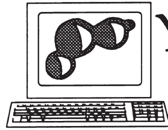
YEAST
Yeast **15**, 1775–1796 (1999)



Yeast Functional Analysis Reports

Systematic Analysis of *S. cerevisiae* Chromosome VIII Genes

RAINER NIEDENTHAL[1]**†, LINDA RILES[2]†, ULRICH GÜLDENER[1]†, SABINE KLEIN[1], MARK JOHNSTON[2] AND JOHANNES H. HEGEMANN[1]*

[1]*Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, Geb. 26.12.01.64, 40225 Düsseldorf, Germany*
[2]*Washington University, Medical School, Department of Genetics, Box 8232, 660 S. Euclid, St. Louis, MO 63110, U.S.A.*

To begin genome-wide functional analysis, we analysed the consequences of deleting each of the 265 genes of chromosome VIII of *Saccharomyces cerevisiae.* For 33% of the deletion strains a growth phenotype could be detected: 18% of the genes are essential for growth on complete glucose medium, and 15% grow significantly more slowly than the wild-type strain or exhibit a conditional phenotype when incubated under one of 20 different growth conditions. Two-thirds of the mutants that exhibit conditional phenotypes are pleiotropic; about one-third of the mutants exhibit only one phenotype. We also measured the level of expression directed by the promoter of each gene. About half of the promoters direct detectable transcription in rich glucose medium, and most of these exhibited only low or medium activity. Only 1% of the genes are expressed at about the same level as *ACT1*. The number of active promoters increased to 76% upon growth on a non-fermentable carbon source, and to 93% in minimal glucose medium. The majority of promoters fluctuated in strength, depending on the medium. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS — *Saccharomyces cerevisiae*; genome; functional analysis; gene deletion; homologous integration; green fluorescent protein GFP; FACS; phenotypic analysis; microtiter plate assays

INTRODUCTION

Knowledge of the genome sequence of the yeast *Saccharomyces cerevisiae* (Goffeau *et al.*, 1996) allows us to imagine reaching the goal of describing the function of all genes that specify this eukaryotic cell. This is a major challenge, because the sequence reveals that the majority of genes of this organism have resisted detection, despite many years of intensive investigation (Dujon, 1996; Garrels, 1996). To reach a complete understanding of the structure and function of a yeast cell, the roles of these 'occult' genes must be revealed.

Many of the previously hidden genes have probably escaped detection because their inactivation does not cause phenotypes that geneticists have used to screen and select mutants. Previous comprehensive genetic searches for mutants yielded only a minority of genes that exhibited one of the few phenotypes that was tested (Burns *et al.*, 1994;

*Correspondence to: Dr J. Hegemann, Institut für Mikrobiologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, Geb. 26.12.01.64, 40225 Düsseldorf, Germany. Tel.: (+49)-211-81-13733; fax: (+49)-211-81-13567; e-mail: hegemann@uni-duesseldorf.de
**Present address: Institut für Biochemie, Medizinische Hochschule Hannover, OE 4310, 30623 Hannover, Germany.
†These authors contributed equally to this work.
Contract/grant sponsor: McDonnell Foundation, U.S.A.
Contract/grant sponsor: US National Center for Human Genome Research; Contract/grant number: HG00956.
Contract/grant sponsor: FAZIT-Stiftung, Germany.
Contract/grant sponsor: BMBF Project, Network for the functional analysis of unknown gene products, Germany; Contract/grant number: FKZ 0310577.
Contract/grant sponsor: EUROFAN Project of the EU.

CCC 0749–503X/99/161775–22$17.50
Copyright © 1999 John Wiley & Sons, Ltd.
Received 30 April 1999
Accepted 10 August 1999

Goebl and Petes, 1986). A recent systematic search for phenotypes caused by mutations in 261 chromosome V genes was more encouraging: a detectable phenotype was observed for mutants of 60% of the genes (Smith *et al.*, 1996). Whether or not these phenotypes provide clues to the function of the encoded protein, they will undoubtedly be beneficial for future genetic analysis of these genes.

Knowledge of how expression of genes is regulated can contribute to our understanding of the relationships of genes to each other and to cellular processes. A large amount of effort has been invested in analysis of the regulation of expression of a relatively small number of yeast genes. New approaches that enable rapid, genome-wide analysis of gene expression promise to yield a wealth of information on expression of each yeast gene (DeRisi *et al.*, 1997; Velculescu *et al.*, 1997; Wodicka *et al.*, 1997).

As a pilot study for these kinds of genome-wide analyses of yeast cell function, we have deleted all the genes of *S. cerevisiae* chromosome VIII and tested the resultant mutants for several phenotypes. In addition, we measured the level of promoter activity of all chromosome VIII genes under several growth conditions. Based on our experience, we believe that this kind of analysis of all 6000 yeast genes is feasible, and will contribute to achieving the goal of a complete understanding of the function of all genes in this simple eukaryotic cell.

MATERIALS AND METHODS

Yeast strains

All work was done in the diploid strain YM4587 (*MAT***a***/MATα can^R/CAN^S his3Δ200/his3Δ200 lys2-801/lys2-801 leu2-3,112/leu2-3,112 trp1-903/trp1-903 tyr1-501/tyr1-501*). The haploid derivative strains YM4585 (*MAT***a** *CAN^S his3Δ200 lys2-801 leu2-3,112 trp1-903 tyr1-501*) and YM4586 (*MATα can^R his3Δ200 lys2-801 leu2-3,112 trp1-903 tyr1-501*) were used as controls in the phenotypic assays.

Gene deletion and sporulation

The ORFs on chromosome VIII were deleted via homologous recombination by integration of a gene replacement cassette carrying the *gfp* gene and the *HIS3* gene, as described previously (Niedenthal *et al.*, 1996). Briefly, the replacement cassette was generated by PCR using plasmid pBM2983 and two ORF-specific chimeric primers, each 64 nucleotides long (Figure 1). The first (upstream) oligonucleotide carried 5′ the 45 nucleotides (nt) immediately 5′ to the START codon of the ORF, followed by the first 19 nt of the *gfp* gene (5′-ATGAGTAAAGGAGAAGAAC-3′); the second (downstream) oligonucleotide directed in the opposite orientation carried 5′ the 45 nt immediately downstream of the STOP codon of the ORF (reading 5′ to 3′ on the ‘bottom’ strand, toward the STOP codon) followed by 19 nt of the 3′ untranslated region of the *HIS3* gene (5′-GCGCGCCTCGTTCAGAATG-3′) (The ORF-specific primer sequences are available upon request). Yeast strain YM4587 was transformed to His$^+$ with 2–5 μg of the PCR product that includes *gfp* and *HIS3* (up to 40 His$^+$ transformants were obtained). Pure clones of 3–4 transformants were analysed by PCR for correct integration using the ORF-specific primer P1, located approximately 500 bp upstream of the ORF START codon (P1 primer sequences available on request) and the primer P2 located within the *gfp* gene (5′-GTATAGTTCATCCATGCC-3′) (Figure 1).

For four ORFs, no correct deletants were obtained. In two cases the sequence immediately flanking one side of the gene was apparently too monotonous to direct efficient recombination (28 As upstream of *YHR071w*; 27 nt of AT downstream of *YHR115c*). These genes were successfully deleted by amplifying *gfp–HIS3* with oligonucleotides that contain more sequence flanking the gene (an additional 18 nt flanking *YHR115c*; an additional 44 nt flanking *YHR071w*). In another case (*YHL021c*), the sequence of the test oligonucleotide differed at only one nucleotide from another region of the genome, a problem that was solved by using a test oligonucleotide with a unique sequence. One ORF (*YHL050w*) is repeated many times in the genome, so we abandoned efforts to delete this gene. As a control for the promoter studies, the *ACT1* gene was also replaced by the *gfp–HIS3* cassette. The RNA level of the *ACT1* gene had recently been quantified by Northern analysis (Planta *et al.*, 1999).

All 265 heterozygous (*ORF$^+$/Δorf$^-$::gfp-HIS3*) diploid strains were sporulated and random canavanine-resistant spores were analysed (Sherman, 1991). In 89 cases where no or only a small number of His$^+$ segregants could be identified, tetrad analysis of the corresponding heterozygous diploid strains was performed to confirm

Copyright © 1999 John Wiley & Sons, Ltd.

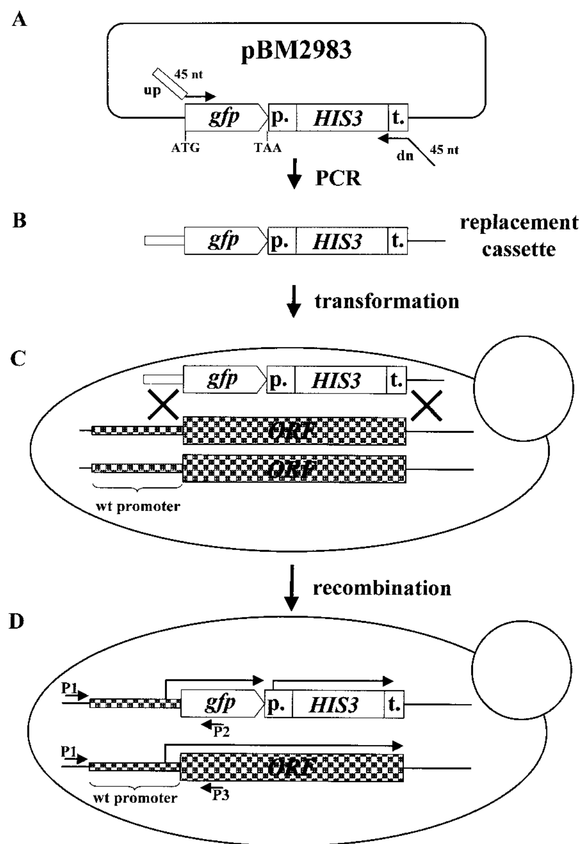


Figure 1. Gene replacement strategy. (A) Map of plasmid pBM2983 (Niedenthal *et al.*, 1996). For each gene replacement experiment, two specific primers were used: the upstream primer (up) carries at its 3′ end 19 nt homologous to the *gfp* gene, starting with the ATG, followed 5′ by 45 nt homologous to the sequence immediately 5′ of the ATG of the gene to be deleted (boxed); the downstream (dn) primer carries at its 3′ end 19 nt homologous to *HIS3*, followed 5′ by 45 nt homologous to sequences immediately downstream of the stop codon of the gene to be deleted (thin line). (B) The gene deletion/replacement cassette was generated by PCR on plasmid pBM2983. (C) Gene disruption using the replacement cassette. Approximately 3–5 μg cassette were transformed into the diploid yeast strain YM4587 and His$^+$ transformants were selected. Homologous recombination between the two 45 nt-long gene-specific DNA segments flanking the cassette and the corresponding homologous regions left and right of the gene of interest results in replacement of one of the two ORF alleles by the cassette. The *gfp* gene is now precisely positioned behind the endogenous promoter of the ORF. (D) Verification of gene replacement event by diagnostic PCR. Two ORF-specific (P1, P3) and 1 *gfp*-specific (P2) verification primers were used to verify correct integration of the *gfp-HIS3* cassette by PCR. Primer pair P1 and P2 yields a PCR product only if the cassette is integrated correctly. After sporulation the viable His$^+$ haploid segregants were tested for the absence of the wt ORF allele by PCR, using primer pair P1 and P3. p. and t.=*HIS3* promoter and terminator regions.

