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Genome-Wide Screening of *Saccharomyces cerevisiae* To Identify Genes Required for Antibiotic Insusceptibility of Eukaryotes

Alexandra S. Blackburn and Simon V. Avery*

School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom

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The adverse reactions provoked by many antibiotics in humans are well documented but are generally poorly understood at the molecular level. To elucidate potential genetic defects that could give rise to susceptibility to prokaryote-specific antibiotics in eukaryotes, we undertook genome-wide screens using the yeast *Saccharomyces cerevisiae* as a model of eukaryotes; our previous work with a small number of yeast mutants revealed some specific gene functions required for oxytetracycline resistance. Here, the complete yeast deletion strain collection was tested for growth in the presence of a range of antibiotics. The sensitivities of mutants revealed by these screens were validated in independent tests. None of the ~4,800 defined deletion strains tested were found to be sensitive to amoxicillin, penicillin G, rifampin, or vancomycin. However, two of the yeast mutants were tetracycline sensitive and four were oxytetracycline sensitive; encompassed among the latter were mutants carrying deletions in the same genes that we had characterized previously. Seventeen deletion strains were found to exhibit growth defects in the presence of gentamicin, with MICs for the strains being as low as 32 μg ml^{-1} (the wild type exhibited no growth defects at any gentamicin concentration tested up to 512 μg ml^{-1}). Strikingly, 11 of the strains that were most sensitive to gentamicin carried deletions in genes whose products are all involved in various aspects of vacuolar and Golgi complex (or endoplasmic reticulum) function. Therefore, these and analogous organelles, which are also the principal sites of gentamicin localization in human cells, appear to be essential for normal resistance to gentamicin in eukaryotes. The approach and data described here offer a new route to gaining insight into the potential genetic bases of antibiotic insusceptibilities in eukaryotes.

To be effective as chemotherapeutic agents, antibiotics not only should inhibit target microorganisms but also should not exert adverse effects on host organisms. Thus, the well-documented spread of antibiotic resistance among pathogenic microorganisms is one important obstacle to effective antibiotic treatment (12). At the same time, adverse reactions to antibiotics arise in about 5 to 10% of patients to whom they are prescribed (3), and this further erodes the perception that many antibiotics are "magic bullets." Adverse reactions range from mild effects such as hypersensitivity, rashes, and gastrointestinal intolerance to more serious complications such as toxicity to various organs and in some cases death (3, 20). Despite this incidence of adverse responses to antibiotics among humans, the underlying causes of these effects at the molecular level are in many cases unknown (unlike the causes of bacterial resistance). This is an important gap in our knowledge, as a clearer understanding of adverse effects is a prerequisite if these are to be averted in the future. For example, if it was possible to predict (e.g., genetically) which patients might be susceptible to the adverse effects of particular antibiotics, then it should be possible to tailor antibiotic prescriptions accordingly (or develop modified antibiotics with lower levels of toxicity), so improving the overall efficacy of antibiotic therapy.

In order to be able to use genetic tools to predict potential drug susceptibilities in humans, it is first necessary to have established any genetic bases for such conditions. However, as for inheritable susceptibilities to diseases, this remains a challenging task, despite the recent availability of the human genome sequence and improved techniques for single-nucleotide-polymorphism analysis (16). The yeast *Saccharomyces cerevisiae* provides a relatively simple model system for eukaryotes that is very well understood genetically and has been at the forefront of recent advances in functional genomics technologies (6, 9, 14, 21). Moreover, there is remarkable conservation of gene functions between the yeast and humans. For example, greater than 40% of single-gene determinants of human heritable diseases have yeast homologs (5). Therefore, with *S. cerevisiae* it is possible to gain valuable insight into eukaryotic cell biology and genetics that would be very difficult to accomplish with higher eukaryotic cell systems.

Using *S. cerevisiae*, we recently identified antioxidant functions that were essential for normal resistance to certain tetracycline antibiotics (1, 2). Whereas the growth of wild-type *S. cerevisiae* was unaffected at concentrations of tetracyclines close to the antibiotics' limits of solubility, deletion mutants deficient in Sod1p (Cu,Zn superoxide dismutase), Ctr1p (high-affinity Cu transporter), and Mac1p (metalloregulatory transcription factor) exhibited marked sensitivities to oxytetracycline and doxycycline. These susceptibilities were shown to be due to a novel mode of oxytetracycline and doxycycline action that was dependent on oxidative damage and that is normally suppressed in cells by Sod1p and copper (1, 2). It was considered likely that the insusceptibilities of humans to these antibiotics may well also rely on these functions (1).

