MOLECULAR AND CELLULAR BIOLOGY, Nov. 1985, p. 3069–3073
0270-7306/85/113069-05$02.00/0

Vol. 5, No. 11

Transcriptional Control of the Sporulation-Specific Glucoamylase Gene in the Yeast *Saccharomyces cerevisiae*

ICHIRO YAMASHITA* AND SAKUZO FUKUI

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Shitami, Saijo-cho, Higashi-Hiroshima 724, Japan

Received 27 March 1985/Accepted 29 July 1985

In the yeast *Saccharomyces cerevisiae*, glucoamylase activity appears specifically in sporulating cells heterozygous for the mating-type locus (*MAT*). We identified a sporulation-specific glucomylase gene (*SGA*) and show that expression of *SGA* is positively regulated by the mating-type genes, both *MATa1* and *MATα2*. Northern blot analysis revealed that control of *SGA* is exerted at the level of RNA production. Expression of *SGA* or the consequent degradatin of glycogen to glucose in cells is not required for meiosis or sporulation, since *MATa*/*MATα* diploid cells homozygous for an insertion mutation at *SGA* still formed four viable ascospores.

The genetic and biochemical controls of meiosis in the yeast *Saccharomyces cerevisiae* is of interest as a model system for the control of this process, because the basic events in yeast are extensively homologous to those in higher plant and animal cells.

The mating-type locus (*MAT*) regulates meiosis or spore formation, because heterozygosity at *MAT* is essential to initiate meiosis; genetic analyses confirmed that two (*MATa1* and *MATα2*) of four mating-type alleles are required for this process (7, 11, 22). Isolation of sporulation-defective mutants (4) and the findings that more than half of *CDC* genes are also required for sporulation (16) have presented a number of genetic constituents essential for sporulation. However, relatively little knowledge of their biochemical function has yet been obtained.

Meiosis and sporulation are accompanied by the extensive accumulation of intracellular carbohydrates, including trehalose and glycogen as reserve or storage materials and mannan and glucan as the major carbohydrate components of yeast cell walls (6). The carbohydrates are synthesized to the same extent in both sporulating and nonsporulating cells; however, only sporulating cells exhibit a period of glycogen degradation coinciding with the appearance of mature spores (6). Colonna and Magee (2) have reported that the glycogenolytic activites appear specifically in sporulating cells.

Recently, we have cloned a structural gene (*STA*) for an extracellular glucoamylase from a starch-fermenting yeast, *Saccharomyces diastaticus*, and have shown that both *S. diastaticus* and *S. cerevisiae* contain a DNA segment (Δ*sta*) that is highly homologous to *STA* DNA (25, 26). In this study we identified the Δ*sta* DNA to code for sporulation-specific glucoamylase and show that expression of the gene (*SGA*) is regulated at the level of transcription. However, we could not obtain any evidence for the significance of *SGA* in sporulation; on the contrary, we observed that diploid cells homozygous for an insertion mutation at *SGA* did not degrade glycogen but were able to undergo sporulation.

MATERIALS AND METHODS

Strains, media, and genetic methods. The yeast strains used and their relevant genotypes are listed in Table 1. The YEPD medium contained 10 g of yeast extract, 20 g of polypeptone, and 20 g of glucose in 1 liter of deionized water. The PSP medium contained 10 g of yeast extract, 10 g of polypeptone, and 10 g of potassium acetate in 1 liter of deionized water. The SPM medium contained 3 g of potassium acetate in 1 liter of deionized water. Genetic techniques including tetrad analysis have been described by Mortimer and Hawthorn (10). The procedure used for the transformation of yeast has been described previously (20).

