Cloning of the *STA2* and *SGA* genes encoding glucoamylases in yeasts and regulation of their expression by the *STA10* gene of *Saccharomyces cerevisiae*

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ABSTRACT

The *Saccharomyces STA2* and *SGA* genes, encoding the extracellular and intracellular sporulation-specific glucoamylase respectively, have been cloned and their transcription and regulation studied. The *STA2* gene differs from the *SGA* gene in that it contains an extra piece of DNA, which encodes the domain for exportation of the extracellular glucoamylase. The *STA2* gene produces a single 2.85 kb transcript. Transcription of the *SGA* gene is initiated from two different sites, yielding two transcripts of 1.95 and 2.40 kb. Transcription of both *STA2* and *SGA* genes is repressed by the *STA10* gene of *Saccharomyces cerevisiae*.

INTRODUCTION

Saccharomyces diastaticus expresses an extracellular glucoamylase which is encoded by either the *STA1*, *STA2* or *STA3* nuclear genes (1,2). Recently, the *STA1* and *STA3* genes from *S. diastaticus* have been cloned and the *STA1* gene sequenced (3,4). It has been suggested that the extracellular glucoamylase encoded by this last gene is translated as a polypeptide of 778 aminoacids containing two domains: one comprising the NH₂-terminal 388 aminoacids that would serve as a signal peptide eliminated during export, and the other one corresponding to the two subunits of the mature extracellular glucoamylase (5). In contrast to *S. diastaticus*, *S. cerevisiae* lacks these genes and, therefore, can not use starch as a carbon source (6). Nevertheless, both *S. cerevisiae* and *S. diastaticus* contain a common DNA sequence highly homologous to the *STA1* gene (4,7). It was shown that this homologous sequence encoded the sporulation-specific glucoamylase of *S. cerevisiae* (8,9,4,10). Moreover, it has been proposed that the extracellular glucoamylase gene of *S. diastaticus* could have evolved from the sporulation-specific glucoamylase gene (*SGA*) of

S. cerevisiae (4) by the acquisition of extra DNA to encode the export domain of the extracellular glucoamylase.

Interestingly, S. cerevisiae contains the STA10 gene which, by genetic studies, was shown to dominantly and epistatically repress the expression of the STA1, 2 and 3 genes in S. cerevisiae x S. diastaticus haploid hybrid strains (6).

In this work we have studied the transcriptional products of both STA2 and SGA genes. Moreover, we have examined, both biochemically and genetically, the STA10-dependent repression of these two genes at the level of transcription in a variety of yeast strains.

MATERIALS AND METHODS

Yeast and bacterial strains and growth conditions

The yeast strains used in this work, their origins and genotypes are listed in Table I. STA⁺ strains were derivatives of those described by Tamaki (1). Escherichia coli HB101 (11) was used for transformation and plasmid amplification.

Yeast cells were grown on YEPD (1% yeast extract, 2% peptone, 2% glucose) or YNB (0.7% yeast nitrogen base w/o aminoacids, 2% glucose, supplemented with 40 µg/ml aminoacids when required) media.

STA⁺ phenotype was scored on plates containing YPS medium (1% yeast extract, 2% peptone, 2% soluble starch, 2% agar). Colonies were transferred to this medium and incubated for 3-5 days before starch was precipitated by keeping the plates at 4°C for 1-2 days. A clear halo appeared around the STA⁺ colonies.

L medium (12) was used for culturing bacterial cells and, when appropriate, it was supplemented with 100 µg/ml ampicillin or 15 µg/ml tetracycline.

Genetic procedures

Standard yeast genetic procedures were followed for crossing, sporulation and tetrad analysis (13).

DNA isolation and cloning procedures

Yeast transformation was carried out as described by Hinnen et al. (14). Genomic and plasmid DNA of yeast were isolated as described by Sherman et al. (15). Bacterial transformation and

plasmid isolation were performed as described by Maniatis et al. (11).

