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Cloning and heterologous expression of glycosidase genes from *Saccharomyces cerevisiae*

(α-mannosidase/glucanase/chitinase/*Schizosaccharomyces pombe*)

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ABSTRACT **Genomic clones were isolated that code for three glycosidases proposed to be involved in the catabolism of cell wall components in *Saccharomyces cerevisiae*. α-Mannosidase (*AMS1*), exoglucanase (*BGL1*), and endochitinase (*CTS1*) genes were isolated with the aid of filter assays based on the hydrolysis of 4-methylumbelliferyl glycosides, which permitted the *in situ* monitoring of these glycosidase activities in yeast colonies. Uracil prototrophs resulting from transformation with a multicopy YEp24 yeast genomic library were screened, leading to the identification of transformants possessing high levels of glycosidase activity. Restriction maps of plasmids from multiple isolates were used to localize glycosidase-overproduction genes, which were subcloned into a *Schizosaccharomyces pombe/S. cerevisiae* shuttle vector. Transformation of *Sch. pombe* with *BGL1* and *CTS1* subclones resulted in the appearance of these activities in this organism, and an *AMS1* plasmid caused a 2-fold increase in endogenous α-mannosidase levels. Insertion of the marker gene *LEU2* into putative *AMS1* sequences disrupted plasmid-encoded α-mannosidase overproduction. *S. cerevisiae* strains that incorporated a restriction fragment containing *ams1*::*LEU2* into their chromosomal DNA by homologous recombination expressed no detectable α-mannosidase activity in either the haploid or homozygous recessive diploid states, whereas heterozygous and wild-type cells exhibited levels proportional to *AMS1* gene dosage. No readily apparent phenotype was associated with the α-mannosidase deficiency; however, labeling experiments utilizing [2-^{3}H]mannose suggest that α-mannosidase may function in mannan turnover.**

The cell wall of *Saccharomyces cerevisiae* is composed of a series of widely studied polysaccharides. Glucan, a major component, is a glucose homopolymer consisting of repeating (1→3)-β-D residues. Differing degrees of (1→6)-β-D branching characterize subclasses of glucan, which are empirically defined by their solubility properties (1). In addition to structural glucans, the cell wall contains roughly equal amounts of "mannan," a mannose-containing polymer. Mannans are complex glycoproteins containing short mannosyl chains linked to serine and/or threonine residues, as well as large mannose polysaccharides linked to asparagine residues via a diacetylchitobiosyl unit (2). Chitin, a (1→4)-β-D-linked polymer of *N*-acetylglucosamine, comprises only about 1% of the total cell wall. This minor component has drawn interest due to its localized deposition in the septa of budding cells (3).

The rigid architecture of the wall, which dictates the characteristic shape of the cell, must accommodate changes in morphology that accompany processes such as budding, sporulation, or "shmoo" formation. Structural alterations essential to these processes may be accomplished in part through the regulated catabolism of the cell wall. Consistent with this hypothesis, a number of autolytic hydrolases have been reported to be associated with the yeast cell envelope, including several exo- and endoglucanases (4) and an endochitinase activity (5). Additionally, α-mannosidase activities capable of hydrolyzing mannose oligosaccharides or *p*-nitrophenyl α-D-mannopyranoside have been found in *S. cerevisiae* (6–8). In this paper, we report the cloning of yeast structural genes coding for an α-mannosidase, exoglucanase, and endochitinase. These probes should permit elucidation of the physiologic role these hydrolases play in the dynamics of cell wall metabolism.

MATERIALS AND METHODS

Strains and Plasmids. The following *Saccharomyces cerevisiae* strains and plasmids were obtained from the laboratory of D. Botstein (Massachusetts Institute of Technology): DBY1034 (*MATa ura3-52 lys2-801 his4-539*), DBY1315 (*MATα ura3-52 lys2-801 leu2-3,112*), DBY2068 (*MATa ura3-52 his4-619 leu2-3,112*), the YEp24 genomic library, and vector plasmids YEp24 and YEp13. *Schizosaccharomyces pombe* strain F642 (*ura4-294*) was obtained from J. Boeke (Johns Hopkins University). The 1.1-kilobase *Sch. pombe* autonomously replicating sequence (ARS) element used in construction of YEAp24 was donated by F. Lacroute (Laboratoire de Genetique Physiologique, Strasbourg, France). *Escherichia coli* strain HB101 (9) was used in all bacterial transformations. Conditions for crosses and sporulation used in strain constructions were standard (10).

