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Transcriptional Control of Glucoamylase Synthesis in Vegetatively Growing and Sporulating *Saccharomyces* Species

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Three unlinked, homologous genes, *STA1*, *STA2*, and *STA3*, encode the extracellular glycosylated glucoamylase isozymes I, II, and III, respectively, in *Saccharomyces* species. *S. cerevisiae*, which is *sta*^{0} (absence of functional *STA* genes in haploids), does carry a glucoamylase gene, Δ*sta*, expressed only during sporulation (W. J. Colonna and P. T. Magee, J. Bacteriol. 134:844–853, 1978; I. Yamashita and S. Fukui, Mol. Cell. Biol. 5:3069–3073, 1985). In this study we examined some of the physiological and genetic factors that affect glucoamylase expression. It was found that *STA2* strains grown in synthetic medium produce glucoamylase only in the presence of either Maltrin M365 (a mixture of maltooligosaccharides) or starch. Maximal levels of glucoamylase activity were found in cells grown in rich medium supplemented with glycerol plus ethanol, starch, or Maltrin. When various sugars served as carbon sources they all supported glucoamylase synthesis, although at reduced levels. In any given growth medium glucoamylase isozyme II synthesis was modulated by functionality of the mitochondria. Synthesis of glucoamylase is continuous throughout the growth phases, with maximal secretion taking place in the early stationary phase. In the various regimens, the differences in enzyme accumulation are accounted for by differences in the levels of glucoamylase mRNA. Both glucoamylase mRNA and enzyme activity were drastically and coordinately inhibited in *MAT*a/*MAT*α diploids and by the presence of the regulatory gene *STA10*. Both effects were partially overcome when the *STA2* gene was present on a multicopy plasmid. The *STA2* mRNA and glucoamylase were coinduced in sporulating *STA2*/*STA2* diploids. A smaller, coinduced RNA species was also detected by Northern blotting with a *STA2* probe. The same mRNA species was detected in sporulating *sta*^{0} diploids and is likely to encode the sporulation-specific glucoamylase.

In *Saccharomyces diastaticus* three unlinked homologous *STA* genes (*STA1*, *STA2*, and *STA3*) encode the extracellular glycosylated glucoamylase (GA) isozymes I, II, and III, respectively (30). *S. diastaticus* is closely related to the GA-nonsecreting *S. cerevisiae*, a major difference being the acquisition of the *STA* genes (33). Haploid cells of the opposite mating type of these two species can diploidize and give rise to viable spores. Some laboratory strains of *S. cerevisiae* carry a gene(s) (*STA10* [24], *INH1* [31, 33]) inhibitory to GA production. Although haploid or diploid *S. cerevisiae* strains do not synthesize extracellular GA, they produce an intracellular sporulation-specific GA (6, 7). The GA gene of *S. cerevisiae* that encodes the sporulation-specific GA (Δ*sta*) has been characterized (32, 33) and shown to be closely related to extensive regions of the *STA* genes.

We have recently cloned the *STA2* gene from *S. diastaticus* (25a) and confirmed that the cloned fragment carried the *STA2* gene by one-step integrative gene disruption and gene fusion experiments. Bacterial strains transformed with plasmids bearing gene fusions between *Escherichia coli lacZ* and yeast *STA2* produced hybrid proteins of the expected molecular weight that were detected by immunoblotting with anti-GAII antibodies.

In this paper we analyze some of the genetic and physiological factors affecting GA expression. We show that GA synthesis is continuous throughout the growth phases and that any of the following modulates GA expression primarily at the level of mRNA accumulation: the carbon and nitrogen sources, the presence of functional mitochondria, the presence of the *STA10* gene (found in most *S. cerevisiae* strains), and the mating-type configuration. In *STA2*/*STA2* *MAT*a/*MAT*α diploids GA expression is repressed, but when sporulated, GA mRNA and enzyme activity are restored in a time-dependent manner. A 2.0-kilobase (kb) mRNA and its encoded sporulation-specific GA are coinduced and detectable in *sta*^{0} sporulating diploids as well.

MATERIALS AND METHODS

Strains, media, and genetic methods. The yeast strains used and their relevant genotypes are listed in Table 1.

YP medium containing 1% yeast extract and 2% Bacto-Peptone (Difco Laboratories, Detroit, Mich.) was supplemented with 2% glucose (YPD), 3% glycerol plus 2% ethanol (YPGE), 3% soluble starch (YPS), 2% raffinose (YPR), 2% sucrose (YPSuc), 2% Maltrin M365 (YPMLT), 2% maltose (YPM), or 2% galactose (YPGal). Synthetic complete (SC) media (27) SCD, SCGE, SCS, and SCMLT contained the same carbon sources as YPD, YPGE, YPS, and YPMLT, respectively. Maltrin M365 was obtained from Grain Processing Corp., Muscatine, Iowa. Cultures of cells transformed with plasmid YEp13 or derived vectors were grown on SC media lacking leucine. The Sta$^+$ phenotype was detected by halo formation on either a solid complex medium (YPSB) or a solid synthetic medium (SCSB) by incubating the plates at 30°C for 4 to 6 days and then at 4°C for 1 day. YPSB and SCSB are identical to YPS and SCS, respectively, except for the addition of 3.3×10^{-3}% bromocresol purple. Solid medium contains 2% agar.

