Two Sporulation-specific Chitin Deacetylase-encoding Genes Are Required for the Ascospore Wall Rigidity of Saccharomyces cerevisiae*

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The formation of the ascospore wall of Saccharomyces cerevisiae requires the coordinate activity of enzymes involved in the biosynthesis of its components such as chitosan, the deacetylated form of chitin. We have cloned the CDA1 and CDA2 genes which together account for the total chitin deacetylase activity of the organism. We have shown that expression of these genes is restricted to a distinct time period during sporulation. The two genes are functionally redundant, each contributing equally to the total chitin deacetylase activity. Diploids disrupted for both genes sporulate as efficiently as wild type cells, and the resulting mutant spores are viable under standard laboratory conditions. However, they fail to emit the natural fluorescence of yeast spores imparted by the dityrosine residues of the ascospore wall. Consistent information on the composition and formation of the more inner layers is produced by the deacetylation of nascent chains of chitin, a β-(1→4)-N-acetyl-d-glucosamine homopolymer produced by the action of chitin synthases (12–14). The deacetylation reaction is catalyzed by the enzyme chitin deacetylase (CDA)1 (15, 16).

Chitin deacetylases are involved either in the formation of the cell wall (12, 14, 17) or in the deacetylation of chitin oligosaccharides following the action of an endochitinase on cell walls during autolysis (18). The involvement of this enzyme in plant-pathogen interactions has also been proposed (19, 20).

Isolation of the first CDA-encoding gene from the fungus Mucor rouxii revealed a remarkable sequence similarity to the rhizobial nodB proteins, suggesting functional homology of these evolutionary distant proteins (21). Subsequently, it was verified biochemically that nodB proteins are chitooligosaccharide deacetylases (22). In this report we describe the identification and molecular characterization of two sporulation-specific genes, CDA1 and CDA2, whose products account for the total chitin deacetylase activity in S. cerevisiae. Mutational analysis of the two genes revealed that CDA contributes to spore wall resistance.

EXPERIMENTAL PROCEDURES

Strains and Growth Media—The strain used for temporal analysis of CDA expression was the DK896 diploid (MATα/MATα, ura3/ura3, leu2/leu2, his4/1is4) kindly provided by Dr. D. Bishop. The strains used for the construction of the disruption alleles of CDA1 and CDA2 were FY104 (MATα, ura3, trp1, his3), the S288C derivative GT44 (MATα, gcn4, ura3, trp1) and their diploid derivative GT44D (MATα/MATα, gcn4/GCN4, HIS3/his3, ura3/ura3, trp1/trp1). Complete, minimal, and sporulation media were as described previously (23). For synchronous sporulation cells were grown in sporulation medium to a density of 3 × 10⁶ cells/ml, washed, and resuspended in SPM (0.5% potassium acetate, 0.02% glucose, and appropriate supplements) at the same density. The efficiency of sporulation of DK896 strain was >95% after 36 h and 15% for all other strains, after 3 days under the same conditions. Ascus formation was monitored by light microscopy using phase-contrast optics.

Genes and Plasmids—The CDA1 and CDA2 genes were isolated from a YCP50 yeast genomic library (24) using a synthetic DNA oligonucleotide as a probe. Two overlapping clones were chosen, one of which was used for the isolation of a 1.2-kb XbaI fragment of CDA1 and the other for the isolation of a 1.3-kb EcoRI fragment of CDA2. These were used for disruption experiments. For the expression experiment, two DNA fragments carrying the entire coding region of CDA1 and CDA2 were generated by a polymerase chain reaction using specific primers and

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1 The abbreviations used are: CDA, chitin deacetylase; bp, base pair(s); kb, kilobase pair(s).
were cloned into the pYES2 (Invitrogen) expression vector carrying the GAL1 promoter region.

