Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1287–1291, February 1995
Cell Biology

Yeast glycosylation mutants are sensitive to aminoglycosides

NETA DEAN

Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794-5215

Communicated by William J. Lennarz, State University of New York, Stony Brook, NY, September 30, 1994 (received for review July 13, 1994)

ABSTRACT **Aminoglycosides are a therapeutically important class of antibiotics that inhibit bacterial protein synthesis and a number of viral and eukaryotic functions by blocking RNA–protein interactions. Vanadate-resistant *Saccharomyces cerevisiae* mutants with defects in Golgi-specific glycosylation processes exhibit growth sensitivity to hygromycin B, an aminoglycoside [Ballou, L., Hitzeman, R. A., Lewis, M. S. & Ballou, C. E. (1991) *Proc. Natl. Acad. Sci. USA* 88, 3209–3212]. Here, evidence is presented that glycosylation is, in and of itself, a key factor mediating aminoglycoside sensitivity in yeast. Examination of mutants with a wide range of glycosylation abnormalities reveals that all are sensitive to aminoglycosides. This effect is specific to aminoglycosides and not merely a consequence of increased permeability of the yeast mutants to drugs. Furthermore, inhibition of glycosylation in wild-type cells leads to a marked increase in their sensitivity to aminoglycosides. These results establish that a defect in glycosylation is sufficient to render yeast cells susceptible to these clinically important drugs. Further, they suggest that a molecule which prevents the uptake or mediates removal of aminoglycosides requires glycosylation for its activity. Perhaps more importantly, this finding on drug sensitivity provides the most powerful screen to date to identify mutants and thereby to isolate genes involved in all aspects of N-linked glycosylation.**

Aminoglycosides are known to interfere with RNA–protein interactions. These antibiotics inhibit bacterial translation by interacting with the 16S ribosomal RNA (1–3). In eukaryotes, aminoglycosides block splicing of group I introns by binding to a specific RNA motif (4, 5). Certain members of the aminoglycoside family selectively block the binding of the human immunodeficiency virus (HIV) Rev protein to its cognate RNA recognition element (6). Inhibition of HIV replication by members of this family has been demonstrated (6), qualifying aminoglycosides as important antiviral agents that likely mediate their effect through sequence-specific RNA interactions. However, the efficacy of these drugs as therapeutic agents is limited because of poor intracellular uptake in eukaryotic cells.

Yeast mutants that have defects in Golgi-specific glycoprotein processing are selectively enriched by growth resistance to sodium vanadate and sensitivity to the aminoglycoside hygromycin B (7). Five hygromycin-sensitive complementation groups that have Golgi-specific glycosylation defects have been identified previously. Three of these are allelic to the well-characterized *mnn8, mnn9*, and *mnn10* mutations, and the remaining two are allelic to the previously unidentified mutations *vrg1* and *vrg4* (7). All of these mutants are defective in glycoprotein modifications that affect the elongation of outer-chain carbohydrates in the Golgi complex (8). It has been proposed that these gene products function in an energy-dependent, Golgi-specific function. To identify more mutants defective in Golgi-specific functions, additional vanadate-resistant/hygromycin B-sensitive cells were isolated. (These mutants and their genetic analyses will be described elsewhere.) Surprisingly, two mutants were isolated that are defective in very early steps of glycosylation that take place in or before the endoplasmic reticulum (ER). The isolation of mutants with defects in early steps in the glycosylation pathway suggested that hygromycin B sensitivity is not due to defects in Golgi-specific functions. To understand how a molecule that binds to RNA impinges upon glycosylation within the secretory pathway, I examined the effect of aminoglycosides on the growth characteristics of yeast glycosylation mutants. In the present work, I report the results of experiments that demonstrate that aminoglycoside hypersensitivity is due, at least in part, to defects in glycosylation. These experiments demonstrate that hygromycin B sensitivity is a phenotype that is not restricted to mutants with Golgi-specific glycosylation defects, but rather is common to a large number of yeast mutants with defects in all aspects of glycosylation. Drug sensitivity, therefore, provides a powerful selection technique that can be used for the cloning of the wild-type genes defined by these mutations.