Media. *S. cerevisiae* strains were grown in YPD medium (1% Bacto-yeast extract/2% Bacto-peptone/2% glucose) or SD medium (0.67% Bacto-yeast nitrogen base/2% glucose) with nutritional supplements appropriate for selections and complementation of strain auxotrophies. *Sch. pombe* was grown in SD or in 2% yeast extract/3% glucose. *E. coli* was grown in Luria–Bertani (LB) medium containing 1% Bacto-Tryptone/0.5% Bacto-yeast extract/0.5% NaCl. LB medium was supplemented when appropriate with ampicillin (100 μg/ml) or tetracycline (5 μg/ml).

DNA Manipulations. *S. cerevisiae* DNA was isolated by the rapid minipreparation protocol described by Sherman *et al.* (10). Plasmid DNA from *E. coli* was isolated by banding in CsCl density gradients or by minipreparation (11). The lithium acetate procedure of Ito *et al.* (12) was used for transformation of both *S. cerevisiae* and *Sch. pombe*. *E. coli* was transformed as described by Maniatis *et al.* (11). Plasmid constructions were performed according to Crouse *et al.* (13).

Colony Screens for Glycosidase Overproduction. Transformants were grown for 3 days on SD plates containing the relevant supplements. Replicas for screening were made by pressing dry sterile filter paper (Schleicher and Schuell no. 497) onto the agar surfaces of the plates. The master plates were allowed to regrow. Each filter was incubated colony-side-up in a Petri dish containing a second filter saturated with a solution appropriate to the detection of a given

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glycosidase. α-Mannosidase was detected by first lysing colonies by incubation in 50 mM Tris/HCl/2 mM $MgCl_2$, pH 7.5, containing 25 mM 2-mercaptoethanol and lyticase (500 units/ml, Sigma) at 30°C for 60 min. Filters were then blotted on Whatman no. 3 paper and transferred to another Petri dish containing filter paper saturated with 0.2 M sodium phosphate/1.0 mM 4-methylumbelliferyl α-D-mannopyranoside (Sigma)/0.1% Triton X-100, pH 7.0. The colonies were incubated at 30°C and viewed with a hand-held long-wavelength UV lamp. Those that displayed increased fluorescence could be easily discerned after 20 min and were identified as putative overproducers. These were recovered from the master plates, streaked onto fresh plates, and rescreened for glycosidase overproduction. Candidates that were positive in a second round of screening were grown in supplemented SD media and assayed for α-mannosidase activity and protein.

Exoglucanase and endochitinase screens were performed in a similar manner but without colony lysis. Filters screened for elevated exoglucanase activity were transferred directly to 10 mM Tris/HCl/1.0 mM 4-methylumbelliferyl β-D-glucoside (Sigma), pH 8.0. These were incubated at room temperature and viewed under UV light for 20 min. Endochitinase filters were incubated initially in 0.1 M sodium citrate/0.5 mM 4-methylumbelliferyl β-D-*N,N',N''*-triacetylchitotrioside (Sigma), pH 3.0, for 30 min at 30°C and then transferred to 0.5 M glycine/NaOH (pH 10.4) to observe fluorescence.

Glycosidase Assays. Assays were initiated by the addition of 10 μl of cell lysate to 40 μl of substrate solution: 0.2 M sodium phosphate/0.1% Triton X-100/1.25 mM 4-methylumbelliferyl α-D-mannopyranoside, pH 7.0, for α-mannosidase; 0.1 M sodium citrate/1.25 mM 4-methylumbelliferyl β-D-glucoside, pH 5.0, for exoglucanase; or 0.1 M sodium citrate/125 μM 4-methylumbelliferyl β-D-*N,N',N''*-triacetylchitotrioside, pH 3.0, for endochitinase. Assays were run in 96-well polystyrene microtiter plates, which made possible qualitative monitoring of reaction progress by viewing with a hand-held UV lamp. Quantification was performed by terminating the reaction by the addition of 100 μl of 0.5 M glycine/NaOH buffer, pH 10.4. The mixture was then diluted to a total volume of 3 ml with the same buffer, and liberated 4-methylumbelliferone was measured with a fluorescence spectrophotometer (excitation at 350 nm, emission at 440 nm). Units of activity are defined as nanomoles of 4-methylumbelliferone released per minute. Assay of endochitinase activity with radiolabeled chitin was performed according to Correa *et al.* (5).