Gene Disruptions in the Yeast Genome—(i) For CDA1 disruption, a NcoI-Hpel 572-bp fragment (−441 to +131) was replaced by a 1.1-kb HindIII URA3 DNA fragment. The resulting 1.8-kb XbaI fragment was excised and used to transform ura3 strains of both mating types. (ii) For CDA2 disruption, a 346-bp StyI-NdeI fragment (+288 to +634) was replaced by the 1.1-kb HindIII URA3 DNA fragment. A 2140-bp EcoRI fragment was excised and used to transform ura3 strains of both mating types. (iii) For CDA1 and CDA2 double disruption, the Smal-XbaI 550-bp fragment of the CDA1 disruption plasmid (the Smal site was created after the insertion of URA3 gene at its end) was replaced by a Ndel-EcoRI 300-bp fragment of the CDA2 region. The resulting construct had the URA3 gene inserted between the 5′ of the CDA1 and the 3′ of the CDA2 regions. The resulting 1950-bp XbaI-EcoRI was excised and used to transform ura3 strains of both mating type.

DNA Blot Hybridization Analysis—Yeast DNA was prepared as described previously (23), digested with EcoRI or XhoI, electrophoresed on 1% agarose gel, and transferred to GenScreen membranes (Dupont NEN). Membranes were hybridized with two different α-32P-labeled probes, either a 1.2-kb XbaI CDA1 fragment or a 1.3-kb EcoRI CDA2 fragment. No cross-hybridization of the two genes was observed using these probes.

Yeast Chitin Deacetylases

FIG. 1. Multiple sequence alignment of CDA1, CDA2, chitin deacetylase from M. rouxii (MrCDA), nodB protein from Rhizobium leguminosarum bv. visiae (Rl/NodB), and the deduced amino acid sequence of the presumed deacetylase from B. stearothermophilus (BstPDA). The black regions indicate residues identical between all five compared sequences and the gray areas those shared only by the three eucaryotic sequences. Amino acid numbers for each protein sequence are given on the right.

RNA Blot Hybridization Analysis—Cells (at a concentration of 5 × 10^7/ml) were collected by centrifugation at 2-h intervals following transfer to sporulation medium and total RNA was isolated by the glass bead/phenol procedure (23). For Northern hybridization analysis RNA was electrophoresed through a 1.5% agarose gel containing 15% formaldehyde and transferred to GenScreen membrane (23). The same membrane filter was sequentially hybridized with [32P]-labeled 1.2-kb XhoI CDA1 DNA fragment and with [32P]-labeled 1.3-kb EcoRI CDA2 DNA fragment.

Cell Extract Isolation—Cell extracts were isolated, at the same time intervals as for the RNA analysis, disrupted with glass beads in a 50 mM Tris-HCl (pH 8.0) buffer at 55°C for 30 min. One unit of enzymatic activity releases 1.0 μmol of [3H]acetic acid from glycol chitin per min.

DNA Blot Hybridization Analysis—Yeast DNA was treated with Glusulase (Sigma) with a mixture of 200 μl of H_2O, 70 μl of undiluted Glusulase, and 15 μl of β-mercaptoethanol. After 5 h at 30°C, filters were transferred to 30% NH_4OH, and their fluorescence was visualized after exposure to UV light (315 nm) (10).

FIG. 2. Sporulation specific transcription of CDA1 and CDA2 genes as determined by Northern blot analysis of total RNA samples isolated from vegetatively grown cells (VEG) and at different times after transfer to sporulation medium (SPO). Ethidium bromide stain was used to normalize RNA levels. The 25 S large ribosomal RNA is shown as control.

FIG. 3. Sporulation specific synthesis of CDA1 and CDA2 enzymes determined by CDA activity assays before and at various hours following transfer to sporulation medium. CDA activity values are given (dark bars). In parallel, the percentage of sporulation (light bars) was also monitored by light microscopy.

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Cell Extract Isolation—Cell extracts were isolated, at the same time intervals as for the RNA analysis, disrupted with glass beads in a 50 mM Tris-HCl (pH 8.0) buffer, and centrifuged at 10,000 rpm to remove cell debris.