MATERIALS AND METHODS

Strains, Plasmids, and Media. The following *Saccharomyces cerevisiae* strains were used in this study: DBY747-3D2 (*MAT***a** *rad52-8::TRP1 trp1-289 leu2-3,112 his3-Δ1 ura3-52*) (R. Rothstein, Columbia University, New York); PRY55 (*MAT***a** *alg1-1 ura3-52*) (9); PRY212 (*MATα alg2-1 ura3-52*) (10); PRY90 (*MATα alg3-1 ade2-101 ura3-52*) (10); PRY95 (*MATα alg4-4 ura3-52*), PRY98 (*MATα alg5-1 ade2-101 ura3-52*) (10) (P. Robbins, Massachusetts Institute of Technology, Cambridge); RSY4 (*MAT***a** *sec18-1 gls1-1*) (11), LB10B (*MATα mnn1*), LB116A (*MAT***a** *mnn2*), and TH210D (*MAT* **a** *mnn1 mnn2 ura3-52*) (Berkeley Genetics Stock Center); mnn8 (*MAT***a** *mnn8 gal1 CUP1*), *mnn9* (*MAT***a** *mnn9 gal1 CUP1*), and mnn10 (*MAT***a** *mnn10 gal1 CUP1*) (C. Ballou, University of California, Berkeley); MA9-D (*MAT***a** *wbp1-1 ade2-101 his3-Δ200 ura3-52 lys2-801*) (12) (M. Aebi, Eidgenössiche Technische Hochschule, Zurich); and 5762-6.2 (*MAT***a** *cyh2 ura3-1 trp1 ade2-101*) (N. Hollingsworth, State University of New York). NDY1.3, -5.3, -9.3, -1.4, -13.4, and -17.4 are spontaneous vanadate-resistant mutants derived from MCY1093 (*MAT* **a** *his4-539 lys2-801 ura3-52*) or MCY1094 (*MATα ade2-101 ura3-52*). NDY1.3, -9.3, and -13.4 may be novel mutants in that these are unable to complement previously identified vanadate-resistant glycosylation mutants, or, in the case of NDY13.4, any early glycosylation mutants. NDY5.3 is allelic to mnn9. NDY1.4 and -17.4 are allelic to m163, an oligosaccharyltransferase mutant isolated from the Hartwell collection of mutants that are temperature sensitive for growth (13). These are derived from A364 (*MAT***a** *ade1 ade2 ura1 lys2 tyr1 his7 gal1-1*). Wild-type strains used in this study include RSY255 (*MATα ura3-52 leu2-2111*) (R. Schekman, University of California, Berkeley) and W303 1a (*MAT***a** *ade2.1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100*), A364 (*MAT***a** *ade1 ade2 ura1 lys2 tyr1 his7 gal1-1*); and SEY6210 (*MATα ura3-52 his3-Δ200 trp1-Δ901 lys2-801 leu2-3,112 suc2-Δ9*) (S. Emr, University of California, San Diego); SS328 (*MATα ade2-101 his3Δ200 ura3-52 lys2-*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: ER, endoplasmic reticulum.

Table 1. Yeasts glycosylation mutants which are hygromycin B sensitive

Mutant	Affected wild-type gene product or function	Affected site of action	Ref(s).
alg1	β-1,4-Mannosyltransferase	ER	9
alg2	Mannosyltransferase	ER	10
alg3	Dol-*P*-Man-Man transferase?	ER	10
alg4/sec53	Phosphomannomutase	Cytosol	10, 23
alg5	Dol-*P*-Glc synthase?	ER	18
dpg1	Dol-*P*-Glc synthase?	ER	19
*ndy17.4** (= m163)	Oligosaccharyltransferase (enzyme subunit or suppressor)	ER	13
*ndy13.4**	Lipid-linked oligosaccharide?	?	
gls1	Glucosidase I	ER	11
wbp1	Oligosaccharyltransferase	ER	12
mnn1; mnn2	Outer chain branching (α-1,2- and α-1,3-mannosyltransferase)	Golgi	8
mnn8†	Outer chain elongation	Golgi	7, 8
mnn9†	Outer chain elongation	Golgi	7, 8
mnn10†	Outer chain elongation	Golgi	7, 8
vrg1†	Outer chain elongation	Golgi	7
vrg4†*	Outer chain elongation	Golgi	7

These yeast mutants failed to grow on YPAD plates supplemented with 50 μg of hygromycin B per ml, a concentration that did not inhibit the growth of the parental, wild-type strains. Dol, dolichyl.

