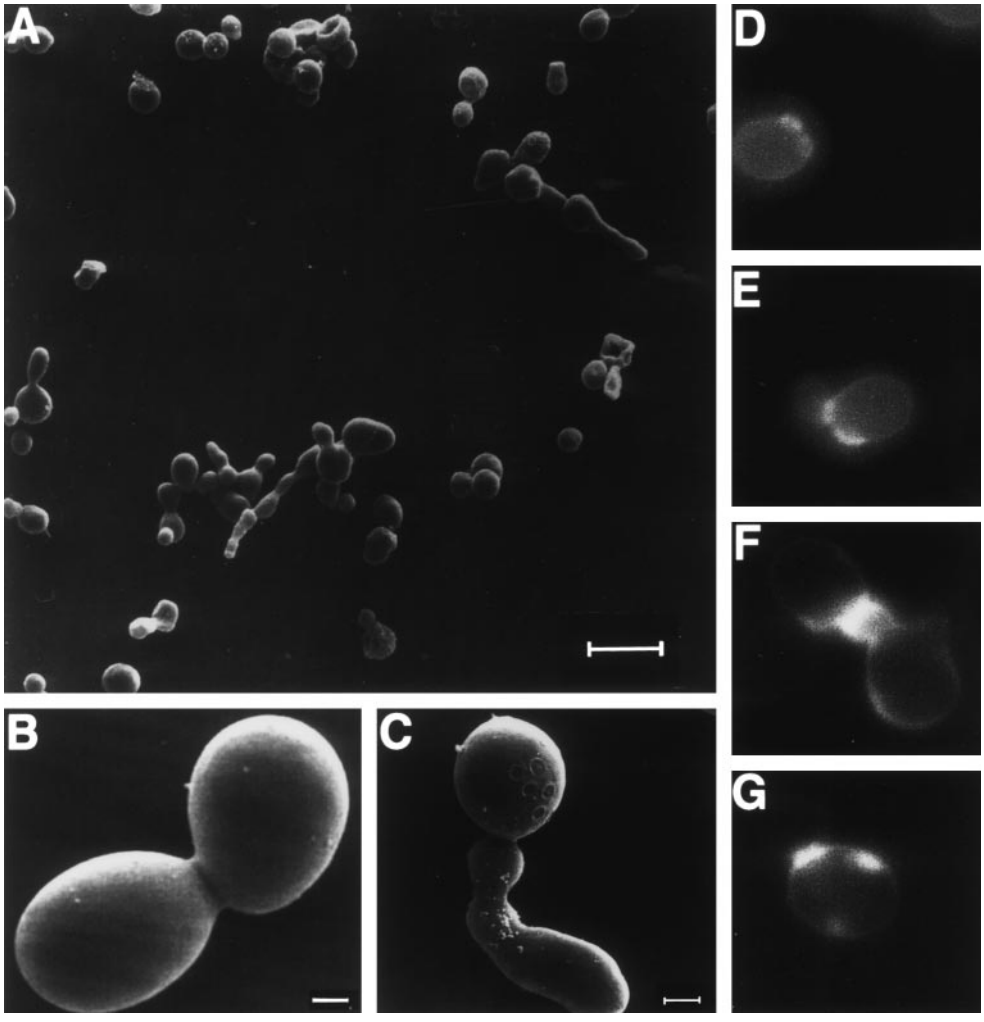

Fig. 4A–G Unipolar budding behavior and randomly distributed chitin spots at the cell surface of mutant strain 117 (*YBR152w*). Scanning electron micrographs show the frequent appearance of a unipolar budding pattern in mutant 117 (**A** and **C**). In addition, these mutant cells tend to form long protrusions which are not observed in case of wild-type cells (**B**). The scale bars indicate 10 μm (**A**), 1 μm (**B**) and 2 μm (**C**). Furthermore, Calcoflour white-stained mutant cells exhibit chitin spots in the cell wall which are randomly distributed over the whole cell (**F** and **G**). In case of wild type cells this staining co-localises only with bud scars (**D** and **E**)

compound thiabendazole (see below), suggesting that the corresponding proteins are not involved in aspects of spindle assembly and/or activity. Finally, it should be noted that none of the non-pleiotropic mutants showed any change in the cell cycle profile of cells in an exponentially growing culture. Database searches did not turn up any significant homologies to other proteins of known function. Thus, further tests will be needed to define the function of these proteins, which may be involved in the chromosome cycle. In particular, it will be interesting to ascertain whether these proteins are involved in DNA replication or mitotic chromosome segregation.

A variety of mechanisms have evolved to cope with chemical and physical damage to DNA and errors made during DNA replication, which could lead to mutations; these include base excision, nucleotide excision, double-strand break and DNA mismatch repair (Friedberg et al. 1995). Mutations in proteins involved in these repair pathways lead to increased mutation rates and thus to an increase in genetic instability.

All 121 mutants were screened for defects in nucleotide excision repair and in other systems that control the fidelity of replication by measuring UV resistance and spontaneous mutation rates. In addition, they were tested for UV-induced mutability and screened for spontaneous mutations in mitochondrial DNA. Deletion strain 69 showed increased mutation rates compared to wild type (Table 6). Its genomic forward mutation rate was increased 19-fold and the reversion rate 16-fold. Likewise the mitochondrial mutation rate – measured as the rate of mutation to chloramphenicol resistance – was enhanced eightfold, while the incidence of erythromycin resistance was not elevated. The deduced amino acid sequence of the ORF deleted in mutant 69 includes most of the conserved regions defined for the DEAH/DEXH family of RNA helicases (Pause and Sonnenberg 1992). From the functions of other members of this family it is not apparent how the lack of this protein could directly affect mutation rates. It may, however, exert an indirect effect by reducing expression or splicing of transcripts required for control of replication fidelity. On the other hand, assuming such a mechanism, one might expect this mutation to have a pleiotropic effect. But no other phenotype was found for this mutant in the tests employed in this project.