that the gene is essential. For each deletant, one haploid *MAT***a** and one *MATα* deletion strain was identified. In case of a 2^+:2^- segregation (indicating that an essential gene was knocked out), the terminal germination phenotype of the deletion strain was determined (see Table 1). In four cases (1·5%) about half of the two viable spores were His$^+$, indicating that the strain had acquired a second mutation in an essential gene unlinked to the *Δorf$^-$::HIS3*. In these cases the gene deletion was repeated. The 219 viable haploid deletion strains were tested for the absence of the wt ORF by a PCR using the 2 wt ORF-specific primers P1 (see above) and P3 (located within the ORF approximately 500 bp downstream of the START codon) (P3 primer sequences available upon request). Fresh cells (incubated for not more than 2 days) were picked up with an Eppendorf tip attached to a pipette set at 15 μl by barely touching the surface of the colony. The cells were inoculated by pipetting once in a 50 μl reaction mix containing 200 μM dNTPs, 1 μM each primer, and 2 units of Taq DNA polymerase (Boehringer). The tubes were kept on ice, then added to a thermal cycler at 94°C and incubated for 2 min, followed by 35 cycles of 94°C 1·5 min, 50°C 2·0 min, 72°C 3·0 min. In the 17 cases (6·4%) for which a PCR product of the expected size was found (indicating that the wt ORF was present), a new gene deletion was successfully carried out.

Media

The standard medium YP plus 2% glucose (YPD$^+$) and synthetic medium plus 2% glucose (SD) plus the necessary amino acids and bases were prepared as described (Sherman, 1991).

Phenotypic testing A series of different solid media were prepared and microtiter plates prepared (Rieger *et al.*, 1997). (A) One series of media were based on standard complete glucose medium (YPD$^+$), containing: 1% yeast extract (20047-056, Gibco, Heidelberg, Germany), 2% peptone (50014-034, Gibco), 0·7% agar (20001-020, Gibco), 2% glucose (6780, Roth, Karlsruhe, Germany), 4 mg/l adenine and 20 mg/l tryptophan. The following compounds and growth inhibitors were added to YPD$^+$ at 65°C with the following final concentrations (order number, Company and stock concentration are given in brackets): 90 mg/l thiabendazole (T5535, Sigma, Deisenhofen, Germany; 1 mg/ml); 4·5 ml/l dimethylformamide

Copyright © 1999 John Wiley & Sons, Ltd.

Table 1. List of essential and 'nearly essential' (slow growth) genes.

Systematic name	Gene	Function or homology	Terminal phenotype	Growth
Nucleic acid metabolism				
YHL025w	*SNF6*	Component of SWI/SNF global transcription activator complex	12	Essential[a]
YHR058c	*MED6*	RNA polymerase II transcription regulating mediator	200	Essential
YHR062c	*RPP1*	Required for processing of tRNA and 35S rRNA	20	Essential
YHR065c	*RRP3*	Similarity to DEAD box family RNA helicases	Spore	Essential
YHR069c	*RRP4*	3′→5′ exoribonuclease; required for 3′ end formation of 5·8S rRNA	6	Essential
YHR089c	*GAR1*	Protein associated with snoRNA and involved in 35S rRNA processing	60	Essential
YHR118c	*ORC6*	Origin recognition complex (ORC); sixth subunit	1–4	Essential
YHR164c	*DNA2*	DNA helicase required for DNA replication	10–20	Essential
YHR165c	*PRP8*	U5 snRNP; pre-mRNA splicing factor	3–6	Essential
YHR169w	*DBP8*	Similar to DEAD box family of RNA helicases	20	Essential
YHR170w	*NMD3*	Nonsense-mediated mRNA decay protein; Nam7p/Upf1p-interacting protein	6	Essential
YHR178w	*STB5*	Protein with similarity to transcription factors; has Zn[2]-Cys[6] fungal-type	5	Essential
YHR084	*wSTE12*	Transcription factor binds to pheromone response element (PRE)	—	Slow growth
YHR120w	*MSH1*	Homolog of *E. coli MutS*; involved in mitochondrial DNA mismatch repair	—	Slow growth
Protein synthesis				
YHL015w	*RPS20*	40S ribosomal protein Urp2p; *E. coli* S10, human S20	1–3	Essential
YHR019c	*DED81*	Asparaginyl-tRNA synthetase	Spore	Essential
YHR020w		Similarity to prolyl-tRNA synthetases; putative class II tRNA synthetase	Spore	Essential
YHR148w		Similar to ribosomal protein Sup46p/Rps13p/YS11; low codon bias	5	Essential
YHL004w	*MRP4*	Mitochondrial ribosomal protein of the small subunit	—	Slow growth
YHR010w	*RPL27A*	Ribosomal protein RPL27p	—	Slow growth
YHR038w	*KIM4*	Killed in mutagen; similarity to ribosome recycling factor	—	Slow growth
Metabolism and biosynthesis				
YHR007c	*ERG11*	Cytochrome P450, lanosterol 14α-demethylase; required for biosynthesis of ergosterol	1–4	Essential
YHR025w	*THR1*	Homoserine kinase; first step threonine biosynthesis pathway	Spore	Essential
YHR027c	*RPN1*	26S proteasome regulatory subunit	4	Essential
YHR072w	*ERG7*	Lanosterol synthase; cyclization of squalene to lanosterol in ergosterol biosynthesis	8–12	Essential
YHR183w	*GND1*	6-phosphogluconate dehydrogenase	4–16	Essential
YHR190w	*ERG9*	Squalene synthetase; branch point for isoprenoid biosynthesis pathway	4–8	Essential
YHR216w	*PUR5*	Inosine-5′-monophosphate dehydrogenase; converts inosine 5′-phosphate and NAD	1–2	Essential
YHL011c	*PRS3*	Phosphoribosyl pyrophosphate synthetase	—	Slow growth
YHR174w	*ENO2*	Enolase 2 (2-phosphoglycerate dehydratase)	—	Slow growth

Continued

Copyright © 1999 John Wiley & Sons, Ltd.

Table 1. Continued

Systematic name	Gene	Function or homology	Terminal phenotype	Growth
Other functions				
YHR005c	*GPA1*	Guanine nucleotide-binding protein α subunit; pheromone response	100	Essential
YHR024c	*MAS2*	Mitochondrial processing peptidase; catalytic (α) subunit	15–30	Essential
YHR026w	*PPA1*	Proteolipid protein of vacuolar proton-transporting ATPase	2–8	Essential
YHR042w	*VMA16*	NADPH-cytochrome P450 reductase; regulated coordinately with ERG11	4–26	Essential
YHR068w	*DYS1*	Deoxyhypusine synthase; first step in hypusine biosynthesis	60	Essential
YHR107c	*CDC12*	Component of 10 nm filaments of mother-bud neck (septin)	3 (hyphal)	Essential
YHR166c	*CDC23*	Component of anaphase-promoting complex with Cdc16p and Cdc27p	12	Essential
YHR172w	*SPC97*	Spindle pole body component	4	Essential
YHR002w		Mitochondrial carrier; similar to Graves' disease protein (human)	—	Slow growth
YHR023w	*MYO1*	Myosin heavy chain (myosin II)	—	Slow growth
YHR051w	*COX6*	Cytochrome *c* oxidase chain VI; located on mitochondrial inner membrane	—	Slow growth
YHR064c	*PDR13*	Similarity to Hsp70 heat shock family of proteins	—	Slow growth
Unknown function				
YHR036w		Similar to hypothetical protein *YGL247w*	6	Essential
YHR052w			12	Essential
YHR070w		Strong similarity to *N. crassa* met-10$^+$ protein	60	Essential
YHR074w		Protein with weak similarity to *B. subtilis* NH3-dependent NAD($^+$) synthetase	15–30	Essential
YHR083w			500	Essential
YHR085w			8–12	Essential
YHR088w		Similar to hypothetical protein *YNL075w*	4–10	Essential
YHR090c	*NBN1*	Contains PHD finger; weak similarity to human retinoblastoma binding protein	60	Essential
YHR099w	*TRA1*	Strong similarity to human TRRAP protein	30	Essential
YHR101c	*BIG1*	Required for normal growth on glucose	150–200	Essential
YHR122w			5–20	Essential
YHR186c		Weak similarity to Cdc39p; has β-transducin (WD-40) domain	Spore	Essential
YHR188c			2–8	Essential
YHR196w			10–16	Essential
YHR197w			10–12	Essential
YHR205w	*SCH9*	Serine/threonine protein kinase that is activated by cAMP	Spore	Essential
YHL031c	*GOS1*	SNARE protein of Golgi compartment	—	Slow growth
YHR040w		Weak similarity to Hit1p in the N-terminal region	—	Slow growth
YHR059w		Weak similarity to *Ustilago hordei* B east mating protein 2	—	Slow growth
YHR067w			—	Slow growth
YHR098c	*SFB3*	Similarity to hypothetical human protein	—	Slow growth
YHR168w		Has GTP-binding motifs	—	Slow growth

The genes have been grouped according to known, proposed or unknown function, as listed. Heterozygous (*ORF*$^+$/*Δorf*$^-$*::gfp-HIS3*) diploid cells were sporulated as described in Material and Methods and 3–10 tetrads were dissected. Spores were germinated and analysed after 3 and 5 days of incubation at 30°C on YPD. For the essential genes, the terminal germination phenotype was determined and the average number of cells present counted (terminal phenotype). 'Essential' indicates no growth or limited number of cell divions after germination on YPD; 'slow growth' means haploid segregants grew significantly slower than wild-type (after restreaking on YPD). [a]snf6 mutations are synthetically lethal with leu2 mutations, probably because *SNF6* is required for expression of a leucine transporter (F. Winston, personal communication).

Copyright © 1999 John Wiley & Sons, Ltd.