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TABLE 1. *Saccharomyces* strains used in this study

Laboratory designation	Sta phenotype	Relevant genotype	Source
JM2099	+	*MATa STA2 sta10 arg4*	H. Tamaki (5206-1B)
JM2340	+	*MATα STA2 arg4 thr1*	M. Wiggs (SPX101-6D)
JM2773-15B[a]	−	*MATα sta⁰ sta10 his3*	J. Marmur
JM2769-4D	−	*MATa STA2 STA10*	J. Marmur
DH22	−	*MATa STA2 sta10* / *MATα STA2 sta10*	J. Marmur
DMV49	−	*MATa sta⁰ sta10 leu2 ura3* / *MATα sta⁰ sta10 leu2 ura3*	This work
DP20	+	*MATα STA2 sta10* / *MATα sta2 sta10*	This work
DP210	−	*MATα STA2 sta10* / *MATα sta2 sta10*	This work

[a] Strain JM2773-15B and the haploid parents of DMV49 are Sta⁻ segregants of a cross between a strain carrying *STA2* and a strain carrying *STA3*. *STA10* was derived initially from S288C *STA10*.

Presporulation medium contained 6.7 g of yeast nitrogen base, 1 g of yeast extract, and 10 g of potassium acetate in 1 liter of 50 mM potassium phthalate buffer, pH 5.1. Sporulation medium contained 3 g of potassium acetate and 0.2 g of raffinose in 1 liter of deionized water (7). Standard genetic methods of mating, purifying, and selecting diploids, sporulation, and dissection were carried out by the methods of Sherman et al. (27).

All the *sta10* strains used in this study were derived from strains described by Tamaki (30). Diploid strains homozygous for *MATα* were constructed from haploids with complementary nutritional requirements by a modification of the protoplast fusion technique described by Gunge (12), followed by selection for prototrophy.

Haploid and diploid strains (Table 1) were transformed with the plasmid pSA3 by the lithium sulfate procedure of Ito et al. (14). Plasmid pSA3 was constructed by standard procedures (18) by inserting an 8.3-kilobase-pair *Bgl*II fragment containing the *STA2* gene (25a) into the *Bam*HI cloning site of the multicopy plasmid YEp13.

Respiratory-deficient, [*rho*⁰] petites were generated by treating the *STA2 Saccharomyces* strains (Table 1) JM2099 and JM2340 with ethidium bromide (27).

Assay for GA activity. Cells were grown at 30°C in a shaking water bath in various media for 60 h. Samples of culture supernatants or cell extracts, prepared by vortexing yeast cells with glass beads, were assayed for extracellular and cell-associated GA activity, respectively. The glucose released from 10 mM maltotriose in 100 mM sodium acetate buffer (pH 4.6) was monitored as described previously (D. Modena, M. Vanoni, S. Englard, and J. Marmur, Arch. Biochem. Biophys., in press). Values obtained for both extracellular and cell-associated activity were normalized for the number of cells and expressed either as the percentage of total GA activity or that of an appropriate control. Spheroplasts were prepared as described by Ide and Saunders (13), using Zymolyase 60000 (Kirin Brewery, Tokyo, Japan). GA activity was assayed in spheroplasting buffer, in intact spheroplasts (buffer supplemented with 0.6 M KCl), and in the osmotically lysed spheroplast pellet. Protein content was estimated by using a Bio-Rad assay kit based on the method of Bradford (4). Bovine serum albumin was used as a standard.

Electrophoresis and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described by Laemmli (16). Mixtures of five proteins were used as size markers (myosin, 200 kilodaltons [kDa]; phosphorylase *b*, 94 kDa, bovine serum albumin, 67 kDa, catalase, 60 kDa; lactic dehydrogenase, 36 kDa). Proteins were electrophoretically transferred to nitrocellulose from SDS-PAGE in a Bio-Rad Transblot Cell in 25 mM Tris (pH 8.3)–192 mM glycine–20% methanol (vol/vol). The proteins were immunologically detected on nitrocellulose with polyclonal anti-GAII antibodies (Modena et al., in press) and horseradish peroxidase-conjugated antibodies. Nonfat milk powder (5%) in phosphate-buffered saline was used as a blocking agent.

Northern and slot-blot hybridizations. RNA for Northern and slot-blot analyses was isolated as described by Elion and Warner (9). Separation of total RNA was carried out on 1.5% agarose gels in 0.01 M sodium phosphate buffer (pH 6.5) containing 6% formaldehyde, followed by blotting onto nitrocellulose; DNA-RNA hybridizations were carried out as described by Pearson et al. (23).

For slot-blot analyses, appropriate amounts of RNA were adsorbed to nitrocellulose with a slot-blot apparatus (Schleicher & Schuell, Inc., Keene, N.H.) according to the instructions of the supplier. The drying, prehybridization, and hybridization procedures were the same as for Northern blot analysis. Each slot blot was probed with both a 1.45-kb *Eco*RI-*Bam*HI *STA2* probe internal to the *STA2* structural gene and a 1.75-kb *Bam*HI-*Hin*dIII actin gene probe (20) (kindly provided by C. Michels). The fragments were isolated from a 1% low-gelling-temperature agarose gel (Sigma Chemical Co., St. Louis, Mo.) and purified with the Elutip-d column (Schleicher & Schuell, Inc.) as described by the supplier. The purified DNA fragments were labeled with ^{32}P by nick translation (26) and heat denaturated before their use as probes. Quantitation of *STA2* and actin mRNA was carried out by densitometric scanning of the slot-blot autoradiographs with a Joyce Loebel Chromoscan 3 densitometer. Steady-state levels of *STA2* mRNA were normalized relative to the actin mRNA levels. The data reported in Tables 2 and 3 are expressed as ratios relative to the values found for the appropriate control, as explained in the table legends and in the text.

