JOURNAL OF BACTERIOLOGY, Jan. 1981, p. 404-409
0021-9193/81/010404-06$02.00/0

Vol. 145, No. 1

Structural Gene for the Alkaline Extracellular Protease of *Saccharomycopsis lipolytica*

PAULA C. SIMMS AND DAVID M. OGRYDZIAK*

Institute of Marine Resources, University of California, Davis, California, 95616

Mutants of *Saccharomycopsis lipolytica* temperature sensitive for alkaline extracellular protease production, but not for growth, were isolated. Thirty-three isolates were temperature sensitive for protease production, and one (*xpr-32*) produced a temperature-sensitive protease. Genetic analysis indicated that *xpr-32* was located in gene *XPR2*, and allele *xpr2-7* was found to also produce a temperature-sensitive protease. None of five independently isolated *xpr2* mutations affects the production of extracellular ribonucleases and acid protease(s). Diploids with zero, one, or two active alleles of the *XPR2* locus were constructed, and the *XPR2* locus was shown to exhibit a gene dosage effect on alkaline extracellular protease synthesis (enzyme activity/cell protein). These results suggest that the *XPR2* gene is the structural gene for the alkaline extracellular protease of *S. lipolytica*.

Yeasts which are amenable to a combined biochemical and genetic approach can be useful organisms for the study of secretion in eucaryotes. In *Saccharomyces cerevisiae*, 23 complementation groups required for post-translational events in the secretory pathway have been identified (10). Genetic studies of extracellular protease production by *Saccharomycopsis lipolytica* have established that mutations (*xpr*) in at least 16 genes result in reduced ability to produce extracellular protease (14; R. D. Mehta and R. C. von Borstel, Genetics **91**:s80-s81, 1979). Biochemical studies indicated that under conditions used for the genetic studies a single alkaline and no neutral extracellular proteases are produced (unpublished data). The *xpr* mutations might be located in the alkaline extracellular protease structural gene, regulatory genes, or genes involved in post-translational modification (processing) or secretion of the protease.

To characterize some of the *xpr* genes, mutants temperature sensitive for appearance of extracellular protease activity, but not for growth, were isolated. Mutants producing a temperature-sensitive protease and mutants temperature sensitive for protease production were found. A mutation resulting in production of a protease with altered thermal stability is often considered a structural gene mutation (6, 16, 17), but it could also be a mutation in a gene involved in post-translational modification of the protease (3, 5, 9). Mutations in genes regulating protease production or in genes affecting secretion would not be expected to result in a temperature-sensitive protease but could result in the production of the protease being temperature sensitive. In *S. cerevisiae*, mutations in genes affecting secretion resulted in temperature sensitivity for protein secretion and cell growth (10, 11).

To determine whether the mutants producing temperature-sensitive extracellular protease were structural gene or processing gene mutants, gene dosage effects were examined. A structural gene would be expected to exhibit a gene dosage effect (6, 17), whereas in most cases, a processing gene would not (3, 5, 9) (because its product is present in excess). Additive gene dosage effects were found, suggesting that the mutations were in the structural gene for the alkaline extracellular protease.

MATERIALS AND METHODS

Strains. The strains used are listed in Table 1. The parental strain CX161-1B was developed in R. Mortimer's laboratory by inbreeding *Candida lipolytica* YB-421 and YB-423-12 obtained from L. Wickerham.

Media, culture maintenance, and scoring of genetic markers. Cultures were maintained on YMS and YM agar as described previously (12). Procedures for preparation of synthetic minimal, synthetic complete, and omission media and for scoring auxotrophic and *uvs* (UV sensitivity) genetic markers and mating type were as described previously (12). SKM plates (14) contain 1% skim milk, yeast nitrogen base without ammonium sulfate and amino acids, adenine, and uracil at pH 6.8. SKM^{++} plates were prepared by supplementing SKM plates with 0.05% ammonium sulfate and 0.2% glycerol. SKM-azide plates are SKM plates containing 625 mg of sodium azide per liter. Glycerol-proteose peptone (GPP) medium was prepared as described earlier (14) except that the phosphate buffer concentration was 40 mM.