Genomic DNA from yeast strain SPX101-1c (a starch-fermenting, STA10 repressor-free strain) was partially digested with Sau3AI and the resulting fragments were separated by low melting point agarose gel electrophoresis. DNA fragments larger than 10 kb were extracted following a cetyl-trimethyl-ammonium chloride-assisted method (16) and were ligated to BamHI-restricted and calf intestinal alkaline phosphatase-treated YEpl3 (17). The ligation mixture was used to transform E. coli HB101. Clones were selected on ampicillin plates. Transformants were pooled and plasmid DNA was isolated from them as indicated above. Yeast spheroplasts from strain MCCX1-5d (a starch-non fermenting, STA10 repressor-free strain; Table I) were transformed with the gene bank. LEU⁺ STA⁺ transformants were selected on regeneration agar plus starch plates (0.7% yeast nitrogen base w/o amino-acids, 0.4% YEPD, 2% soluble starch, 0.5% glucose, 1.2 M sorbitol, 3% agar). Plates were incubated 10-15 days at 30°C and then cooled at 4°C for 2-3 days after which starch-fermenting colonies were detected by the clear halos of solubilized starch.

Internal glucoamylase assay

Cells were grown in liquid YEPD medium at 30°C with shaking. At intervals, 20 ml samples were collected and filtered on Millipore membranes, washed with 50 ml of 50 mM phosphate buffer, pH 5.0, and frozen at -20°C until required. For the glucoamylase assay, cells were thawed in phosphate buffer and permeabilized by vortex-mixing with a 1/10 vol of toluene/ethanol (1:1). Glucoamylase activity was estimated by glucose production from 1% maltotriose (18) in 50 mM phosphate buffer, pH 5.0, after 60 min incubation at 40°C. Reactions were stopped by boiling for 10 min after which the samples were clarified by centrifugation and glucose was measured in the supernatants with a coupled assay system containing glucose oxidase, peroxidase and O-dianisidine (Sigma Technical Bulletin NQ 510) for 30 min at 40°C.

Preparation of Poly(A)⁺ RNA

Spheroplasts of late exponential phase cells were lysed by mixing in a Vortex mixer with 25 mM Tris/HCl pH 7.4, 0.1 M NaCl,

10 mM EDTA, 1% SDS and 1mg/ml heparin. The lysate was extracted three times with phenol and chloroform. The supernatant was made 0.3M with NH_4Cl and nucleic acids were precipitated with 2 vols of ethanol. The RNA was dissolved in water and reprecipitated by making the solution 3 M with respect to NaAc and incubating it at 0°C overnight. Poly(A)⁺ RNA was purified by oligo (dT)-cellulose chromatography (19).

RNA blot analysis of Poly(A)⁺ RNA

Poly(A)⁺ RNA was denatured by heating to 60°C in 17% formaldehyde/50% formamide and subjected to electrophoresis in 2M formaldehyde/1% agarose gels (11). The RNA was transferred to nitrocellulose and hybridized to the ³²P-labeled DNA probes as described by Thomas (20). ³²P-labeled probes were obtained by nick-translation according to the procedure of Rigby *et al* (21) using (α -³²P)dATP at 3000 Ci/mmol specific activity. DNA fragments were purified from low melting point agarose gels (16).

A single-stranded RNA probe was synthesized using SP6 polymerase and (α -³²P)CTP (400 Ci/mmol) as described by Melton *et al.* (22). The 0.7 kb BamHI-SalI DNA fragment from the SGA gene was cloned in the SP6 promoter-containing plasmid pGEM-1 (Promega Biotec). In this construction the riboprobe is an antisense RNA with respect to that from the SGA gene. Conditions for hybridization and filter washing were as described (22).

Isolation of the intracellular glucoamylase gene

The SGA gene was cloned from the gene library of strain SPX101-1c DNA (see above). Colonies of E. coli cells harboring this library were transferred to nitrocellulose filters and their DNA was hybridized with ³²P-labeled probes by nick-translation of the 1.45 kb BamHI-EcoRI DNA fragment from the STA2 gene (11). Several clones hybridizing with the probe were isolated and, in order to identify the SGA gene, the restriction map of their plasmid DNA inserts was determined.

S1 nuclease mapping

The 2.0 kb EcoRI-SalI fragment from the SGA gene DNA was purified from low melting point agarose gel and its 5' ends were dephosphorylated by calf intestinal alkaline phosphatase treatment. It was then labeled at its 5' ends using (γ -³²P)ATP and polynucleotide kinase (11). Probe DNA (50 ng, 2 x 10⁵ cpm) was

hybridized overnight to 15 µg of poly(A)⁺ RNA from strain MCCX1-5d transformed with plasmid JMp79 (a YEpl3 plasmid containing the SGA gene), and then digested with S1 as described by Maniatis *et al.* (11). The reaction products were fractionated by electrophoresis in a 1.2% agarose gel. The gel was dried and autoradiographed at -70°C with an intensifying screen.