RESULTS AND DISCUSSION

Effect of growth medium composition on *STA2* gene expression. The accumulation of GA was examined in culture supernatants and cell extracts of strain JM2099 carrying the *STA2* gene and grown in rich and synthetic media supplemented with various carbon sources. Cells were inoculated from a fresh culture and grown at 30°C in a shaking water bath. After 60 to 70 h the cells were collected by centrifugation, and the levels of GA activity released in the growth medium and associated with the cells were determined. GA activity was barely detectable when cultures were grown in SC media supplemented with either glucose or glycerol plus ethanol as a carbon source. GA activity was induced when a maltooligosaccharide mixture, Maltrin, or soluble starch was added as a carbon source. When added to complex media, however, neither Maltrin nor starch was found to induce GA synthesis above the levels found in YPGE-grown cells. GA was also synthesized when cells were grown in rich medium supplemented with sucrose, glucose, maltose, galactose, or raffinose. In all cases the total activity was reduced (25 to 70%) compared with the maximal activity found in YPGE-grown cells (Table 2). The fraction of GA released into the

growth medium was also dependent on the carbon source. Typical values are reported in Table 2. In no case did stationary-phase yeast cells release more than 40% of the total GA synthesized into the growth medium.

To determine the basis for this carbon-source-dependent accumulation of GA protein, we examined the levels of *STA2* mRNA. Total RNA was prepared from cells grown for 60 to 70 h in the different media and hybridized with *STA2* and actin gene probes. This was carried out either after electrophoretic separation and blotting onto nitrocellulose membranes (Northern analysis) or directly by adsorbing onto nitrocellulose membranes (slot-blot analysis). An example of the Northern and slot-blot analysis of RNA samples prepared from cells grown in YPGE, YPMLT, YPS, and SCGE is shown in Fig. 1. The steady-state levels of *STA2* mRNA were calculated after densitometric scanning of the autoradiograms and normalization relative to the actin mRNA levels determined in the same way. The levels of *STA2* mRNA reported in Table 2 are relative to the levels found in YPGE-grown cells and indicate that the levels of both *STA2* mRNA and GA activity were coordinately regulated. It would thus appear that *STA2* gene regulation is primarily at the level of mRNA accumulation. This conclusion is supported by the coordinate reduction of GA mRNA and cell-associated GA enzymatic activity observed when 2% glucose is added to cells grown for 48 h in YPGE. When the cells were transferred into a medium containing starch, no effect on GA expression was observed (data not shown).

TABLE 2. Effect of growth medium composition on *STA2* expression in [*rho*$^+$] and [*rho*^{0}] strains[a]

Medium	[*rho*] genotype	Cell density ($\times 10^8$)	% Cell-associated GA activity	Levels relative to *STA2* haploid in YPGE (%)	
				Total GA activity	GA mRNA
YPD	+	7.0	86	30	40
	0	2.7	81	10	12
YPMal	+	6.6	71	60.2	ND[b]
	0	0.8	60	45	ND
YPGal	+	6.3	75	61	38
	0	0.8	74	23	ND
YPSuc	+	4.8	61	10	9
	0	3.1	63	27	10
YPS	+	2.5	69	89	95
	0		NG[c]		
YPR	+	0.5	86	36	24
	0		NG		
YPMLT	+	3.7	66	100	98
	0	0.8	66	71	63
SCMLT	+	1.0	43	22	16
	0	0.4	73	51	23
SCGE	+	1.6	100	5	6
	0		NG		
SCS	+	0.9	100	22	18
	0		NG		

[a] Both strains were grown in the various media for 60 to 70 h and processed for enzyme activity and mRNA levels as indicated in the text. The values for the GA activity have been normalized on a per cell basis, and the levels of *STA2* mRNA have been normalized to the levels of actin mRNA. Both are expressed as a percentage of either the levels of GA activity (3.3×10^{-10} units per cell) or *STA2* mRNA found in strain JM2099 grown for 60 h in YPGE medium.

[b] ND, Not determined.

[c] NG, No growth.