Preparation of extracts and assay for glucoamylase activity. For analysis of glucoamylase activity in SPM, cells were cultured in 100 ml of PSP at 28°C to an optical density at 660 nm of 1.5 to 2.0, harvested, washed with sterile water, suspended in 100 ml of SPM at an optical density of 1.0, and then incubated at 28°C, usually for 24 h. For analysis of glucoamylase activity in either YEPD or PSP, cells were cultured at 28°C in 100 ml of either YEPD or PSP to an optical density of 1.0. These cells were harvested, washed with water, and suspended in 0.5 ml of 30 mM Tris hydrochloride buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride. To the cell suspension was added 1.5 g of acid-washed glass beads (0.5 mm in diameter). The mixture was vortexed for 5 min with occasional chilling on ice. Breakage was monitored microscopically and was always more than 95%. The homogenate was centrifuged successively for 10 min at 20,000 × *g* and for 10 min at 10,000 × *g*. The resulting supernatant was dialyzed overnight against 30 mM Tris hydrochloride buffer (pH 7.5) and used for the enzyme assay. Glucoamylase activity was assayed at 50°C using soluble starch as a substrate as described previoulsy (24). The activities (as shown in the tables) are presented as total activities (U) of the cells in 100-ml cultures.

Determination of the percentage of sporulation. The percentage of sporulation was determined by scoring the asci with more than one spore in each ascus under a microscope with an oil immersion lens. Most asci contained four spores under the sporulation conditions described above.

Glycogen determinations. The procedures for extraction, isolation, and measurement of cellular glycogen were as described by Colonna et al. (1).

Immunoprecipitation. Hybridoma cells (F3.2C7 and F3.2E12) (25) were cultured in RPMI 1640 medium (Nissui Seiyaku Co.), and the culture supernatants were used for the experiments. The culture supernatants were diluted with the same medium. Glucoamylase (0.4 U) was incubated at 0°C

* Corresponding author.

TABLE 1. Strains

Strain	Genotype	Source
AH22	*MATa leu2-3,112 his4 can1*	A. Toh-e
YIYD	*MATa leu2-3,112 his4 lys7*	Our collection
YIY6B-2	*MATα leu2-3,112 his7 ura1*	Our collection
EG1-23	*MATa leu2 his4 ura3 trp1*	K. Tatchell
23a20	*mata1 leu2 his4 ura3 trp1*	K. Tatchell
23a7-12	*mata2 leu2 his4 ura3 trp1*	K. Tatchell
23α113	*matα1 leu2 his4 ura3 trp1*	K. Tatchell
23α182	*matα2 leu2 his4 ura3 trp1*	K. Tatchell
YIY3A	*MATa his7 ura1*	Our collection
YIY3B	*MATα his7 ura1*	Our collection

TABLE 3. Both *MATa1* and *MATα2* are required for glucoamylase production

Strain cross	*MAT* genotype	Glucoamylase activity (U)	% sporulation
EG1-23 × YIY3B	*MATa/MATα*	25	55
23a20 × YIY3B	*mata1/MATα*	0	0
23a7-12 × YIY3B	*mata2/MATα*	18	47
YIY3A × 23α113	*MATa/matα1*	14	18
YIY3A × 23α182	*MATa/matα2*	0	0

for 60 min with a series of diluted antibodies which had been attached to staphylococcal protein A (21) and then centrifuged. Samples of the resulting supernatants were assayed for the remaining glucoamylase activity.

Southern blot analysis. Total genomic DNA was isolated by a modification of the method of Cryer et al. (3). Transfer of DNA fragments from agarose gels to nitrocellulose paper was as described by Southern (17). Hybridization conditions and the labeling of DNA for hybridization probes by nick translation were as described by Rigby et al. (13). Plasmid pBR-STA1SS1.15 (25) was used as a probe.

Northern blot analysis. Cells were cultured in any of the media (YEPD, PSP, or SPM) as described above, harvested, and washed with sterile water. RNA was extracted from the cells as described by Jensen et al. (5). Poly(A)$^+$ RNA was isolated with oligo(dT) cellulose as recommended by the supplier (Collaborative Research Inc., Waltham, Mass.). The RNA (20 μg per each lane) was fractionated in two separate agarose (1%) gels as described by McMaster and Carmichael (9), transferred to nitrocellulose paper, and hybridized as described by Thomas (23) with a nick-translated probe, either the plasmid pBR-STA1SS1.15 (25) or a plasmid YIp5 (19), for detecting the *SGA* or *URA3* transcripts, respectively.