For the full-length DNA protection against S1 digestion experiment, a 5.4 kb BglII-BglII, comprising all the SGA gene was hybridized to 15 µg of poly(A)⁺ RNA from transformant MCCX1-5d-(JMp79) as above. After S1 nuclease digestion, the sample was subjected to electrophoresis in an alkaline agarose gel (11). Then the RNA was alkali-hydrolyzed by soaking the gel in 0.1 M NaOH, 1mM EDTA at 37°C for 1 hour. The DNA was transferred to a nitrocellulose filter after neutralization and hybridized to the 32-P labeled 5.4 kb BglII-BglII DNA fragment. After washing, the filter was autoradiographed as above.

RESULTS

Construction of STA2 stal0° and sta2° stal0° haploid hybrids from S. cerevisiae x S. diastaticus strains.

Initially, it was important to construct a yeast strain carrying a well characterized STA2 gene and lacking the starch-fermenting repressor STA10 gene. Therefore, S. diastaticus 5206-1b (Table I) was crossed with S. cerevisiae S288C and 4836-8c strains (Table I). From these crosses the haploid strain SPX101-1c (STA2 stal0°) (Table I) was obtained. The STA2 character of this strain was confirmed by crossing it with haploid strains carrying either the STA1 or STA3 genes. The results of these crosses showed that SPX101-1c contained a STA2 gene, which was further shown to segregate independently from both STA1 and STA3 (not shown). Similarly, the starch-non fermenting and STA10 repressor-free strain MCCX1-5d (Table I) was isolated. This strain was used as a host to clone the STA2 gene.

Cloning of the STA2 gene

The gene library of strain SPX101-1c (see Materials and Methods) was used to transform spheroplasts of the starch-non fermenting and STA10 repressor-free yeast strain MCCX1-5d (Table I). Selection for both STA⁺ and LEU⁺ transformants resulted in

Table I. Yeast strains used in this work.

STRAIN	GENOTYPE	ORIGIN
S228C	α sta ^o STA10 (wild type)	YGSC*
4836-8c	a leu1 thr1 sta ^o STA10	(6)
M1-2b	α trp1 ura3 gal2 sta ^o STA10	(25)
SC483	a leu2 his4 can ^r sta ^o STA10	(26)
5206-1b	a arg4 STA2 stal ^o	Tamaki H.
SPX101-1c	a arg4 leu1 STA2 stal ^o	This work
MCCX1-5d	a leu2 his4 sta ^o stal ^o	This work
YP2-4a	α trp1 STA2 stal ^o	This work
YP2-4b	α trp1 arg4 sta ^o stal ^o	This work
YP2-4c	α ura3 STA2 STA10	This work
YP2-4d	a arg4 ura3 sta ^o STA10	This work

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the isolation of one clone (MCCX1-5d, JMpl) with this phenotype. Total DNA from this clone was used to transform *E. coli*, selecting for ampicillin resistance. Several transformants were shown to carry plasmid JMpl (Fig 1), which consists of plasmid YEpl3 with an insertion of 10 kb of foreign DNA. When yeast spheroplasts from strain MCCX1-5d were transformed with JMpl, all LEU⁺ clones were also STA⁺. This phenotype had a mitotic instability in the absence of selection, indicating that the STA⁺ character was conferred by a STA2 gene which was present in plasmid JMpl.

By subcloning experiments, the STA2 gene was shown to be included in the 2.9 kb fragment between the left Sau3A1-BamHI insertion site and the BglII restriction site of the inserted yeast DNA (plasmid JMpl1, Fig 1). The restriction map of the DNA containing the STA2 gene is identical to that of a fragment of DNA from *S. diastaticus* containing the STA1 gene (4).