Table 5 Differentiation phenotypes

Strain No.	Systematic gene name	Gene designation	Reduced mating efficiency[a]	Enhanced mating efficiency[a]	Invasive growth[b,c]	NaCl/KCl sensitivity[c]	Additional phenotypes[d]
16	*YJR117w*	*STE24*	**a**				Copper resistance
29	*YJL079c/078c*	*PRY1/PRY3*		**a**			–
30	*YJR120w*		α			+	Pleiotropic
41	*YBR095c*		**a**		+		Pleiotropic
42	*YBR096w*				+	+	H_2O_2 sensitivity
46	*YBR103w*			α			–
51	*YKL135c*	*APL2*		α			Growth
52	*YBR296c*			**a** and α			Growth
55	*YJR144w*	*MGM101*			+	+	Pleiotropic
62	*YBR017c*	*KAP104*	α		+	+	Pleiotropic
82	*YKL132c*		**a**				–
85	*YJR101w*				+	+	Pleiotropic
86	*YJR102c*		**a** and α		+	+	Pleiotropic
89	*YLR098c*	*CHA4*	**a** and α			+	–
96	*YGR056w*				+	+	Pleiotropic
106	*YBR111c*		a				Copper sensitivity
117	*YBR152w*		**a** and α			+	Pleiotropic
119	*YJR132w*	*NMD5*	α			+	Pleiotropic
122	*YJL076w*		α			+	Pleiotropic
124	*YGR045c*				+		Growth
140	*YOL054w*			**a**		+	–
143	*YBR114w*	*RAD16*		α			Genetic stability

[a] Reduced or enhanced mating efficiency of the mutants is indicated for *MAT***a** as well as for *MAT*α cells
[b] Altered invasive growth behaviour and salt sensitivity is marked by "+"
[c] Salt sensitivity data were included to underline the possible correlation between differentiation and signalling of salt stress
[d] Mutants which fall into two or more phenotypic classes are defined as being pleiotropic (see Discussion and Table 7)

One mutant strain (143) was found to be moderately UV-sensitive (Table 6). This confirmed our previous finding that the gene deleted in this mutant (*RAD16*) is involved in nucleotide excision repair (Mannhaupt et al. 1992; Schild et al. 1992).

Mutants affecting heterologous signal transduction

Nuclear hormone receptors of higher eukaryotic cells belong to the family of ligand-dependent transcription factors. After entry into the cell, the ligand binds to a receptor, thereby changing its conformation; the receptor-ligand complex enters the nucleus and recognizes specific DNA sequences known as hormone response elements, and activates transcription of the target genes (Tsai and O'Malley 1994). This signal transduction system has been established in yeast, and components have been identified that influence this signaling pathway (Nawaz et al. 1992; Yoshinaga et al. 1992; Baniahmad et al. 1995; Kralli et al. 1995). In this work, the human estrogen receptor was used to screen the deletion mutants for alterations in the response to addition of estrogen to the cells (Picard et al. 1990).

Of 77 deletion strains analyzed 13 showed a reduced response to the addition of estradiol (reduction of more than 40% as compared to wild type) (Table 7). In five mutants (2, 84, 123, 126, 129) this was the only detect-

Table 6 Genetic stability

Strain No.[a]	Systematic gene name	Gene designation	Plasmid loss	Spontaneous mutator phenotype	Mitochondria resistant to chloramphenicol	UV sensitivity	Additional phenotypes[b]
49	*YJR106w*		+				Pleiotropic
53	*YJR138w*		+				None
62	*YBR017c*	*KAP104*	+				Pleiotropic
63	*YJL082w*		+				None
69	*YIR002c*			+	+		None
119	*YJR132w*	*NMD5*	+				Pleiotropic
133	*YBR107c*		+				None
143	*YBR114w*	*RAD16*				+	Enhanced mating efficiency

[a] Mutants that show an altered phenotype relative to wild type are indicated by +. Please note that, in addition to the test results tabulated, assays for UV-induced canavanine resistance and resistance of mitochondria to erythromycin were also performed, but none of the mutants listed showed any effect
[b] Mutants which fall into two or more phenotypic classes are defined as being pleiotropic (see Discussion and Table 7)

able phenotype, suggesting that the corresponding gene products are likely to be involved in some step of this signal transduction pathway. In four cases (114, 118, 119, 147), the mutants showed either sensitivity or resistance to $CuSO_4$ in addition to the reduced signal response, suggesting a link between stress and the estrogen signalling pathway. Finally, five pleiotropic mutants (1, 49, 119, 122, 125) also showed a reduced signal response, with one (122) not responding to estradiol at all.

Discussion

This investigation presents a preliminary characterization of 149 ORFs from *S. cerevisiae* which were identified by genome sequencing and whose function was unknown when this project was started. For the first time, an attempt was made to dissect gene function by a systematic approach using a large variety of test systems which were implemented in eleven different laboratories. In contrast to genetic approaches which discover genes by complementation of a genetic defect, this work started with the deletion of genes, followed by systematic investigation of the deletion mutants for possible malfunctions. This approach has uncovered a large number of phenotypes, most of which would not have been discovered by the classical approach. We have been able to attribute phenotypes to more than half of the viable mutants analysed. If these mutants had been identified by classical means, in most cases they would have been described on the basis of a single primary phenotype. However, here we show that the systematic analysis reveals pleiotropic phenotypes in many cases. This complicates the interpretation of the results and demonstrates that many of the known yeast genes, which have already been characterized by a specific phenotype, need systematic reinvestigation. This, of course, is obvious to geneticists and is confirmed in many cases by the variety of different names assigned to the same gene. It also emphasizes the importance of the systematic analysis of gene function. The data resulting from such an approach will be of increasing value for the future understanding of physiological and genetic interactions.

The test systems

In addition to dissecting gene functions, the present study demonstrates that some straightforward test systems are capable of uncovering a large variety of gene functions, thereby reducing redundant labour to a minimum. Simple tetrad analysis after sporulation of the heterozygous deletion mutants proved to be very powerful, since it revealed differences from wild-type segregants for one-third of the deletion strains (53 of 150 tested; of the genes affected, 29 were essential; see legend of Table 2). Tetrad analysis in conjunction with the rapid and simple plating test systems, which examine growth behaviour and analyse growth under different stress conditions, detected phenotypes in 68% of the viable deletion mutants (82 of 121). The growth and stress tests alone therefore revealed phenotypes for 54 of the 121 viable mutants (45%), including 24 already identified by tetrad analysis. Together with the essential phenotypes, these tests, in conjunction with more sophisticated morphological and cytological studies and the mating assays, assigned at least one phenotype to 105 of the 150 deletion mutants (70%).

Based on these observations, an efficient functional analysis system would apply the more sophisticated test systems first to those mutants which exhibit malfunctions in the rapid tests. The deletion mutants that still fail to show phenotypes can be subjected to the remaining tests; based on our experience, this should uncover phenotypes for a further 20% of the mutants.