*These mutants will be described in detail elsewhere. *ndy1.4* and -17.4 are allelic to the oligosaccharyltransferase mutant, *m163* (13). *ndy13.4* affects early glycosylation in an, as yet, undetermined mechanism.

†Hygromycin B sensitivity reported previously (7).

801) and MCY1094 (*MATα ade2-101 ura3-52*) (M. Carlson, Columbia University, New York).

All yeast strains were grown in YPAD medium [1% yeast extract, 2% peptone, 2% dextrose, (all wt/vol) and 50 mg of adenine sulfate per liter], supplemented with 0.5 M KCl. Most of the glycosylation mutants required the addition of KCl for optimal growth (7).

With the exception of camptothecin (see below), all drugs were resuspended in water or 50% ethanol to prepare concentrated stock solutions. Hygromycin B (Sigma) was purchased as an aqueous solution supplied at 460,000 units/ml. According to the manufacturer, this represents a concentration of 460 mg/ml, and the stock was diluted as required by using this number as a conversion factor. All drugs were appropriately diluted into cooled, autoclaved medium just before pouring the agar plates.

Assay of Camptothecin and Cycloheximide Sensitivity. To measure drug sensitivity to camptothecin and cycloheximide, approximately 10^7 yeast cells were spread as a lawn on YPAD agar plates. A 5-mm diameter plug of agar from the center of the plate was removed to create a well. To measure camptothecin sensitivity, 100 μl of 20 mM camptothecin (kindly provided by Rolf Sternglanz, State University of New York) dissolved in dimethyl sulfoxide/methanol (1:1) was placed in the well and plates were incubated at 30°C for 2 days, as described (14). To measure cycloheximide sensitivity, 10 μl of a 10 mg/ml solution of cycloheximide dissolved in ethanol was placed in the well, and plates were incubated as described above. In both cases, the zone of inhibition produced around the well is a measure of the degree of cell sensitivity toward the drug.

Inhibition of Glycosylation by Tunicamycin. Overnight cultures of wild-type yeast cells (strain RSY255) were grown to