Yeast cell lysates were prepared by mechanical disruption with glass beads. One milliliter of saturated culture was transferred to a 1.5-ml microcentrifuge tube and collected by centrifugation. The pellet was resuspended in 200 μl of buffer (0.2 M sodium phosphate/0.1% Triton X-100, pH 7.0, for α-mannosidase; 0.1 M sodium citrate, pH 5.0, for exoglucanase; 25 mM Mes/0.1% digitonin, 0.1% 2-mercaptoethanol, pH 6.3, for endochitinase) and glass beads (0.45–0.5 mm diameter; Braun, Melsungen, F.R.G.) were added to a level ≈2 mm below the meniscus. The suspension was chilled on ice and then mixed vigorously on a Vortex for 2 min. The broken-cell slurry was recovered with a pipette and centrifuged for 2 min in a microcentrifuge to remove cell debris. The supernatant was collected and used in glycosidase assays.

RESULTS

Isolation of Transformants that Overproduce Glycosidase Activities. The experimental approach used was based on the hypothesis that a glycosidase structural gene carried on a multicopy vector would result in elevated levels of activity in yeast cells harboring the plasmid. Uracil prototrophs resulting from transformation of yeast strain DBY1034 (*ura*$^-$) with a YEp24 yeast genomic library were screened for enhanced levels of glycosidase activity. This process was facilitated by a series of filter screens based on the hydrolysis of 4-methylumbelliferyl glycosides. After transfer of colonies to filter paper, hydrolase activities were monitored qualitatively *in situ* by following the time-dependent appearance of blue fluorescence resulting from the release of 4-methylumbelliferone. Colonies displaying increased fluorescence could be easily discerned over the uniform background of colonies having wild-type levels of activity (Fig. 1). Conditions for each assay were based on previously reported properties for each glycosidase. For instance, detection of α-mannosidase, a component of the vacuolar membrane (14), required lysis of the colonies before a fluorescent signal could be detected. Exoglucanase and endochitinase, both periplasmic enzymes (15, 16), did not require colony lysis. Colonies screened for exoglucanase activity gave an almost instantaneous signal when incubated near the reported pH optimum range of 5.0–6.0 (4), which precluded the discrimination of overproducers. To circumvent this problem, exoglucanase activity was assayed at pH 8.0. In addition to attenuating activity, this modification also enhanced fluorescence, which is optimum in alkaline solutions. Endochitinase activity was assayed with a 4-methylumbelliferyl glycoside of *N,N',N''*-triacetylchitotriose. Cleavage of this compound by yeast cell lysates occurred optimally at pH 3.0, which parallels a previously reported endochitinase activity monitored by the hydrolysis of radiolabeled chitin (5). Screens for this activity were performed by incubation of filters in 4-methylumbelliferyl substrate under acidic conditions followed by transfer to alkaline buffer, which terminated the reaction and allowed viewing of fluorescence.

Seven thousand transformants were screened for each hydrolase. Eight isolates were found that significantly overproduced glycosidases (three α-D-mannosidase, three exoglucanase, and two endochitinase) as assayed with 4-methylumbelliferyl substrates. These exhibited a 4- to 22-fold higher specific activity than cells containing the vector without insert. Elevated chitinase levels were confirmed by an assay using radiolabeled chitin as substrate (data not shown). Plasmids isolated from glycosidase overproducers were used to transform *E. coli*. Plasmids purified from bacteria and reintroduced into yeast in each case conferred the anticipated overproduction phenotype, thus demonstrating that the insert encoded a gene responsible for the increased activity. Individual plasmids were restriction-mapped (Fig. 2). No duplicate plasmids were found. However, each glycosidase group was found to share a common subset of restriction fragments that roughly delineate the boundaries of a gene responsible for the overexpression. The isolated genes were designated *AMS1*, *BGL1*, and *CTS1* for α-mannosidase, exoglucanase, and endochitinase, respectively.