* Corresponding author. Mailing address: School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom. Phone: 44-115-9513315. Fax: 44-115-9513251. E-mail: Simon.Avery@nottingham.ac.uk.

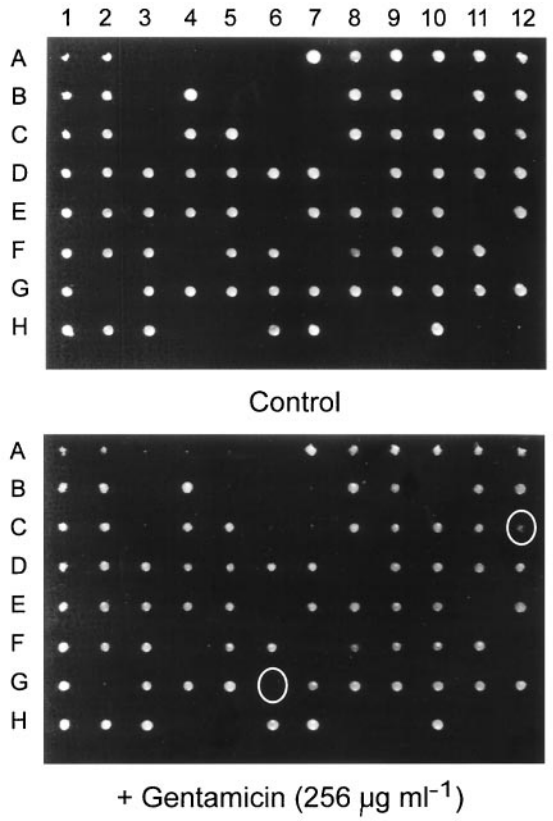

FIG. 1. Screening for gentamicin sensitivity using the *S. cerevisiae* deletion strain collection. Strains were cultured in liquid YEPD medium in a 96-well format and replica inoculated onto YEPD agar supplemented with gentamicin (256 μg ml^{-1}). The results are for 1 strain set (strain set 4_3; Euroscarf) of a total of 76 strain sets examined with each antibiotic after incubation for 3 days at 30°C. Circles highlight strains that exhibited slight (position C12; *gcs1*Δ) and strong (position G6; *luv1*Δ) sensitivities to gentamicin relative to their growth on the control plate lacking gentamicin. The gentamicin sensitivities of these strains were subsequently validated (Fig. 2 and Table 1). Empty inocula on the control plate correspond to positions at which essential open reading frames were originally deleted, producing nonviable mutants.

To build on the findings described above and broaden the work beyond antioxidant gene functions alone, in this study we present the results of the first genome-wide screen for eukaryotic gene functions that may be required to avert the adverse effects of antibiotics. This is possible with the availability of the complete yeast deletion strain collection, which has been generated through an international effort to delete systematically every yeast open reading frame (21). We screened the collection with a range of antibiotics and report here several new gene functions that are required for normal antibiotic resistance in this yeast model of eukaryotes. In particular, the data reveal that normal vacuolar and Golgi complex functions are essential for insusceptibility to the aminoglycoside antibiotic gentamicin in eukaryotes.

MATERIALS AND METHODS

S. cerevisiae* deletion strain collection.** The *S. cerevisiae* deletion strain collection, constructed in the BY4741 background (*MATa** *his3*Δ*1 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*), was obtained from Euroscarf (Frankfurt, Germany) in a 96-well format. Each deletion strain (of a total of ~4,800 in the library) carries a defined deletion of a characterized or putative open reading frame, in which the open reading frame has been replaced with the *kanMX4* marker by PCR (21). Strains were routinely stored in the 96-well format at −80°C in YEPD medium (8): 2% (wt/vol) bacteriological peptone (Oxoid), 1% yeast extract (Oxoid), 2% glucose supplemented with 15% (vol/vol) glycerol, and 150 μg of Geneticin (G418; Sigma) ml^{-1} for selection.