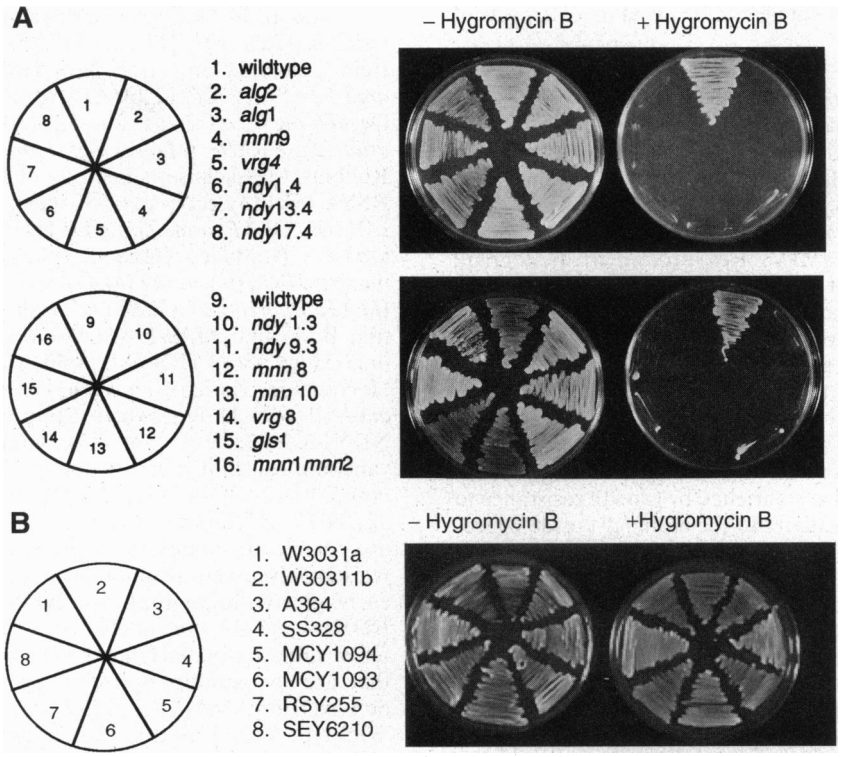

FIG. 1. Both early and late-stage glycosylation mutants are sensitive to hygromycin B. (*A*) Representative yeast mutants containing lesions that affect various steps in glycoprotein processing were grown at 25°C on YPAD (–) or YPAD supplemented with 50 μg of hygromycin B per ml. A diagram of the pattern in which the 16 strains (listed 1–16) are plated is at the left. The wild-type function that is affected in each of the strains is listed in Table 1. *ndy1.4, ndy13.4, ndy17.4, ndy1.3*, and *ndy9.3* are vanadate-resistant/hygromycin B-sensitive strains that have not been described previously. *ndy1.4, ndy13.4, ndy17.4* are early glycosylation mutants, while *ndy1.3* and *ndy9.3* contain mutations that affect outer-chain processing in the Golgi complex. (*B*) Wild-type yeast strains that are parental to those in *A* (sectors 3, 5, 6, and 7) as well as those parental to other hygromycin-sensitive strains listed in Tables 1 and 2 were plated on YPAD or YPAD supplemented with 50 μg of hygromycin B per ml, demonstrating that hygromycin B sensitivity is not due to strain variation.

Table 2. Effect of different antibiotics on growth of wild-type and glycosylation-defective yeast

Antibiotic conc., μg/ml	Growth of yeast strains					
	Wild type	*alg2*	*ndy1.4*	*alg4*	*vrg4*	*mnn9*
Hygromycin B						
10	+	−	−	−	+	+
50	+	−	−	−	−	−
100	+	−	−	−	−	−
200	−	−	−	−	−	−
Gentamicin (G418)						
10	+	−	−	−	+	+
20	+	−	−	−	−	−
50	+	−	−	−	−	−
200	−	−	−	−	−	−
Kanamycin						
100	+	−	−	−	+	+
250	+	−	−	−	−	−
500	+	−	−	−	−	−
1000	+/−	−	−	−	−	−
Neomycin						
20	+	−	−	−	+	+
50	+	−	−	−	+/−	+/−
100	+	−	−	−	−	−
500	−	−	−	−	−	−
Erythromycin						
500	+	+	+	+	+	+
1000	+	+	+	+	+	+
2000	+	+	+	+	+	+
Chloramphenicol						
500	+	+	+	+	+	+
1000	+	+	+	+	+	+
2000	+	+	+	+	+	+

Representative early and late glycosylation mutants and wild-type yeast were plated on YPAD agar supplemented with the indicated amount of antibiotics.

midlogarithmic phase (10^7 cells per ml) in liquid YPAD medium. These cultures were then diluted into equal aliquots, to approximately 5×10^6 cells per ml and growth continued in the absence or presence of a sublethal concentration of tunicamycin (5 μg/ml; Boehringer Mannheim) for 5 h or overnight at 30°C to inhibit glycosylation. Cultures were then diluted and plated onto YPAD plates or YPAD supplemented with 50 μg of hygromycin B per ml, and incubated 16–20 h at 30°C.

RESULTS AND DISCUSSION

A number of glycosylation mutants have been isolated which are reported to be hygromycin sensitive (7). All of these mutants are defective in processes that affect glycosylation reactions that occur in the Golgi complex. This suggested a relationship between abnormal glycosylation and hygromycin B sensitivity. To examine this further, other well-characterized glycosylation mutants were tested for drug sensitivity. The mutants examined are defective in glycosylation steps that range from those catalyzed in or before the ER, to those catalyzed in the Golgi complex (15, 16). With two exceptions, described below, every glycosylation mutant that was tested exhibited hygromycin B sensitivity. Table 1 is a list of glycosylation mutants that are sensitive to hygromycin B, including those five that were previously described (7). Of the individual glycosylation mutants that were tested, only two failed to display sensitivity: *mnn1* and *mnn2*. These mutants are defective in the addition of α-1,3-linked (*mnn1*) (22) and α-1,2-linked (mnn2) (17) mannose side chains, which are components of the carbohydrate outer chain (8). Although neither single mutant displayed growth inhibition (7), the *mnn1 mnn2* double mutant was sensitive, suggesting that a minimal loss of sugar modification is required for sensitivity. Growth of a representative subset of yeast mutants on medium supplemented with or without 50 μg of hygromycin B per ml is displayed in Fig. 1*A*. All of the wild-type parental yeast strains