RESULTS

Glucoamylase activity appears specifically in sporulating cells. Haploid (*MATa* or *MATα*) and diploid (*MATa/MATα*) cells were cultured in YEPD and PSP, or the cells pregrown in PSP were incubated in SPM. Extracts were prepared from these cells and assayed for glucoamylase activity (Table 2). The enzyme activity appeared specifically in the extract from *MATa/MATα* cells which had been sporulating in SPM. The results agree with the observations of Colonna and Magee (2).

To identify the mating-type genes that are essential for this regulation, we constructed diploid cells in which one of four

TABLE 2. Appearance of glucoamylase activity in sporulating cells

Strain	*MAT* genotype	Medium	Glucoamylase activity (U)	% sporulation
YIYD	*MATa*	YEPD	0	0
		PSP	0	0
		SPM	0	0
YIY6B-2	*MATα*	YEPD	0	0
		PSP	0	0
		SPM	0	0
YIYD × YIY6B-2[a]	*MATa /MATα*	YEPD	0	0
		PSP	0	0
		SPM	17	75

[a] A diploid strain constructed by the cross between YIYD and YIY6B-2.

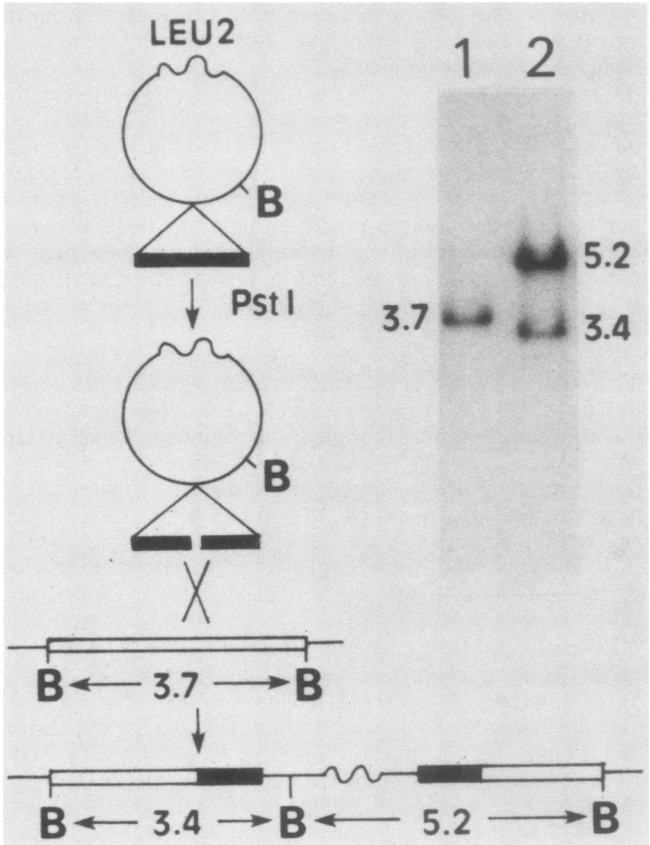

FIG. 1. Gene disruption. The plasmid YIpSTA1SBa10.7 was digested with *Pst*I and was used for the transformation of *leu2* haploid strains (either *MATa* or *MATα*). Schematic diagrams represent integration of the plasmid DNA into Δ*sta* DNA on chromosomal DNA (see text for details). Southern blot analysis of *Bam*HI-digested genomic DNA from both transformants and recipients was carried out as described in the text. Lane 1, AH22 (a recipient); lane 2, AH22TF3 (a transformant). The other recipient strains (YIYD and YIY6B-2) and their Leu$^+$ transformants (YIYDTF3 and YIY6B-2TF1) showed the same hybridization patterns as those of AH22 and AH22TF3, respectively. Sizes of *Bam*HI (B) fragments are shown in kilobases.

TABLE 4. Cross-reactivity of sporulation-specific glucoamylase with anti-*S. diastaticus* glucoamylase monoclonal antibodies

Monoclonal antibody	Dilution (×)	Remaining activity (% that of initial)
F3.2C7	8	37
	4	0
	2	0
	1	0
F3.2E12	8	32
	4	0
	2	0
	1	0
1G6D10[a]	1	100

[a] In a control experiment with a culture fluid of hybridoma cells (1G6D10) which produced no antiglucoamylase antibody, nonspecific adhesion could not be detected.

codominant alleles of *MAT* (*MAT***a***1*, *MAT***a***2*, *MATα1*, and *MATα2*) is mutated. These cells were cultured in PSP and then incubated in SPM. Diploid cells carrying the mutations in *MAT***a***1* and *MATα2* could neither undergo sporulation nor produce glucoamylase activity, while the *mat***a***2* and *matα1* mutations affected neither sporulation nor enzyme activity (Table 3). These results indicate that both *MAT***a***1* and *MATα2* are essential for enzyme production.