Isolation of temperature-sensitive extracellular protease (xpr) mutants. Mutants were induced by UV irradiation at a dose of 480 ergs/mm^2 at the surface of the plates. This treatment resulted in ap-

proximately 20% survival of cells. The treated cells were plated on SKM^{++} medium and incubated at 30°C for 72 h. Colonies that exhibited no surrounding zone of clearing were selected. These isolates were transferred to YM plates, which were incubated at 23°C for 24 h. The YM-grown isolates were inoculated onto SKM plates by making a small stab with a toothpick and were replica plated onto SM-plus-adenine plates. Duplicate plates were incubated at 23 and 34°C for 48 h.

Isolates which produced zones of clearing (indicative of protease activity) significantly smaller than wild-type zones at 34°C but comparable in size at 23°C were selected as possible *xpr*(Ts) mutants. Only those isolates which grew on the SM-plus-adenine plates at 34°C were characterized further.

Characterization of *xpr*(Ts) mutants. An agar diffusion assay was used to distinguish between mutants that were temperature sensitive for protease production and mutants that produced a temperature-sensitive protease. Isolates were grown in GPP medium at 23°C on a rotary shaker, and the samples were collected during exponential growth. Cell density was determined with a Klett-Summerson photoelectric colorimeter with a green filter. One gram (dry weight) of cells per liter yielded a value of 250 Klett units. Cells were removed by centrifugation at 10,000 × *g* for 10 min, and 35-μl aliquots of the supernatants were deposited into 6-mm wells made in SKM-azide plates. Zones of clearing produced after 48 h at 23 and 40°C were compared. Isolates producing wild-type zones of clearing at 23°C but reduced zones at 40°C produced a temperature-sensitive protease.

Thermal stability. Isolates that produced a temperature-sensitive protease were grown on a rotary shaker in 50 ml of GPP medium in 500-ml baffled flasks at 23°C. Supernatant samples were taken during exponential growth and diluted in 0.03 M phosphate buffer (1 mM $CaCl_2$, pH 6.8), heated at 52°C for 0, 5, 15, and 30 min, and then diluted further in 0.03 M borate-KCl buffer (1 mM $CaCl_2$, pH 9.0). The protease activities remaining in the heated supernatants were measured by using a modification of the casein hydrolysis assay described previously (13). The modifications were a change from pH 6.8 to 9.0 (20 mM borate-KCl buffer with 1 mM $CaCl_2$) and a change of incubation temperature from 40 to 25°C.

Genetic analysis. Genetic procedures have been described (12). Haploid strains used for construction of diploids for gene dosage studies contained complementing auxotrophic markers so that the prototrophic diploids could be selected on synthetic minimal medium plates after mating. Diploids were confirmed by sporulation on YM.

Complementation analysis of newly isolated *xpr* mutants was performed as described previously (14) except that protease production of diploids containing an *xpr*(Ts) mutation was examined on SKM plates at 23 and 34°C. Mutants from 13 *xpr* complementation groups were used as tester strains. All diploids were confirmed by sporulation.

Gene dosage. Diploids constructed for gene dosage analysis were grown in GPP medium, and supernatant samples were collected during exponential growth by centrifugation at 10,000 × *g* for 10 min. Samples were diluted with borate-KCl buffer (1 mM $CaCl_2$, pH 9.0), and protease activity was determined at 25 and 33°C, using the modified casein hydrolysis assay described above. Total cell protein for specific activity calculations was assayed by the biuret method (7).