Screening for antibiotic-sensitive deletion mutants. Deletion strains were inoculated from frozen stocks into Geneticin-supplemented YEPD medium in 96-well plates by using a 96-pin tool (1 to 2 μl of inoculum per pin). The strains were cultured for 2 days at 30°C and then replica inoculated onto YEPD agar supplemented or not supplemented with the appropriate antibiotic at 256 μg ml^{-1}. All antibiotics were purchased from Sigma. The plates were incubated at 30°C for 3 to 5 days before they were examined for growth. A positive result was scored when the growth of a mutant in antibiotic-supplemented plates was visibly diminished compared to its growth in control plates (e.g., see Fig. 1). The functions of genes that were deleted in mutants of interest were derived from databases on the World Wide Web (http://genome-www.stanford.edu and http://mips.gsf.de/proj/yeast).

Validation of antibiotic sensitivity. The antibiotic sensitivities of mutant strains of interest, identified during screening of the deletion strain collection (see above), were validated by spotting tests. Strains of interest were cultured in 96-well plates under the same conditions described above and then adjusted to an optical density at 600 nm (OD_{600}) of ~0.01 with sterile water. Aliquots (4 μl) were spotted onto plates supplemented with the appropriate antibiotic, supplied at the same concentration used for initial screening (256 μg ml^{-1}). Growth was examined after incubation at 30°C for 3 to 5 days. All plates were prepared and inoculated at least in duplicate.

Determination of MICs. Strains of interest were cultured in 96-well plates under the same conditions described above and then adjusted to an OD_{600} of ~0.03 with sterile water. These cell suspensions were replica inoculated by using a 96-pin tool (~400 to 500 cells per inoculum) to YEPD agar supplemented or not supplemented with antibiotics; antibiotics were supplied in twofold dilution series at final concentrations ranging between 1 and 512 μg ml^{-1}. The plates were examined after incubation for 3 to 5 days at 30°C. The MICs for each sensitive mutant strain were determined as the lowest antibiotic concentrations that resulted in full inhibition of visible growth in replicate incubations.

RESULTS

Screening the *S. cerevisiae* deletion strain collection for antibiotic-sensitive mutants. To elucidate the gene functions that may be required for normal antibiotic resistance in eukaryotes, the full collection of haploid *S. cerevisiae* deletion strains was screened for growth in the presence of a range of test antibiotics. The antibiotics selected for this study (Table 1) are well characterized and are in use for human therapy, and most are also commonly associated with adverse effects in humans (3, 10). It was considered worthwhile to include two tetracycline antibiotics in the study since the oxytetracycline-sensitive mutants that we identified previously were tetracycline resistant

TABLE 1. Numerical breakdown of strains of interest identified during screening of the deletion strain collection and subsequent validation

Antibiotic	No. of strains		
	Putative sensitive strains identified in initial screen	Strains confirmed as sensitive	Strains for which MIC was ≤512 μg ml^{-1}
Amoxicillin	8	0	0
Gentamicin	19	17	15
Penicillin G	9	0	0
Oxytetracycline	12	4	4
Rifampin	2	0	0
Tetracycline	9	2	2
Vancomycin	2	0	0

(1, 2), indicating that these similar antibiotics can exert different effects on eukaryotic cells.

It was confirmed in preliminary experiments that the growth of wild-type *S. cerevisiae* was unaffected by each of the test antibiotics at concentrations up to 512 μg ml^{-1} (the highest concentration tested). To screen for antibiotic-sensitive mutants, 256 μg ml^{-1} was used as the test antibiotic concentration. Putative antibiotic-sensitive yeast mutants were identified in the screens outlined in the Materials and Methods. These mutants exhibited various degrees of diminished growth in the presence of antibiotic compared with the growth in the control incubations lacking antibiotic. In this way, screens with oxytetracycline (12 strains) and gentamicin (19 strains) yielded the greatest number of putative sensitive mutants (Table 1). Furthermore, many of the putative gentamicin-sensitive strains exhibited complete inhibition of growth in the presence of gentamicin (for example, see Fig. 1). In contrast, only two putative rifampin-sensitive mutants and two putative vancomycin-resistant mutants were identified, and these strains still exhibited some (albeit apparently diminished) growth in the presence of the antibiotics (data not shown). A total of 61 putative antibiotic-sensitive mutants were identified in the screens with the seven test antibiotics (Table 1).