(6251, Roth); 0·3 M $CaCl_2$ (5239, Roth); 100 mM CsCl (7878, Roth; 3 M); 0·15% caffeine (C0750, Sigma; 5% in water); 1·3 M NaCl (3957, Roth); 1·5 M sorbitol (S1876, Sigma, added directly to media before autoclaving); 1 mM EGTA (E4378, Sigma; 100 mM in water). (B) The following complete media without a carbon source (YP) or with different carbon sources were prepared: plus 2% glycerol (YPGly), plus 3% ethanol (YPEtOH), plus 2% galactose (YPGal), plus 2% raffinose (YPRaf). (C) A YPGly/Na_3VO_4 medium was produced by supplementing YPGly with 0·5 mM Na_3VO_4 (S6508, Sigma; 50 mM). (D) The synthetic medium plus 2% glucose (SD) is composed of: 1·7 g/l Yeast Nitrogen Base without amino acids (0335-15-9, Difco, Augsburg, Germany), 2% glucose (Roth), 0·7% Bitek agar (0138-17-6, Difco), 5 g/l ammonium sulphate (Roth), 30 mg/l tyrosine, 20 mg/l histidine, 20 mg/l tryptophan, 30 mg/l leucine and 30 g/l lysine. The following compounds were added to the SD medium: 4 mM NaF (S1504, Sigma; 1 M in water); 78 mM hydroxyurea (H8627, Sigma). All media (65°C) were placed, using an automatic multichannel pipette, in flat-bottomed 96-well microtiter plates (250 µl/well) (82.1581.001, Sarstedt, Numbrecht, Germany).

Gene expression analysis The following media were used: complete glucose medium $YYPD^+$ (1% yeast extract, Gibco; 2% peptone, Gibco; 2% glucose, Roth; 1·7 g/l Yeast Nitrogen Base, Difco; 5 g/l ammonium sulphate, 4 mg/l adenine, 40 mg/l tryptophan, 30 mg/l tyrosine, 20 mg/l histidine, 30 mg/l leucine and 30 mg/l lysine, pH 5·0, adjusted with HCl); selective glucose media, SD (1·7 g/l Yeast Nitrogen Base, Difco; 5 g/l ammonium sulphate, Roth; 2% glucose, Roth; 30 mg/l tyrosine, 20 mg/l histidine, 20 mg/l tryptophan, 30 mg/l leucine and 30 mg/l lysine); selective ethanol medium SE (1·7 g/l Yeast Nitrogen Base, Difco; 5 g/l ammonium sulphate, Roth; 0·1% glucose, Roth; 2% ethanol, Roth; 30 mg/l tyrosine, 20 mg/l histidine, 20 mg/l tryptophan, 30 mg/l leucine and 30 mg/l lysine).

Phenotypic assays in microtiter plates

The phenotypic assays were performed in microtiter plates as described (Rieger *et al.*, 1997). *MAT***a** and *MATα* haploid deletion strains and the wild-type (wt) control strains were recovered from glycerol stock (−70°C) and streaked out onto YPD^+ plates. Then strains were incubated overnight at 30°C in 700 µl liquid YPD^+ media in microtiter plates (1·2 ml well volume, Polylabo, Paris, France). Using multichannel pipettes, 10 µl of the pre-cultures were added to 700 µl YPD^+ media in microtiter plates and incubated overnight at 30°C to saturation (average of 2×10^7 cells/ml). These cultures were diluted 70-fold twice (in total, 4900-fold) in Ringer solution (1.15525, Merck, Darmstadt, Germany) and 20 µl of both dilutions (approx. 80 and 5700 cells) were placed on the surface of the solid medium in two adjacent wells of 96-well microtiter plates. The plates were covered with a lid and incubated at 30°C for 5 days. Photographs were taken after 3 and after 5 days. No growth of cells in both wells compared to the wt strain was scored as a lethal phenotype, while reduced growth in both wells or only growth in the well containing the higher number of cells was noted. Only those phenotypes that were shown by both haploid strains were listed.

Gene expression analysis

Growth of strains The amount of GFP produced from the endogenous promoters in the diploid heterozygous ($ORF^+/\Delta orf^-::gfp\text{-}HIS3$) deletion strains was quantified by flow cytometry. Strains were streaked out from glycerol stock onto YPD^+ plates. For promoter studies with cells grown in complete glucose media, $YYPD^+$, the following growth regime was followed. The cells were incubated overnight in 5 ml $YYPD^+$ media at 30°C and again inoculated in 5 ml $YYPD^+$ (starting OD_{600}, 0·1) up to an OD_{600} of 0·8–1·0 (approximately 6 h). Cells were diluted to an OD_{600} of 0·03 in 10 mM Tris, pH 5, and analysed directly by flow cytometry. For promoter analysis of cells grown in synthetic minimal glucose medium (SD), cells were inoculated overnight in 5 ml SD medium, again inoculated in 5 ml SD (starting OD_{600} 0·01) up to an OD_{600} of 0·5–0·7 (approximately 15 h). The cells were diluted and analysed as described previously (Niedenthal *et al.*, 1996). Promoter analysis of cells grown on non-fermentable carbon sources was done as follows: cells were inoculated in 5 ml SE medium overnight to an OD_{600} of 0·7–0·8, diluted into SE media (starting OD_{600} 0·1) and inoculated to an OD_{600} of 0·2–0·4 (7–8 h), and again inoculated in 5 ml SE media (starting OD_{600} 0·025) to an OD_{600} of 0·45–0·55 (approximately 20 h). Cells were diluted and analysed as described previously.

Copyright © 1999 John Wiley & Sons, Ltd.

FACS analysis The amount of green fluorescent protein in living yeast cells was quantified by flow cytometry, as previously described (Niedenthal *et al.*, 1996). Yeast strains not expressing GFP have a low level of autofluorescence when irradiated with a laser at 488 nm. To check whether the presence of the *gfp* gene *per se* (without a promoter in front of the gene) in yeast changes the fluorescence profile of the cells, two control strains were constructed in which the *gfp–HIS3* cassette was integrated at two different chromosome VIII locations in such a way that the *gfp* gene was not fused to a promoter (*YHR047c-YHR048w*: deletion of approximately 3 kb intergenic region between both ORFs; *YHR211w-YHR216w*: deletion of approximately 30 kb including the ORFs *YHR211w* and *YHR216w*). Analysis of these strains for green fluorescence in the three different growth media revealed that their fluorescence was not increased above the autofluorescence level found for the wt strain, indicating that presence of the *gfp* gene *per se* did not lead to an increase in green fluorescence.

The level of autofluorescence is dependent on the medium in which the cells were grown (Niedenthal *et al.*, 1996). Therefore, for sets of experiments, all strains were grown in the same medium under the same conditions and always analysed in parallel with the wt strain: deletion strains and wt strains alternated during the FACS measurements. For each deletion strain the following procedure was used: the fluorescence intensity values of the two wt strains flanking a particular deletion strain were used to calculate the mean value, which was set to zero, and the fluorescence intensity of the deletion strain was calculated relative to this, yielding the relative green fluorescence (RGF) value given in Table 5 (see below).

Flow cytometry was carried out using a FACSort system (Becton Dickinson, Heidelberg, Germany). Illumination was with a 200 mW 488 nm argon-ion laser. Emission was detected through a 530/30 nm filter (FL1-H filter). 10 000 particles (living cells) were analysed per sample (flow rate=300 cells/s). The autofluorescence obtained for the wt strain was set electronically to channel 200 and the deletion strains were then analysed using the same parameters. The standard deviation for the autofluorescence of the yeast strain YM4587 (no *gfp* gene integrated into the genome) was determined to be 0·19 RGF units for growth in YPD^+, 0·10 RGF units for growth in SD and 0·23 RGF units for growth in SE. The promoter activity of diploid heterozygous strains exhibiting RGF units within these standard deviations, or exhibiting negative RGF units, were set to zero.

RESULTS AND DISCUSSION

Gene deletion

All but four of the 269 (non-overlapping) ORFs on chromosome VIII predicted to encode a protein of at least 100 amino acids were disrupted (Johnston *et al.*, 1994). This was achieved by transforming diploid yeast cells to His^+ with a DNA fragment carrying *HIS3* and sequences encoding green fluorescent protein (GFP) of *Aequorea victoria,* flanked by 45 nt of sequence immediately adjacent to each end of the ORF. This DNA fragment was generated by a PCR (see Figure 1) as described previously (Baudin *et al.*, 1993; Niedenthal *et al.*, 1996). Homologous recombination of this DNA fragment with the yeast genome precisely removes the ORF (from the ATG translational initiation codon through the translational termination codon) and fuses the ATG of the GFP coding sequence to the ATG of the ORF. The *HIS3* gene, downstream of *gfp,* is expressed from its own promoter. For each gene deletion we verified by PCR that *gfp–HIS3* correctly replaced the ORF (using primers P1 and P2 shown in Figure 1; see Materials and Methods). Most genes were easily deleted: 74% (199) of the disruptions were obtained by testing three or four transformants (average of 3·8) from an average of 15 transformants (range 0–40) obtained; two or three (average of 2·7) transformants were correctly disrupted. An additional 13% (36) of the disruptions required testing seven or eight transformants (average of 7·7, range 6–20); an average of two (range 1–11) of these were correct. Further transformation and testing, using the same primers, were required for 27 (10%) of the disruptions. Only four (1·5%) of the disruptions required new primers and extensive work (see Materials and Methods). We were unable to disrupt *YHL050*, because it lies in the left subtelomeric region of the chromosome that is precisely duplicated on several other chromosomes (we could not design unique primers to test for its disruption). Three other genes that are part of the *CUP1* repeat: *YHR053c, −54c and −055c* (Karin *et al.*, 1984) were excluded from the analysis (although we were able to delete *YHR053 and YHR054c*).

Copyright © 1999 John Wiley & Sons, Ltd.

The heterozygous diploid strains ($ORF^{+}/\Delta orf^{-}$::*gfp-HIS3*) were sporulated, and haploid His^{+} mutants were identified among random spores (see Materials and Methods). If approximately half of the spores produced His^{+} colonies, it was concluded that the deleted gene is not essential for growth. Tetrads of the 89 mutants (33% of the total) that yielded few or no His^{+} spore clones were dissected to verify that the mutant is non-viable or slowly growing; 64 (72%) of these mutants produced tetrads with only two viable spores that were His^{-} (47 essential genes, 18% of the total) or with two fast-growing His^{-} and two slow-growing His^{+} spore colonies (17 'nearly essential' genes, 6% of the total). In four cases, about half of the two viable spores were His^{+}, indicating that the deleted gene is not essential, and that the diploid strain carried another mutation in an essential gene, probably induced during transformation of yeast with the *gfp–HIS3* deletion cassette. We do not understand why these appeared as potential essential genes in the random spore analysis. In these cases the gene deletion was successfully repeated.

All viable haploid mutants were tested for the absence of the ORF by a PCR using primers P1 and P3 (Figure 1). Nineteen (8·7%) of the 218 viable mutants yielded a PCR product of the expected size, indicating that they retain a normal, undeleted copy of the ORF in addition to the deleted copy (in these cases a mutant strain lacking the ORF was obtained by repeating the gene deletion). This phenomenon has been observed previously (B. Dujon, personal communication). Nearly all of the His^{+} spores (82 spores from eight different mutants) of the original diploid deletants that we tested retained the undeleted ORF, indicating that the deleted and undeleted ORFs are closely linked. This suggests that these mutants carry a local duplication of the ORF, which could have been induced by the transformation that generated the deletion, or could have pre-existed in the population before transformation.