TABLE 1. *Strain list*

Strain	Description	Genotype
CX161-1B	Parental strain (from J. Bassel)	*ade1 A*
DO7	UV mutagenesis of CX161-1B	*ade1 xpr2-7 A*
DO9	UV mutagenesis of CX161-1B	*ade1 xpr2-9 A*
PS2011	UV mutagenesis of CX161-1B	*ade1 xpr2-32 A*
PS3065	UV mutagenesis of CX161-1B	*ade1 xpr2-33 A*
PS6069	UV mutagenesis of CX161-1B	*ade1 xpr2-34 A*
PX7-45		*ura1 xpr2-7 B*
PX100	PS2011 × PX7-45	$\frac{ade1 \; + \; xpr2\text{-}32 \; A}{+ \; ura1 \; xpr2\text{-}7 \; B}$
DX266-8B		*ura1 uvs2 pro1 B*
PX103	PS2011 × DX266-8B	$\frac{ade1 \; + \; +}{+ \; ura1 \; uvs2}$ $\frac{+ \; xpr2\text{-}32 \; A}{pro1 \; + \; B}$
PX103-14B		*ura1 uvs2 xpr2-32 B*
PX103-15B		*ura1 B*
DX271	PS2011 × PX203-14B	$\frac{ade1 \; + \; +}{+ \; ura1 \; uvs2}$ $\frac{xpr2\text{-}32 \; A}{xpr2\text{-}32 \; B}$
DX271-2A		*ura1 xpr2-32 B*
DX272	PS2011 × DX271-2A	$\frac{ade1 \; +}{+ \; ura1}$ $\frac{xpr2\text{-}32 \; A}{xpr2\text{-}32 \; B}$
DX273	CX161-1B × DX271-2A	$\frac{ade1 \; +}{+ \; ura1}$ $\frac{+ \; A}{xpr2\text{-}32 \; B}$
DX274	PS2011 × PX103-15B	$\frac{ade1 \; +}{+ \; ura1}$ $\frac{xpr2\text{-}32 \; A}{+ \; B}$
DX275	CX161-1B × PX103-15B	$\frac{ade1 \; + \; A}{+ \; ura1 \; B}$

Extracellular RNase and acid protease assays. The plate assay for RNase production based on the size of the zone of RNA hydrolysis surrounding the colony has been described previously (14). The procedures for determining acid protease production will be described in more detail (D. M. Ogrydziak and S. J. Scharf, manuscript in preparation). Briefly, strains were grown in GPP medium at pH 3.4, the supernatant was collected at a cell density of 600 to 750 Klett units, and protease activity was assayed at pH 3.5, using acid-denatured hemoglobin as the substrate.

RESULTS

Mutant isolation. At the pH of SKM medium the extracellular acid protease of *S. lipo-*

lytica is inactive (unpublished data), so the Xpr phenotype on SKM^{++} or SKM medium does not require an acid protease deficiency.

Over 18,000 colonies were screened for reduced extracellular protease activity at 30°C on SKM^{++} plates, which support limited growth of *xpr* mutants. The 30°C temperature for isolation of *xpr*(Ts) mutants was chosen because recovery of irradiated cells at 34°C (maximum temperature for growth) on SKM^{++} plates was low. Five hundred eighty-seven isolates that showed no clearing zones on the SKM^{++} plates were screened on SKM plates. The wild type produced clearing zones of 7 to 9 mm at 34°C and 12 to 14 mm at 23°C on SKM plates. Mutants were chosen on the basis of smaller zones (0 to 5 mm) at 34°C and near-wild-type zone size (10 to 14 mm) at 23°C. Isolates were also tested for growth on synthetic minimal plates containing adenine at 34°C to eliminate mutants that were temperature sensitive for growth or which had temperature-sensitive nutritional requirements. Of the 587 isolates screened on SKM plates, 34 *xpr*(Ts) mutants and 27 nonconditional *xpr* mutants were obtained.

The high percentage (~90%) of false *xpr* isolates selected from SKM^{++} plates was probably because, to avoid complete clearing of the plates by the *XPR*$^{+}$ survivors, the isolates had to be selected after only 72 h. After UV mutagenesis many cells undergo an extended lag period before resuming growth. At 72 h many of these late starting colonies, although capable of protease production, would score as *xpr*, especially considering that, compared with SKM medium, SKM^{++} medium has a slight repressive effect on protease production.