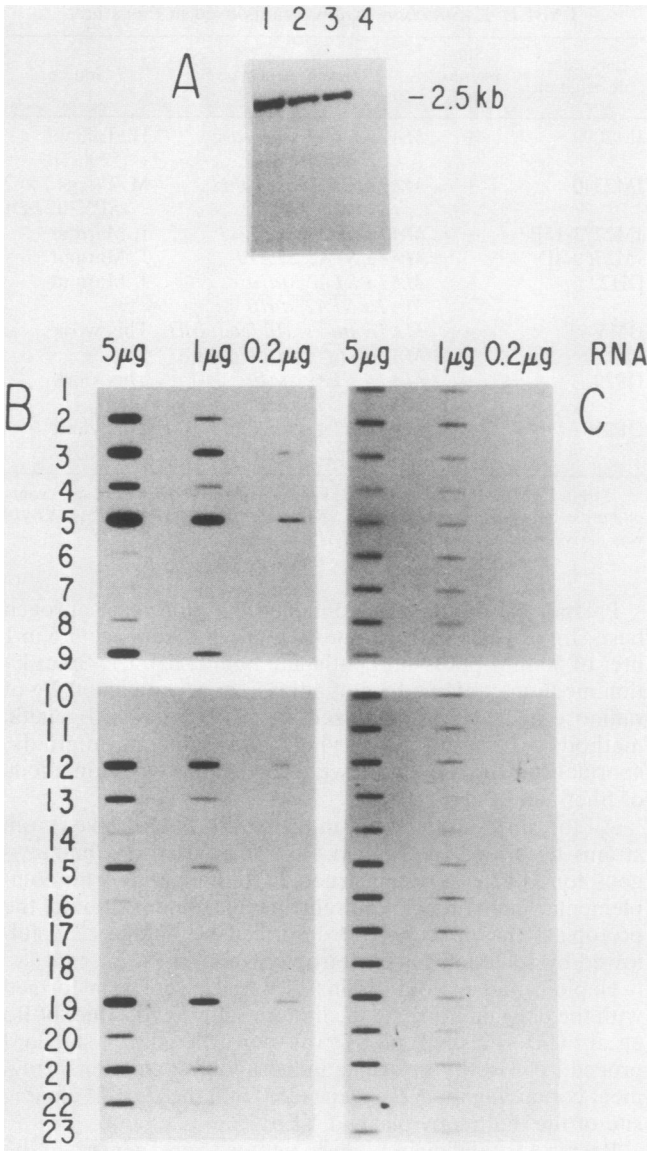

FIG. 1. (A) Northern blot analysis of *STA2* transcripts prepared from yeast cells grown under different growth conditions. Lanes: 1, YPS; 2, YPGE; 3, YPMLT; 4, SCGE. (B) Slot-blot analysis of *STA2* mRNA prepared from the strains listed in Table 3 (lanes 1 to 9) and from [*rho*$^+$] and [*rho*$^-$] strains listed in Table 2 (lanes 10 to 23) and grown on different media. Three concentrations of RNA (5.0, 1.0, and 0.2 μg) from each strain were loaded onto the slot-blot apparatus, transferred to nitrocellulose, dried, and hybridized to the *STA2* gene probe. All the strains in lanes 1 to 9 were grown on YPGE. Lanes: 1, JM2773-15B; 2, DH22(pSA3); 3, JM2099; 4, DP20; 5, JM2773-15B(pSA3); 6, DH22; 7, JM2769-4D; 8, DP210; 9, JM2769-4D. All the strains in lanes 10 to 23 were either *STA2* [*rho*$^+$] or *STA2* [*rho*^{0}]. Lanes: 10, [*rho*$^+$] in SCGE; 11, [*rho*$^+$] in YPSuc; 12, [*rho*^{0}] in YPSuc; 13, [*rho*$^+$] in YPGE; 14, [*rho*$^+$] in YPD; 15, [*rho*^{0}] in SCMLT; 16, [*rho*^{0}] in YPMLT; 17, [*rho*$^+$] in SCS; 18, [*rho*^{0}] in YPD; 19, [*rho*$^+$] in SCMLT; 20, [*rho*$^+$] in YPMLT; 21, [*rho*$^+$] in YPR; 22, [*rho*$^+$] in YPS; 23, [*rho*$^+$] in YPGal. (C) Identical slot blots were hybridized to the actin gene probe that served as an internal standard.