Mutant phenotypes

Genes essential for growth and/or germination
Eighteen percent (47 of 265) of the genes are essential for growth on rich glucose (YPD^{+}) growth medium. This is similar to estimates of the number of essential yeast genes obtained from other studies (Burns *et al.*, 1994; Entian *et al.*, 1999; Goebl and Petes, 1986; Smith *et al.*, 1996; Winteler *et al.*, 1999). An additional 17 (6%) of the genes are 'nearly essential': their mutants grow noticeably slower than wt on YPD^{+} medium. Thus, almost a quarter of the genes on chromosome VIII are necessary for normal growth on YPD medium.

For mutants of essential genes, the terminal germination phenotype of the orf^{-}::*gfp-HIS3* spores was determined (see Table 1). Among the 47 heterozygous $ORF^{+}/\Delta orf^{-}$::*gfp-HIS3* diploid strains, six strains (13%) produced normal-looking spores that did not germinate, suggesting that the corresponding gene products might be involved in the germination process. The remaining 41 heterozygous strains produced spores which germinated and produced microcolonies of 1–200 cells, depending on the deleted ORF. In 16 cases (34%) the germinated cells underwent up to three cell divisions, while in the remaining 25 cases (53%) more cell divisions took place.

The essential and 'nearly essential' genes appear to be randomly distributed on the chromosome (Figure 2): the number of clusters (75) of essential, 'nearly essential' and non-essential genes is almost precisely the expected mean (75·7) for this number of genes. Analysis of these results with a non-parametric statistical test called the Runs test (Katz, 1988; Mood, 1940) makes us 95% confident that the essential and 'nearly essential' genes are randomly arranged on the chromosome.

A seemingly larger fraction of known genes than of genes not identified prior to determination of the sequence of the yeast genome are essential for growth on YPD^{+} (24 of 106, or 24%, of known genes vs. 22 of 158, or 13·9%, of unknown genes). Intuitively, this result seems reasonable, since lethality is a clear phenotype that leads to the identification of many genes, causing essential genes to be over-represented among 'known' genes relative to uncharacterized genes. It is not surprising that only two of the 38 genes (5·3%) that encode proteins with close yeast homologues (as described by Wolfe *et al.*, 1997) are essential (*YHR183/GND1* and *YHR216/PUR5*) and the essential nature of these genes is in question (see below).

The requirement of some of the essential genes for normal cell growth can be easily reconciled with the known or predicted function of their encoded proteins (Table 1). For example, several are known or predicted to be involved in protein synthesis or nucleic acid metabolism. However, most essential genes encode proteins of unknown

Copyright © 1999 John Wiley & Sons, Ltd.

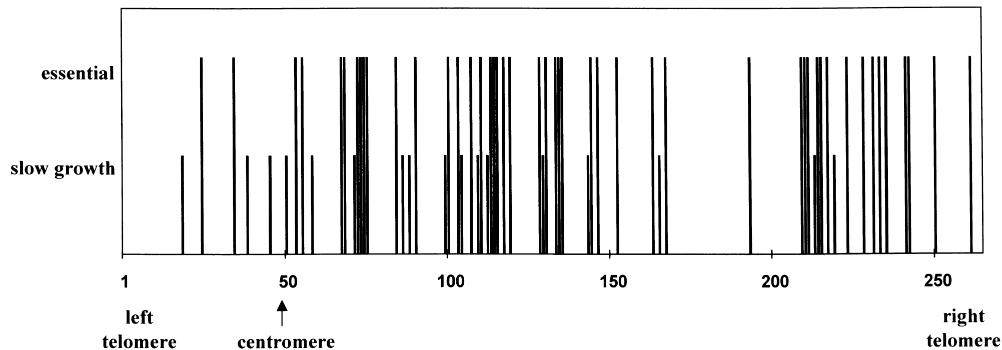


Figure 2. Essential and 'nearly essential' (slow growth) genes on chromosome VIII are not clustered. The positions of each of the 47 essential and 17 'nearly essential' genes on chromosome VIII, listed in Table 1, are presented. Essential genes and slow growth genes are indicated by long and short vertical bars, respectively.

function. Some of the genes are likely to be essential for growth on YPD for trivial reasons. For example, three of the genes, *YHR007c, YHR072w* and *YHR190w*, are involved in ergosterol biosynthesis and are essential for growth on YPD^+ because this growth medium lacks this nutrient. Also, *SNF6,* which encodes a transcription factor known to be dispensable, was essential for growth of our strain. This is probably because the strain also carries a *leu2* mutation, which is known to be synthetically lethal with *snf6* mutations, possibly because Snf6 is required for transcription of a gene encoding a leucine permease (F. Winston, personal communication). These cases emphasize that the results must be interpreted with caution.

It is difficult to understand how three of the genes can be essential for growth. We found *THR1* (*YHR025w*), which encodes homoserine kinase, the first enzyme of the threonine biosynthetic pathway, to be essential for growth on YPD^+ medium. This result has not been observed previously (Schultes *et al.*, 1990) and is difficult to fathom, since threonine is available in YPD^+ medium. One possibility is that *THR1* coding sequences harbour promoter elements for one or both of the adjacent genes (*YHR024c* and *YHR026w*), which are essential for growth. We have no explanation for the requirement of *GND1* (*YHR183w*) for viability on YPD^+ medium. This gene encodes the enzyme (6-phosphogluconate dehydrogenase) that catalyses the third step of the pentose phosphate pathway. The gene encoding the first enzyme in the pathway (*ZWF1*, encoding glucose-6-phosphate dehydrogenase) is dispensable for growth on YPD^+ medium (Nogae and Johnston, 1990; Thomas *et al.*, 1991). In addition, *GND1* is apparently a duplicated gene (Wolfe and Shields, 1997). It is likewise surprising that *YHR216w* is essential for growth, since this gene, which probably encodes an inosine-5′-monophosphate dehydrogenase involved in purine biosynthesis, is almost precisely duplicated on chromosome I. However, the copy of this gene on chromosome I is probably non-functional, because it is not expressed (Barton *et al.*, 1997). We also do not know why two genes that we found to be essential (*BIG1/YHR101* and *NCP1/YHR042*) are not essential in the hands of others (Bickle *et al.*, 1998; Urban *et al.*, 1997).

Conditional phenotypes The 218 viable deletion strains were grown under the 20 different growth conditions listed in Table 2. About 18% (39) of those strains exhibited a growth phenotype under one of these 20 conditions. These strains are listed in Tables 3 and 4. Interestingly, most (14, or 82%) of the 17 slowly growing deletion strains exhibited a conditional phenotype, indicating that these strains are particularly sensitive to perturbation. In contrast, a small percentage (25, or only 12%) of the 201 non-growth impaired deletion strains showed a conditional phenotype. In total, 33% of the 265 chromosome VIII mutants exhibit a detectable phenotype (64 essential or 'nearly essential' genes, plus 25 'conditional' genes). Growth on five different media (stationary phase/YPD^+, dimethylformamide/YPD^+, Na_3VO_4/YPGly, NaF/SD and sorbitol/YPD^+) did not affect growth of any of the 218 strains tested.

Altogether, 13 mutants were found to be defective for growth when tested on five different carbon sources (Table 3). Some of the phenotypes

Copyright © 1999 John Wiley & Sons, Ltd.

Table 2. List of growth conditions tested.

Growth condition	Relevant supplement	Relevant cellular process
YPD$^+$	Complete 2% glucose	
SD	Synthetic 2% glucose	
YPGly	Complete 2% glycerol	Respiration
Ts/YPD$^+$	YPD 37°C	
YP	Complete, no C-source	
Stationary phase/YPD$^+$	15 days 30°C; then plate on YPD	
$CaCl_2$/YPD$^+$	0·3 M $CaCl_2$	Ion-transport; cell cycle regulation
NaCl/YPD$^+$	1·3 M NaCl	Osmotic stability
CsCl/YPD$^+$	100 mM CsCl	Transport; growth inhibition, K^+ replacement
Hydroxyurea/SD	78 mM Hydroxyurea	DNA synthesis
Thiabendazole/YPD$^+$	90 mg/l Thiabendazole	Microtubule function
Dimethylformamide/YPD$^+$	4·5 ml/l Dimethylformamide	Solvent of thiabendazole
Caffeine/YPD$^+$	0·15% Caffeine	e.g. cAMP-phosphodiesterases; MAP kinase signalling pathways
Na_3VO_4/YPGly	0·5 mM Sodium orthovanadate	Protein glycosylation; protein secretion
NaF/SD	4·0 mM NaF	Inhibits various phosphatases
EGTA/YPD$^+$	1·0 mM EGTA	Stability of Ca^+ level
Sorbitol/YPD$^+$	1·5 M Sorbitol	Osmotic stability
YPEtOH	3% Ethanol	Respiration
YPGal	2% Galactose	Galactose utilization
YPRaf	2% Raffinose	Raffinose utilization

A detailed description of the conditional phenotypes and their corresponding functional implications has been described elsewhere (Hampsey, 1997). The media were prepared as described (Rieger *et al.*, 1997) with modifications as described in Materials and Methods.

Table 3. List of mutant strains showing respiration defects.

Phenotype	Gene	Function/homology
Respiration 1[a]		
YHL004w	*MRP4*	Mitochondrial ribosomal protein of the small subunit
YHL038c	*CBP2*	Apo-cytochrome *b* pre-mRNA processing protein 2
YHR038w	*KIM4*	Protein with similarity to ribosome recycling factor
YHR051w	*COX6*	Cytochrome *c* oxidase chain VI; located on mitochondrial inner membrane
YHR060w	*VMA22*	Vacuolar ATPase assembly protein
YHR091c	*MSR1*	Arginyl-tRNA synthetase of mitochondria
YHR120w	*MSH1*	Homologue of *E. coli MutS*; involved in mitochondrial DNA mismatch repair
YHR147c	*MRP-L6*	Mitochondrial ribosomal protein of the large subunit (YmL6)
YHR168w		Protein of unknown function; GTP-binding motifs
Respiration 2[b]		
YHR067w		Protein of unknown function
YHR116w		Protein of unknown function
YHR129c	*ARP1*	Centractin
Respiration 3[c]		
YHR142w		Protein of unknown function; has seven potential transmembrane domains

The mutants are classified according to their level of impairment in respiration: [a]no or slow growth on Gly, YP, EtOH, Raf and Gal; [b]no or slow growth on Gly, YP, EtOH and Raf (Gal$^+$); [c]no or slow growth on YP and EtOH.