The 34 *xpr*(Ts) isolates were grown at permissive temperature (23°C) in GPP medium, which supports extracellular protease production. The thermal stability of the protease produced was screened by comparing clearing zone sizes produced at 23 and 40°C, using an agar diffusion assay on SKM-azide plates. The supernatant from a wild-type culture at a cell density of 1 g (dry weight) per liter produced zones of clearing of about 16 mm at 23°C and 18 mm at 40°C. Mutants that produced a temperature-sensitive protease were identified by substantially smaller zones (8 mm or less) at 40°C. Only 1 of 34 isolates tested, *xpr-32*, produced a temperature-sensitive extracellular protease. Use of the same agar diffusion screening method to determine thermal stability of protease in supernatants (concentrated by ultrafiltration) from the original *xpr* mutants (14) revealed that *xpr2-7* also produced a temperature-sensitive extracellular protease (S. Scharf, personal communication).

Thermal stability. The temperature sensitivity of the extracellular protease produced by *xpr-32* and *xpr2-7* was confirmed (Fig. 1). Partial purification of the protease activity from *xpr-32* resulted in a slight increase in stability, but the mutant protease was still significantly less stable than the wild type.

Complementation analysis. A temperature-sensitive gene product can be a result of a mutation in the structural gene itself or in a gene involved in post-translational modification of the gene product. To determine whether *xpr-32* and *xpr2-7* were in the same gene, the diploid PX100 was constructed. Both *xpr-32* (see below) and *xpr2-7* (14) are recessive. The diploid produced little protease activity on SKM plates at 23°C and no detectable activity at 34°C. The lack of complementation indicates that *xpr-32* is located in gene *XPR2*.

Gene dosage. A mutation in the extracellular protease structural gene would be expected to exhibit a gene dosage effect. To examine the dosage effect, a set of diploids was constructed:

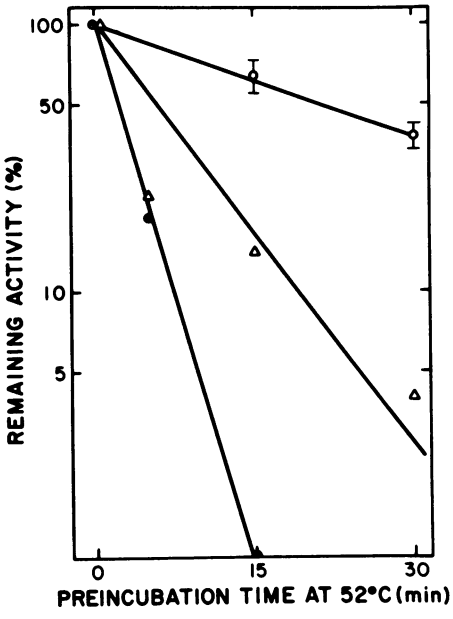

FIG. 1. *Thermosensitivity of alkaline extracellular proteases. Symbols:* (○) *wild-type protease from CX161-1B; each point represents the mean value of five experiments with different protease preparations (bars indicate ± standard error). One of the preparations was partially purified by gel filtration on Sephadex G-75, and activity remaining after 15 and 30 min was 65 and 35%.* (△) *xpr2-32 and* (●) *xpr2-7 proteases partially purified on Sephadex G-75. Unpurified xpr2-32 and xpr2-7 preparations had no detectable activity after 15 and 30 min.*

DX275, homozygous for the wild-type extracellular protease gene; DX272, homozygous for the *xpr2-32* mutation; DX273 and DX274, both heterozygous for the allele.

The results in Table 2 show a clear-cut gene dosage effect and are consistent with *XPR2* being the extracellular protease structural gene. The results for the heterozygous diploids were close to the values expected for complete additivity of the gene doses. The absolute value for protease production of the wild-type diploid DX275 varied from experiment to experiment (values at 25°C were 179, 113, 118, and 165 U/mg of cell protein), but protease production (as a percentage of DX275) by the diploids containing *xpr2-32* was consistent within a given experiment.