Deletion of already known genes

In the course of our investigation, phenotypes and functions have been described by other authors for 26 of the 149 genes investigated here. These data can be used as a control for the results obtained in our tests. Only six of these genes (*UBS1*, *SSE2*, *SHE2*, *TOK1*, *RGT2*, and *PRPS5*) failed to show a phenotype in our screen. Deletion of one of them, *UBS1*, which has been described as a positive regulator of Cdc34p, causes a phenotype only in conjunction with a temperature-sensitive *cdc34* allele (Prendergast et al. 1996). *SHE2* is required for daughter cell-specific repression of *HO* expression, a function which was not covered by our test systems (Jansen et al. 1996). For the same reason deletion of *PRPS5*, which encodes a phosphoribosylpyrophosphate synthetase, would not be expected to reveal a phenotype in our analysis. Likewise, no function has been detected for *SSE2* (Mukai et al. 1993), either in our work or that of others. Although *TOK1* has been shown to code for a putative potassium channel protein (Ketchum et al. 1995), its deletion did not lead to increased sensitivity to KCl in our tests. Likewise, deletion of *RGT2*, a regulator of glucose transport (Oezcan et al. 1996), did not result in reduced growth in glucose-containing medium. A possible explanation for these results is the presence of redundant functional genes in the genetic background. Only additional deletion of those genes would then be expected to cause a phenotypic change.

Deletion of 12 of the remaining 20 genes revealed phenotypes which coincided with those described by others (*STE24*, *SEC66*, *YML30*, *BUD4*, *MGM101*, *MIP1*, *HIR3*, *KAP104*, *SAT4*, *NMD5*, *RAD16*, *MUP1*). *STE24* encodes a membrane-associated metalloprotease which is required for the N-terminal processing of the yeast **a**-factor precursor (Fujimura-Kamada et al. 1997). In fact, our results show that *MAT***a** cells of mutant 16, which lacks *STE24*, show reduced mating, while *MAT*α cells do not. Surprisingly, the same mutant also exhibits

resistance to copper. *SEC66* codes for an integral membrane protein complex of the endoplasmic reticulum, which is required for translocation of presecretory proteins (Feldheim et al. 1993). Mutant 9, which is deleted for *SEC66*, shows a defect in glycosylation of invertase in our test. The temperature-sensitive phenotype observed in mutant 9 has also been confirmed in the meantime (Feldheim et al. 1993). Mutant 48 (*BUD4* deletion) exhibits a bipolar budding pattern as reported (Chant and Herskowitz 1991). *YML30* has been found to be a mitochondrial ribosomal protein (Grohmann et al. 1991). The phenotype of the deletion mutant – respiration deficiency – is compatible with this finding. The deletion of *MGM101* (Chen et al. 1993) or *MIP1* (Isaya et al. 1994), which both code for mitochondrial proteins, also leads to respiration deficiency. Deletion of *KAP104* or *NMD5* results in pleiotropic phenotypes. This is expected since *KAP104* encodes a karyopherin required for mRNA export from the nucleus (Aitchison et al. 1996), and *NMD5* specifies a putative Upf1p-interacting component of the yeast nonsense-mediated mRNA decay pathway (Yeast Proteome Handbook 1997). Defects in RNA transport and metabolism should lead to a general reduction in growth rate.

SAT4 deletion mutants have been reported to be sensitive to NaCl but not KCl, a phenotype we were able to verify (Skala et al. 1991). Also for the *HIR3* deletion we were able to observe this phenotype. While *SAT4* codes for a kinase with similarity to Npr1p, the *HIR3* gene is known as one of the negative transcriptional regulators of histone genes *HTA1* and *HTB1* (Osley and Lycan 1987; Xu et al. 1992), but may be involved in the regulation of other genes as well. Therefore, it is conceivable that the lack of this transcriptional repressor gene leads to an altered expression pattern of the histone genes that influences growth behavior.

Mup1p is a high-affinity methionine permease involved in amino acid transport through the plasma membrane (Isnard et al. 1996). The deletion of the gene leads to a decrease in the reporter gene response in our heterologous signal transduction test. Since this experiment uses estradiol to activate the signal transduction pathway, the result suggests that the permease may also be involved in transport of steroids. Finally, the decrease in genetic stability noted with *RAD16* deletion mutants might be caused by a defect in nucleotide excision repair (Wang et al. 1997), however, the enhanced mating behavior of *MAT*α but not *MAT***a** cells, which we also observe in this mutant, is difficult to explain.

In seven cases (*HOM6*, *MYO3*, *SUL1*, *SUL2*, *CHA4*, *PHO88*, *VPS15*), gene deletion gave rise to phenotypes which do not correspond to the functions previously described . For example, Hom6p catalyses the third step in the common pathway of methionine and threonine biosynthesis (Thomas et al. 1993). Our *HOM6* deletion mutant, however, exhibits thiabendazole sensitivity and a reduced germination rate. Deletion of *MYO3*, which encodes the cytoskeletal protein myosin type I (Goodson and Spudich 1995), leads to H_2O_2 and NaCl sensitivity. A similar phenotype is found for *vps15* mutants lacking a vacuole sorting protein (Stack et al. 1993); in our investigation, the corresponding deletion mutant was found to have enlarged vacuoles.

Although *SUL1* and *SUL2* code for sulphate transporters (Cherest et al. 1997), *SUL1* deletion mutants contain fewer actin patches than wild-type cells and *SUL2* mutants generate many anucleate cells. Cha4p is a Zn finger-containing transcription factor that activates *CHA1*, which encodes the catabolic L-serine (L-threonine) deaminase responsible for the utilisation of serine and threonine as nitrogen sources (Holmberg and Schjerling 1996). According to our data, its deletion leads to sensitivity to high concentrations of NaCl, and the mutant shows reduced mating efficiency of *MAT***a** as well as of MATα cells. A possible explanation for these additional phenotypes might be that Cha4p transmits certain stress signals. It may also bind to promoters of other genes and may therefore affect the expression of specific stress response and mating-dependent genes. Finally, the *PHO88* gene is involved in the transport of inorganic phosphate (Yompakdee et al. 1996). However, its deletion affects not only phosphate metabolism but also results in increased copper sensitivity, retarded germination and in shorter mitochondria than observed in wild-type cells.