Expression of *AMS1*, *BGL1*, and *CTS1* in *Sch. pombe*. Heterologous-expression experiments were performed by

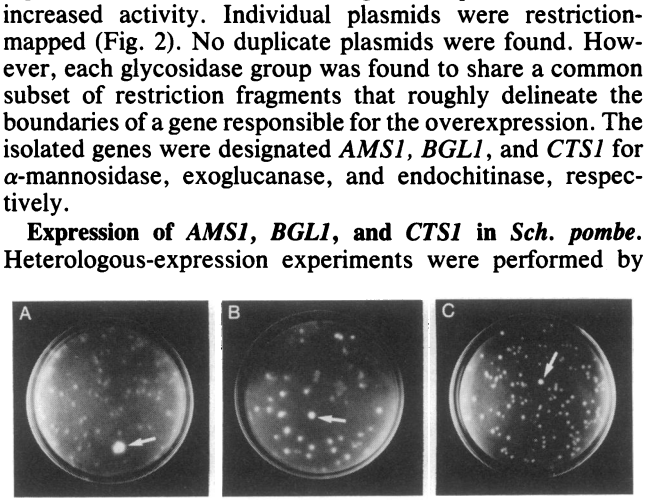

FIG. 1. Colony screen for glycosidase overproduction. Transformants were transferred onto filter paper and incubated with 4-methylumbelliferyl glycosides as described in *Materials and Methods*. Filters were photographed under UV light. Colonies displaying elevated levels of glycosidase activity are indicated by arrows. (*A*) α-Mannosidase. (*B*) Exoglucanase. (*C*) Endochitinase.