Other *xpr2* mutations. The 27 nonconditional *xpr* mutants were tested for complementation with *xpr2-7* and *xpr2-9*, a nonconditional recessive mutation (14). Two additional *xpr2* alleles, *xpr2-33* and *xpr2-34*, were found. These strains were grown in GPP medium, the supernatants were concentrated 100-fold by ultrafiltration, and the concentrated supernatants were assayed at 23 and 40°C, using the agar diffusion assay. No activity could be detected for *xpr2-33*, and the protease from *xpr2-34* was not temperature sensitive.

Concentrated supernatants from strains containing each of the five *xpr2* alleles were examined by polyacrylamide gel electrophoresis. In all cases, the band corresponding to the protease was either not detectable or much less intense than from the wild type (Ogrydziak and Scharf, in preparation). No differences in mobility compared with the wild-type protease were found for the *xpr2-32* protease on 6 and 9% polyacrylamide native gels and for *xpr2-7* and *xpr2-9* on isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gels (data not shown).

Production of other extracellular enzymes. Some of the *xpr* mutations affected production of extracellular RNases (14) and extracellular acid protease(s) (Ogrydziak and Scharf, in preparation). Neither *xpr2-7* nor *xpr2-9* had been shown to affect production of extracellular RNases (14), and based on the results from RNA agar plate assays, the other three *xpr2* alleles also do not affect production of extracellular RNases. All five *xpr2* alleles had little affect on production of extracellular acid protease(s) (Table 3).

TABLE 2. *Gene dosage effect of xpr2-32 on extracellular protease production*

Strain	Relevant genotype	n[a]	Protease production[b] (U/mg of cell protein[c]) assayed at:	
			25°C	33°C
DX275	+/+	4	100	100
DX274	+/*xpr2-32*	4	64 ± 5	53 ± 9
DX273	+/*xpr2-32*	1	59	47
DX272	*xpr2-32/xpr2-32*	4	10 ± 3	5 ± 2

[a] Number of experiments upon which the calculation is based.

[b] Values were calculated as the percentage of protease produced compared with the wild-type diploid DX275 within a given experiment; results are expressed as the mean (± standard deviation).

[c] Cell protein was determined by the biuret method.

DISCUSSION

Mutations in several genes affect production of the alkaline extracellular protease (14; Mehta and von Borstel, Genetics 91:s80–s81, 1979). Such genes might include the structural gene for the protease, genes involved in regulation of protease production, or genes involved in post-translational modification (processing) or secretion of the protease. Only a structural gene mutation or a mutation involved in post-translational modification of the protease would be expected to cause changes in the thermal stability of the protease.

We infer that *XPR2* is the structural gene for the alkaline extracellular protease because (i) alleles *xpr2-7* and *xpr2-32* cause production of alkaline extracellular protease activities with altered thermal stability compared with the wild type, (ii) the *XPR2* locus exhibited a gene dosage effect on alkaline extracellular protease production (enzyme activity/cell protein), and (iii) the *xpr2* alleles do not affect the production of other extracellular enzymes.

S. lipolytica produces a single alkaline extracellular protease and one or more acid extracellular proteases (unpublished data). At the pH (6.8) used for mutant isolation and screening,

TABLE 3. *Production of extracellular acid protease(s) by xpr2 mutants*

Strain	n[a]	Acid protease (U/mg of dry wt)[b]
CX161-1B	4	31.0 ± 3.6
xpr2-7	1	25.7
xpr2-9	2	32.5
xpr2-32	1	28.2
xpr2-33	1	29.5
xpr2-34	1	34.7

[a] Number of experiments upon which the calculation is based.

[b] Results are expressed as the mean (± standard deviation).

little acid protease is produced and it is not active, so only alkaline protease activity would be detected (unpublished data). At the pH (9.0) used for protease activity assay in the thermal stability and gene dosage studies, all of the protease activity is attributable to the single alkaline extracellular protease (unpublished data).