Analysis of the transcripts for glucoamylases

We have studied the transcripts encoding the glucoamylase enzymes from a variety of yeast strains to obtain further information on the regulation of the SGA and STA2 genes by the STA10 locus. Poly(A)⁺ RNA from strains SPX101-1c (STA2 SGA stal^o), MCCX1-5d (sta2^o SGA stal^o), SC483 (sta2^o SGA STA10) and MCCX1-5d(JMpl) (as a positive control), were subjected to agarose gel electrophoresis and then transferred to a nitrocellulose membrane. The blotted RNA was hybridized to the ³²P-labeled BamHI-SphI DNA fragment (Probe 1, Fig 2), which is internal to the STA2

Figure 1. Restriction map of plasmid JMpl and derived plasmids. + and - indicate starch-fermenting or non fermenting ability respectively, of yeast strain MCCX1-5d carrying the indicated plasmids.

gene and contains sequences for both the unprocessed and mature forms of the external glucoamylase (3). It was found that four distinct mRNAs of 1.95, 2.40, 2.85 and 5.4 kb from the starch-fermenting strain SPX101-lc, hybridized to the probe (Fig 2). All of these except the 2.85 kb transcript were also apparent in the starch-non fermenting strain MCCX1-5d. In addition transformant MCCX1-5d(JMpl) superproduced the 2.85 kb transcript. All these results indicate that transcription from the STA2 gene results in a mRNA of 2.85 kb. This finding correlates well with the size (2.35 kb) of the STA1 structural gene (3). S. cerevisiae (strain SC483) only shows a mRNA of 1.95 kb, giving a very

Figure 2. Analysis of yeast transcripts homologous to the STA2 gene. Poly(A)⁺ RNA from strains STA2 SGA stal0⁰ (1), sta2⁰ SGA stal0⁰ (2), sta2⁰ SGA STA10 (3) and sta2⁰ SGA stal0⁰ (JMpl) (4) were obtained. Samples of 5 μ g (except in channel 4, where only 0.25 μ g was used) were submitted to agarose gel electrophoresis and were then transferred to nitrocellulose sheets, which were subsequently hybridized to the indicated DNA probes.

weak hybridization. In this respect, it is interesting to note that the yeast strains MCCX1-5d and SC483 differ in the presence of a STA10 gene in the latter. Therefore, it could be implied that this gene represses the production of the 1.95 and 2.40 kb transcripts in S. cerevisiae.

It was important at this point to know the relationship between the different mRNAs which were homologous to the STA2 gene. Therefore, we performed new hybridizations using as probes the DNA fragments from the two different domains of the external glucoamylase gene, coding for the export region (not present in the mature protein) and the extracellular form of the enzyme. These two probes were selected according to the published sequence of the STA1 gene, whose restriction map is identical to

that of the STA2 gene (Fig 1 and (3,4)).

When the poly(A)⁺ RNA was hybridized to the 0.6 kb BamHI-PvuII DNA fragment (probe 2, Fig 2), which is internal to the export domain of the unprocessed form of the extracellular glucoamylase, only the 5.4 kb band from strains SPX101-1 and MCCX1-5d, and the 2.85 Kb band, from the former, appeared. In contrast, no hybridization band was apparent in the case of S. cerevisiae. On the other hand, when the probe was the 0.92 kb SalI-HinfI DNA fragment (probe 3, Fig 2), which is internal to the domain for the mature extracellular glucoamylase, the 5.4 kb band did not appear in any of the RNA tracks. In the track corresponding to the SPX101-1C strain (STA2 SGA stal0^o), the 1.95, 2.4 and 2.85 kb transcripts were present, while only the 1.95 and the 2.40 kb bands appeared in the MCCX1-5d (sta2^o SGA stal0^o) track. Again a weakly hybridizing 1.95 kb transcript was present in the mRNA from S.cerevisiae SC483 (sta2^o SGA STA10) (track 3). Therefore, the 5.4 kb transcript was not related to the mature form of the extracellular glucoamylase. Moreover, these results suggested that the 1.95 and 2.40 kb transcripts might encode an internal glucoamylase, which would then be expressed in the SPX101-1c and MCCX1-5d strains. This was found to be the case in the following experiment. Strains SPX101-1c (STA2 SGA stal0^o), MCCX1-5d (sta^o SGA stal0^o) and SC483 (sta2^o SGA STA10) were grown in rich medium and samples taken at several time intervals were assayed for glucoamylase activity. The results (Fig 3A) showed that in both the STA2 SGA stal0^o and sta2^o SGA stal0^o strains the expression of the glucoamylase activity was triggered at the middle of the log phase. The much higher levels of glucoamylase activity in the STA2-containing strain may have been due to a higher level of expression of this gene with respect to the SGA gene. No significant glucoamylase activity could be detected in the sta2^o SGA STA10 strain.