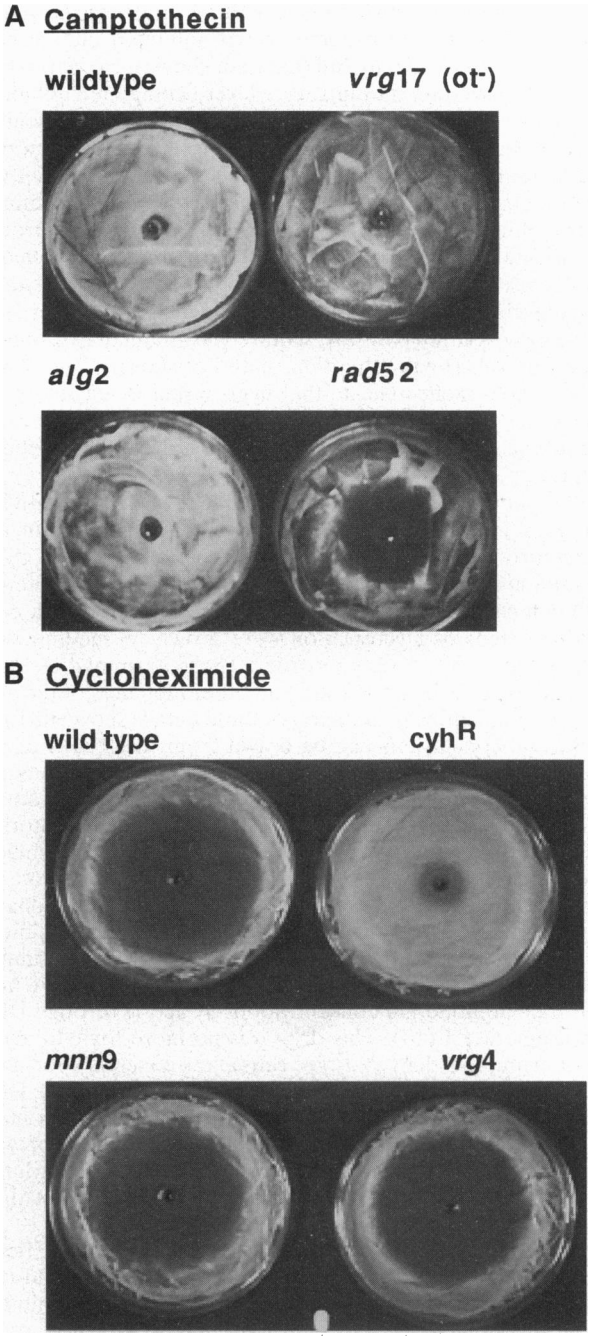

FIG. 2. Yeast glycosylation mutants are not sensitive to nonaminoglycosides. To measure sensitivity to camptothecin or cycloheximide, approximately 10^7 yeast cells were spread as a lawn on YPAD agar plates. A 5-mm plug of agar was removed from the center of the plate to create a well. The zone of inhibition produced around the well is a measure of the degree of sensitivity toward the drug. (*A*) One hundred microliters of a 20 mM solution of camptothecin dissolved in dimethyl sulfoxide/methanol (1:1) was placed in the well, as described (22). Shown are plates spread with wild-type yeast, the early glycosylation mutants *vrg17* (also known as *ndy17.4*) and *alg2*, and the DNA topoismerase mutant *rad52* (22). (*B*) Ten microliters of a 10 mg/ml solution of cycloheximide in ethanol was placed in the well. Shown are plates spread with wild-type yeast, *mnn9, vrg4*, and a cycloheximide-resistant strain, 5762-6.2 (cyh^R). Plates were incubated at 30°C for 2 days. See Table 1 for a description of the various lesions in glycosylation mutants.