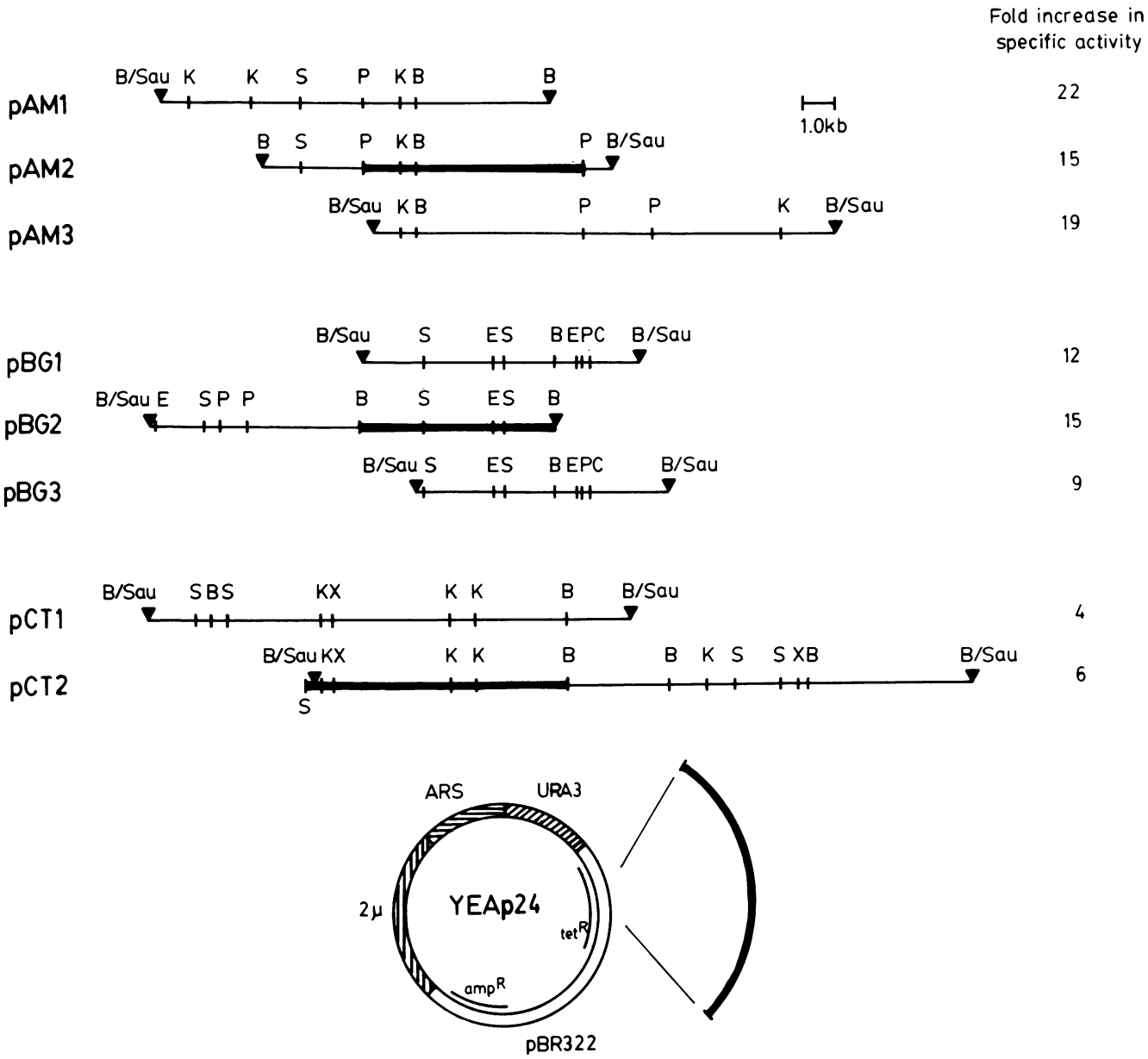

FIG. 2. Restriction maps of yeast genomic inserts giving glycosidase overproduction. Plasmids isolated from yeast glycosidase overproducers were used to transform *E. coli*, reisolated, and restriction-mapped. pAM1-3, pBG1-3, and pCT1-2 were original plasmids giving elevated levels of α-mannosidase, exoglucanase, and endochitinase, respectively. Indicated levels of specific activity (units/mg of protein) were normalized to cells containing a vector without insert. pAM4, pBG4, and pCT3 were produced by subcloning fragments indicated by heavy lines into the shuttle vector YEAp24, which allows plasmid maintenance in both *S. cerevisiae* and *Sch. pombe*. B, *Bam*HI; C, *Cla* I; E, *Eco*RI; K, *Kpn* I; P, *Pst* I; S, *Sal* I; Sau, *Sau*3A1. Endpoints of genomic DNA inserts are indicated (▼). kb, Kilobase.

subcloning restriction fragments into the shuttle vector YEAp24, which allowed plasmid maintenance in *S. cerevisiae*, *Sch. pombe*, and *E. coli*. YEAp24 was constructed by cloning a 1.1-kilobase segment containing *Sch. pombe* ARS sequences into the *S. cerevisiae* vector YEp24 at the *Eco*RI site located between the 2-μm ("2-μ") circle DNA and *URA3* regions. This construction conserved the tetracycline-resistance gene, allowing selection of inserts by acquisition of drug sensitivity. Common restriction fragments from each glycosidase group (Fig. 1) were subcloned into YEAp24. Transformation of *S. cerevisiae* followed by assay (Table 1) showed that the overproduction phenotype was conserved in each construction. The same plasmids were then used to transform a *Sch. pombe* uracil auxotroph. Transformation resulted in an insert-dependent appearance of exoglucanase and endochitinase activities. Transformation of *Sch. pombe* with a plasmid containing *AMS1* showed a >2-fold increase in the specific activity of α-mannosidase compared to the vector alone.

Disruption of *AMS1*. Data obtained from restriction mapping and subcloning experiments were used to localize *AMS1*. A series of restriction-fragment deletions of pAM1 (Fig. 3), together with maps of the other original isolates, pAM2 and pAM3 (Fig. 1), demonstrated that single *Kpn* I and *Bam*HI sites are located within a region necessary for retention of α-mannosidase overexpression. A YEp13 *Bgl* II fragment, containing the selectable marker *LEU2*, was ligated with partially *Bam*HI-digested (linearized) pAM2. Insertion of *LEU2* into the *Bam*HI site on the boundary of the insert resulted in retention of elevated α-mannosidase activity, whereas ligation into the site internal to the putative *AMS1* sequence (pAM9, Fig. 3) yielded wild-type levels of activity after transformation of the leucine auxotroph DBY1315. The marker gene, with flanking glycosidase se-

Table 1. Glycosidase activities in *S. cerevisiae* DBY1034 and *Sch. pombe* F642 after transformation with YEAp24 subclones of plasmids giving glycosidase overproduction