Chitin Deacetylase Assays—For the determination of chitin deacetylase activity we have employed a radiometric assay using as substrate partially O-hydroxyethylated chitin (glycol chitin), radiolabeled in N-acetyl glycine. The substrate was prepared according to Araki and Ito (25). Enzyme assays were performed as described previously (16) in 50 mM Tris-HCl (pH 8.0) buffer at 55°C for 30 min. One unit of enzymatic activity releases 1.0 μmol of [3H]acetic acid from glycol chitin per min.

Dityrosine Fluorescence of Sporulated Colonies—Wild type and mutant strains were streaked in YPD plates, grown at 30°C, and replated onto nitrocellulose filters. Filters were grown in YPD plates for 1 day and then transferred to sporulation plates for 3 days. Filters were treated with Glusulase (Sigma) with a mixture of 200 μl of H_2O, 70 μl of undiluted Glusulase, and 15 μl of β-mercaptoethanol. After 5 h at 30°C, filters were transferred to 30% NH_4OH, and their fluorescence was visualized after exposure to UV light (315 nm) (10).
Glusulase Treatment, Ether Test, and Heat Shock Resistance—Sporulated cells from the above filters were resuspended in undiluted Glusulase at 30 °C in a hemocytometer chamber, and their lysis was observed by light microscopic examination. Resistance of spores to diethylether was assessed as described in Law and Segall (26), and the viability of cells after exposure to 55 °C was determined as described by Briza et al. (9).

DNA and Protein Sequence Analysis—The M. rouxii CDA sequence was compared against the GenBank™/EMBL data base using FastA (27) of the Genetics Computer Group package software (28). Multiple alignment of similar protein sequences was performed using the PILEUP algorithm.

RESULTS

Identification of Two Yeast Genes Encoding Proteins Similar to Chitin Deacetylases—Comparison of the deduced amino acid sequence of M. rouxii chitin deacetylase (21) with the EMBL data base revealed the existence of two similar and closely linked open reading frames (EMBL accession no. for the region, U17247) on chromosome XII of S. cerevisiae. The homology exhibited with M. rouxii CDA was 24.5% over 284 amino acids for the first and 27.4% over 274 amino acids for the second open reading frame, CDA1 and CDA2, respectively. The predicted proteins (34.6 and 35.6 kDa, respectively) have an identity of 57% and a similarity of 72% throughout their entire amino acid sequence, with a gap of 10 amino acids starting at position 39 of CDA1. The two open reading frames are separated by 1411 nucleotides, and they are transcribed in the same orientation. No other genes were predicted in the intergenic region or in the 800-bp region upstream of CDA1.

Multiple sequence alignments between a central region of M. rouxii CDA protein (extending from amino acids 121 to 325), Bacillus stearothermophilus putative deacetylase and the NodB protein family, which includes chitooligosaccharide (Nod factor precursors) deacetylases (22), revealed conservation at specific residues (21). We performed a new alignment with the inclusion of the newly identified ScCDA1 and ScCDA2 genes, which confirmed the conservation for most of these residues, designating more accurately the invariant ones (Fig. 1). The above sequence comparison studies prompted us to investigate the function of CDA1 and CDA2 genes. Toward this goal the two genes were isolated from a yeast genomic plasmid library using an oligonucleotide probe, designed according to the published sequence (for the restriction map, see Fig. 5A).

Sporulation Specific Expression of CDA1 and CDA2—We

Fig. 4. Expression of CDA1 and CDA2 genes under the control of a GAL1 promoter during growth in glucose containing medium (glu) and after transfer in galactose containing medium (gal). A, CDA1 and CDA2 activity assays. CDA activity of sporulating cells (15 h in sporulation medium) is given as positive control. B, transcription of CDA1 and CDA2 genes measured by Northern blot hybridization analysis. ACT1 was used as control for vegetatively grown cells.