Validation of antibiotic sensitivity. Since ~4,800 different strains were involved in the screens described above, it was not feasible to standardize conditions (e.g., cell densities) rigorously during screening. Therefore, to validate the antibiotic sensitivities of the strains of interest identified above, organisms were applied to agar supplemented with the appropriate antibiotic (at 256 μg ml^{-1}) as spots of standardized cell density. These 4-μl spots also diffused further in the agar than the smaller inocula that were necessary for the screening tests (described above), thereby giving a better resolution of individual cell colonies in this case and a greater sensitivity of detection (Fig. 2B). Of the 61 putative sensitive mutants identified in the screens described above, 23 strains were confirmed to be sensitive when retested with the relevant antibiotic under these more uniform conditions. None of the putative amoxicillin-, penicillin G-, rifampin-, or vancomycin-sensitive mutants identified in the screens were found to exhibit genuine sensitivities to these antibiotics. In contrast, 17 of the 19 putative gentamicin-sensitive strains were confirmed to be gentamicin sensitive in spotting tests (Fig. 2A and B). The growth of each of these 17 strains was completely or almost completely inhibited by gentamicin at 256 μg ml^{-1}, with the exception of *S. cerevisiae chs1*Δ, which did grow in the presence of gentamicin at this concentration, but with a diminished colony density (Fig. 2B). Strikingly, 11 of the 17 gentamicin-sensitive mutants were defective in gene functions that are involved directly with organellar protein sorting or processing (Table 2). These included genes important for Golgi complex or endoplasmic reticulum (ER) functions (e.g., *CAX4*, *GCS1*, *MNN9*, and *SAC1*) as well as several *VPS* (*PEP*) genes that are involved specifically with vacuolar protein sorting or biogenesis (*PEP3*, *PEP5*, *VPS15*, *VPS16*, *VPS33*, *VPS34*). Other gentamicin-sensitive mutants were defective in various other types of function or had no characterized function.

Mutants that appeared to be sensitive to the tetracycline antibiotics during screening were also tested by spotting. From a total of 21 putative sensitive strains, 4 were confirmed to be oxytetracycline sensitive and 2 were confirmed to be tetracycline sensitive; the last 2 strains (*erg28*Δ, *adh1*Δ) were also among the oxytetracycline-sensitive strains (Table 3). The two mutants that were confirmed to be oxytetracycline sensitive but not tetracycline sensitive carried deletions in the *SOD1* and *MAC1* genes, in keeping with our previous findings (1, 2). However, the *ctr1*Δ mutant described above was not among those identified by screening for oxytetracycline sensitivity in this study.