Glycosidase	Strain	Specific activity, (units/mg of protein) × 10^3
α-Mannosidase	DBY1034/pAM4	34,000
	DBY1034/YEAp24	1,500
	F642/pAM4	820
	F642/YEAp24	360
Exoglucanase	DBY1034/pBG4	2,700
	DBY1034/YEAp24	260
	F642/pBG4	1,300
	F642/YEAp24	<1
Endochitinase	DBY1034/pCT3	110
	DBY1034/YEAp24	26
	F642/pCT3	13
	F642/YEAp24	<1

quences, was removed from the plasmid by restriction digestion, purified, and used to disrupt the chromosomal *AMS1* gene according to the method of Rothstein (17). Transformation of a *leu2* homozygous diploid (DBY1315 × DBY2068) with the *LEU2* fragment resulted in transformants that were LEU2$^+$. Five independent diploid transformants were sporulated. Six asci derived from each transformant were dissected and analyzed for α-mannosidase activity by the filter assay. All five diploids yielded some fraction of haploids that were α-mannosidase minus. Three of the five diploids were determined to be heterozygous at the *AMS1* locus by the 2:2 segregation of α-mannosidase activity in the resulting haploids. In addition, these haploids, when scored for nutritional markers, showed cosegregation of the α-mannosidase-negative and LEU2$^+$ phenotypes, indicating successful disruption of the *AMS1* gene. Mannosidase activity was quantitated in disrupted haploids, using the 4-methylumbelliferyl substrate. No activity was detected. Homozygous (*ams1*::*LEU2*/*ams1*::*LEU2*) and heterozygous (*AMS1*/*ams1*::*LEU2*) diploids were constructed from the appropriate haploids. Mannosidase activity in these strains was dependent on *AMS1* gene dosage (Table 2).

There was no readily apparent phenotype associated with

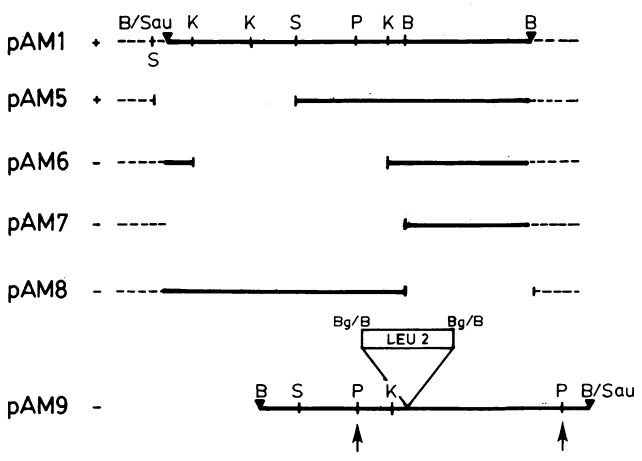

FIG. 3. Localization and disruption of the yeast α-mannosidase gene. Deletions of plasmid pAM1 were made by excising the indicated restriction fragments. Retention (+) or loss (−) of the α-mannosidase overproduction after transformation of *S. cerevisiae* is indicated. Map of pAM9 indicates insertion of a *Bgl* II (Bg) fragment containing the *LEU2* gene into a *Bam*HI site of pAM2 postulated to be located within the α-mannosidase gene. Restriction enzyme abbreviations and symbols are as in Fig. 1. Broken lines represent the vector plasmid YEp24. Arrows indicate the endpoints of the restriction fragment used in the *AMS1* disruption experiment.

Table 2. Levels of α-mannosidase in diploid strains containing *ams1*::*LEU2*

Strain	Genotype	Specific activity, (units/mg of protein) × 10^3
XMK2	*ams1*::*LEU2*/*ams1*::*LEU2*	<1
XMK3	*AMS1*/*ams1*::*LEU2*	1200
XMK4	*AMS1*/*AMS1*	1900

α-mannosidase deficiency. The homozygous mannosidase-negative strain was found to sporulate normally. No significant differences in growth or morphology were observed between the mannosidase-deficient or wild-type strains, demonstrating that *AMS1* is a nonessential gene.