Specificity of the phenotypes

Our network approach to functional gene analysis resulted in a large variety of individual phenotypes. We assigned these phenotypes to seven classes (Table 7). The Growth class combines all data displayed in Table 2. The Stress class includes the data collected in Table 3 (NaCl sensitivity and resistance, KCl sensitivity, H_2O_2 sensitivity, copper resistance and sensitivity, ts and cs phenotypes), with the exception of the data listed for respiration deficiency. The Morphology class contains all data obtained by our light and electron microscopy studies (Table 4). The Differentiation class includes the data on reduced and enhanced mating as well as on deficiency in invasive growth behavior (Table 5), while the Genetic stability set merges the data presented in Table 6. The phenotypes are summarized in Table 7. In addition, we included two extra classes Glycosylation and secretion, and Heterologous signal transduction in Table 7, the data for which are not presented in any of the other Tables. Mutants that exhibit phenotypes that fall into only one class show a single specific phenotype, while mutants that are members of two or more classes are considered to be pleiotropic.

Based on this definition, 33 out of 77 viable deletion mutants belong to the single-phenotype class (2, 8, 11, 18, 29, 39, 46, 48, 53, 58, 61, 63, 68, 69, 71, 82, 84, 88, 92, 95, 98, 101, 115, 116, 121, 123, 126, 129, 132, 133, 135, 146, 148). Six of the corresponding genes have already been described (48: *BUD4*; 58: *MYO3*; 61: *HIR3*; 88: *SUL2*; 98: *BIO5*; 101: *SUL1*). While four of the latter

Table 7 Phenotypic classification

Strain number[a]	Systematic gene name	Gene designation	New gene name[b]	Stress	Growth	Morphology	Differentiation	Gene stability	Glycosylation/ secretion	Heterologous signal transduction	Features/homology of product (from Proteome June 97)
1[S]	*YBR163w*		*DEM1*	+	+	+				+	Weak similarity to Pta1p (pre-tRNA process. protein)
8	*YGL236c*		*IPS1*	+							Strong similarity to *E. coli* GidA
9	*YBR171w*	*SEC66*			+	+			+		Component of ER protein translocation subcomplex
11	*YNL265c*		*IST1*	+							Similar to Nuf1p (spindle pole body component)
12	*YNL264c*		*ISS1*	+	+						Similar to Ynl231p (weak sim to Sec14p + Ylr380p)
16	*YJR117w*	*STE24*		+			+				Processing of farnesylated proteins (a-factor conversion)
18	*YBR150c*		*TBS1*				+				
29	*YJL079c/ 078c*		*PRY1/ PRY3*				+				Both show similarity to plant pathogenesis related proteins. *PRY1* is expressed under starvation conditions
30[S]	*YJR120w*			+	+	+	+				–
32[S]	*YJR118c*		*ILM1*		+	+					–
37[S]	*YNL252c*	*YML30*		+	+	+					Mitochondrial ribosomal protein
39	*YBR086c*		*IST2*	+							Similar to calcium and sodium channel proteins
41[S]	*YBR095c*				+	+	+				Similar to Drosophila cyclin B
42	*YBR096w*			+			+				
43[S]	*YBR098w*			+	+	+					Similar to clathrin heavy chain
44[S]	*YBR101c*				+	+					Weak similarity to Ypt/Rab interacting protein
46	*YBR103w*		*EMB1*				+				WD-40 repeats
48	*YJR092w*	*BUD4*				+					Involved in axial but not bipolar budding pattern
49[S]	*YJR106w*	*ECM27*		+	+	+		+		+	Similar to *C. elegans* C07A9.4. Yeast mutant is Calcofluor sensitive; integral membrane protein
51	*YKL135c*	*APL2*			+		+				*β*-Adaptin, large subunit of the clathrin- associated protein (AP) complex, role in Golgi function
52	*YBR296c*		*ITN1*		+		+				Probably a Na+-phosphate symporter
53	*YJR138w*		*IML1*					+			–
55[S]	*YJR144w*	*MGM101*		+	+	+	+				Mitochondrial genome maintenance
56	*YJR139c*	*HOM6*			+	+					Homoserine dehydrogenase, biosynthesis of serine and threonine
58	*YKL129c*	*MYO3*		+							Myosin type 1/function similar to Myo5p
60[S]	*YKL134c*	*MIP1*		+	+	+					Mitochondrial intermediate peptidase. Involved in processing of Rip1p and Cox4p
61	*YJR140c*	*HIR3*		+							Probably a membrane protein
62[S]	*YBR017c*	*KAP104*		+	+	+	+	+			Transportin, karyopherin. Involved in nuclear transport of mRNA binding proteins
63	*YJL082w*		*IML2*					+			Similar to Ykr018p
65[S]	*YJL094c*			+	+				+		Similar to *Enterococcus hirae* Na+/H+ antiporter NapA
68	*YIR001c*		*SGN1*		+						Contains one RNA recognition (RRM) domain
69	*YIR002c*		*MPH1*					+			Similar to ATP-dependent RNA helicases
71	*YIR004w*		*ICS1*	+							Similar to *E. coli* DnaJ and other DnaJ-like proteins
75[S]	*YIR005w*		*IST3*	+	+						Contains one RNA recognition (RRM) domain
80	*YJL096w*			+	+	+					Suppresses formation of red pigment in W303 *ade2* cells
82	*YKL132c*		*RMA1*				+				Similar to folyl-polyglutamate synthase
85[S]	*YJR101w*			+	+	+	+				Similar to superoxide dismutase
86	*YJR102c*			+	+		+		+		–
87	*YIR009w*	*NAM2/ MSL1*		+	+	+					Similar to U1 type A and U2 type B snRNPs
88	*YLR092w*	*SUL2*				+					High affinity sulfate transporter, similar to Sul1p
89	*YLR098c*	*CHA4*		+			+				Zn-finger protein (Zn2-Cys6 type)