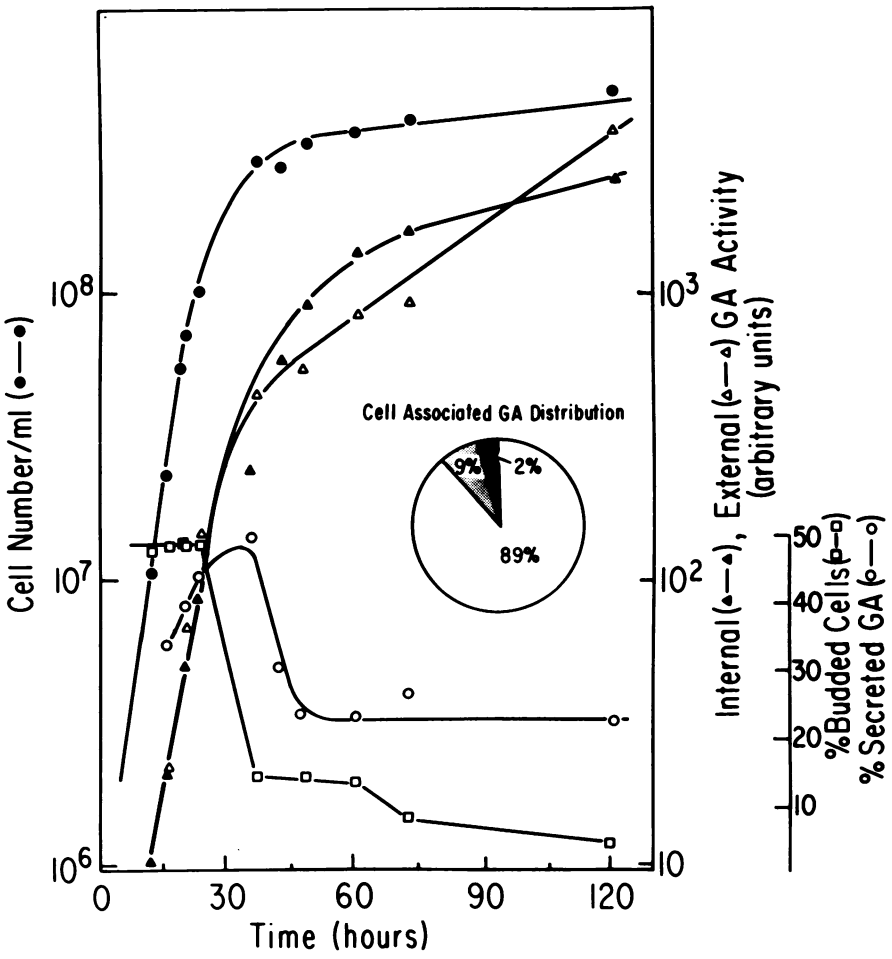

FIG. 2. Synthesis and secretion of GA during the growth cycle of *S. diastaticus* JM2099 (*STA2*). Cells were grown in shake flasks containing YPGE medium. Growth of the cultures was monitored by counting the cell number (●) and the fraction of budded cells (□). GA activity was measured both in the cell medium (△) and in cell extracts (▲). The units used for cell-associated and external GA activity are different so they can fit onto the same scale. The percentage of secreted GA (○) was calculated from the actual values. The insert shows the distribution of GA activity in the periplasmic space (white), membrane (grey), and cytosol (black) of a sample collected after 36 h.

The fact that all the Sta^+ strains examined in this study were found to synthesize and secrete significant amounts of GAII in YPD medium, albeit at reduced levels compared with those of the same strain grown in YPGE medium, was surprising since glucose brings about carbon catabolite repression of a variety of yeast enzymes and permeases involved in sugar catabolism. These include invertase (11, 22), alcohol dehydrogenase (17), maltase and maltose permease (3, 8), α-galactosidase, and the enzymes of the Leloir pathway (1, 25). Enzymes involved in the metabolism of starch and related compounds are known to be subject to catabolite repressions in several yeast genera, and mutants derepressed for the synthesis of the starch-degrading enzyme α-amylase have been isolated in *Schwannomyces* species (19). GA synthesis is severely carbon catabolite repressed by glucose in *Neurospora crassa* (28), whereas *Aspergillus awamori* produces significant amounts of enzyme when grown in glucose-containing media. In this organism, as in *Saccharomyces* species, the differences in enzyme levels among cells grown in different media are accounted for by differences in the level of GA mRNA (21).

An analysis of GA expression was also performed on a [*rho*^{0}] petite derivative of strain JM2099. The strain did not grow on nonfermentable carbon sources or on YPS, SCS, or YPR medium. When the samples were collected for GA and mRNA determination, the cell density was always lower than that of the grande parent grown in the same medium that supported their growth (Table 2). The total GAII activity and *STA2* mRNA levels of the respiratory-deficient strain were reduced compared with those of the [rho^+] parent in YPD, YPM, YPGal, and YPMLT media. In contrast, respiratory-deficient cells reproducibly produced more GAII than grande cells when grown in both SCMLT and YPSuc. The involvement of mitochondrial functions in the metabolism of several fermentable carbon sources, e.g., galactose and maltose, has been previously reported (2, 10, 15). Although the biochemical basis of this nuclear-mitochondrial interaction has not been elucidated, the role of specific nuclear genes has been clearly established.

GA synthesis: distribution and relationship to growth phase. The synthesis and secretion of GA by the JM2099 (*STA2*) strain were studied as a function of the growth curve. Cells were grown in YPGE medium on a rotary shaker at 30°C. The levels of GA activity released into the growth medium and associated with the cells were assayed over a 5-day period. Figure 1 shows that GA activity was detectable as early as 12 and 16 h after inoculation in broken cells and in the growth medium, respectively. The fraction of secreted

TABLE 3. Effect of *STA10* and mating-type configuration on *STA2* gene expression[a]

Strain	Relevant genotype	Ploidy	% Cell-associated GA activity	Levels relative to *STA2* haploid (%)	
				Total GA activity	GA mRNA
JM2773-15B	*sta2 sta10*	n	ND[b]	ND	ND
JM2769-4D	*STA2 STA10*	n	>95	<5	<5
DP210	$\frac{MAT\alpha\ STA2\ STA10}{MAT\alpha\ sta2\ sta10}$	2n	82	50	30
DP20	$\frac{MAT\alpha\ STA2\ sta10}{MAT\alpha\ sta2\ sta10}$	2n	83	70	50
DH22	$\frac{MATa\ STA2\ sta10}{MAT\alpha\ STA2\ sta10}$	2n	95	<10	<10
JM2773-15B(pSA3)	*sta2 sta10*(pSA3)[c]	n	92	140	125
JM2769-4D(pSA3)	*STA2 STA10*(pSA3)	n	>95	43	55
DH22(pSA3)	$\frac{MATa\ STA2\ sta10}{MAT\alpha\ STA2\ sta10}$(pSA3)	2n	88	56	60

[a] Cells were grown for 60 h in YPGE medium unless otherwise indicated. The levels of GA activity and *STA2* mRNA have been normalized and expressed as indicated in Table 2, footnote *a*.

[b] ND, Not detectable.

[c] Transformants were grown to the early stationary phase in SCGE minus leucine, transferred for about 12 h in YPGE, collected, and assayed for enzyme activity and mRNA levels.