grow on medium supplemented with 50 μg of hygromycin B per ml (Fig. 1*A*) and become growth inhibited only at concentrations above 100 μg/ml (data not shown). In contrast, all of the glycosylation mutants tested were completely inhibited on plates containing 50 μg of hygromycin B per ml, demonstrating that hygromycin B sensitivity is a mutant phenotype and is not due to strain variation. The degree of sensitivity at other concentrations varied among the different mutants. Those mutants affected at the earliest steps of glycoprotein modifications, such as the *alg* mutants and the oligosaccharyltransferase mutants, were inhibited by less than 10 μg of hygromycin B per ml. *mnn* mutations, which affect later steps of the glycosylation pathway, required 30–50 μg of hygromycin B per ml for growth inhibition (data not shown). The results of this experiment indicate that hygromycin B sensitivity is a previously unrecognized phenotype common to a wide range of mutants that have different degrees of glycosylation abnormalities.

Why should defective glycosylation lead to drug sensitivity? The yeast cell wall is composed primarily of glucan, chitin, and mannoproteins (20). Therefore, one possibility is that glycosylation mutants may be permeable to many drugs due to cell wall defects. To test this, the mutants defective at both early and late steps of glycosylation were grown on medium supplemented with a variety of drugs. Those examined included nonaminoglycoside antibiotics and other members of the aminoglycoside family. A summary of these data is shown in Table 2. Several conclusions can be drawn from the results of this survey. None of the nonaminoglycosides tested had any differential effect upon the mutants. Nonaminoglycoside antibiotics that have related modes of action, such as inhibitors of protein synthesis (e.g., erythromycin and chloramphenicol), had no effect on either the wild type or mutants at any concentration used. As a test for hyperpermeability to nonaminoglycosides that are known to effect yeast, growth inhibition by camptothecin and cycloheximide was examined. Camptothecin is a cytotoxic alkaloid known to damage DNA in *S. cerevisiae* at moderate concentrations by acting through DNA topoisomerase I (14). This drug was no more toxic to glycosylation mutants than wild-type cells at the concentration used, though it inhibited the growth of a known hypersensitive DNA repair mutant, *rad52* (14) (Fig. 2*A*). Similarly, cycloheximide, an inhibitor of protein synthesis, inhibited the growth of wild-type cells to the same degree as any of the glycosylation mutants tested (Fig. 2*B*). These results suggested that these mutants are not hypersensitive to drugs in general.

Of the drugs tested, only the aminoglycosides specifically inhibit the growth of glycosylation mutants but not wild-type cells (Table 2). The most potent aminoglycoside inhibitors were hygromycin B and gentamicin (G418). Gentamicin blocked growth of early glycosylation mutants at concentrations below 10 μg/ml. Inhibition of wild-type cells requires 200 μg/ml to inhibit growth (Fig. 3). Other aminoglycosides that were effective included neomycin and kanamycin, though these required higher concentrations to fully inhibit the growth of mutants defective at late stages of glycosylation. While the possibility cannot be ruled out that the effect of aminoglycosides may be due to a generalized change in the permeability properties of these cells, these results suggest that glycosylation mutants are specifically sensitive to aminoglycosides.

All of the mutants have glycosylation defects. This does not, however, establish that drug sensitivity is a result of abnormal glycosylation. Whether or not abnormal glycosylation is sufficient to cause drug sensitivity was examined by testing the effect of glycosylation inhibition in wild-type cells. Wild-type cells were grown in suspension in the presence or absence of a sublethal concentration of tunicamycin (5 μg/ml), a specific inhibitor of N-linked glycosylation. Aliquots of the cell suspension were then plated on solid medium, with or without added hygromycin B. Cells that had been exposed to tunicamycin completely failed to grow on plates supplemented with hygromycin B. Tunicamycin, at the concentration used, did not lead to cell death, as these same tunicamycin-treated cells grew well on plates lacking hygromycin B (Fig. 4). This result strikingly demonstrates that inhibition of glycosylation in wild-type cells results in drug sensitivity. Furthermore, it implies that in mutant cells, drug sensitivity is caused by abnormal glycosylation.