The effect of the STA10 gene on the expression of glucoamylases

The results presented above suggested that the STA10 gene of S. cerevisiae might repress the intracellular glucoamylase, in addition to its known repressor activity of the extracellular glucoamylase at the genetic level (6). Therefore, to determine whether this was the case, we constructed a heterozygous diploid

Figure 3. (A) Glucoamylase activity of several yeast strains during growth. Yeast cells were grown as indicated in Materials and Methods. Samples were taken at time intervals, permeabilized with toluene:ethanol and their glucoamylase activity was determined. (□) cell growth; (▲) *STA2 SGA sta10*⁰; (●) *sta2*⁰ *SGA sta10*⁰; (○) *sta2*⁰ *SGA STA10*. (B) Coregulation by the *STA10* gene of the mRNAs for glucoamylases. Poly(A)⁺ RNA (5 μg) from the progeny of the spores of a tetatype tetrad for *STA2* and *STA10* genes (spore a is YP2-4a; b is YP2-4b; c is YP2-4c and d is YP2-4d see Table I) and the parental strains (5206-1b in the first channel and M1-2b in the second one) were subjected to agarose gel electrophoresis, transferred to a nitrocellulose sheet and hybridized to the *SalI-HinfI* probe from the *STA2* gene (probe 3; Fig 2). Only the relevant genotype of the different strains is given.

for both *STA2* and *STA10*, which was then sporulated and subjected to tetrads dissection. One tetrad, having a tetatype segregation (1 fermenting:3 non-fermenting spores), was selected. This tetrad contained the four combinations of the two genes (Table I). Indeed, the genotype of each spore was tested by appropriate genetic crosses (not shown). Poly(A)⁺ RNA was isolated from the progeny of spores and was analyzed by hybridization to the 0.92 kb *SalI-HinfI* DNA fragment (probe 3, Fig 2), which is internal

to the domain encoding the mature extracellular glucoamylase. As control, poly(A)⁺ RNAs from the parental *S. diastaticus* 5206-1b and *S. cerevisiae* M1-2b strains (Table I) were included. As expected, a bulk 2.85 kb transcript was present in the sample from *S. diastaticus* (*STA2 SGA stal0^o*) which corresponded to the *STA2* gene, together with two other minor transcripts, of 1.95 and 2.40 kb (Fig 3B). In contrast, none of these transcripts were seen in the sample from *S. cerevisiae* (*sta^o SGA STA10*). The results obtained from the progeny of spores showed that in cells lacking the *STA10* gene (spores a and b, Fig 3B) the 1.95 and 2.40 kb transcripts were present and suggested that they corresponded to the *SGA* gene. In addition, the poly(A)⁺ RNA derived from spore a also contained the 2.85 kb transcript, corresponding to the *STA2* gene product. In contrast, the *STA10* gene repressed the transcription of these three mRNAs irrespective of their *STA2* genotype (spores c and d, Fig. 3B). These results show that both the *STA2* and *SGA* genes are coordinately regulated at the transcriptional level by *STA10*.

A transcript of about 7.0 kb was also present in the *S. diastaticus* 5206-1b sample (Fig 3B) which was not found in the poly(A)⁺ RNA from the SPX101-1c strain (Fig 2, line 1, probe 3). This transcript did not appear to be present in *S. cerevisiae* and was presumably repressed by the *STA10* gene, since it was only present in the cells with a *STA2 stal0^o* genotype (spore a, Fig 3B). The biological significance of this transcript is not known.

Initiation of transcription at the SGA gene

The two mRNAs homologous to the intracellular glucoamylase gene could be encoded by two different *SGA* genes, each transcribing a different mRNA. Alternatively, they could arise from a single *SGA* gene by either different transcription initiation and/or termination sites or by differential splicing. To determine which of these possibilities was the most likely, the *SGA* gene was isolated from the gene library of strain SPX101-1c, using the 1.49 kb *Bam*HI-*Sph*I DNA fragment from the *STA2* gene (Fig 2) as a probe. The restriction analysis of the different plasmids obtained (a representative example is plasmid JMp79, Fig 4) showed that several of them carried a DNA stretch with an