GA increased during the exponential phase of growth, reaching a maximum of 50% of total GA synthesized after 36 h (Fig. 2). At this time the yeast culture was just entering the stationary phase as judged by the sharp drop in the fraction of budded cells (Fig. 2, open squares). Thereafter, the amount of GA secreted in the growth medium decreased to a level of about 20% of total enzyme and then remained constant until at least 120 h (Fig. 2, open circles). The cell wall of stationary-phase cells is known to undergo complex modifications, becoming more rigid and less accessible to glucanases (5, 35), and may account, at least partially, for the reduced fraction of synthesized GA that is released in the growth medium. A simple sieving hypothesis cannot explain the pattern of secretion of glycoproteins, since proteins smaller than GA such as invertase remain in the periplasmic space and are not released into the medium. It is possible, though, that invertase forms large aggregates in the periplasmic space, preventing its excretion into the medium. Whether the information required to release a glycoprotein in the growth medium or for it to be retained in the periplasmic space is inherent in the amino acid sequence or due to posttranslation modification of the protein or the nature of carbohydrate moiety is not known.

To localize cell-associated GAII, yeast cells were collected in the early stationary phase of growth (at 36 h), treated with Zymolyase to remove the cell wall, and assayed for enzyme activity in the spheroplasting buffer, in the intact spheroplast, and in the pellet of osmotically lysed spheroplasts. The spheroplast treatment resulted in the release of about 90% of cell-bound GAII activity in the buffer after centrifugation, indicating that the cell-associated enzyme is primarily periplasmic. The remaining activity was mostly membrane bound. In fact, only about 2% of the cell-associated activity was found in the lysis buffer after disruption of the spheroplast and centrifugation of the membranes (Fig. 2, insert). The values for the intracellular enzyme levels determined by enzymatic and mechanical disruption were found to correspond closely, differing by less than 10%.

Effect of *STA10* gene on GA expression. To study the effect of the *STA10* gene on GA expression, haploid and diploid strains homozygous for the mating-type locus carrying both a *STA2* and a *STA10* gene were constructed by standard techniques. The relevant genotypes of the strains are listed in Table 1. JM2769-4D (*STA2 STA10*) has a level of *STA2* mRNA and a total GA activity less than 5% of that found in JM2099 (*STA2 sta10* (Table 3). Since the levels of GA activity in the growth medium were barely detectable, it is uncertain whether the altered distribution of the greatly reduced total GA activity is physiologically significant, although these values have been reproducibly found in at least three independent experiments.

When the *STA10* gene was present in the heterozygous state in the *MATa*/*MAT*α strain DP210, a less dramatic inhibition of *STA2* expression (expressed both as mRNA and GA enzyme activity levels) was found, suggesting a gene dosage effect for the *STA10* gene. The repressive effect by the *STA10* gene was overcome when the *STA2* gene was introduced into the *STA2 STA10* haploid strain JM2769-4D on the YEp13-based multicopy plasmid pSA3. The *STA2 STA10* haploid strain transformed with pSA3 showed a level of *STA2* mRNA that was at least 10-fold higher than the level found in the untransformed recipient strain, but still significantly lower than that of the haploid *sta*^{0} strain JM2773-15B harboring the same plasmid. One interpretation of this effect is that the *STA10* gene encodes a protein that represses GA synthesis and whose intracellular concentration is not high enough to fully control the multicopy *STA2* genes. Another possibility, that *STA10* is the inactive allelic form of a positive activator of GA expression rather than a repressor, cannot at present be rigorously ruled out. If *STA2* is in fact transcribed at low levels in the absence of any functional