Three important observations are described here. First, almost all glycosylation mutants tested, both early-stage and late-stage mutants, are sensitive to the aminoglycoside hygromycin B. The only exceptions, *mnn1* and *mnn2*, proved sensitive when the double mutant was tested. Among the different mutants, the increased sensitivity to this drug is substantial, varying from 4- to 20-fold relative to wild-type yeast. On the basis of these results, a strong prediction is that other yeast mutants with mutations that affect glycosylation will also be hygromycin B sensitive. Second, these same mutants are sensitive to other aminoglycosides but not to other drugs tested. Third, in wild-type cells, inhibition of glycosylation by tunicamycin results in sensitivity to hygromycin B. This last observation demonstrates that abnormal glycosylation, specifically glycosylation of an N-linked glycoprotein, is sufficient to render cells susceptible to the effects of aminoglycosides.

The conclusion from these data is that abnormal glycosylation results in an altered sensitivity or permeability to these clinically important drugs. This may be due to a defect in a cell wall or plasma membrane component(s) that either allows uptake or prevents export of aminoglycosides. Alternatively, an N-linked glycoprotein within the endomembrane system may be involved in the detoxification of aminoglycosides once they have entered the cell. If this were the case, the data presented here suggest that the functional activity or localization of this molecule is exquisitely sensitive to its glycosylation state. Accordingly, we are using aminoglycoside sensitivity as a powerful tool for the isolation of genes defined by novel and

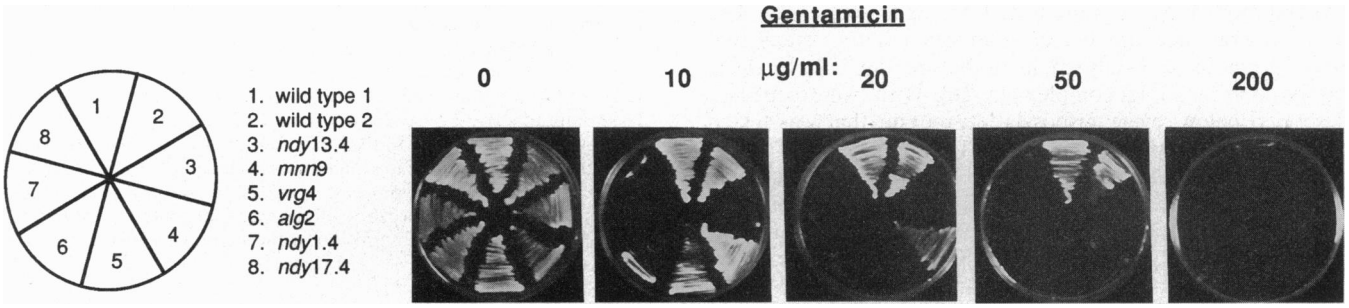

FIG. 3. The growth of glycosylation mutants as a function of gentamicin concentration. Various mutants, depicted schematically at left, were grown on YPAD plates supplemented with 0, 5, 10, 20, 50, and 200 μg of gentamicin per ml. See Table 1 for a description of the various lesions in glycosylation mutants.

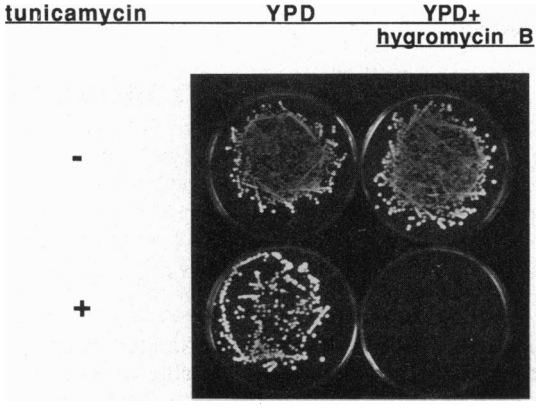

FIG. 4. Inhibition of glycosylation results in hygromycin B sensitivity. Wild-type yeast cells were grown in liquid YPAD medium in the absence (−) or presence (+) of tunicamycin (5 μg/ml) for 5 h at 30°C. Cultures were then diluted and plated onto either YPAD plates or plates with YPAD supplemented with 50 μg of hygromycin B per ml. The plates were incubated 16–20 h at 30°C.

preexisting mutants that affect both early and late steps in glycoprotein processing.