Δ*sta* DNA codes for sporulation-specific glucoamylase. We have reported that *S. cerevisiae* contains Δ*sta* DNA that is highly homologous by Southern hybridization to a functional domain of *STA1* which codes for the extracellular glucoamylase of *S. diastaticus* (25, 26). The glucoamylase activity in sporulating cells was completely immunoprecipitated with monoclonal antibodies (25) raised by the extracellular glucoamylase of *S. diastaticus* (Table 4). This result suggests that sporulation-specific glucoamylase activity consists of a single enzyme, and that Δ*sta* DNA codes for sporulation-specific glucoamylase.

To confirm this, we have carried out a gene-disruption experiment (15) using the integrating plasmid YIpSTA1SBa10.7 that carries *LEU2* and part (*SalI-BalI* fragment) of *STA1* (Fig. 1). To stimulate insertion at homologous sequences of Δ*sta* DNA (12), the plasmid DNA was cleaved at one site of the *STA1* fragment with *Pst*I. The linear full-length DNA was used to transform *leu2* haploid strains (either *MAT***a** or *MATα*). The plasmid DNA was integrated at Δ*sta* DNA in all Leu$^+$ tranformants obtained, which was shown by Southern blot analysis (Fig. 1). The *MAT***a** tranformants were crossed with the *MATα* transformants, and the resulting diploid cells were examined for glucoamylase activity, glycogen accumulation, and sporulation. The diploid cells homozygous for the disrupted Δ*sta* showed no glucoamylase activity (Table 5), indicating that

TABLE 5. Δ*sta* DNA codes for sporulation-specific glucoamylase

Strain of cross[a]	Glucoamylase activity (U)	% sporulation
AH22 × YIY6B-2	38	78
YIYD × YIY6B-2	17	75
AH22TF3 × YIY6B-2TF1	0	95
YIYDTF3 × YIY6B-2TF1	0	93

[a] Haploid *leu2* strains AH22 (*MAT***a**), YIYD (*MAT***a**), and YIY6B-2 (*MATα*) are used as recipients for transformation with the integrating plasmid YIpSTA1SBa10.7. Strains AH22TF3, YIYDTF3, and YIY6B-2TF1 are Leu$^-$ transformants in which Δ*sta* DNA is disrupted by insertion of the plasmid.

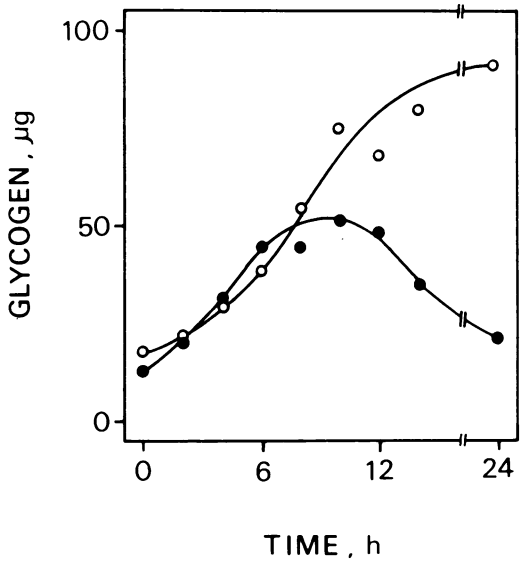

FIG. 2. Glycogen accumulation. Diploid strains, AH22 × YIY6B-2 (wild type, ●) and AH22TF3 × YIY6B-2TF1 (transformant, ○), were cultured in 100 ml of SPM. At intervals, 5-ml fractions were removed, and the glycogen content in the cells was determined as described in the text. Ascus formation was more than 95% in both wild-type and transformant diploids at 24 h.

only Δ*sta* DNA codes for sporulation-specific glucoamylase. The transformant diploid cells accumulated glycogen progressively, while the wild-type diploid cells degraded the glycogen which had been synthesized at an early stage of incubation (Fig. 2). However, sporulation-specific glucoamylase activity and the consequent degradation of glycogen are not required for spore formation, at least under the conditions used in this study, because more than 90% of the transformant diploid cells still had undergone meiosis or formed four spores in each ascus (Table 5), most of which were estimated to be viable (Table 6).