92	*YLR099c*		*ICT1*	+								Similar to Ecm18p; mutant shows increased sensitivity to Calcofluor white
95	*YCR008w*	*SAT4*		+								Member of Hal5p subfamily, mutant shows increased salt sensitivity. Similar to Npr1p protein kinase
96[S]	*YGR056w*			+	+		+					Similar to Ylr357p
98	*YNR056c*	*BIO5*			+							Transmembrane regulator of KAPA/DAPA transport, involved in biotin biosynthesis
101	*YBR294w*	*SUL1*				+						High-affinity sulfate transporter, similar to Sul2p
102[S]	*YJR108w*		*BM1*		+	+						–
103[S]	*YKL137w*			+	+	+						–
105	*YBR106w*	*PHO88*		+	+	+						Membrane protein involved in inorganic phosphate transport
106	*YBR111c*		*RMA2*	+			+					Weak similarity to Drosophila serendipity protein
114[S]	*YBR242w*			+	+						+	Contains purine-nucleotide binding domain
115	*YBR244w*		*MI1*			+						Similar to gluthatione peroxidase
116	*YBR245c*		*SGN2*		+							
117[S]	*YBR152w*			+	+	+	+		+			–
118	*YBR151w*		*APD1*	+		+					+	–
119[S]	*YJR132w*	*NMD5*		+	+		+	+			+	Nam7p/Upf1p-interacting protein (mRNA decay pathway)
121	*YJR134c*		*SGM1*		+							
122[S]	*YJL076w*			+	+	+	+				+	Similar to Ykr010cp
124	*YGR045c*				+		+					–
125	*YGR054w*								+		+	Similar to *C. elegans* C14B9.6 product
130[S]	*YLR087c*			+	+				+			Contains Myb DNA binding domain repeat signatures, probably a membrane protein
132	*YLR093c*		*MAM2*			+						Similar to the C-terminal region of synaptobrevens
133	*YBR107c*		*IML3*					+				Weak similarity to chitin synthase
135	*YBR125c*		*GCT1*						+			Ser/Thr protein phophatase (PP2 C family)
137	*YOL060c*		*MAM3*		+	+						Similar to *C. elegans* R13G10.4, 3; potential transmembrane domains
140	*YOL054w*			+			+					Contains C3HC4-type Zn-finger, and cytochrome *c* heme-binding motif
142[S]	*YBR097w*	*VPS15*		+	+	+						Ser/Thr protein kinase for vacuole protein sorting.
143	*YBR114w*	*RAD16*					+	+				Nucleotide excision repair protein
146	*YBR157c*		*ICS2*	+								–
147	*YJL077c*		*ICS3*	+							+	–
148	*YBR158w*		*ICS4*	+								Weak similarity to *C. elegans* CNTF receptor alpha. The null mutant shows slow growth and reduced germination rates
150[S]	*YGR057c*			+	+							–

[a] The + sign indicates that the mutant phenotype falls into the indicated class(es). Deletion of the following 45 genes did not lead to any detectable phenotype: *YBR053c*, *YBR063c*, *YBR094w*, *YBR105c*, *YBR108w*, *YBR147w*, *YBR165w*, *YBR169c*, *YBR176w*, *YBR295w*, *YIR002c*, *YIR004w*, *YJL036w*, *YJL043w*, *YJL060w*, *YJL078c*, *YJL079c*, *YJL083w*, *YJL093c*, *YJL098w*, *YJL185c*, *YJR107w*, *YJR111c*, *YJR115w*, *YJR126c*, *YJR127c*, *YJR129c*, *YJR130c*, *YJR142w*, *YKL130c*, *YKL146w/147c*, *YL138w*, *YLR089c*, *YLR094c*, *YLR095c*, *YNL249c*, *YNL253w*, *YNL254c*, *YNL255c*, *YNR057c*, *YNR058w*, *YOL056w*, *YOL057w*, *YOL061w*

[b] New gene designations: *ABM*, Aberrant microtubules; *AMJ*, Aberrant mitochondria; *APD*, Actin patches distal; *DEM*, Defects in morphology; *EMB*, Enhanced mating B (in *MATα* cells); *GCT*, Glycosylation chain truncated; *ICS*, Increased copper sensitivity; *ICT*, Increased copper tolerance; *ILM*, Increased loss of mitochondrial DNA; *IML*, Increased minichromosome loss; *IPS*, Increased potassium sensitivity; *ISS*, Increased sodium sensitivity; *IST*, Increased sodium tolerance; *ITN*, Increased in 2n; *MPH*, Mutator phenotype; *RMA*, Reduced mating A (in *MAT**a*** cells); *SGM*, Slow growth on galactose and mannose; *SGN*, Slower growth on non-fermentable carbon sources; *TBS*, Thiabendazole sensitive

[c] The following mutants were affected only in heterologous signal transduction: 2 (*YBR164c*), 84 (*YBR293w*), 123 (*YGR043c*), 126 (*YGR055w*) and 129 (*YGR042w*). All gene names have been reported to SGD

mutants exhibit phenotypes which were in accordance with our data, the phenotypes of two of them (*SUL1* and *SUL2*) were different. Of the previously undescribed genes, single and multiple phenotypes, which were clearly defined, qualified them for the name shown in Table 7 (all gene names have been registered at SGD). Phenotypic changes pertaining to two classes were found in 19 mutants (12, 16, 32, 42, 44, 51, 52, 56, 75, 89, 102, 106, 124, 125, 137, 140, 143, 147, 150). For nine of them a function can be predicted due to their phenotypic properties (12, 32, 51, 52, 75, 102, 106, 137, 147). These genes were named for their most prominent phenotypes (see Table 7). Fifteen mutants exhibited phenotypic changes in three different classes (9: *SEC66*, 37: *YML30*, 41, 43, 60, 65, 80, 87, 96, 103, 105: *PHO88*, 114, 118, 130, 142: *VPS15*), one of which was named for its predominant phenotypic behaviour (118). Phenotypes in more than three classes were found for 10 mutants (1, 30, 49, 55: *MGM101*, 62: *KAP104*, 85, 86, 117, 119: *NMD5*, 122).

The heterologous signal transduction test differs from all other tests used, because it interferes with a number of cellular functions, such as non-specific substrate uptake by multiple drug resistance proteins, gene activation and nuclear transport. Five of 77 mutants tested (2, 84, 123, 126, 129) revealed a decrease in estrogen response as their sole phenotype. The gene deleted in one of the mutants (84) shows homology to multidrug resistance proteins, which is compatible with the phenotype we observe. *MUP1*, which is deleted in mutant 126, encodes a methionine permease (Isnard et al. 1996), suggesting that this permease may also be able to transport steroids. These four genes were named for their homology to multidrug resistance proteins or for their decreased ability to transduce an estrogen signal.