The frequency of *xpr2* mutations (3 in 18,000) is comparable to the frequency (2 in 6,587) found in an earlier study (14). That *xpr2* mutants grew on minimal medium under conditions where no protease activity was produced indicates that the alkaline extracellular protease activity is not essential for growth on minimal medium. This result is consistent with the earlier proposal (13) that the extracellular protease is produced to provide low-molecular-weight nutrients from peptides and proteins in the environment. Mehta and von Borstel (Genetics **91**: S80–S81, 1979) screened *S. lipolytica* mutants temperature sensitive for growth and found that several were affected for alkaline extracellular protease production. None of these mutations was in *xpr2*.

The wild-type alkaline extracellular protease exhibits maximum caseinolytic activity at pH 9 to 10 and 40°C (unpublished data). However, since activities of temperature-sensitive proteases had to be determined in this study, assays were done at 25°C and pH 9.0. Inactivation of the protease can be caused by thermal denaturation and by self-proteolysis. To limit self-proteolysis as a factor in the thermal stability studies, the studies were performed in the presence of 1 mM Ca^{2+}, which stabilizes the protease (15; unpublished data), at the pH (6.8) at which the protease is most stable (15), and at the temperature (52°C) that was as high as possible above the temperature of maximum activity (40°C) but at which the protease was only partially inactivated. After 15 min of incubation at pH 9.0 at 52°C, the wild-type alkaline extracellular protease was completely inactivated (unpublished data).

Since proteins can protect proteases from inactivation (2) and since the protease preparations used for the thermal stability studies were not completely purified, differences in thermal stability of the alkaline extracellular proteases possibly reflect differences in purity of the protease preparations. Several results do not support this interpretation. First, partial purification of the protease preparations had little effect on thermal stability. After the partial purification, about 85% of the protein in a wild-type preparation is protease (unpublished data). Second, since the *xpr2* mutants all produce less alkaline extracellular protease protein than the wild type, the *xpr2* protease preparations would have a greater percentage of contaminating protein and should be stabilized at least to the same extent as a wild-type preparation. We conclude that the differences in thermal stability reflect differences in the alkaline extracellular protease molecules.

Mutations that alter the physical properties of an enzyme, such as thermal stability, have often been considered as evidence for identification of the structural gene for the enzyme (6, 16, 17). But mutations in genes that affect post-translational modifications can also alter the physical properties of enzymes (3, 5, 9). The alkaline extracellular protease is a secreted glycoprotein (unpublished data), and several types of post-translational modifications might be expected. The protease may start with a signal sequence (1), and it may exist intracellularly as a zymogen. Mutations affecting removal of the signal sequence, zymogen activation, carbohydrate addition, or processing could result in altered thermal stability of the protease. Studies of gene dosage have been used to distinguish between structural gene mutations and mutations that affect processing of the enzyme. In most cases, the structural gene mutations show a dosage effect: the specific activity of the enzyme is proportional to the number and type of alleles present (6, 17). In most cases, processing activity is in excess and the processing gene mutations are recessive and exhibit no gene dosage effects (3, 5, 9). For a processing gene mutation to show a gene dosage effect, correct processing must be essential for activity. Then, in the heterozygote, either the processing activity is strictly limiting and about half the protease molecules are processed correctly and half are not processed or, perhaps more likely, the wild-type and mutant processing activities compete for unprocessed protease molecules with resulting production of about half correctly processed and half incorrectly processed protease molecules. A possible example of a gene dosage effect for a mutation thought to affect post-translational modification of human neuraminidase and β-galactosidase has been reported (8). In the heterozygote the neuraminidase activity exhibited a gene dosage effect but the β-galactosidase activity did not.

In several cases a mutation in a enzyme involved in processing results in altered properties of several different proteins (4, 8). Although several of the *xpr* mutations affect production of extracellular RNases and acid protease(s), the *xpr2* mutations do not. If the *xpr2* mutations are processing gene mutations, it seems that they are fairly specific for the alkaline extracellular protease.

Although definitive results such as proof of a

difference in the amino acid sequence have not been obtained, the above results strongly suggest that *XPR2* is the structural gene for the alkaline extracellular protease. Since the protease is a single polypeptide (unpublished data), these results suggest that only one structural gene for the alkaline proteose exists in the haploid strain and the *XPR2* is this structural gene. Presently, we are investigating the *xpr* mutations to determine whether they are involved in processing, regulation, or secretion.