Transcriptional control of the sporulation-specific glucoamylase gene. To examine transcriptional control of the sporulation-specific glucoamylase gene (*SGA*), we used as a probe the subcloned *Sal*I fragment of *STA1* (25) that hybridizes specifically to *SGA*. Haploid (either *MAT***a** or *MATα*) and diploid (*MAT***a**/*MATα*) cells were cultured in YEPD and PSP, or the cells which had been grown in PSP were incubated in SPM. Poly(A)$^+$ RNA was isolated from these cells and hybridized with the probe pBR-STA1SS1.15. Only *MAT***a**/*MATα* cells in SPM accumulated a species of RNA (ca. 2.0 kilobases) complementary to *SGA* (Fig. 3). The content of stable *SGA* RNA increased until 12 h and then was reduced at 24 h.

TABLE 6. Spore viability[a]

Strain cross	No. of viable spores	No. of four-spored asci dissected	% viable spores
AH22 × YIY6B-2	42	12	88
YIYD × YIY6B-2	42	11	95
AH22TF3 × YIY6B-2TF1	89	27	82
YIYDTF3 × YIY6B-2TF1	103	28	92

[a] Diploid cells (see footnote *a* in Table 5) were sporulated in SPM. Four-spored asci were dissected with a micromanipulator and cultured on plates with YEPD. Spores which formed colonies were estimated to be viable.

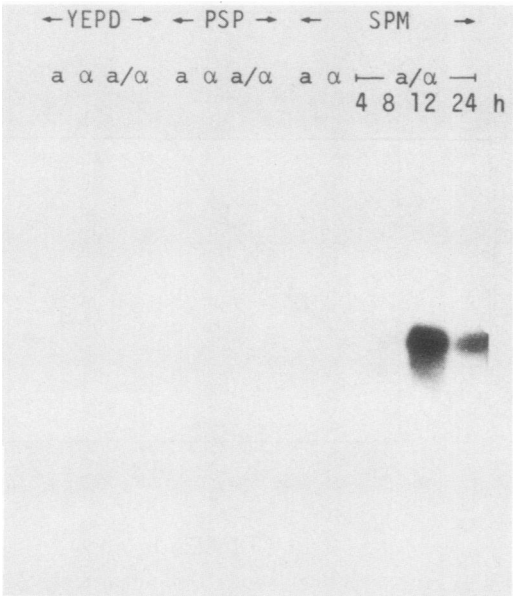

FIG. 3. Only sporulating diploid cells produced *SGA* RNA. Haploid strains YIYD (*MAT***a**) and YIY6B-2 (*MATα*) and the diploid strain AH22 × YIY6B-2 (*MAT***a**/*MATα*) were cultured in any of the media (YEPD, PSP, and SPM). Haploid cells were cultured in SPM for 12 h. Diploid cells were cultured in SPM for the indicated times. Poly(A)$^+$ RNA was isolated from these cells, and Northern blot analysis was carried out as described in the text.

To examine whether positive regulation of *SGA* by *MAT* (*MAT***a***1* and *MATα2*) occurs at the transcriptional level, poly(A)$^+$ RNA was isolated from diploid cells in which one of four *MAT* genes was mutated and hybridized with the probe. The content of stable *SGA* RNA was greatly reduced in diploid cells carrying the *mat***a***1* or *matα2* mutation, whereas diploid cells carrying the *mat***a***2* or *matα1* mutation produced an equivalent amount of *SGA* RNA to that of wild-type diploid cells (Fig. 4).

These results indicate that control of *SGA* is exerted at the level of RNA production and that both *MAT***a***1* and *MATα2* gene products are required for expression of this gene. When the *SGA* RNA disappeared or was present at a reduced level, the possibility that it was due to the degradation of RNA during isolation can be excluded, because all *URA3* RNAs detected in these samples appeared as discrete bands on Northern blots (data not shown).