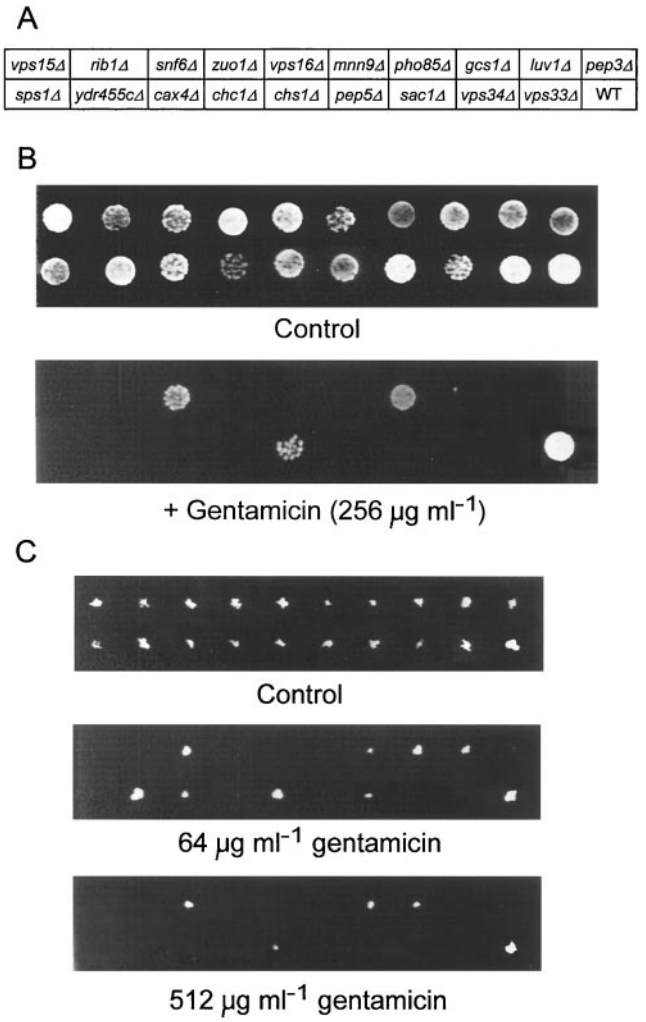

FIG. 2. Validation and quantification of antibiotic (gentamicin) sensitivity. All 19 putative gentamicin-sensitive strains identified during initial screening of the deletion strain collection were tested quantitatively for antibiotic sensitivity. (A) Grid of putative gentamicin-sensitive mutants identified from screening (WT, wild type). (B) Mutants of interest were cultured in liquid YEPD medium and adjusted to an OD_{600} of ~0.01 before they were spotted (4 μl) onto unsupplemented and gentamicin-supplemented YEPD agar (the strains in the grid correspond to those shown in panel A). (C) MIC determination. Mutants were cultured as described above for panel B and adjusted to an OD_{600} of ~0.03 before replica inoculation with a 1- to 2-μl pin tool onto YEPD agar supplemented with a range of gentamicin concentrations (1 to 512 μg ml^{-1}); the results obtained with 0, 64, and 512 μg of gentamicin ml^{-1} are shown. All plates were incubated for 3 days at 30°C before examination. Typical results from one of several replicates are shown.

TABLE 2. Gentamicin-sensitive *S. cerevisiae* mutants

Deleted open reading frame	Gene name	Gene product	Main function	MIC (μg ml^{-1})[a] for deletion mutant
YBR097w	*VPS15*	Ser/Thr protein kinase	Vacuolar protein sorting	32
YLR240w	*VPS34*	Phosphatidylinositol 3-kinase	Vacuolar sorting and segregation	32
YBL033c	*RIB1*	GTP cyclohydrolase II	Riboflavin biosynthesis	64
YDR523c	*SPS1*	Ser/Thr protein kinase	Meiosis	64
YGR285c	*ZUO1*	Zuotin	Chaperone	64
YGL206c	*CHC1*	Clathrin heavy chain	Protein sorting, internalization	64
YLR148w	*PEP3 (VPS18)*	Vacuolar membrane protein	Vacuolar protein sorting	64
YLR396c	*VPS33*	Vacuolar sorting protein	Vacuolar protein sorting	64
YMR231w	*PEP5 (VPS11)*	Vacuolar biogenesis protein	Vacuolar protein biogenesis	64
YPL045w	*VPS16*	Vacuolar sorting protein	Vacuolar protein sorting	64
YPL050c	*MNN9*	Uncharacterized	N-glycosylation	64
YKL212w	*SAC1*	ER and Golgi membrane protein	Golgi function and actin organization	128
YDR027c	*LUV1*	Uncharacterized	Microtubule function regulation	256
YDR455c		Uncharacterized	Unknown	256
YGR036c	*CAX4*	Possible phosphatase	Cell wall biogenesis and ER function	512
YDL226c	*GCS1*	ADP-ribosylation factor GTPase-activating protein	ER to Golgi transport	>512
YNL192w	*CHS1*	Chitin synthase I	Cell wall biogenesis	>512

[a] The MIC refers to the lowest concentration of gentamicin that completely inhibited visible growth of the mutant on agar in replicate incubations.

MICs for antibiotic-sensitive yeast mutants. To provide a more quantitative analysis of antibiotic sensitivity, the MICs were determined as outlined in Materials and Methods for each of the mutants that were confirmed to be antibiotic sensitive in the spotting tests (described above). Gentamicin MICs were between 32 and 64 μg ml^{-1} for the majority of the gentamicin-sensitive mutants, and these included all of the *VPS* mutants (Fig. 2C; Table 2). Gentamicin MICs were greater than 512 μg ml^{-1} for two mutants (*gcs1*Δ and *chs1*Δ): they were not inhibited fully at this concentration, the highest concentration tested. Note that the growth of these mutants in the MIC tests could be attributable to the outgrowth of only one or two cells, as suggested by the spotting test for the *gcs1*Δ mutant, in which just one colony was apparent in the presence of gentamicin at 256 μg ml^{-1} (Fig. 2B). Overall, the gentamicin sensitivities of the test strains (Table 2) were more marked than the oxytetracycline or tetracycline sensitivities, with the MICs of the last two antibiotics ranging between 128 and 512 μg ml^{-1} (Table 3). It should be noted that for most of the antibiotic-sensitive mutants, some degree of growth inhibition was evident at concentrations considerably lower than the MICs for full inhibition (data not shown).