Fig. 5. A, restriction map for selected enzymes of the DNA region containing the CDA1 and CDA2 genes. Abbreviations of the different restriction enzyme names are as follows: X, XbaI; H, HpaI; Nc, NcoI; E, EcoRI; S, StyI; Nd, NdeI. The coding regions of CDA1 and CDA2 are boxed. For Southern blot analysis, CDA1 and CDA2 genes were detected using probe 1 and 2, respectively, from regions indicated by two-pointed arrows. Horizontal bars denote regions of the genome replaced by a 1.1-kb HindIII fragment containing the URA3 gene in order to construct the mutant strains Δcda1, Δcda2, and Δcda1Δcda2. B, Southern blot analysis of CDA1 and CDA2 gene disrupted strains. Samples of genomic DNA isolated from diploid strains containing a disruption and deletion of CDA1 (Δcda1), CDA2 (Δcda2), or both (Δcda1Δcda2) as well as the parental wild-type strain were analyzed as described under “Experimental Procedures.”
were not able to detect any CDA transcripts in vegetatively grown cells. Since expression of many genes encoding sporulation specific functions is developmentally regulated, we examined the transcription of the putative CDA genes during the process of sporulation, when the chitosan layer of spore walls is formed (6). Total RNA from MATa/MATα diploid cells was isolated from vegetatively grown cells and at various time intervals following transfer to sporulation medium. RNA blot hybridization analysis using either probe 1 or 2 (Fig. 5A) revealed that transcripts for both genes began to accumulate 9 h after transfer to sporulation medium (Fig. 2). At that time mature ascii had not yet appeared (Fig. 3). Maximal accumulation of CDA transcripts occurred at 15 h, and the transcripts almost disappeared after 19 h. The two genes exhibited exactly the same transcriptional pattern and accumulated to comparable levels, a conclusion based on the use of probes of similar specific activity. The appearance of these transcripts was diploid specific since they were not detected in either vegetative or starved haploid cells (not shown).

CDA Activity in Sporulating Cells—The samples used for the RNA analysis were also assayed for CDA activity (Fig. 3). No activity was detected during vegetative growth. However, during the process of sporulation CDA activity accumulated in parallel with the transcriptional pattern, but with a 2-h delay. Thus, the progressive increase of CDA activity was initiated at 9 h and reached maximal levels at 17 h. Approximately half of this activity persisted at 27 h, when sporulation was completed and one third of maximal activity remained even after 36 h.

Expression of CDA1 and CDA2 Genes in Vegetatively Grown Cells—In order to examine the effects of unregulated expression of each CDA gene, the coding regions of the CDA1 and CDA2 genes were placed downstream of a GAL1 promoter region. These constructs were introduced into haploid cells which were grown first in glucose and then transferred to galactose minimal medium, to induce CDA gene expression. RNA blot hybridization analysis showed that transcripts of the two genes were accumulated in cells grown on galactose (Fig. 4B). Moreover, CDA activity, at levels comparable to that found in sporulating cells, was detected in the transformed cells, indicating that indeed these two genes encode for chitin deacetylases (Fig. 4A). Furthermore, it was concluded that an unregulated expression at these levels had no effect on haploid cell viability.

CDA1 and CDA2 Account for the Total CDA Activity of S. cerevisiae—In order to analyze the contribution of the two genes in the total CDA activity of S. cerevisiae, we constructed the disrupted mutant strains Δcda1, Δcda2, and Δcda1Δcda2 as shown in Fig. 5A. The disruptions were confirmed by DNA blot hybridization analysis (Fig. 5B). Normal growth of the disrupted strains in minimal medium indicated that the two genes were not essential for cell viability.

CDA assays were performed in wild type, Δcda1, Δcda2, and Δcda1Δcda2 diploid strains during vegetative growth and at various stages of sporulation. Fig. 6 shows CDA activity detected just prior the formation of ascii (0% sporulation) and after it reached a maximum when 30% of the cells were sporulated. The double disrupted Δcda1Δcda2 strain exhibited no CDA activity at any time during spore formation. Moreover, Δcda1 and Δcda2 exhibited approximately half of the activity measured in the wild type strain at the same time point. These results demonstrated that the proteins produced by these two genes account for the total S. cerevisiae CDA activity and that the two genes contribute essentially equally to the total CDA activity of the cell.