DISCUSSION

The mating-type locus regulates the mating ability of haploid yeast cells and meiosis or spore formation in diploid cells (7, 11, 22). Both *MAT***a***1* and *MATα2* gene products (**a**1-α2) are essential to sporulation, since *mat***a***1*/*MATα* and *MAT***a**/*matα2* cells cannot undergo sporulation. **a**1-α2 are considered to repress haploid-specific genes directly or indirectly and to activate sporulation-specific genes. The level of control exerted by **a**1-α2 is known to be at the level of RNA production for only three cases of negative regulation. **a**1-α2 negatively regulate the synthesis of RNA from the *MATα1* gene (7, 11), resulting in repression of the α-specific *STE3* gene (18); **a**1-α2 negatively regulate the *HO* gene as well (5). We have shown here that **a**1-α2 positively regulate the *SGA* gene. This conclusion comes from the following observations: (i) *MAT***a**/*MATα* diploid cells produced *SGA* RNA, but *MAT***a** or *MATα* haploid cells did not; and (ii) *mat***a***2*/*MATα* and *MAT***a**/*matα1* cells produced *SGA* RNA, but *mat***a***1*/*MATα* or *MAT***a**/*matα2* cells did not. Thus, it is clear that both *MAT***a***1* and *MATα2* are required to express *SGA*.

How do **a**1-α2 activate sporulation-specific genes (*SSG*)? The following hypotheses are proposed. (i) The simplest explanation is that both *MAT***a***1* and *MATα2* gene products are required for transcription of *SSG*. (ii) By the analogy of a regulatory mechanism in sporulation of *Bacillus subtilis* (8), **a**1-α2 activate the transcription of sigma factor-like genes, and then sporulation-specific RNA polymerase transcribes *SSG*. (iii) *MAT***a** and *MATα* haploid cells contain inhibitors that repress the transcription of *SSG*, but **a**1-α2 repress the transcription of inhibitor genes or inactivate the inhibitors (14).

*MAT***a**/*MATα* diploid cells initiate sporulation and synthesize glucoamylase only when incubated in the nitrogen-free, acetate-containing medium. Induction of the enzyme by this medium can be explained by the fact that *MAT***a**/*MATα* cells synthesize *SGA* RNA only in the sporulation medium (SPM), but not in YEPD or PSP.

Results of the gene disruption experiment indicate that expression of *SGA* or the consequent degradation of glycogen to glucose is not essential to spore formation at least under the conditions used in this study. It is obscure whether other glucose-supplying mechanisms are present or whether endogenous glucose is required for sporulation. We cannot exclude the possibility that cells pregrown in PSP contain enough endogenous glucose to undergo sporulation. It can be seen from the results that the glucoamylase activity appearing in sporulation consists of a single enzyme that is encoded by *SGA*, because the enzyme activity was completely immunoprecipitated by monoclonal antibodies, and

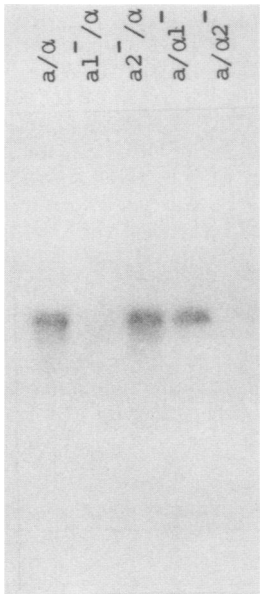

FIG. 4. Both *MAT***a***1* and *MATα2* are required for *SGA* RNA production. Diploid cells carrying *MAT***a**/*MATα* or diploid cells in which one of four *MAT* alleles is mutated were cultured in SPM for 12 h. Poly(A)$^+$ RNA was isolated from these cells, and Northern blot analysis was carried out as described in the text. The following strains were used: EG1-23 × YIY3B (*MAT***a**/*MATα*); 23a20 × YIY3B (*mat***a***1*/*MATα*); 23a7-12 × YIY3B (*mat***a***2*/*MATα*); YIY3A × 23α113 (*MAT***a**/*matα1*); and YIY3A × 23α182 (*MAT***a**/*matα2*).

diploid cells homozygous for the mutant *SGA* produced no glucoamylase activities.