DISCUSSION

This is the first study in which a eukaryotic genome has been screened to identify genes that are required for normal resistance to antibiotics in eukaryotes. This was possible with the yeast model thanks to the strides in functional genomics technologies for this organism that have arisen since the completion of its genome sequence in 1996. In particular, we exploited the yeast deletion strain collection, which provides an outstanding resource for addressing biological questions such as this (7, 15, 21).

The molecular mechanisms underlying the broad range of adverse effects that can arise during antibiotic administration are unknown in many cases. Our previous demonstration that three nonessential antioxidant genes in yeast (which have human homologs) are essential for resistance to certain tetracyclines was consistent with the argument that susceptibility to adverse effects of antibiotics, like susceptibility to many diseases, can be determined genetically (1, 2). There are already certain known examples of genetically determined antibiotic susceptibility in humans. Thus, patients deficient in the enzyme glucose-6-phosphate dehydrogenase can develop acute hemolysis when they are prescribed sulfonamides or certain other antibiotics (3). In this study, we have identified 21 new gene functions that are required for normal resistance of the yeast model of eukaryotes to prokaryote-specific antibiotics, in particular, gentamicin. The same or similar functions in humans may well also be required for insusceptibility to the same antibiotics.

Since none of the ~4,800 yeast mutants exhibited susceptibility to amoxicillin, penicillin G, rifampin, or vancomycin, it seems less likely that the common adverse reactions that these antibiotics may elicit (3) are dependent on the defective activities of specific gene products. However, our results do not

TABLE 3. Tetracycline- and oxytetracycline-sensitive *S. cerevisiae* mutants

Sensitivity[a]	Deleted open reading frame	Gene name	Gene product	Main function	MIC (μg ml^{-1})[b] for deletion mutant
TET, OTC	YER044c	*ERG28*	Uncharacterized	Ergosterol biosynthesis	128
TET, OTC	YOL086c	*ADH1*	Alcohol dehydrogenase I	Ethanol from acetaldehyde	256
OTC	YJR104c	*SOD1*	Cu,Zn superoxide dismutase	Antioxidant defense	512
OTC	YMR021c	*MAC1*	Transcription factor	Regulation of Cu and Fe uptake	512

[a] TET, tetracycline; OTC, oxytetracycline.
[b] MIC refers to the lowest concentration of antibiotic that completely inhibited visible growth of the mutant on agar in replicate incubations.

fully rule out that possibility since susceptibility to these antibiotics could be (i) a result of polygenic traits, which are much more difficult to elucidate; (ii) dependent on human gene functions or processes that do not occur in yeast; (iii) dependent on partial loss (e.g., due to heterozygosity) of essential gene functions which are not encompassed in the haploid yeast deletion strain collection (due to lethality); (iv) manifested in subtler ways that are not detectable as inhibition of growth; or (v) influenced by any differences in antibiotic uptake between mammalian and yeast cells. In addition, while our screens erred on the side of saturation—more than half of the mutants that were scored as potentially sensitive from the initial screens proved to have normal resistance when they were examined further—some moderately sensitive mutants may have been missed. For example, only two (*sod1*Δ and *mac1*Δ) of the three previously identified oxytetracycline-sensitive mutants were detected here (the screens were performed blind). However, the oxytetracycline MICs for these mutants were high at 512 μg ml^{-1}, and the third mutant, *ctr1*Δ, appeared to be slightly less sensitive than the *sod1*Δ and *mac1*Δ mutants in the previous study (1). Thus, it can be estimated that an MIC of ~512 μg ml^{-1} is the approximate limit above which any slight sensitivity may, in many cases, not have been detected by our screening methodology, and this is borne out by the data in Tables 2 and 3. Of course, the antibiotic concentration used here for screening (256 μg ml^{-1}) could be raised or lowered to adjust the sensitivity of the screens, although the potential relevance to adverse reactions of gene defects that yield antibiotic MICs greater than 512 μg ml^{-1} is questionable: the peak concentrations of most antibiotics in the plasma or serum of treated patients are typically less than about 10 to 20 μg ml^{-1} (18). Nonetheless, it should be noted that even though the oxytetracycline MIC for the *sod1*Δ mutant, for example, was high (~512 μg ml^{-1}), some inhibition of *sod1*Δ mutant growth is still readily evident at 100 μg ml^{-1} and can be detectable in the presence of oxytetracycline at a concentration as low as 10 μg ml^{-1} (2). Inhibitory effects commencing at antibiotic concentrations lower than the MICs presented for full inhibition were also detected against most other mutants of interest in this study.