Double Disrupted Strains Produced Sensitive Spores—In the Δcda1Δcda2 strain both the efficiency of spore formation and spor viability, as judged by light microscopy and growth assays, were not affected. Since mutated spores lacking each of the two outermost layers are sensitive to lytic enzymes and stress conditions (9, 29, 30), we examined the sensitivity of the double and single disrupted strains to such treatments. The sensitivity to Glusulase was measured by monitoring either the natural fluorescence of yeast spores imparted by the dityrosine
containing macromolecule of the outermost layer (10), or by observing spore lysis. CDA mutants in parallel with wild type strains were allowed to sporulate on filters which were then transferred to undiluted Glusulase. After prolonged treatment with the enzyme (see “Experimental Procedures”) double mutants of both types and the wild type diploid strain exhibited normal fluorescence levels (Fig. 7). Moreover, double mutant spores were lysed by the action of this enzyme whereas single mutant and wild type spores were resistant, as revealed by observation of Glusulase exposed mutant spores under a phase contrast microscope (not shown). The fact that one of the two genes was sufficient to restore normal resistance of the spore wall suggested that the two genes are functionally redundant.

Thesensitivityofmutantsporesoutelevatedtemperatureand ether was also tested. We examined the spore thermostolerance of the wild type and the double disrupted strain after exposure to 55 °C for various periods of time (Fig. 8B). Mutant spore viability was two orders of magnitude lower following a 40-min exposure to elevated temperature, as compared to the wild type spores. We also examined the sensitivity of the above spores on ether exposure. As shown in Fig. 8A double disrupted spores were significantly more sensitive than wild type spores. However, in both tests, mutant strains were more resistant than stationary phase cells. These results indicated that chitin deacetylases contributed to the formation of an ascospore wall, resistant to stress conditions.

DISCUSSION

Chitin deacetylase is one of the components of the binary enzyme system leading to chitosan formation in the cell walls of the Zygomycete M. rouxii. The other component is chitin synthase, the enzyme that catalyzes the synthesis of nascent chitin chains upon which CDA acts, removing the N-linked acetyl groups (12). It has been shown that the second of the four layers forming the ascospore wall in S. cerevisiae consists of chitosan (6), and it is reasonable to assume that a similar mode to that of M. rouxii operates for its biosynthesis, i.e. the coordinated involvement of chitin synthase and chitin deacetylase (6, 31). Yeast chitin synthase has been extensively studied (32). Three different enzymes have been detected, each one being nonessential, but lack of all three leads to cell lethality (32). Although none of these genes is strictly sporulation specific, chitin synthase III is the possible candidate for chitin synthesis in spores since the levels of CSD2 message, the gene presumed to encode for its catalytic subunit (33, 34), increase during sporulation (35) and no chitosan is detected in mutants lacking this gene (30). On the other hand, no study concerning chitin deacetylase has ever been reported.

We have cloned and characterized the CDA1 and CDA2 genes encoding two proteins with significant similarity to the M. rouxii CDA protein. These genes indeed encode for chitin deacetylase as shown by (a) the lack of CDA activity in Δcda1 and Δcda2 double disrupted strains and (b) the presence of such an activity upon expression of the cloned genes during vegetative growth. In addition, the fact that the total CDA activity from sporulating cells is abolished when both genes are deleted strongly suggests that no other gene in yeast encodes for chitin deacetylase. Deletion of each gene separately reduces the levels of CDA activity to approximately half indicating that the activities of these enzymes are additive.

The assumption that yeast chitin deacetylase activity has a unique role in sporulation, came from studies on the expression of the CDA1 and CDA2 genes. Both are expressed specifically during sporulation, in accordance with the existence of chitosan only in this developmental stage of the S. cerevisiae life cycle (6). Substantial accumulation of CDA1 and CDA2 mRNA is observed 9 h following transfer to sporulation medium, just prior to the formation of asci. Thus, these two genes probably belong to the mid-late class of sporulation specific genes (10, 36). Two other genes, DIT1 and DIT2, that are involved in the formation of the ascospore wall are also expressed with similar developmental specificity as the CDA genes (10). Although we have not detected any potential common promoter elements among these four genes, we expect that the key regulators of
sporulation specific genes, including DIT1 and DIT2, IME1 and IME2, should play an important role in the expression of the CDA genes (36, 37). Within 2 h after the appearance of IME2, should play an important role in the expression of the spore wall. On the contrary, a strain in which both Glusulase sensitivity, heat shock and ether, indicating that D most layer of the ascospore wall (8). Wild type cells and single the bulk of dityrosine residues which are located in the outer-
zymes, ether, and heat shock (8, 10). The fluorescence is due to the case for the M. rouxii enzyme (14).