Copyright © 1999 John Wiley & Sons, Ltd.

correlate with the known or predicted function of the protein. For example, four of the seven mutants that exhibit the most severe respiration defect (respiration phenotype 1, see Table 3) are missing genes encoding known mitochondrial proteins. The other, previously unrecognized proteins required for normal growth on non-fermentable carbon sources are likely to be required for function of the mitochondrial respiratory chain.

In addition to the 13 respiration-defective mutants, another 34 mutant strains exhibited a phenotype when tested under other growth conditions (Table 4). Of these strains only a minority (12 mutants) were monotropic: most (22) showed pleiotropic phenotypes, making it difficult to predict the function of the corresponding gene products. The number of strains with pleiotropic phenotypes is likely to increase in the future, as more phenotypic tests are employed. Many strains (21) were found to be sensitive to the microtubule-destabilizing drug thiabendazole. This phenotype had been described for a deletion of *ACT5*/*YHR129c*, while for many other genes this phenotype cannot easily be explained. Another 13 deletion strains showed sensitivity to caffeine, a drug known to affect various cellular pathways (Hampsey, 1997).

Promoter activity

Since each gene was deleted so as to fuse the GFP coding sequence to the ATG of the ORF, transcription of the *gfp* gene is regulated by the promoter of the disrupted gene (see Figure 1). The amount of GFP protein produced, which can be quantified by flow cytometry, is thus a direct measure of the strength of a given promoter (Niedenthal *et al.*, 1996). The relative green fluorescence (RGF) of four control strains (*GAL1*, *ACT1*, *URA3* and *GAL4*) is presented in Table 5. For all four promoters the RGF levels are consistent with the strength of these promoters, measured by different methods (Niedenthal *et al.*, 1996; Planta *et al.*, 1999).

All 265 heterozygous diploid deletion strains were grown under three different growth conditions (rich glucose medium, YYPD$^+$; synthetic glucose medium, SD; and synthetic ethanol medium, SE) and analysed for their promoter activities (for details, see Materials and Methods). Green fluorescence was observed in about 50% (131) of the strains grown in YYPD$^+$ (Figure 3). Fluorescence intensity spanned approximately two orders of magnitude (range 0–13 RGF units), with the majority of the promoters exhibiting low (approximate level of *URA3* expression) or intermediate (approximate level of *ACT1* expression) activity. No clustering of genes with particularly high or low promoter activity was apparent. Not surprisingly, the highly expressed genes seem to be excluded from the telomeric regions: no medium or high level promoters are included in the first 16 genes from the left telomere and the first 12 genes from the right telomere, probably due to silencing of gene expression by telomeres (Lustig, 1998) (Figure 3). This pattern of expression did not significantly change when the strains were grown in a synthetic minimal glucose or ethanol medium (see below), supporting previous results (Planta *et al.*, 1999).

About half of the strains (134) exhibited no significant green fluorescence, indicating that the corresponding promoters are either inactive, or active at a very low level on YYPD$^+$ (Figure 3, Table 5). Among this group are 31 essential or ‘nearly essential’ genes (see Tables 1 and 5), which must be expressed, but apparently at a level we are unable to detect. As expected, the haploid-specific genes *STE12* and *GPA1* show no promoter activity in the diploid cells used in this study. Of the promoters, most (103) function at a low level (below 0·93 RGF units, *URA3* promoter level). Twenty-seven promoters exhibit intermediate activity, yielding RGF levels between 5·53 (*ACT1* promoter level) and 0·93 (*URA3* promoter level), while only three promoters are stronger than the promoter of *ACT1*. Seven of the 10 genes found on chromosome VIII that encode ribosomal proteins (*RPL8A, RPS20, RPL14B, RPL27A, RPS27B, MRPS20* and *RPS4B*) have promoters of high or intermediate strength. This was also observed in a genome-wide analysis of yeast gene expression, in which the 30 most highly expressed genes included 16 encoding ribosomal proteins (Velculescu *et al.*, 1997). This is expected, since the protein synthesis machinery is very active in cells growing on rich media. The three strongest promoters are found for *YHR143w*, encoding a serine/threonine-rich protein of unknown function (13 RGF units), *ENO2* (*YHR174w*), encoding enolase 2 (12·4 RGF units) and *RPL4A* (*YHL033c*), encoding a 60S ribosomal protein (7·6 RGF units) (Table 5).

Growth on synthetic minimal glucose medium (SD) increased the number of active promoters to 93% (246) of all chromosome VIII genes (Table 5). The strength of the three strongest promoters

Copyright © 1999 John Wiley & Sons, Ltd.

Table 4. List of mutant strains with conditional phenotypes.

Systematic name	Gene	Growth	YPD⁺ 30°C	YPD⁺ 37°C	SD	TBZ	Caffeine	CsCl	$CaCl_2$	NaCl	HU	EGTA	Function or homology
YHL038c	*CBP2*					X			X				Apo-cytochrome *b* pre-mRNA processing protein 2
YHL031c	*GOS1*	Slow				O	O	O					SNARE protein of Golgi compartment
YHL017w						O							Strong similarity to Ptm1p
YHL011c	*PRS3*	Slow				X	O						Ribose-phosphate pyrophosphokinase
YHR002w		Slow				O		X					Mitochondrial carrier; similar to Graves' disease protein (human)
YHR004c	*NEM1*					O	X		O				Involved in nuclear morphology; similarity to *YLL010c* and *YLR019w*
YHR006w	*STP2*											O	Involved in pre-tRNA splicing
YHR011w						O	X						Strong similarity to seryl-tRNA synthetases
YHR013c	*ARD1*					X	O			O			Protein N-acetyltransferase subunit
YHR023w	*MYO1*	Slow					O	X					Myosin-1 isoform (type II myosin) heavy chain
YHR030c	*SLT2*						O					O	Ser/thr protein kinase of MAP kinase family
YHR034c						X	O						Protein of unknown function
YHR038w	*KIM4*	Slow						X					Killed in mutagen; similarity to ribosome recycling factor
YHR041c	*SRB2*					O		X					DNA-directed RNA polymerase II subunit
YHR050w	*SMF2*											O	Probable manganese transporter
YHR059w		Slow							X				Weak similarity to *Ustilago hordei* B east mating protein 2
YHR060w	*VMA22*					O	O		O	O	X		Vacuolar ATPase assembly protein
YHR064c	*PDR13*	Slow						O	O				Regulator protein involved in pleiotropic drug resistance
YHR066w	*SSF1*					O							Mating protein
YHR067w		Slow				O		O	X		X		Protein of unknown function
YHR071w	*PCL5*								X	O			Cyclin like protein interacting with Pho85p
YHR075c	*MRPS2*					O							Ribosomal protein of the small subunit, mitochondrial
YHR081w						X	O						Weak similarity to human C1D protein
YHR084w	*STE12*	Slow				X		X					Transcriptional activator
YHR098c	*SFB3*	Slow				O					X		Similarity to human hypothetical protein
YHR100c				O				X					Protein of unknown function
YHR119w	*SET1*						X						Involved in chromatin-mediated gene regulation
YHR120w	*MSH1*	Slow				X		X					DNA mismatch repair protein, mitochondrial
YHR129c	*ARP1*					X							Centractin
YHR142w						X	O						Weak similarity to cytochrome *c* oxidases
YHR151c										O			Protein of unknown function
YHR152w	*SPO12*						O						Sporulation protein
YHR168w		Slow							X				Similarity to GTP-binding proteins
YHR194w							X	X					Similarity to hypothetical protein *YOR147w*

The growth conditions tested are listed in Table 2. For each strain, the slow-growth (X) or the no-growth phenotype (O) is indicated. Five growth conditions for which no mutant strain was positive are not listed here.

Copyright © 1999 John Wiley & Sons, Ltd.

Table 5. Flow cytometric quantification of fluorescence in the 265 heterozygous diploid mutant strains grown in the three different media: YYPD^{+} (complete plus glucose), SD (synthetic minimal plus glucose) or SE (synthetic minimal plus ethanol).

Systematic name	Gene	RGF		
		YYPD^{+}	SD	SE
YPL248c	*GAL4*	0·68	2·20	8·40
YEL021w	*URA3*	0·93	4·67	1·57
YFL039c	*ACT1*	5·53	7·48	3·92
YOL051w	*GAL1*	0·56	2·06	7·52
Left telomere				
YHL049c		0·00	0·26	2·02
YHL048w	*COS8*	0·68	1·43	1·46
YHL047c		0·24	1·79	1·79
YHL046c		0·00	0·22	2·29
YHL045w		0·00	0·11	1·11
YHL044w		0·22	0·26	1·47
YHL043w	*ECM34*	0·50	1·51	1·36
YHL042w		0·34	1·35	0·63
YHL041w		0·58	1·48	5·67
YHL040c		0·69	1·26	0·34
YHL039w		0·00	0·47	4·07
YHL038c	*CBP2*	0·53	2·58	1·34
YHL037c		0·45	0·70	4·15
YHL036w	*MUP3*	0·00	0·21	4·48
YHL035c		0·81	1·17	7·60
YHL034c	*SBP1*	0·35	2·77	2·30
YHL033c	*RPL8A*	7·60	4·59	4·97
YHL032c	*GUT1*	0·00	1·77	0·00
YHL031c	*GOS1*	0·80	1·75	1·25
YHL030w	*ECM29*	1·00	2·00	7·35
YHL029c		0·82	1·31	5·19
YHL028w	*WSC4*	0·00	0·36	3·21
YHL027w	*RIM101*	0·33	3·60	1·84
YHL026c		0·00	0·25	3·08
YHL025w	*SNF6*	0·91	1·81	1·40
YHL024w		0·68	0·67	4·88
YHL023c		0·00	0·68	1·12
YHL022c	*SPO11*	0·00	3·37	0·58
YHL021c		1·15	2·46	0·00
YHL020c	*OPI1*	0·00	1·17	0·00
YHL019c	*APM2*	0·23	0·45	2·06
YHL018w		0·25	0·22	0·63
YHL017w		0·00	0·18	0·00
YHL016c	*DUR3*	0·00	1·46	0·25
YHL015w	*RPS20*	1·00	5·07	1·20
YHL014c	*YLF2*	0·00	0·58	1·76
YHL013c		0·00	0·22	2·43
YHL012w		0·00	0·44	2·07
YHL011c	*PRS3*	0·25	0·65	2·48
YHL010c		0·00	0·35	2·05
YHL009c	*YAP3*	0·00	0·39	3·51
YHL008c		0·00	0·61	2·94
YHL007c	*STE20*	0·22	0·73	0·81

Continued

Copyright © 1999 John Wiley & Sons, Ltd.

Table 5. Continued.