ACKNOWLEDGMENTS

We thank Glenn Tanimoto for preliminary work on establishing conditions for the thermal stability studies.

This work was supported by National Science Foundation grant PCM 78-03191 and Agriculture Experiment Station fund CA-D-FST-3590-H.

LITERATURE CITED

1. **Blobel, G., and B. Dobberstein.** 1975. Transfer of proteins across membranes. Presence of proteolytically processed and unprocessed nascent immunoglobin light chains on membrane-bound ribosomes of murine myeloma. J. Cell Biol. **67**:835–851.
2. **Feder, J., D. Kochavi, R. G. Anderson, and B. S. Wildi.** 1978. Stabilization of proteolytic enzymes in solution. Biotechnol. Bioeng. **20**:1865–1872.
3. **Finnerty, V., and G. Johnson.** 1979. Post-translational modification as a potential explanation of high levels of enzyme polymorphism: xanthine dehydrogenase and aldehyde oxidase in *Drosophila melanogaster*. Genetics **91**:695–722.
4. **Free, S. J., and R. T. Schimke.** 1978. Effects of a post-translational modification mutation on different developmentally regulated glycosidases in *Dictyostelium discoideum*. J. Biol. Chem. **253**:4107–4111.
5. **Free, S. J., R. T. Schimke, H. Freeze, and W. F. Loomis.** 1978. Characterization and genetic mapping of *modA* a mutation in the post-translational modification of the glycosidases of *Dictyostelium discoideum*. J. Biol. Chem. **253**:4102–4106.
6. **Free, S. J., R. T. Schimke, and W. F. Loomis.** 1976. The structural gene for α-mannosidase-1 in *Dictyostelium discoideum*. Genetics **84**:159–174.
7. **Herbert, D., P. J. Phipps, and E. R. Strange.** 1971. Chemical analysis of microbial cells, p. 209–344, *In* J. R. Norris and D. W. Ribbins (ed.), Methods in microbiology, vol. 5B. Academic Press, Inc., New York.
8. **Hoogeveen, A. T., F. W. Verheijen, A. d'Azzo, and H. Galjaard.** 1980. Genetic heterogeneity in human neuraminidase deficiency. Nature (London) **285**:500–502.
9. **Loppes, R.** 1978. A mutation altering some properties of the neutral phosphatase in *Chlamydomonas reinhardi*: possible post-translational modification of phosphatase structure. J. Bacteriol. **135**:551–558.
10. **Novick, P., C. Field, and R. Schekman.** 1980. The identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell **21**:205–215.
11. **Novick, P., and R. Schekman.** 1979. Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **76**:1858–1862.
12. **Ogrydziak, D., J. Bassel, R. Contopulou, and R. Mortimer.** 1978. Development of genetic techniques and the genetic map of the yeast *Saccharomycopsis lipolytica*. Mol. Gen. Genet. **163**:229–239.
13. **Ogrydziak, D., A. L. Demain, and S. R. Tannenbaum.** 1977. Regulation of extracellular protease production in *Candida lipolytica*. Biochim. Biophys. Acta **497**:525–538.
14. **Ogrydziak, D., and R. K. Mortimer.** 1977. Genetics of extracellular protease production in *Saccharomycopsis lipolytica*. Genetics **87**:621–632.
15. **Tobe, S., T. Takami, S. Ikeda, and K. Mitsugi.** 1976. Production and some enzymatic properties of alkaline proteinase of *Candida lipolytica*. Agric. Biol. Chem. **40**:1087–1092.
16. **Uehara, H., K. Yamane, and B. Maruo.** 1979. Thermosensitive, extracellular neutral proteases in *Bacillus subtilis*: isolation, characterization, and genetics. J. Bacteriol. **139**:583–590.
17. **Zubenko, G. S., A. P. Mitchell, and E. W. Jones.** 1979. Septum formation, cell division, and sporulation in mutants deficient in proteinase B. Proc. Natl. Acad. Sci. U.S.A. **76**:2395–2399.