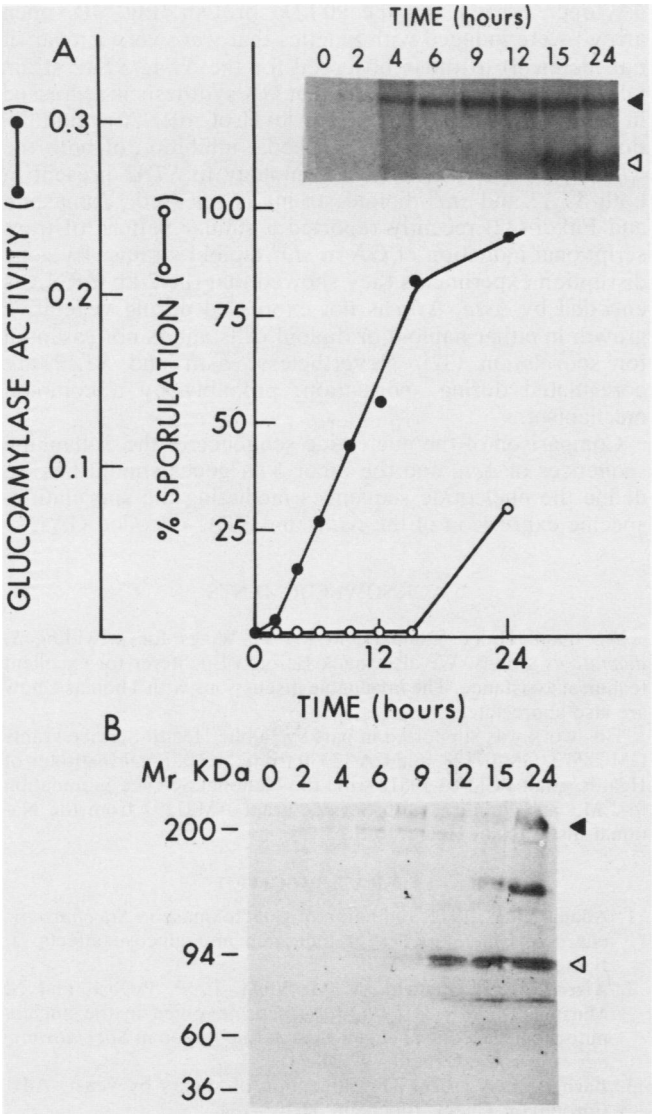

FIG. 3. Sporulation-specific induction of GA. (A) GA-specific activity (●) and the fraction of four-celled asci (○) were monitored in the *STA2/STA2* diploid strain DH22 at the indicated time intervals after cells were shifted into sporulation medium (t = 0). Parallel samples were processed for mRNA isolation and subjected to Northern analysis with a *STA2* structural gene fragment as a probe (insert). (B) Total cell proteins were prepared at the same time, separated by SDS-PAGE, and transferred to nitrocellulose. The blots were probed with anti-GAII polyclonal antibodies. GA activity is expressed as micromoles of glucose released per hour per milligram of protein. The dark arrows are *STA2* GA mRNA or GA; the open arrows are Δ*sta* GA mRNA or its encoded GA.

positive activator, additive basal transcription of the *STA2* gene on a multicopy plasmid would result in increased levels of both *STA2* mRNA and GA activity. These data again indicate that *STA10* affects GA synthesis mainly at the level of *STA2* mRNA accumulation, although minor effects on secretion cannot at present be ruled out.

Effect of mating-type configuration on vegetatively growing and sporulating diploid Sta$^+$ and Sta$^-$ cells. *STA2* diploids either homozygous or heterozygous at the *MAT* locus were grown in YPGE medium as described above, and the levels of GA activity and *STA2* mRNA were determined. The results of these experiments are summarized in Table 3. In the diploid strain DH22 (*MAT***a**/*MAT*α *STA2/STA2*), inhibition of the levels of GA activity and of *STA2* mRNA were detected. These levels were less than 10% of those found in the *STA2* haploid strain JM2099 grown under the same conditions. No inhibition of activity or of *STA2* mRNA accumulation was found in the diploid strain DP20 that is homozygous for the mating-type locus but heterozygous for the *STA2* gene (Table 3). Transformation of DH22 with a *STA2* gene on a YEp13-derived multicopy plasmid resulted in an almost complete release of the inhibition of *STA* gene expression in *MAT***a**/*MAT*α diploids, monitored by both *STA2* mRNA levels and GA activity (Table 3). These increased levels are probably the result of the titration of a negative factor(s) that represses *STA2* gene transcription in the diploids. In *MAT***a**/*MAT*α diploids the role of the *MAT* locus has not as yet been fully elucidated. Current models propose that the products of the *MAT***a***1* and *MAT*α*2* genes act to repress mRNA production, probably at the level of transcription, of several *MAT***a**/*MAT*α-controlled genes including *HO*, *STE5*, and *MAT*α*1* (whose product is required to activate α-specific genes), as well as the mRNAs produced from some Ty1 elements and from genes that have become linked to Ty1 elements (reviewed in reference 29).

Recently, a possible role for the *MAT***a***2* gene product in repression of *MAT***a**/*MAT*α-controlled genes has been proposed by Yamashita et al. (34). They reported that a functional *MAT***a***2* gene turns off *STA1* expression in *MAT***a**/*MAT*α diploids at the posttranscriptional level. In the case of *STA2* gene expression in *MAT***a**/*MAT*α *STA2/STA2* diploids, we found that both GA mRNA and GA activity were severely repressed (Table 3). Possibly some regulatory sequence(s) upstream of the GA genes has been altered in such a way that transcription of *STA1* is no longer inhibited in *MAT***a**/*MAT*α *STA1/STA1* diploids. The original cosmid isolate carrying the cloned *STA2* gene (25a) was examined to determine whether it also has Ty1 sequences associated with it; however, none were detected (I. Pretorius, unpublished data).

Release from the mating-type-dependent inhibition of GA synthesis was also achieved by inducing sporulation in the *MAT***a**/*MAT*α *STA2/STA2* diploid. Strain DH22 was grown to the mid-logarithmic phase in YPD and transferred to presporulation medium at a cell density of 10^5/ml. After six to seven generations (at 1×10^7 to 2×10^7 cells per ml), the cells were collected by centrifugation, washed with sterile water, and suspended in sporulation medium at the same density. Samples were collected after various intervals and processed in the usual way for Northern and GA activity analyses. The results (Fig. 3) indicate that GA activity and a 2.5-kb *STA2* mRNA are coinduced and clearly detectable within 4 h after transfer to the sporulation medium. A second, smaller-sized mRNA species with homology to the *STA2* probe was also induced. The latter is most likely the transcript of the Δ*sta* sporulation-specific GA gene (6, 7, 32, 33) and will be discussed later.