Systematic name	Gene	YYPD⁺	RGF SD	SE
YHL006c		0·00	0·41	3·62
YHL005c		0·00	0·63	3·30
YHL004w	*MRP4*	0·00	2·77	0·00
YHL003c	*LAG1*	0·82	0·00	1·44
YHL002w		0·54	1·45	4·72
YHL001w	*RPL14B*	2·62	3·43	0·35
Centromere				
YHR001w		0·22	1·64	0·28
YHR002w		0·58	3·04	0·00
YHR003c		0·26	1·41	0·70
YHR004c	*NEM1*	0·00	0·89	0·51
YHR005c	*GPA1*	0·00	0·00	0·00
YHR006w	*STP2*	0·77	3·50	0·87
YHR007c	*ERG11*	2·11	3·58	4·10
YHR008c	*SOD2*	0·29	2·12	1·64
YHR009c		0·59	1·30	0·66
YHR010w	*RPL27A*	4·00	5·17	3·28
YHR011w		0·41	4·39	3·20
YHR012w	*VPS29*	0·20	0·35	0·53
YHR013c	*ARD1*	0·00	1·71	0·78
YHR014w	*SPO13*	0·00	2·25	0·00
YHR015w	*MIP6*	0·00	0·27	0·00
YHR016c	*YSC84*	1·07	2·15	1·67
YHR017w	*YSC83*	1·60	2·80	0·00
YHR018c	*ARG4*	0·59	1·37	1·03
YHR019c	*DED81*	0·75	1·76	1·07
YHR020w		1·11	2·81	5·58
YHR021c	*RPS27B*	0·93	1·33	0·67
YHR022c		0·38	0·00	0·00
YHR023w	*MYO1*	0·49	3·54	0·00
YHR024c	*MAS2*	0·20	1·13	1·49
YHR025w	*THR1*	0·00	2·76	0·00
YHR026w	*VMA16*	0·58	3·21	0·00
YHR027c	*RPN1*	1·31	1·44	6·35
YHR028c	*DAP2*	0·00	2·77	1·15
YHR029c		0·37	0·24	0·00
YHR030c	*SLT2*	0·21	2·96	1·56
YHR031c		0·24	0·52	0·00
YHR032w	*ERC1*	0·00	0·00	1·04
YHR033w		0·00	1·07	0·00
YHR034c		0·00	0·75	0·00
YHR035w		0·00	1·06	1·95
YHR036w		0·00	1·63	0·78
YHR037w	*PUT2*	0·00	2·31	0·00
YHR038w	*KIM4*	0·00	1·29	0·00
YHR039c		0·23	0·73	0·00
YHR040w		0·00	1·47	0·00
YHR041c	*SRB2*	0·56	1·28	0·93
YHR042w	*NCP1*	0·18	3·36	0·73
YHR043c	*DOG2*	0·33	0·59	0·00

Continued

Copyright © 1999 John Wiley & Sons, Ltd.

Table 5. Continued.

Systematic name	Gene	YYPD+	RGF SD	SE
YHR044c	*DOG1*	0·21	0·61	0·38
YHR045w		0·00	1·21	0·29
YHR046c		0·19	2·96	0·00
YHR047-YHR048[m]		0·00	0·00	0·00
YHR047c	*AAP1*	0·35	2·53	0·84
YHR048w		0·00	1·19	0·00
YHR049w		0·81	0·00	0·00
YHR050w	*SMF2*	1·84	0·14	0·68
YHR051w	*COX6*	0·00	2·18	1·47
YHR052w		0·00	0·52	6·02
YHR056c		0·00	2·35	0·00
YHR057c	*CYP2*	0·00	1·56	0·33
YHR058c	*MED6*	0·45	2·95	0·44
YHR059w		0·00	0·00	0·00
YHR060w	*VMA22*	0·00	0·00	3·07
YHR061c	*GIC1*	0·64	0·38	0·86
YHR062c	*RPP1*	0·38	0·38	0·00
YHR063c		0·31	0·23	0·00
YHR064c	*PDR13*	0·49	0·25	0·00
YHR065c	*RRP3*	0·00	0·20	0·93
YHR066w	*SSF1*	0·00	0·00	1·06
YHR067w		0·00	0·00	0·42
YHR068w	*DYS1*	0·00	0·25	0·48
YHR069c	*RRP4*	0·00	0·15	0·00
YHR070w		0·87	1·81	1·32
YHR071w	*PCL5*	0·21	0·22	0·34
YHR072w	*ERG7*	0·00	2·31	0·00
YHR073w		0·00	0·97	0·81
YHR074w		0·00	0·75	2·04
YHR075c	*MRPS2*	2·52	5·32	3·89
YHR076w		0·00	0·80	3·39
YHR077c	*NMD2*	3·98	0·00	0·83
YHR078w		0·00	0·67	3·69
YHR079c	*IRE1*	0·00	1·11	0·30
YHR080c		0·50	0·00	0·00
YHR081w		0·00	1·20	4·46
YHR082c	*KSP1*	0·91	0·35	0·00
YHR083w		0·42	1·31	3·44
YHR084w	*STE12*	0·00	2·45	0·00
YHR085w		0·00	0·63	3·05
YHR086w	*NAM8*	0·00	1·60	0·00
YHR087w		0·23	0·75	6·89
YHR088w		0·00	0·19	1·40
YHR089c	*GAR1*	0·99	1·82	1·33
YHR090c	*NBN1*	0·50	1·26	0·66
YHR091c	*MSR1*	0·00	0·11	0·65
YHR092c	*HXT4*	0·00	0·30	1·21
YHR093w	*AHT1*	0·00	0·58	2·49
YHR094c	*HXT1*	0·49	0·47	1·54
YHR095w		0·00	1·08	5·77

Continued

Copyright © 1999 John Wiley & Sons, Ltd.

Table 5. Continued.

Systematic name	Gene	YYPD+	RGF SD	SE
YHR096c	*HXT5*	0·43	0·43	7·07
YHR097c		0·41	0·32	2·47
YHR098c	*SFB3*	1·33	2·47	1·50
YHR099w	*TRA1*	0·52	2·30	0·35
YHR100c		0·00	0·00	0·72
YHR101c	*BIG1*	0·00	0·00	1·10
YHR102w	*NRK1*	0·00	1·17	4·81
YHR103w	*SBE22*	0·00	0·64	4·74
YHR104w	*GRE3*	0·39	1·51	6·32
YHR105w		0·00	0·87	3·36
YHR106w	*TRR2*	0·00	0·93	5·42
YHR107c	*CDC12*	1·94	1·67	5·24
YHR108w		0·00	1·00	3·71
YHR109w		0·00	0·43	3·43
YHR110w	*ERP5*	0·18	0·11	1·83
YHR111w		0·00	1·72	3·36
YHR112c		0·00	0·38	3·99
YHR113w		0·00	1·66	1·22
YHR114w		0·00	0·51	2·16
YHR115c		0·54	1·07	1·49
YHR116w		0·00	0·00	2·17
YHR117w	*TOM71*	0·00	0·63	4·87
YHR118c	*ORC6*	0·53	1·47	0·00
YHR119w	*SET1*	0·00	1·23	4·81
YHR120w	*MSH1*	0·00	2·08	0·24
YHR121w		0·00	0·11	2·36
YHR122w		0·00	0·00	2·66
YHR123w	*EPT1*	0·31	0·39	0·61
YHR124w	*NDT80*	0·00	0·14	1·24
YHR125w		1·15	2·43	3·22
YHR126c		0·00	0·63	1·53
YHR127w	*HSN1*	0·94	1·64	1·16
YHR128w	*FUR1*	0·56	1·38	0·00
YHR129c	*ARP1*	0·00	0·82	0·00
YHR130c		0·31	0·00	0·88
YHR131c		0·27	0·00	0·53
YHR132c	*ECM14*	0·00	1·47	1·30
YHR133c		0·00	0·99	2·52
YHR134w		0·00	0·14	0·00
YHR135c	*YCK1*	0·00	0·88	0·67
YHR136c	*SPL2*	0·00	0·42	1·38
YHR137w	*ARO9*	0·79	3·44	11·83
YHR138c		0·00	0·62	0·02
YHR139c	*SPS100*	0·64	1·29	0·93
YHR140w		0·72	1·98	1·58
YHR141c	*RPL42B*	0·37	2·09	0·86
YHR142w		0·54	0·86	1·29
YHR143w		13·02	14·58	30·16
YHR144c	*DCD1*	0·00	1·04	0·90
YHR145c		0·00	0·48	0·34

Continued

Copyright © 1999 John Wiley & Sons, Ltd.

Table 5. Continued.

Systematic name	Gene	YYPD⁺	RGF SD	SE
YHR146w		0·21	1·47	2·79
YHR147c	*MRPL6*	0·00	1·19	0·60
YHR148w		0·80	0·00	1·36
YHR149c		0·00	0·39	1·10
YHR150w		0·35	3·68	3·53
YHR151c		0·24	1·17	2·25
YHR152w	*SPO12*	0·00	2·54	0·00
YHR153c	*SPO16*	0·00	1·00	0·00
YHR154w	*ESC4*	0·56	1·51	0·00
YHR155w		1·14	2·27	3·48
YHR156c		0·00	0·38	0·00
YHR157w	*REC104*	0·31	2·48	0·00
YHR158c	*KEL1*	0·49	1·18	0·00
YHR159w		0·55	1·12	0·62
YHR160c		0·00	0·83	0·00
YHR161c	*YAP1801*	0·00	0·53	2·31
YHR162w		1·92	5·47	12·00
YHR163w	*SOL3*	0·00	0·48	2·44
YHR164c	*DNA2*	0·00	1·13	0·47
YHR165c	*PRP8*	0·00	1·20	0·42
YHR166c	*CDC23*	0·00	1·14	0·00
YHR167w		0·00	1·25	0·00
YHR168w		0·00	1·36	0·00
YHR169w	*DBP8*	1·11	1·39	0·85
YHR170w	*NMD3*	0·49	2·98	0·00
YHR171w	*APG7*	0·70	2·79	0·57
YHR172w	*SPC97*	1·59	3·11	0·00
YHR173c		0·00	0·95	1·77
YHR174w	*ENO2*	12·41	9·94	15·40
YHR175w	*CTR2*	0·00	1·07	1·50
YHR176w	*FMO*	0·23	2·11	1·66
YHR177w		0·00	1·15	0·00
YHR178w	*STB5*	0·00	1·32	0·00
YHR179w	*OYE2*	0·19	1·71	5·32
YHR180w		0·39	1·51	0·00
YHR181w		2·55	5·22	4·06
YHR182w		0·45	1·44	0·83
YHR183w	*GND1*	1·86	4·83	9·41
YHR184w	*SSP1*	0·00	0·00	2·84
YHR185c		0·00	0·20	0·00
YHR186c		0·67	2·85	4·68
YHR187w	*IKI1*	0·00	0·41	0·00
YHR188c		0·00	0·52	1·71
YHR189w		0·00	2·76	0·41
YHR190w	*ERG9*	0·34	2·65	0·00
YHR191c	*CTF8*	0·00	0·32	0·77
YHR192w		0·38	2·15	0·00
YHR193c	*EGD2*	0·00	0·64	0·49
YHR194w		0·00	0·77	2·34
YHR195w		0·46	2·14	2·03

Continued

Copyright © 1999 John Wiley & Sons, Ltd.