In order to investigate the importance of the chitin deacetylation process to cell wall assembly, we took advantage of the natural fluorescence that wild type spores emit when excited by ultraviolet light, as well as their resistance to hydrolytic enzymes, ether, and heat shock (8, 10). The fluorescence is due to the bulk of dityrosine residues which are located in the outer-
most layer of the ascospore wall (8). Wild type cells and single Δcda disruptants exhibit no differences in fluorescence or in Glusulase sensitivity, heat shock and ether, indicating that even one CDA gene is sufficient for the proper formation of spore wall. On the contrary, a strain in which both CDA genes are deleted, is sensitive to the above treatments and its natural fluorescence is lost after enzymatic hydrolysis.

The unequivocal existence of a chitin deacetylase in S. cerevisiae and its importance to cell wall resistance against glu-
colytic enzymes and stress conditions, reinforces the hypothesis that a mechanism of chitosan biosynthesis similar to that of M. rouxii operates and that chitosan is produced by deacetylation of chitin (6, 31). Similar phenotypes to those of the Δcda null mutants are also exhibited by mutations that affect chitin synthesis, such as glucosamine auxotrophies (29), the forma-
tion of the chitosan layer, such as dit101 (30) and the formation of dityrosine bridges, such as dit1 and dit2 (9, 10). The lack of chitin deacetylation in a ΔcdaΔcda2 strain presumably leaves the second ascospore wall layer untransformed, consisting of pure chitin instead of chitosan. Briza et al. (6) proposed that the chitosan layer is somehow associated with the dityrosine-contain-
ging macromolecules of the outermost layer and appears to be prerequisite for dityrosine incorporation. Possibly, these interactions are disrupted when the positively charged amino groups are acetylated, leading to a loosely packed structure susceptible to hydrolytic enzymes and stress conditions.

Recent information about chitin deacetylases from different species revealed that although these enzymes have the same catalytic activity and stringent specificity for chitinous sub-
strates, they are involved in many different biological processes depending on the individual organism. Thus in fungi M. rouxii and Absidia coerulea, CDAs are constitutively expressed en-
zymes (16, 17), localized in the periplasmic space (14) in order to perform their task in cell wall construction, whereas in the pathogen Colletotrichum lindemuthianum and in Asper-
gillus nidulans CDAs are secreted enzymes postulated to act on chitin oligomers released by fungal cell walls, in order to pro-
mote plant invasion (20) or cell wall degradation (18). In pro-
caryotes, the NodB family of genes encodes CDA which partic-
ipates in the biosynthesis of the Nod factors that promote plant nodulation (21, 22), while putative CDA homologs are found in nonendosymbiotic bacteria (21). Given this diversity in the biological functions of CDA, the availability of the S. cerevisiae Δcda mutant phenotype as well as the easily measurable CDA activity offer important tools not only toward understanding of cell wall formation but also toward the elucidation of the structure-function relationships of chitin deacetylases.