Outlook

The functional analysis of 150 deletion mutants has revealed a surprisingly large number of phenotypes. Nearly 70% of the mutants exhibited at least one phenotype. This harvest of phentoypes was made possible by the choice of 20 different test systems used for this analysis. Moreover, a significant number of deletion strains exhibited pleiotropic phenotypes, making it often very difficult to predict in what pathway the corresponding gene product might primarily be involved. Indeed it can be anticipated that in further functional analysis programs, like this one or the European EUROFAN programs, many deletants will show pleiotropic phenotypes. This may reflect the complexity of interactions and dependencies to which each single protein is subject within a cell. Thus, it will not only be important to understand a specific phenotype but also to consider the assays which did not reveal a phenotype. It will be a great challenge for bioinformatics to build up phenotypic networks describing the "phenotypic footprint" of each of the approximately 6000 yeast proteins. Finally, such phenotypic networks may result in functional networks, and at some point the sum of all biochemical activities may describe what constitutes a living cell.

Acknowledgements This work was supported by the Bundesministerium für Bildung und Forschung. The authors would like to thank Dr. S. Kieffer for all the encouraging help which she gave to the project and Dr. Johnston for his support. We also thank Dr. U. Luhmann and Mrs. B. Wiehl for their excellent financial administration of the project and Drs. W. Ansorge and T. Pohl for providing DNA sequences to the different laboratories involved in the functional analysis approach. Furthermore, the excellent technical assistance and experimental support provided by G. Fiedler, B. Habbig, A. Meuche, M. Ninkovic, S. Pfränger, K.W. Prohl, O. Reimer, H. Schreer, and S. Schulte is gratefully acknowledged. Finally, the authors would like to express their special thanks to Dr. P. Hardy for excellent handling and editing of the manuscript.

References

Adams AE, Pringle JR (1991) Staining of actin with fluorochrome-conjugated phalloidin. Methods Enzymol 194:729–731

Aitchison JD, Blobel G, Rout MP (1996) Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. Science 274:624–627

Ammerer G (1994) Sex, stress and integrity: the importance of MAP kinases in yeast. Curr Opin Genet Dev 4:90–95

Baniahmad C, Nawaz Z, Baniahmad A, Gleeson MA, Tsai MJ, O'Malley BW (1995) Enhancement of human estrogen receptor activity by SPT6: a potential coactivator. Mol Endocrinol 9:34–43

Bell SP (1995) Eukaryotic replicators and associated protein complexes. Curr Opin Genet Dev 5:162–167

Brewster JL, Gustin MC (1994) Positioning of cell growth and division after osmotic stress requires a MAP kinase pathway. Yeast 10:425–439

Brewster JL, de Valoir T, Dwyer ND, Winter E, Gustin MC (1993) An osmosensing signal transduction pathway in yeast. Science 259:1760–1763

Burns N, Grimwade B, Ross-Macdonald PB, Choi EY, Finberg K, Roeder GS, Snyder M (1994) Large-scale analysis of gene expression, protein localization, and gene disruption in *Saccharomyces cerevisiae*. Genes Dev 8:1087–1105

Butt TR, Sternberg EJ, Gorman JA, Clark P, Hamer D, Rosenberg M, Crooke ST (1984) Copper metallothionein of yeast, structure of the gene, and regulation of expression. Proc Natl Acad Sci USA 81:3332–3336

Byers B (1981) Cytology of the yeast life cycle. In: Strathern JF, Jones EW, Broach JR (eds) The Molecular biology of the yeast *Saccharomyces cerevisiae*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Cairns BR, Lorch Y, Li Y, Zhang M, Lacomis L, Erdjument-Bromage H, Tempst P, Du J, Laurent B, Kornberg RD (1996) RSC, an essential, abundant chromatin-remodeling complex. Cell 87:1249–1260

Chant J, Herskowitz I (1991) Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. Cell 65:1203–1212

Chen XJ, Guan MX, Clark-Walker GD (1993) MGM101, a nuclear gene involved in maintenance of the mitochondrial genome in *Saccharomyces cerevisiae*. Nucleic Acids Res 21:3473–3477

Cherest H, Davidian JC, Thomas D, Benes V, Ansorge W, Surdin-Kerjan Y (1997) Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. Genetics 145:627–635

Dancis A, Haile D, Yuan, DS, Klausner RD (1994) The *Saccharomyces cerevisiae* copper transport protein (Ctr1p). Biochemical characterization, regulation by copper, and physiologic role in copper uptake. J Biol Chem 269:25660–25667

Davenport KR, Sohaskey M, Kamada Y, Levin DE, Gustin MC (1995) A second osmosensing signal transduction pathway in yeast. Hypotonic shock activates the PKC1 protein kinase-regulated cell integrity pathway. J Biol Chem 270:30157–30161

Entian KD, Kötter P (1998) Yeast mutants and plasmid collections. In: Tuite MF, Brown AJP (eds) Yeast gene analysis. Academic Press, London, pp 431–449

Erdmann R, Veenhuis M, Mertens D, Kunau WH (1989) Isolation of peroxisome-deficient mutants of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 86:5419–5423

Feldheim D, Yoshimura K, Admon A, Schekman R (1993) Structural and functional characterization of Sec66p, a new subunit of the polypeptide translocation apparatus in the yeast endoplasmic reticulum. Mol Biol Cell 4:931–939

Fowell R (1952) Sodium acetate agar as a sporulation medium for yeast. Nature 170:578

Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM Press, Washington DC

Fujimura-Kamada K, Nouvet FJ, Michaelis S (1997) A novel membrane-associated metalloprotease, Ste24p, is required for the first step of NH2-terminal processing of the yeast **a**-factor precursor. J Cell Biol 136:271–285

Fürst P, Hu S, Hackett R, Hamer D (1988) Copper activates metallothionein gene transcription by altering the conformation of a specific DNA binding protein. Cell 55:705–717

Gimeno CJ, Ljungdahl PO, Styles CA, Fink GR (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: regulation by starvation and RAS. Cell 68:1077–1090

Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6000 genes. Science 274:546–567

Goldstein A, Lampen JO (1975) Beta-D-fructofuranoside fructohydrolase from yeast. Methods Enzymol. 42:504–511

Goodson HV, Spudich JA (1995) Identification and molecular characterization of a yeast myosin I. Cell Motil Cytoskeleton 30:73–84

Grivell LA, Netter P, Borst P, Slonimski PP (1973) Mitochondrial antibiotic resistance in yeast: ribosomal mutants resistant to chloramphenicol, erythromycin and spiramycin. Biochim Biophys Acta 312:358–367

Grohmann L, Graack HR, Kruft V, Choli T, Goldschmidt-Reisin S, Kitakawa M (1991) Extended N-terminal sequencing of proteins of the large ribosomal subunit from yeast mitochondria. FEBS Lett 284:51–56