Figure 4. Restriction maps of the yeast DNA fragments carrying the STA2 and SGA genes. Both JMp40, a STA2 gene-containing plasmid, and JMp79, a SGA gene-containing plasmid, show the DNA flanking sequences to both genes. For clarity, the structure of the extracellular glucoamylase protein is shown. The open box represents the export domain of the glucoamylase encoded by the STA2 gene and the shaded box represents the structural domain of the mature protein (3) (see fig 2). The arrows indicate the direction of transcription and the approximate size of the mRNAs. Abbreviations for restriction enzymes are as shown in Fig 1.

identical restriction map to that of the STA2 gene domain encoding the mature form of the extracellular glucoamylase and its downstream flanking sequences. However, this newly cloned DNA, lacked a sequence homologous to that encoding the export domain of the extracellular glucoamylase. Moreover, the restriction maps of the upstream DNA flanking sequences of the STA2 gene and the cloned homologous DNA were different. These differences were shown not to be due to a DNA rearrangement but to correspond to an intact sequence of genomic DNA from strain SPX101-1c by a Southern blot experiment. Indeed, an identical sequence was also present in the genome of strains 5206-1b, MCCX1-5d, SC483 and M1-2b. Moreover, MCCX1-5d transformed with JMp79 showed an increased level of intracellular glucoamylase with respect to the untransformed strain (results not shown). Therefore, it could be concluded that this sequence represented the SGA gene which is present in *S. diastaticus* and *S. cerevisiae*.

Poly(A)⁺ RNA from this clone carrying the SGA gene was fractionated by gel electrophoresis and then hybridized to an antisense riboprobe, made as described in Materials and Methods. It was found that the 2.40 and 1.95 kb transcripts were overproduced in a similar fashion from plasmid JMp79 and that

Figure 5. S1 mapping of the transcription initiation sites of the SGA gene. The 2.0 kb EcoRI-SalI DNA fragment (heavy line) was labeled at its 5' ends and then hybridized to poly(A)⁺ RNA from the plasmid JMp79-transformed MCCX1-5d strain. The reaction mixture was digested with the S1 nuclease and then subjected to agarose gel electrophoresis. (A) mRNA-protected DNA fragments from S1 digestion; (B) Molecular weight markers. A schematic view of the results is shown on the left of the figure.

they were transcribed from the same DNA strand as the STA2 gene (not shown), indicating that they proceeded from the same locus and encoded the intracellular glucoamylase.

Taking into account that the DNA encoding the carboxy-terminal end of both the intra and extracellular glucoamylases and their downstream flanking sequences were similar (Fig 4) it could be deduced that the two transcripts from the SGA locus ended in the same site (the same that the STA2 gene transcript). If this were the case, there would be two transcription initiation sites separated by a 0.45 kb DNA fragment and both sites should be included in the 2.0 kb EcoRI-SalI DNA fragment encoding the NH₂-terminal of the intracellular glucoamylase (Fig 5). In contrast, if there were only a single 2.40 kb transcript,

Figure 6. Size analysis of the DNA strand resistant to nuclease *S1* digestion in DNA/RNA hybrids from the *SGA* gene. A 5.4 kb *Bgl*III-*Bgl*III fragment comprising the *SGA* gene was hybridized to poly(A)⁺ RNA from a JMp79-transformed MCCX1-5d strain. These hybrids were then digested with *S1* nuclease and subjected to alkaline agarose gel electrophoresis. The RNA was hydrolyzed by alkali and the DNA probed with the same DNA fragment. (A) *S1* nuclease digested hybrids; (B) undigested sample; (C) molecular weight markers.

processed to give the 1.95 kb transcript, there would be a single transcription initiation event. To decide between these possibilities, the 2.0 kb *Eco*RI-*Sal*I DNA fragment (Fig 5), encoding the NH₂-terminal region of the *SGA* gene, was labeled with

^{32}P at its 5' ends and was hybridized to poly(A)⁺ RNA from the MCCX1-5d strain that had been transformed with plasmid JMp79 carrying the SGA gene. The hybridization products were subjected to digestion with S1 nuclease and fractionated by agarose gel electrophoresis. The resulting autoradiography of the dried gel showed two protected fragments, of 1.03 and 0.60 kb, suggesting that there were two different transcription initiation sites in the SGA gene (Fig 5). Moreover, when an experiment of full-length DNA protection against S1 digestion was performed (see Material and Methods) the result (Fig 6) showed that the majority, if not all, of the 1.95 and 2.40 kb transcripts were colinear with the SGA gene. Nevertheless, we can not rule out the possibility that there may be a small intron at either end of the two transcripts.