ACKNOWLEDGMENTS

We are grateful to A. Toh-e for helpful discussions and advice. We also thank K. Tatchell for providing *mat* mutants.

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Colonna, W. J., J. M. Gentile, and P. T. Magee.** 1977. Inhibition by sulfanilamide of sporulation in *Saccharomyces cerevisiae*. Can. J. Microbiol. **23**:659–671.
2. **Colonna, W. J., and P. T. Magee.** 1978. Glycogenolytic enzymes in sporulating yeast. J. Bacteriol. **134**:844–853.
3. **Cryer, D. R., R. Eccleshall, and J. Marmur.** 1975. Isolation of yeast DNA. Methods Cell Biol. **12**:39–44.
4. **Esposito, M. S., and R. E. Esposito.** 1974. Genes controlling meiosis and spore formation in yeast. Genetics **78**:215–225.
5. **Jensen, R., G. F. Sprague, Jr., and I. Herskowitz.** 1983. Regulation of yeast mating-type interconversion: feedback control of *HO* gene expression by the mating-type locus. Proc. Natl. Acad. Sci. USA **80**:3035–3039.
6. **Kane, S. M., and R. Roth.** 1974. Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118**:8–14.
7. **Klar, A. J. S., J. N. Strathern, J. R. Broach, and J. B. Hicks.** 1981. Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. Nature (London) **289**:239–244.
8. **Losick, R., and J. Pero.** 1981. Cascades of sigma factors. Cell **25**:582–584.
9. **McMaster, G. K., and G. G. Carmichael.** 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA **74**:4835–4838.
10. **Mortimer, R. K., and D. C. Hawthrone.** 1975. Genetic mapping in yeast. Methods Cell Biol. **11**:221–233.
11. **Nasmyth, K. A., K. Tatchell, B. D. Hall, C. Astell, and M. Smith.** 1981. A position effect in the control of transcription at yeast mating type loci. Nature (London) **289**:244–250.
12. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78**:6354–6358.
13. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
14. **Rine, J., G. G. Sprague, and I. Herskowitz.** 1981. *rmel* mutation of *Saccharomyces cerevisiae*: map position and bypass of mating type locus control of sporulation. Mol. Cell. Biol. **1**:958–960.
15. **Shortle, D., J. E. Haber, and D. Botstein.** 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. Science **217**:371–373.
16. **Simchen, G.** 1974. Are mitotic functions required in meiosis? Genetics **76**:745–753.
17. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
18. **Sprague, G. F., Jr., R. Jensen, and I. Herskowitz.** 1983. Control of yeast cell type by the mating type locus: positive regulation of the α-specific *STE3* gene by the *MATα1* product. Cell **32**:409–415.
19. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.
20. **Suzuki, K., Y. Imai, I. Yamashita, and S. Fukui.** 1983. In vivo ligation of linear DNA molecules to circular forms in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **155**:747–754.
21. **Tanaka, S., S.-Z. Hu, T. S.-F. Wang, and D. Korn.** 1982. Preparation and preliminary characterization of monoclonal antibodies against human DNA polymerase α. J. Biol. Chem. **257**:8386–8390.
22. **Tatchell, K., K. A. Nasmyth, B. D. Hall, C. Astell, and M. Smith.** 1981. In vitro mutation analysis of the mating-type locus in yeast. Cell **27**:25–35.
23. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
24. **Yamashita, I., T. Hatano, and S. Fukui.** 1984. Subunit structure of glucoamylase of *Saccharomyces diastaticus*. Agric. Biol. Chem. **48**:1611–1616.
25. **Yamashita, I., T. Maemura, T. Hatano, and S. Fukui.** 1985. Polymorphic extracellular glucoamylase genes and their evolutionary origin in the yeast *Saccharomyces diastaticus*. J. Bacteriol. **161**:574–582.
26. **Yamashita, I., K. Suzuki, and S. Fukui.** 1985. Nucleotide sequence of the extracellular glucoamylase gene *STA1* in the yeast *Saccharomyces diastaticus*. J. Bacteriol. **161**:567–573.