The analyses of inhibition by aminoglycoside antibiotics has led to an understanding of the RNA structures involved in splice-site selection and the decoding of mRNA during translation. As potential therapeutics, aminoglycosides offer great potential as inhibitors of viral replication. A major obstacle in the utility of these polycationic drugs has been in delivering them to the target cells. Increased cellular uptake may be achieved by chemical derivatization of the antibiotic, which can lead to loss of specificity or by the use of a delivery vehicle, such as liposomes. If the relationship between aminoglycoside sensitivity and glycosylation is not specific to yeast, the results described here suggest an alternative strategy. Uptake efficiency may be achieved through a course in which drugs that affect glycosylation are coupled with aminoglycosides. Furthermore, these hypersensitive mutants may afford a means for identifying molecule(s) involved in mediating drug uptake or aminoglycoside sensitivity.

I thank Peter Gergen, Rolf Sternglanz, Deborah Brown, and Bill Lennarz for their enthusiasm, useful ideas, and discussions; Jay Poster for technical support; and Jack Roos, Phil Robbins, Bill Lennarz, and Markus Aebi for yeast strains. This work was supported in part by a grant from the American Heart Association and by an American Cancer Society Junior Faculty Award.

1. Gonzalez, A., Jimenez, A., Vasquez, D., Davies, J. E. & Schindler, D. (1978) *Biochim. Biophys. Acta* **521,** 459–468.
2. Moazed, D. & Noller, H. F. (1987) *Nature (London)* **327,** 389–394.
3. Cundliffe, E. (1987) *Biochimie* **69,** 863–869.
4. von Ahsen, U. & Noller, H. F. (1993) *Science* **260,** 1500–1503.
5. von Ahsen, U., Davies, J. & Schroeder, R. (1991) *Nature (London)* **353,** 368–370.
6. Zapp, M. L., Stern, S. & Green, M. R. (1993) *Cell* **74,** 969–978.
7. Ballou, L., Hitzeman, R. A., Lewis, M. S. & Ballou, C. E. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3209–3212.
8. Ballou, C. E. (1990) *Methods Enzymol.* **185,** 440–470.
9. Huffaker, T. C. & Robbins, P. W. (1982) *J. Biol. Chem.* **257,** 3203–3210.
10. Huffaker, T. C. & Robbins, P. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7466–7470.
11. Esmon, B., Esmon, P. C. & Schekman, R. (1984) *J. Biol. Chem.* **259,** 10322–10327.
12. te Heesen, S., Janetsky, B., Lehle, L. & Aebi, M. (1992) *EMBO J.* **11,** 2071–2075.
13. Roos, J., Sternglanz, R. & Lennarz, W. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1485–1489.
14. Eng, W. K., Faucette, L., Johnson, R. K. & Sternglanz, R. (1988) *Mol. Pharmacol.* **34,** 755–760.
15. Kukuruzinska, M. A., Bergh, M. L. E. & Jackson, B. J. (1987) *Annu. Rev. Biochem.* **56,** 915–944.
16. Herscovics, A. & Orlean, P. (1993) *FASEB J.* **7,** 540–550.
17. Devlin, C. & Ballou, C. E. (1990) *Mol. Microbiol.* **4,** 1993–2001.
18. Runge, K. W., Huffaker, T. C. & Robbins, P. W. (1984) *J. Biol. Chem.* **259,** 412–417.
19. Ballou, L., Gopal, P., Krummel, B., Tammi, M. & Ballou, C. E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3081–3085.
20. Ballou, C. E. (1982) in *The Molecular Biology of the Yeast Saccharomyces cerevisiae: Metabolism and Gene Expression* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 335–360.
21. Gilman, A. G., Rall, T. W., Nies, A. S. & Taylor, P. (1990) *The Pharmacological Basis of Therapeutics* (Macmillan, New York), 8th Ed.
22. Graham, T. R., Verostek, M. F., Mackay, V., Trimble, R. & Emr, S. D. (1992) *Yeast* **8,** S458.
23. Kepes, F. & Schekman, R. (1989) *J. Biol. Chem.* **263,** 9155–9161.