Güldener U, Heck S, Fiedler T, Beinhauer J, Hegemann JH (1996) A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res 24:2519–2524

Hall JP, Cherkasova V, Elion E, Gustin MC, Winter E (1996) The osmoregulatory pathway represses mating pathway activity in *Saccharomyces cerevisiae*: isolation of a *FUS3* mutant that is insensitive to the repression mechanism. Mol Cell Biol 16:6715–6723

Hamer DH (1986) Metallothionein. Annu Rev Biochem 55:913–951

Hanschke R, Schauer F (1996) Improved ultrastructural preservation of yeast cells for scanning electron microscopy. J Microsc 184:81–87

Hartwell LH (1974) *Saccharomyces cerevisiae* cell cycle. Bacteriol Rev 38:164–198

Hegemann JH, Fleig UN (1993) The centromere of budding yeast. Bioessays 15:451–460

Hegemann JH, Klein S, Heck S, Güldener U, Niedenthal RK, Fleig U (1999) A fast method to diagnose chromosome and plasmid loss in *Saccharomyces cerevisiae* strains. Yeast 15: 1009–1019

Holmberg S, Schjerling P (1996) Cha4p of *Saccharomyces cerevisiae* activates transcription via serine/threonine response elements. Genetics 144:467–478

Hutter KJ, Eipel HE (1978) Flow cytometric determinations of cellular substances in algae, bacteria, moulds and yeasts. Antonie van Leeuwenhoek 44:269–282

Isaya G, Miklos D, Rollins RA (1994) *MIP1*, a new yeast gene homologous to the rat mitochondrial intermediate peptidase gene, is required for oxidative metabolism in *Saccharomyces cerevisiae*. Mol Cell Biol 14:5603–5616

Isnard AD, Thomas D, Surdin-Kerjan Y (1996) The study of methionine uptake in *Saccharomyces cerevisiae* reveals a new family of amino acid permeases. J Mol Biol 262:473–484

Jansen RP, Dowzer C, Michaelis C, Galova M, Nasmyth K (1996) Mother cell-specific HO expression in budding yeast depends on the unconventional myosin Myo4p and other cytoplasmic proteins. Cell 84:687–697

Jiang YW, Stillman DJ (1992) Involvement of the SIN4 global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. Mol Cell Biol 12:4503–4514

Jungmann J, Reins HA, Lee J, Romeo A, Hassett R, Kosman D, Jentsch S (1993) MAC1, a nuclear regulatory protein related to Cu-dependent transcription factors is involved in Cu/Fe utilization and stress resistance in yeast. EMBO J 12:5051–5056

Kaiser CA, Botstein D (1986) Secretion-defective mutations in the signal sequence for *Saccharomyces cerevisiae* invertase. Mol Cell Biol 6:2382–2391

Ketchum KA, Joiner WJ, Sellers AJ, Kaczmarek LK, Goldstein SA (1995) A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. Nature 376:690–695

Kralli A, Bohen SP, Yamamoto KR (1995) LEM1, an ATP-binding-cassette transporter, selectively modulates the biological potency of steroid hormones. Proc Natl Acad Sci USA 92:4701–4705

Lashkari DA, DeRisi JL, McCusker JH, Namath AF, Gentile C, Hwang SY, Brown PO, Davis RW (1997) Yeast microarrays for genome wide parallel genetic and gene expression analysis. Proc Natl Acad Sci USA 94:13057–13062

Lea DE, Coulson CA (1949) The distribution of the numbers of mutants in bacterial populations. J Genet 49:264–285

Liu H, Styles CA, Fink GR (1993) Elements of the yeast pheromone response pathway required for filamentous growth of diploids. Science 262:1741–1744

Lussier M, White AM, Sheraton J, diPaolo T, Treadwell J, Southard SB, Horenstein CI, ChenWeiner J, Ram AFJ, Kapteyn JC, Roemer TW, Vo DH, Bondoc DC, Hall J, Zhong WW, Sdieu AM, Davies J, Klis FM, Robbins PW, Bussey H (1997) Large scale identification of genes involved in cell surface biosynthesis and architecture in *Saccharomyces cerevisiae*. Genetics 147:435–450

Mannhaupt G, Stucka R, Ehnle S, Vetter I, Feldmann H (1992) Molecular analysis of yeast chromosome II between *CMD1* and *LYS2*: the excision repair gene *RAD16* located in this region belongs to a novel group of double-finger proteins. Yeast 8:397–408

Matsuzaki F, Matsumoto S, Yahara I (1988) Truncation of the carboxy-terminal domain of yeast beta-tubulin causes temperature-sensitive growth and hypersensitivity to antimitotic drugs. J Cell Biol 107:1427–1435

Mazzoni C, Zarov P, Rambourg A, Mann C (1993) The SLT2 (MPK1) MAP kinase homolog is involved in polarized cell growth in *Saccharomyces cerevisiae*. J Cell Biol 123:1821–1833

Mewes HW, Albermann K, Bahr M, Frishman D, Gleissner A, Hani J, Heumann K, Kleine K, Maierl A, Oliver SG, Pfeiffer F, Zollner A (1997) Overview of the yeast genome. Nature 387(Suppl):7–65

Miosga T, Schaaff-Gerstenschläger, Chalwatzis N, Baur A, Boles E, Fourjier C, Schmitt S, Velten C, Wilhelm N, Zimmermann FK (1995) Sequence analysis of a 33.1 kb fragment from the left arm of *Saccharomyces cerevisiae* chromosome X, including putative proteins with leucine zippers, a fungal Zn(II)2-Cys6 binuclear cluster domain and a putative alpha 2-SCB-alpha 2 binding site. Yeast 11:681–689

Moreno S, Klar A, Nurse P (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol 194:795–826

Mortimer RK, Schild D, Contopoulou CR, Kans JA (1989) Genetic map of *Saccharomyces cerevisiae*, edition 10. Yeast 5:321–403

Mukai H, Kuno T, Tanaka H, Hirata D, Miyakawa T, Tanaka C (1993) Isolation and characterization of *SSE1* and *SSE2*, new members of the yeast *HSP70* multigene family. Gene 132:57–66

Munder T, Fürst P (1992) The *Saccharomyces cerevisiae CDC25* gene product binds specifically to catalytically inactive Ras proteins in vivo. Mol Cell Biol 12:2091–2099

Nawaz Z, Tsai MJ, McDonnell DP, O'Malley BW (1992) Identification of novel steroid-response elements. Gene Expr 2:39–47