In preliminary experiments *AMS1* or *ams1* haploids were labeled with [2-^{3}H]mannose to assess alterations in mannose metabolism resulting from the missing glycosidase. [2-^{3}H]-Mannose can follow two major metabolic routes in yeast: (*i*) incorporation into mannoproteins or lipids or (*ii*) reaction with mannose-6-phosphate isomerase, which converts mannose 6-phosphate to fructose 6-phosphate, causing the loss of label as tritiated water. The latter process can conveniently be monitored by measuring volatile radioactivity in the growth medium, since the labeled water is free to exchange with the large extracellular pool. Following a 30-min pulse with [2-^{3}H]mannose and a 2-hr wash to remove unincorporated label, an *AMS1* strain was found to secrete 3 times more tritiated water than an *ams1* strain even though both strains had incorporated identical amounts of radioactivity during the labeling period. This effect is presumably due to the inability of the mannosidase-negative cells to catabolize a pool of mannose-containing glycoconjugates that were labeled during the initial incubation with [2-^{3}H]mannose.

DISCUSSION

S. cerevisiae strains containing multiple copies of specific resident chromosomal genes were prepared by transforming cells with a multicopy yeast genomic library. The characteristic overproduction of protein product resulting from high gene dosage was used to isolate genomic DNA coding for α-mannosidase (*AMS1*), exoglucanase (*BGL1*), and endochitinase (*CTS1*) genes. Proof for the cloning of the glycosidase structural genes was provided in part by their heterologous expression in the fission yeast *Sch. pombe*. This organism contains neither exoglucanase (18) nor endochitinase (Table 1) activity as measured under the present assay conditions. Transformation of *Sch. pombe* with plasmids containing DNA segments putatively encoding exoglucanase and endochitinase resulted in the expression of these activities (Table 1), which shows that these plasmids encode the respective structural genes. Similarly, transformation with an α-mannosidase plasmid caused a >2-fold increase in specific activity, presumably due to the coexpression of the *S. cerevisiae* and *Sch. pombe* α-mannosidase genes in the latter organism.

Further evidence demonstrating cloning of the α-mannosidase structural gene is provided by results correlating gene dosage with levels of α-mannosidase activity in *S. cerevisiae*. Haploids carrying multiple copies of the α-mannosidase *AMS1* gene exhibited a 20-fold increase in specific activity. This overproduction phenomenon provided the basis for the isolation and subsequent disruption of the corresponding chromosomal *AMS1* gene. Strains integrating a disrupted *ams1* sequence into their genomic DNA expressed no detectable levels of α-mannosidase activity in either the haploid or homozygous recessive diploid states. Heterozygous diploid constructs (*AMS1*/*ams1*::*LEU2*) exhibited reduced levels of activity compared to wild-type

diploids. These results are consistent with *AMS1* being the structural gene for α-mannosidase.

Although previous work has established some properties of the glycosidases studied here, no rigorous proof of hydrolase function has been presented. Several investigators have reported partial purification of an α-mannosidase active toward *p*-nitrophenyl α-D-mannopyranoside from *S. cerevisiae* (6–8). This activity, likely the *AMS1* gene product, has been reported to be a component of the vacuolar membrane (14) and has been catalogued as a marker enzyme for this organelle. α-Mannosidase levels have been shown to be regulated by catabolite repression and to increase significantly during sporulation (7). Since *ams1*::*LEU2* homozygous diploids were found to sporulate normally in this study, it appears that this hydrolase is not essential for this process. Pastor *et al.* (19) have demonstrated by pulse–chase labeling experiments that at least a portion of the mannoprotein pool in the *S. cerevisiae* cell envelope is subject to degradative turnover. The cloned α-mannosidase may play a role in glycoprotein catabolism, analogous to the corresponding hydrolase in mammalian lysosomes, by catalyzing the sequential removal of mannose residues from the nonreducing end of mannan chains.