Table 5. Continued.

Systematic name	Gene	YYPD$^+$	RGF SD	SE
YHR196w		0·00	0·34	1·77
YHR197w		0·00	0·50	2·31
YHR198c		0·00	0·21	1·02
YHR199c		0·00	0·44	1·59
YHR200w	*RPN10*	0·08	0·27	0·86
YHR201c	*PPX1*	0·00	0·45	0·00
YHR202w		0·34	2·38	0·93
YHR203c	*RPS4B*	2·23	3·40	3·09
YHR204w		0·00	0·46	1·22
YHR205w	*SCH9*	0·00	1·35	0·00
YHR206w	*SKN7*	0·00	0·85	1·73
YHR207c		0·00	0·26	1·58
YHR208w	*BAT1*	1·90	8·30	17·43
YHR209w		0·67	1·57	0·98
YHR210c		0·00	1·71	6·61
YHR211w	*FLO5*	0·00	0·85	1·99
YHR211w-216w[m]		0·00	0·00	0·00
YHR212c		0·00	1·21	0·28
YHR213w		0·21	2·61	1·09
YHR214w		0·21	2·24	0·40
YHR215w	*PHO12*	0·21	2·66	0·00
YHR216w	*PUR5*	0·00	0·79	0·95
YHR217c		0·81	1·64	0·88
YHR218w		0·00	0·39	0·39
YHR219w		0·00	1·20	0·29
Right telomere				

Details of the media, growth of cells, preparation of cells and subsequent FACS analysis are described in Materials and Methods. The first column shows the systematic ORF name. The second column shows the SGD database synonym. The last three columns give the relative green fluorescence values (RGF) of strains grown in the media indicated. RGF values were determined as described in Materials and Methods. The first four genes (*GAL4*, *URA3*, *ACT1* and *GAL1*) served as controls and have been analysed previously (Niedenthal *et al.*, 1996). [m]These strains served as negative controls, indicating that presence of the *gfp* gene *per se* did not lead to an increase in green fluorescence. The *gfp–HIS3* cassette was integrated in the intergenic region between ORFs in such a way that no promoter fusion was created. *YHR047c–YHR048w*, integration between ORFs *YHR047c* and *YHR048w*; *YHR211w–YHR216w*, integration between *YHR210c* and *YHR217c*.

found in YYPD$^+$ (*ENO2, YHR143w, RPL4A*) changed only slightly on SD compared to YYPD$^+$ (9·9 RGF units/1·3-fold down, 14·6 RGF units/1·1-fold up and 4·6 RGF units/1·7-fold down, respectively). The fourth-strongest promoter (*BAT1*/*YHR208w*, encoding an aminotransferase) was upregulated 4·4-fold when growing on SD (8·3 RGF units) compared to YYPD$^+$.

The number of active promoters in cells growing in synthetic minimal media containing ethanol as a carbon source (SE) was reduced to 76% (202) (Table 5). Sixteen (6%) of these promoters were strong in cells grown on SE media (Figure 4). The strongest promoter activity on SE was found for *YHR143w,* encoding an unknown protein, which also exhibited high activity on YYPD$^+$ and SD. Likewise, other strong SE-specific promoter activities include the eight unknown ORFs: *YHL041w, YHL035c, YHR020w, YHR052w, Yhr087w, YHR095w, YHR162w* and *YHR210c*.

In summary, the promoter activity studies in three different media revealed that the vast majority of the chromosome VIII promoters are only active at low or intermediate levels (Figure 4). However, we could detect activity of all but two (99%) of the 265 promoters in one of the three

Copyright © 1999 John Wiley & Sons, Ltd.

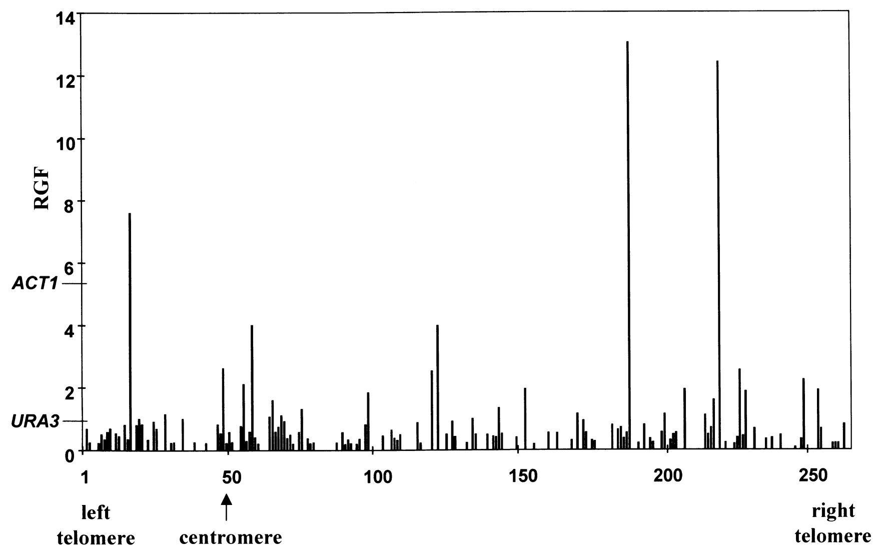


Figure 3. Promoter activity profile of all chromosome VIII genes grown in YYPD$^+$. Flow cytometric quantification of GFP green fluorescence in heterozygous diploid strains (*ORF*$^+$/*Δorf*$^-$*::gfp-HIS3*) grown in YPD (for details of growth and analysis, see Materials and Methods). The relative green fluorescence units (RGF) from each strain as given in Table 5 is plotted relative to the position of the corresponding gene on chromosome VIII. The position of the centromere between genes 49 and 50 is marked. For comparison, the fluorescence levels of the two control strains *URA3* (0·93 RGF units) and *ACT1* (5·53 RGF units) grown in YYPD$^+$ are indicated on the *y* axis.

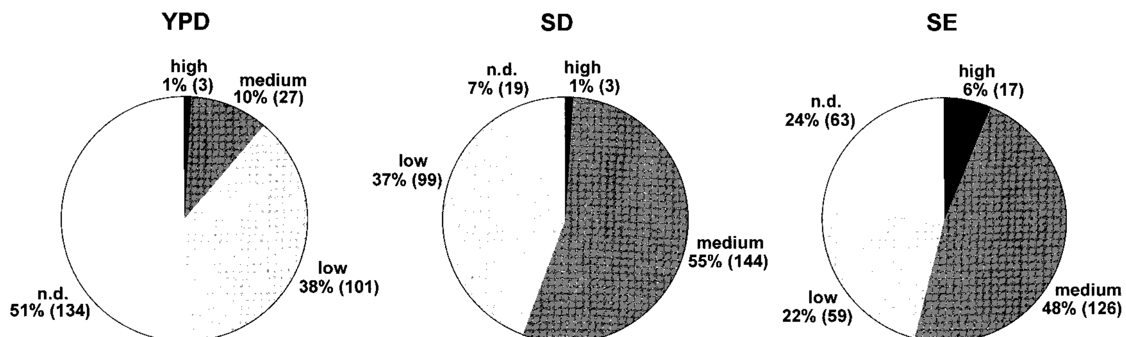


Figure 4. Diagrammatic representation of chromosome VIII promoter strengths in the 3 different media analysed. The RGF values for the 265 strains analysed (Table 5) were classified into four categories: n.d., no fluorescence detected above background; low, low promoter activity with RGF values below *URA3* (0·93 RGF in YYPD+); medium, medium promoter activity with RGF values between *ACT1* and *URA3* levels (5·53 and 0·93, respectively, in YYPD$^+$); high, high promoter activity with RGF values above *ACT1* level (5·53 in YYPD$^+$). The percentage of strains exhibiting high, medium, low or no fluorescence is given. The number of strains in each category is indicated in brackets.

different media. This result indicates that the ORFs encoding proteins of unknown function are very likely to be expressed. A high percentage of active promoters in yeast have also been reported by others. In studies employing Northern blotting, 50–88% of all genes analysed were found to be transcribed under one or more growth conditions (82% of 250 chromosome XIV genes, Planta *et al.*, 1999; 88% of the 182 chromosome III genes, Yoshikawa and Isono, 1990; 53% of the 333 chromosome XI genes, Richard *et al.*, 1997; and 83% of the 126 chromosome VI genes, Naitou *et al.*, 1997). It appears that our promoter–*gfp* fusion assay is more sensitive in measuring promoter

Copyright © 1999 John Wiley & Sons, Ltd.

activity than other techniques used to quantify the mRNA amount of an ORF directly or indirectly (Planta *et al.*, 1999; DeRisi *et al.*, 1997; Hauser *et al.*, 1998; Wodicka *et al.*, 1997). This is probably due to the fact that the number of RNA molecules per cell is orders of magnitude lower than the number of the corresponding protein molecules, e.g. the most highly expressed yeast genes are *TDH2* and *TDH3*, producing 425 RNA molecules per cell (Velculescu *et al.*, 1997), while their translation into protein yields about 1 100 000 protein molecules per cell (Norbeck and Blomberg, 1997).

The level of promoter activity identified for the chromosome VIII genes does not necessarily predict their protein expression levels. New data indicate that quantitative mRNA data and protein levels do not necessarily correlate. For some genes the protein levels varied by more than 20-fold, although the mRNA levels had identical values (Gygi *et al.*, 1999).

Seventy-seven per cent (205) of all chromosome VIII promoters were upregulated during growth on SD compared to $YYPD^+$ (Figure 4). Of those, 124 promoters were induced from an undetectable level in $YYPD^+$, while another 81 promoters were upregulated more than two-fold in SD compared to $YYPD^+$. Only a few promoters were strongly upregulated on SD: 19 promoters were five-fold stronger, and another nine promoters were at least 10-fold stronger on SD compared to $YYPD^+$ (Table 5). This high degree of promoter regulation in cells grown on SD probably reflects the necessity of cells to respond to the low level of nutrients present in synthetic minimal medium. This strong bias towards SD is confirmed by the fact that only 10 promoters were upregulated more than two-fold when grown on $YYPD^+$ compared to growth on SD. Growth on a fermentable (glucose) vs. a non-fermentable (ethanol) carbon source also significantly influenced the promoter activities: 38% (100) of all chromosome VIII promoters were upregulated at least two-fold when grown on SE compared to growth on SD, while 33% (88) of the promoters were downregulated at least two-fold on SE (compared to growth on SD). Similar results were obtained from Northern blot experiments, which revealed that about 30% of 250 chromosome XIV genes are regulated by glucose (Planta *et al.*, 1999).