Osley MA, Lycan D (1987) Trans-acting regulatory mutations that alter transcription of *Saccharomyces cerevisiae* histone genes. Mol Cell Biol 7:4204–4210

Oezcan S, Dover J, Rosenwald AG, Wolfl S, Johnston M (1996) Two glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression. Proc Natl Acad Sci USA 93:12428–12432

Page BD, Snyder M (1993) Chromosome segregation in yeast. Annu Rev Microbiol 47:231–261

Pause A, Sonenberg N (1992) Mutational analysis of a DEAD box RNA helicase: the mammalian translation initiation factor eIF-4A. EMBO J 11:2643–2654

Picard D, Khursheed B, Garabedian MJ, Fortin MG, Lindquist S, Yamamoto KR (1990) Reduced levels of hsp90 compromise steroid receptor action in vivo. Nature 348:166–168

Phalip V. Kuhn I, Lemoine Y, Jeltsch JM (1999) Characterization of the biotin biosynthesis pathway in *Saccharomyces cerevisiae* and evidence for a cluster containing *BIO5*, a novel gene involved in vitamer uptake. Gene 232:43–51

Prendergast JA, Ptak C, Kornitzer D, Steussy CN, Hodgins R, Goebl M, Ellison MJ (1996) Identification of a positive regulator of the cell cycle ubiquitin-conjugating enzyme Cdc34 (Ubc3). Mol Cell Biol 16:677–684

Pringle JR, Preston RA, Adams AE, Stearns T, Drubin DG, Haarer BK, Jones EW (1989) Fluorescence microscopy methods for yeast. Methods Cell Biol. 31:357–435

Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17:208–212

Rieske JS (1967) Preparation and properties of reduced coenzyme Q-cytochrome c reductase (complex III of respiratory chain). Methods Enzymol 10:239–245

Roberts RL, Fink GR (1994) Elements of a single MAP kinase cascade in *Saccharomyces cerevisiae* mediate two developmental programs in the same cell type: mating and invasive growth. Genes Dev 8:2974–2985

Rose MD, Winston F, Hieter P (1990) Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Ross-Macdonald P, Sheehan A, Roeder GS, Snyder M (1997) A multipurpose transposon system for analyzing protein production, localization, and function in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 94:190–195

Rothstein R (1991) Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol 194:281–301

Sanders SL, Herskowitz I (1996) The BUD4 protein of yeast, required for axial budding, is localized to the mother/bud neck in a cell cycle-dependent manner. J Cell Biol 134:413–427

Schild D, Glassner BJ, Mortimer RK, Carlson M, Laurent BC (1992) Identification of *RAD16*, a yeast excision repair gene homologous to the recombinational repair gene *RAD54* and to the *SNF2* gene involved in transcriptional activation. Yeast 8:385–395

Schnell N, Krems B, Entian KD (1992) The *PAR1* (*YAP1*/*SNQ3*) gene of *Saccharomyces cerevisiae*, a *c-jun* homologue, is involved in oxygen metabolism. Curr Genet 21:269–273

Schüller C, Brewster JL, Alexander MR, Gustin MC, Ruis H (1994) The HOG pathway controls osmotic regulation of transcription via the stress response element (STRE) of the *Saccharomyces cerevisiae CTT1* gene. EMBO J 13:4382–4389

Skala J, Purnelle B, Crouzet M, Aigle M, Goffeau A (1991) The open reading frame YCR101 located on chromosome III from *Saccharomyces cerevisiae* is a putative protein kinase. Yeast 7:651–655

Smith V, Chou KN, Lashkari D, Botstein D, Brown PO (1996) Functional analysis of the genes of yeast chromosome V by genetic footprinting. Science 274:2069–2074

Sprague GF (1991) Assay of yeast mating reaction. Methods Enzymol 194:77–93

Stack JH, Herman PK, Schu PV, Emr SD (1993) A membrane-associated complex containing the Vps15 protein kinase and the Vps34 PI 3-kinase is essential for protein sorting to the yeast lysosome-like vacuole. EMBO J 12:2195–2204

Thomas D, Barbey R, Surdin-Kerjan Y (1993) Evolutionary relationships between yeast and bacterial homoserine dehydrogenases. FEBS Lett 323:289–293

Tisdale HD (1967) Preparation and properties of succinic-cytochrome c reductase (complex II–III). Methods Enzymol 10:213–215

Tokuyasu KT (1989) Use of poly(vinylpyrrolidone) and poly(vinyl alcohol) for cryoultramicrotomy. Histochem J 21:163–171

Tsai MJ, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486

Velculescu VE, Zhang L, Zhou W, Vogelstein J, Basrai MA, Bassett DE Jr, Hieter P, Vogelstein B, Kinzler KW (1997) Characterization of the yeast transcriptome. Cell 88:243–251

Wang Z, Wei S, Reed SH, Wu X, Svejstrup JQ, Feaver WJ, Kornberg RD, Friedberg EC (1997) The *RAD7*, *RAD16*, and *RAD23* genes of *Saccharomyces cerevisiae*: requirement for transcription-independent nucleotide excision repair in vitro and interactions between the gene products. Mol Cell Biol 17:635–643

Wharton DC, Tzagoloff A (1967) Cytochrome oxydase from beef heart mitochondria. Methods Enzymol 10:245–250

Xu H, Kim UJ, Schuster T, Grunstein M (1992) Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in *Saccharomyces cerevisiae*. Mol Cell Biol 12:5249–5259

Yeast Proteome Handbook (1997) Proteome, 200 Cummings Center, Suite 425C, Beverly, MA 01915, USA

Yompakdee C, Ogawa N, Harashima S, Oshima Y (1996) A putative membrane protein, Pho88p, involved in inorganic phosphate transport in *Saccharomyces cerevisiae*. Mol Gen Genet 251:580–590

Yoshinaga SK, Peterson CL, Herskowitz I, Yamamoto KR (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science 258:1598–1604

Zarzov P, Mazzoni C, Mann C (1996) The SLT2(MPK1) MAP kinase is activated during periods of polarized cell growth in yeast. EMBO J 15:83–91

Zimmer T, Kaminski K, Scheller U, Vogel V, Schunck W-H (1995) In vivo reconstitution of highly active *Candida maltosa* cytochrome P450 monooxygenase systems in inducible membranes of *Saccharomyces cerevisiae*. DNA Cell Biol 14:619–628