Unlike *AMS1*, the secretory *BGL1* and *CTS1* gene products reside close to their polymeric substrates, suggesting a role in cell wall construction or modification. As many as six different glucanase activities capable of hydrolyzing the cell wall polysaccharide, (1→3)-β-D-glucan, have been reported in *S. cerevisiae* (4). Two classes of these glycosidases have been described based on their mode of substrate hydrolysis. The first, endo-(1→3)-β-glucanases, releases oligosaccharides as products resulting from hydrolysis of internal chain linkages, while the second, exo-(1→3)-β-glucanases, acts at the nonreducing end of the polymer, releasing glucose. The latter group can be assayed conveniently with *p*-nitrophenyl or 4-methylumbelliferyl glucosides, which provided the basis for the cloning of the exoglucanase activity reported here. The various endo- and exoglucanases have been shown to be modulated during phases of vegetative growth, conjugation, and sporulation (20–22), suggesting an important role in these processes. However, it appears that not all of these activities are essential. Santos *et al.* (23) have reported a mutant (*exb1-1*) deficient in secreted exoglucanase activity yet not affected in growth or structure of the cell wall.

The *CTS1* gene encodes an endochitinase. This activity, which is readily extracted from intact cells with a mixture of 2-mercaptoethanol and detergent, solubilizes chitin, resulting in the liberation of different-size oligosaccharides (5). Elevated levels of this chitinase were observed to occur during logarithmic growth (16), a time when localized chitin synthesis is important to the formation of septa marking the points of separation between mother and daughter cells during budding (3). It is not clear what role the chitinase may play in septum formation, regulation of chitin synthesis, or cell fission. Utilization of the cloned glycosidase genes *BGL1* and *CTS1* in disruption experiments similar to those described above for the *AMS1* gene should result in hydrolase-deficient strains that will be useful in determining the function of these glycosidases in cell wall metabolism.

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1. Fleet, G. H. & Phaff, G. H. (1981) in *Encyclopedia of Plant Physiology*, eds. Tanner, W. & Loewus, F. A. (Springer, Heidelberg), Vol. 13B, pp. 416–440.
2. Ballou, C. E. (1982) in *The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 335–360.
3. Cabib, E. & Bowers, B. (1971) *J. Biol. Chem.* **246,** 152–159.
4. Hien, N. H. & Fleet, G. H. (1983) *J. Bacteriol.* **156,** 1204–1213.
5. Correa, J. V., Elango, N., Polacheck, J. & Cabib, E. (1982) *J. Biol. Chem.* **257,** 1392–1397.
6. Kaya, T., Aikawa, M. & Matsumoto, T. (1977) *J. Biochem.* **82,** 1443–1449.
7. Opheim, D. J. (1978) *Biochim. Biophys. Acta* **524,** 121–130.
8. Jelinek-Kelly, S., Akiyama, T., Saunier, B., Tkacz, J. S. & Herscovics, A. (1985) *J. Biol. Chem.* **260,** 2253–2257.
9. Bolivar, F. & Backman, K. (1979) *Methods Enzymol.* **68,** 245–267.
10. Sherman, F., Fink, G. R. & Lawrence, C. W. (1982) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
11. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
12. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
13. Crouse, G. F., Frischauf, A. & Lehrach, H. (1983) *Methods Enzymol.* **101,** 78–89.
14. Van der Wilden, W., Matile, P., Schellenberg, M., Meyer, J. & Wiemkem, A. (1973) *Z. Naturforsch.* **28C,** 416–421.
15. Sanchez, A., Villanueva, J. R. & Villa, T. G. (1982) *FEBS Lett.* **138,** 209–212.
16. Elango, N., Correa, J. U. & Cabib, E. (1982) *J. Biol. Chem.* **257,** 1398–1400.
17. Rothstein, R. J. (1983) *Methods Enzymol.* **101,** 202–211.
18. Reichelt, B. Y. & Fleet, G. H. (1981) *J. Bacteriol.* **147,** 1085–1094.
19. Pastor, F. I. J., Herrero, E. & Sentandreu, R. (1982) *Arch. Microbiol.* **132,** 144–148.
20. Del Rey, F., Villa, T. G., Santos, T., Garcia-Acha, I. & Nombela, C. (1982) *Biochem. Biophys. Res. Commun.* **105,** 1347–1353.
21. Hein, N. H. & Fleet, G. H. (1983) *J. Bacteriol.* **156,** 1214–1220.
22. Olivero, I., Hernandez, L. M. & Larriba, G. (1985) *Arch. Microbiol.* **143,** 143–146.
23. Santos, T., Del Rey, F., Villanueva, J. R. & Nombela, C. (1982) *FEMS Microbiol. Lett.* **13,** 259–263.