The promoters of two of the essential genes did not produce any detectable GFP in all three media. One gene is *GPA1*, encoding the alpha sub-unit of the G protein complex involved in the mating signalling pathway. Expression of this gene is repressed in diploid cells, in which the GFP measurements were done. The other gene (*YHR059w*) encodes a protein of unknown function.

OUTLOOK

GFP proved to be a reliable reporter of gene expression, but because it is fairly labour-intensive to measure, other methods that rely on detecting RNA directly are probably preferred for measuring expression of a large number of genes (DeRisi *et al.*, 1997; Hauser *et al.*, 1998; Wodicka *et al.*, 1997). In addition, our promoter-*gfp* fusions do not report endogenous mRNA stabilities, and may disrupt posttranscriptional regulation. On the other hand, the DNA microarrays are unable to detect translational regulation of gene expression, while our promoter-*gfp* fusions would allow this for genes with translational regulatory signals upstream of the ATG codon). In addition it seems that our promoter–*gfp* fusion technology is more sensitive than assays which rely on mRNA quantification.

We observed a phenotype caused by 33% of the gene disruptions. While these phenotypes may provide clues to gene function in some cases, we believe that the major impact of systematic analysis like this lies in the provision to the scientific community of the resource of the complete set of mutants. It is difficult for one laboratory to analyse adequately many phenotypes in a large set of mutants such as that we have produced, but experts in specific areas of yeast cell biology will be able to analyse the relatively few phenotypes in which they are experts. We hope that the reagents and information provided by our preliminary and relatively superficial analysis of 265 genes on chromosome VIII will catalyse discovery of gene function by others interested in particular aspects of yeast cell function.

ACKNOWLEDGEMENTS

We thank Dr Chalfie for the wt *gfp* DNA-containing plasmid. We thank Dr K.-J. Rieger for teaching us the microtitre plate-based phenotypic assays. Susanne Heck is thanked for excellent technical assistance. We are grateful to Becton Dickinson for support and discussions during the

Copyright © 1999 John Wiley & Sons, Ltd.

GFP measurements. Parts of this project were supported by funds provided by the McDonnell Foundation, and the US National Center for Human Genome Research (HG00956, R. W. Waterston, PI) (to M.J.) and the FAZIT-Stiftung, the German BMBF project, 'Network for the functional analysis of unknown gene products' (No. FKZ 0310577) and the EUROFAN project (to J.H.H.).

REFERENCES

Barton, A. B., Bussey, H., Storms, R. K. and Kaback, D. B. (1997). Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: characterization of the 54 kb right terminal CDC15-FLO1-PHO11 region. *Yeast* **13,** 1251–1263.

Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F. and Cullin, C. (1993). A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **21,** 3329–3330.

Bickle, M., Delley, P. A., Schmidt, A. and Hall, M. N. (1998). Cell wall integrity modulates RHO1 activity via the exchange factor ROM2. *EMBO J.* **17,** 2235–2245.

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

DeRisi, J. L., Iyer, V. R. and Brown, P. O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278,** 680–686.

Dujon, B. (1996). The yeast genome project: what did we learn? *Trends Genet.* **12,** 263–270.

Entian, K.-D., Schuster, T., Hegemann, J. H., Becher, D., Feldmann, H., Güldener, U., Götz, R., Hansen, M., Hollenberg, C. P., Jansen, G., Kramer, W., Klein, S., Kötter, P., Kricke, J., Launhardt, H., Mannhaupt, G., Maierl, A., Meyer, P., Mewes, W., Munder, T., Niedenthal, R. K., Ramazani Rad, M., Röhmer, A., Römer, A., Rose, M., Schäfer, B., Siegler, M.-L., Vetter, J., Wilhelm, N., Wolf, K., Zimmermann, F. K., Zollner, A. and Hinnen, A. (1999). Functional analysis of 150 deletion mutants in *Saccharomyces cerevisiae* by a systematic approach. *Mol. Gen. Genet.* (in press).

Garrels, J. I. (1996). YPD-A database for the proteins of *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **24,** 46–49.

Goebl, M. G. and Petes, T. D. (1986). Most of the yeast genomic sequences are not essential for cell growth and division. *Cell* **46,** 983–992.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H. and Oliver, S. G. (1996). Life with 6000 genes. *Science* **274,** 546.

Gygi, S. P., Rochon, Y., Franza, B. R. and Aebersold, R. (1999). Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* **19,** 1720–1730.

Hampsey, M. (1997). A review of phenotypes in *Saccharomyces cerevisiae*. *Yeast* **13,** 1099–1133.

Hauser, N. C., Vingron, M., Scheideler, M., Krems, B., Hellmuth, K., Entian, K. D. and Hoheisel, J. D. (1998). Transcriptional profiling on all open reading frames of *Saccharomyces cerevisiae*. *Yeast* **14,** 1209–1221.

Johnston, M., Andrews, S., Brinkman, R., Cooper, J., Ding, H., Dover, J., Du, Z., Favello, A., Fulton, L., Gattung, S., Geisel, C., Kirsten, J., Kucaba, T., Hillier, L., Jier, M., Johnston, L., Langston, Y., Latreille, P., Louis, E. J., Macri, C., Mardis, E., Menezes, S., Mouser, L., Nhan, M., Rifkin, L., Riles, L., Peter, H. S., Trevaskis, E., Vaughan, K., Vignati, D., Wilcox, L., Wohldman, P., Waterston, R., Wilson, R. and Vaudin, M. (1994). Complete nucleotide sequence of *Saccharomyces cerevisiae* chromosome VIII. *Science* **265,** 2077–2082.

Karin, M., Najarian, R., Haslinger, A., Valenzuela, P., Welch, J. and Fogel, S. (1984). Primary structure and transcription of an amplified genetic locus: the CUP1 locus of yeast. *Proc. Natl Acad. Sci. U S A* **81,** 337–341.

Katz, B. P. (1988). A computer enhanced runs test for smoothed data. *Stat. Med.* **7,** 795–804.

Lustig, A. J. (1998). Mechanisms of silencing in *Saccharomyces cerevisiae*. *Curr. Opin. Genet. Dev.* **8,** 233–239.

Mood, A. M. (1940). The distribution theory of runs. *Ann. Math. Stat.* **12,** 228–232.

Naitou, M., Hagiwara, H., Hanaoka, F., Eki, T. and Murakami, Y. (1997). Expression profiles of transcripts from 126 open reading frames in the entire chromosome VI of *Saccharomyces cerevisiae* by systematic Northern analyses. *Yeast* **13,** 1275–1290.

Niedenthal, R. K., Riles, L., Johnston, M. and Hegemann, J. H. (1996). Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast. *Yeast* **12,** 773–786.

Nogae, I. and Johnston, M. (1990). Isolation and characterization of the ZWF1 gene of *Saccharomyces cerevisiae*, encoding glucose-6-phosphate dehydrogenase. *Gene* **96,** 161–169.

Norbeck, J. and Blomberg, A. (1997). Two-dimensional electrophoretic separation of yeast proteins using a non-linear wide range (pH3–10) immobilized pH gradient in the first dimension: reproducibility and evidence for isoelectric focusing of alkaline (pH >7) proteins. *Yeast* **13,** 1519–1534.

Planta, R. J., Brown, A. J., Cadahia, J. L., Cerdan, M. E., de Jonge, M., Gent, M. E., Hayes, A., Kolen, C. P., Lombardia, L. J., Sefton, M., Oliver, S. G.,

Copyright © 1999 John Wiley & Sons, Ltd.

Thevelein, J., Tournu, H., van Delft, Y. J., Verbart, D. J. and Winderickx, J. (1999). Transcript analysis of 250 novel yeast genes from chromosome XIV. *Yeast* **15,** 329–350.

Richard, G. F., Fairhead, C. and Dujon, B. (1997). Complete transcriptional map of yeast chromosome XI in different life conditions. *J. Mol. Biol.* **268,** 303–321.

Rieger, K. J., Kaniak, A., Coppee, J. Y., Aljinovic, G., Baudin-Baillieu, A., Orlowska, G., Gromadka, R., Groudinsky, O., Di Rago, J. P. and Slonimski, P. P. (1997). Large-scale phenotypic analysis—the pilot project on yeast chromosome III. *Yeast* **13,** 1547–1562.

Schultes, N. P., Ellington, A. D., Cherry, J. M. and Szostak, J. W. (1990). *Saccharomyces cerevisiae* homoserine kinase is homologous to prokaryotic homoserine kinases. *Gene* **96,** 177–180.

Sherman, F. (1991). Getting started with yeast. *Methods Enzymol.* **194,** 3–21.

Smith, V., Chou, K. N., Lashkari, D., Botstein, D. and Brown, P. O. (1996). Functional analysis of the genes of yeast chromosome V by genetic footprinting. *Science* **274,** 2069–2074.

Thomas, D., Cherest, H. and Surdin-Kerjan, Y. (1991). Identification of the structural gene for glucose-6-phosphate dehydrogenase in yeast. Inactivation leads to a nutritional requirement for organic sulfur. *EMBO J.* **10,** 547–553.

Urban, P., Mignotte, C., Kazmaier, M., Delorme, F. and Pompon, D. (1997). Cloning, yeast expression, and characterization of the coupling of two distantly related *Arabidopsis thaliana* NADPH-cytochrome P450 reductases with P450 CYP73A5. *J. Biol. Chem.* **272,** 19 176–19 186.

Velculescu, V. E., Zhang, L., Zhou, W., Vogelstein, J., Basrai, M. A., Bassett, D. E. Jr, Hieter, P., Vogelstein, B. and Kinzler, K. W. (1997). Characterization of the yeast transcriptome. *Cell* **88,** 243–251.

Winzeler, E A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benido, R., Boeke, J. D., Bussey, H., Chu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., ElBakkoury, M., Foury, F., Friend, S. H., Gentalen, E., Giaever, G., Hegemann, J. H., Jones, T., Laub, M., Liao, H., Liebundguth, N., Lockhart, D. J., Lucau-Danila, A., Lussier, M., M'Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebischung, C., Revuelta, J. L., Riles, L., Roberts, C. J., Ross-Macdonald, P., Scherens, B., Snyder, M., Storms, R. K., Véronneau, S., Voet, M., Volckaert, G., Ward, T. R., Wysocki, R., Yen, G. S., Yu, K., Zimmermann, K., Philippsen, P., Johnston, M. and Davis, R. W. (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285** (in press).

Wodicka, L., Dong, H., Mittmann, M., Ho, M. H. and Lockhart, D. J. (1997). Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nat. Biotechnol.* **15,** 1359–1367.

Wolfe, K. H. and Shields, D. C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **387,** 708–713.

Yoshikawa, A. and Isono, K. (1990). Chromosome III of *Saccharomyces cerevisiae*: an ordered clone bank, a detailed restriction map and analysis of transcripts suggest the presence of 160 genes. *Yeast* **6,** 383–401.

Copyright © 1999 John Wiley & Sons, Ltd.