The screen with the aminoglycoside antibiotic gentamicin yielded the greatest number of sensitive yeast mutants. Gentamicin is an inhibitor of bacterial protein synthesis, but it also has well-documented nephrotoxic and ototoxic side effects in humans. The molecular bases for these adverse effects are not yet fully understood (4, 11), although a mutation in a mitochondrial rRNA gene has been linked to familial aminoglycoside ototoxicity (13). It is known that gentamicin is internalized through endocytosis in mammalian cells and it becomes localized principally to endosomal and lysosomal vacuoles as well as to the Golgi complex (17, 19). Therefore, it is particularly interesting that most of the gentamicin-sensitive strains identified in this study were defective in genes associated with various aspects of vacuolar and Golgi complex (or ER) function. Thus, normal operation of these organelles is required for the insusceptibility of yeast to gentamicin. This evidence supports a previously suggested hypothesis that the normal localization of gentamicin in eukaryotic subcellular compartments such as lysosomes may serve to divert the antibiotic from more critical cellular targets, so helping to avert gentamicin toxicity (11). Presumably, patients with potential defects in functions analogous to those identified here (i.e., the vacuolar and Golgi complex functions as well as certain others listed in Table 2) could be at a high risk of suffering gentamicin toxicity, and our approach has now paved the way for this novel hypothesis to be tested in a mammalian system. It is also of interest that one of the gentamicin-sensitive yeast mutants identified here carried a deletion in a putative open reading frame (YDR455c) with no previously characterized function. Assigning functions to such open reading frames is one of the major challenges in the postgenomics era. By association, there seems a good chance from our results that YDR455c may encode a product that is involved in vacuolar or Golgi complex function.

As well as the antioxidant functions that we previously showed are required for oxytetracycline insusceptibility, two further genes required for both oxytetracycline and tetracycline insusceptibility, *ADH1* and *ERG28*, were identified here. These two genes apparently played a more important role in antibiotic insusceptibility since the oxytetracycline or tetracycline MIC for the relevant deletion mutant was lower (128 μg ml^{-1}). Erg28p is involved in ergosterol biosynthesis in yeast, although its precise role is unknown (9). We hypothesized that a possible defective membrane function in an *erg28*Δ mutant could allow more tetracycline to enter cells. However, in preliminary experiments we found no evidence for elevated levels of tetracycline uptake in this mutant compared to those in wild-type yeast (data not shown). It is interesting that only *ERG28* and none of the other yeast *ERG* genes appeared to be required for tetracycline resistance, and this difference could help pinpoint the role of *ERG28* in conferring tetracycline resistance as the molecular function of Erg28p becomes unraveled in the future. Moreover, such knowledge should also provide the opportunity to determine whether any functions equivalent to that of Erg28 involved in human cholesterol biosynthesis could be important for human responses to tetracycline antibiotics.

In conclusion, by exploiting the yeast model we have established the first data sets from genome-wide screens to catalogue eukaryotic genes that are required for antibiotic insusceptibility. The data obtained for the tetracyclines and gentamicin, in particular, are consistent with models in which the susceptibilities of certain individuals to the well-documented adverse effects of these antibiotics could have a genetic basis. Our data provide the necessary information with which such hypotheses can now be tested in higher systems. They also give new insight into the mechanisms by which these prokaryote-specific antibiotics may be processed in eukaryotic cells.

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