The synthesis of GA and immunologically related proteins was studied by immunoblotting with anti-GAII polyclonal antibodies. Equal amounts of protein (150 μg) were separated by 5 to 12% gradient SDS-PAGE, blotted onto nitrocellulose, and analyzed with rabbit polyclonal anti-GAII antibodies as described in Materials and Methods. Several cross-reacting protein bands were present in nonsporulating cells and are most likely due to cross-reaction of the antiserum with the carbohydrate chains of unrelated yeast glycoproteins (D. Modena et al., Arch. Biochem. Biophys., in press). Two major proteins, however, appear to be

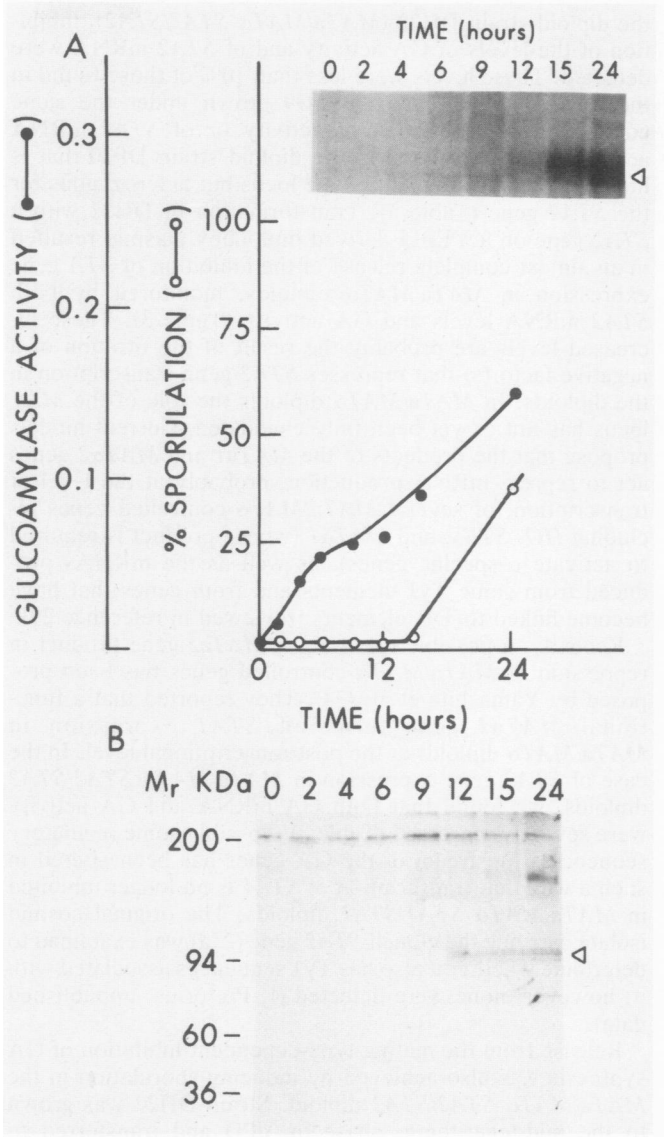

FIG. 4. Sporulation-specific induction of GA. (A) GA-specific activity (●) and the fraction of four-celled asci (○) were monitored in the sta^0/sta^0 strain DMV49 at the indicated time intervals after cells were transferred into sporulation medium (t = 0). Parallel samples were processed for mRNA isolation and subjected to Northern analysis with a *STA2* structural gene fragment as a probe (insert). (B) Total cell proteins were prepared at the same time, separated by SDS-PAGE, and transferred to nitrocellulose. The blots were probed with anti-GAII polyclonal antibodies. The open arrows indicate Δ*sta* mRNA (A) and Δ*sta* GA (B). GA activity is expressed as micromoles of glucose released per hour per milligram of protein.

specifically induced during sporulation: a species of about 300 kDa comigrating with GAII and a polypeptide of about 90 kDa, possibly encoded by the 2.0-kb mRNA. To further investigate this point, the *MAT***a**/*MAT*α sta^0/sta^0 diploid strain DMV49 was induced to sporulate, and the sporulation-dependent accumulation of GA activity, its mRNA, and protein immunoblotted with anti-GAII antibodies were examined. As sporulation proceeded, GA activity was induced (Fig. 4) and the pattern of accumulation was very similar to that observed for the *STA2* strain DH22. As expected, neither the 2.5-kb *STA2* mRNA nor the 300-kDa GAII protein was coinduced. However, the 2.0-kb mRNA (Fig. 4A, open arrow) and the 90-kDa protein (Fig. 4B, open arrow) were induced with kinetics that were very similar (if not identical) to those observed for the *STA2*/*STA2* strain DH22. These results indicate that GA synthesis is repressed in *MAT***a**/*MAT*α diploids at the level of mRNA accumulation. Thus, as sporulation proceeds, inhibition of both the *STA2* gene and a gene with homology to *STA2* present in both *STA2* and sta^0 diploid strains is released. Yamashita and Fukui (32) recently reported a similar pattern of transcriptional induction of GA in sta^0 diploid strains. By gene disruption experiments they showed that the 2-kb mRNA is encoded by Δ*sta*. Δ*sta* is not expressed during vegetative growth in either haploid or diploid cells and is not essential for sporulation (32). Nevertheless, Δ*sta* and *STA2* are coregulated during sporulation, probably by a common mechanism.

Comparison of the nucleotide sequence of the 5′-flanking sequences of Δ*sta* and the other *STA* genes should help to define the nucleotide sequences mediating the sporulation-specific expression of the Δ*sta*- and *STA2*-encoded GAs.

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