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REFERENCES
1. Cid, J. V., Duran, A., del Rey, F., Snyder, P. M., Nombela, C., and Sanchez, M. (1995) Microbial. Rev. 59, 345–384
2. Klis, M. F. (1994) Yeast 10, 851–869
3. Stratford, M. (1994) Yeast 10, 1741–1752
4. Miller, J. J. (1989) in The Yeasts (Rose, A. H., and Harrison J. S., eds), pp. 3489–3550, Academic Press, New York
5. Kregger-van Rij, N. J. W. (1978) Arch. Microbiol. 117, 73–77
6. Briza, P., Ellinger, A., Winkler, G., and Breitenbach, M. (1988) J. Biol. Chem. 263, 11569–11574
7. Kathoda, S., Konno, K., Sasaki, Y., and Sakamoto, S. (1984) Agr. Biol. Chem. 48, 855–901
8. Briza, P., Winkler, G., Kalchhauser, H., and Breitenbach, M. (1986) J. Biol. Chem. 261, 4288–4294
9. Briza, P., Winkler, G., Ellinger, A., and Breitenbach, M. (1990) J. Biol. Chem. 265, 15118–15125
10. Briza, P., Breitenbach, M., Ellinger, A., and Segall, J. (1990) Genes Dev. 4, 1775–1789
11. Bartnicki-Garcia, S., and Nickerson, W. J. (1962) Biochem. Biophys. Acta 58, 102–119
12. Davis, L. L., and Bartnicki Garcia, S. (1983) Biochemistry 22, 1065–1073
13. Davis, L. L., and Bartnicki Garcia, S. (1984) J. Gen. Microbiol. 130, 2095–2102
14. Bartnicki-Garcia, S. (1989) in Chitin and Chitosan: Sources, Chemistry, Bio-
chemistry, Physical Properties and Applications (Skjar: Brack, G., Anhonsen, T., and Sandford, P., eds) pp. 23–35, Elsevier, Essex
15. Araki, Y., and Ito, E. (1975) Eur. J. Biochem. 55, 71–78
16. Kafetzopoulos, D., Martinou, A., and Bouriotis, V. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2564–2568
17. Gan, X., Katsumoto, T., and Onodera, K. (1995) J. Biochem. (Tokyo) 117, 257–263
18. Alfonso, C., Nuero, M. O., Santamaria, F., and Reyes, F. (1995) Curr. Micro-
bio. 30, 49–54
19. Kauss, H., Jeblick, W., and Young, H. D. (1983) Plant Sci. Lett. 28, 231–236
20. Tsanos, I., and Bouriotis, V. (1995) J. Biol. Chem. 270, 26286–26291
21. Kafetzopoulos, D., Thireos, G., Vournakis, N. J., and Bouriotis, V. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 8005–8008
22. John, M., Rohring, H., Schmidt, J., Wieneke, U., and Shell, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2564–2568
23. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, A. J., and Struhl, K. (1987) Current Protocols in Molecular Biology, Wiley InterScience, New York
24. Driscoll-Penn, M., Thireos, G., and Greer, H. (1984) Mol. Cell. Biol. 4, 520–525
25. Araki, Y., and Ito, E. (1988) Methods Enzymol. 161, 510–512
26. Law, D. T. S., and Segall J. (1988) Mol. Cell. Biol. 8, 912–922
27. Pearson, V. R., and Lipman, J. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2444–2448
28. Desreux, J., Haberli, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 9509–9524
29. Ballou, C. E., Maitra, K. S., Walker, W. J., and Whelan, L. W. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 4351–4355
30. Pammer, M., Briza, P., Ellinger, A., Schuster, T., Stucka, R., Feldmann, H., and Breitenbach, M. (1992) Yeast 8, 1089–1099
31. Bulawa, C. E. (1993) Annu. Rev. Microbiol. 47, 505–534
32. Shaw, J. A., Mol, P. C., Bowers, B., Silverman, S. J., Valdivieso, M. H., Duran, A., and Cabib, E. (1991) J. Cell Biol. 114, 111–123
33. Bulawa, C. E. (1992) Mol. Cell. Biol. 12, 1764–1776
34. Valdivieso, M. H., Mol, P. C., Shaw, J. A., Cabib, E., and Duran, A. (1991) J. Cell Biol. 114, 101–109
35. Choi, W., Santos, B., Duran, A., and Cabib, E. (1994) Mol. Cell. Biol. 14, 7685–7694
36. Mitchel, P. A. (1994) Microbiol. Rev. 58, 56–70
37. Hepworth, R. S., Ebiuzsuki, K. L., and Segall, J. (1995) Mol. Cell. Biol. 15, 3934–3944