DISCUSSION

The restriction map of the STA2 gene described in this work is identical to that of the STA1 and STA3 genes reported by Yamashita *et al.* (4) and the DEX1 gene described by Meaden *et al.* (7). These results are in agreement with the finding that these genes, although mapping at different loci of the genetic map of S. diastaticus, encode identical forms of extracellular glucoamylases (2). The analysis of the transcriptional products of the STA2 and SGA genes shows that the STA2 gene, and by extension STA1 and STA3, arose from a recombinational event involving at least two preexisting genes of Saccharomyces. One of them (SGA) transcribes two mRNAs of 1.95 and 2.40 kb that encode the intracellular glucoamylase and the other, whose function is not known at present, is identified by its 5.40 kb transcript and shares with the STA2 gene the region encoding the export domain of the external glucoamylase. A similar conclusion was drawn by Yamashita *et al.* (4) from the study of homologous DNA sequences of the STA1 gene and S. cerevisiae DNA. Yamashita *et al.* (4) and Meaden *et al.* (7) have shown that the S. diastaticus and S. cerevisiae genomes contain sequences homologous, but at different loci, to the STA1 and DEX1 genes. Moreover, Yamashita *et al.* (10) showed that these sequences determine the glucoamylase that is expressed in S. cerevisiae during sporulation

(8,9). Our results support this idea and suggest that the intracellular glucoamylase described here is the SGA gene product.

We have shown that the STA10 gene from S. cerevisiae represses the SGA gene during the vegetative life cycle. The transcription of STA2 is also repressed by STA10, although the nucleotide sequences preceeding the structural gene seems different to those of the SGA gene. Since, S. diastaticus does not contain the STA10 gene it is difficult to link the SGA gene with sporulation. Yamashita *et al.* (10) have postulated that the SGA gene is positively regulated by the MAT α 1 and MAT α 2 gene products which are required for the SGA gene transcription. However, our results are not in full agreement with this proposal since we find that the SGA gene is transcribed in haploid MAT α and MAT α yeast strains which lack the STA10 repressor gene. Moreover, we only find repression of transcription of the STA2 and SGA genes by the STA10 repressor gene. Yamashita *et al.* (10) did not find expression of the SGA gene in S. cerevisiae diploid strains carrying either matal or mat α 2 mutations. However, these diploids did not sporulate since the active forms of these two genes are required for this process. Nevertheless, this finding could not be attributed to a lack of the SGA gene product, since its product was dispensable for sporulation (10). Therefore, it can be postulated that in diploid strains carrying matal or mat α 2 mutations, there is no glucoamylase expression because the mating type dependent mechanism(s) for sporulation is (are) not activated. Hence, the SGA gene, which is sporulation specific, is not expressed. Our results suggest that the SGA gene (and, in addition, the STA1, 2 and 3 genes) are primarily regulated by the STA10 gene, which represses their expression during the vegetative life cycle. In sporulating MAT α /MAT α cells the STA10 gene would be repressed (most probably by a MAT locus gene product) allowing the expresion of the SGA gene to take place. It is also possible that other genes which may be regulated by the STA10 gene (such as the one transcribing the 5.40 kb mRNA) are then expressed. If this were the case, the STA10 gene would be a general repressor of sporulation-specific functions.

As mentioned above, we have found that two poly(A)⁺ RNA of 1.95 and 2.40 kb are transcribed from the SGA locus. This situa-

tion could be common for those genes that are preferentially expressed during sporulation in *S. cerevisiae*. Clancy *et al.* (23) have shown that three out of four cloned genes, which are sporulation-specific, transcribe two poly(A)⁺ RNAs of different sizes. Moreover, Percival-Smith and Segall (24) found that two poly(A)⁺ RNAs of different length were transcribed from three out of fourteen sporulation-specific genes. These facts may have some significance in the control of gene expression during sporulation. It is unclear whether these mRNAs are transcribed from the same gene or clustered genes, all of which could be sporulation specific. In our case, we have shown that both transcripts originate from the same locus, a finding that might be applicable to other genes specific for sporulation, since functionally related genes are not usually clustered in *Saccharomyces*.

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