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Differential regulation of *STA* genes of *Saccharomyces cerevisiae*

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Summary. The single glucoamylase gene (*SGA1*) of the yeast *Saccharomyces cerevisiae* is expressed exclusively during the sporulation phase of the life cycle. Enzymatic studies and nucleic acid sequence comparisons have shown that the *SGA1* glucoamylase is closely related to the secreted enzymes of *S. cerevisiae* var. *diastaticus*. The latter are encoded by any of three unlinked *STA* genes, which have been proposed to derive from the ancestral *SGA1* form by genomic rearrangement. We show that the regulation of *SGA1* is distinct from that of the other members of the *STA* gene family. *SGA1* expression did not respond to *STA10*, the primary determinant of glucoamylase expression from *STA2*. Unlike *STA2*, *SGA1* was not regulated directly by the mating type locus. Expression of *SGA1* depended on the function of the *MAT* products in supporting sporulation and not on the formation of haploid progeny spores or on the composition of the mating type locus per se. We conclude that the *STA* genes acquired regulation by *STA10* and *MAT* by the genomic rearrangements that led to their formation. This regulation is thus distinct from that of the ancestral *SGA1* gene.

Key words: Glucoamylase – Sporulation – *Saccharomyces cerevisiae* – *Saccharomyces cerevisiae* var. *diastaticus* – *STA* genes

Introduction

The sporulation glucoamylase gene (*SGA1* or *Asta*; Yamashita and Fukui 1985; Pretorius et al. 1986a; Erratt and Nasim 1986b) is common to all *Saccharomyces cerevisiae* strains that have been examined. This is one of a group of "late" sporulation-specific genes whose transcripts appear at the time of meiosis I and at no other stage in the life cycle of standard laboratory strains (Clancy et al. 1983; Holaway et al. 1985). Glucoamylase is responsible for the extensive degradation of internal glycogen stores which occurs at the time of spore formation, but is not necessary for sporulation (Yamashita and Fukui 1985). Nonetheless, appearance of enzymatic activity during sporulation depends on early sporulation functions (Clancy et al. 1982) including those directed by the *MAT* gene products (Yamashita and Fukui 1985).

Additional glucoamylase genes (*STA* or *DEX*) exist in *S. cerevisiae* var. *diastaticus* strains. These genes confer on the cells the ability to produce a secreted glucoamylase during vegetative growth and hence, to use starch or dextrins for growth. Three such genes have been described (Tamaki 1978; Erratt and Nasim 1986a) and their physical relationships delineated by hybridization techniques and DNA sequencing (Meaden et al. 1985; Pretorius et al. 1986b; Yamashita et al. 1985c, 1987). All three *STA* genes contain a region common to *SGA1* which corresponds to the catalytic domain of the protein. The secreted glucoamylase proteins contain, in addition, a serine/threonine-rich amino-terminal domain which is probably the site of extensive O-glycosylation and confers at least some of the secretory information of the molecule (Modena et al. 1986; Pugh et al. 1989). It has been suggested that the *STA* genes arose from the ancestral *SGA1* gene by genomic rearrangement (Yamashita et al. 1985a, 1987), and that *SGA1* be regarded as a member of the *STA* gene family.

Despite the divergence at the 5′ ends of the *STA* vs *SGA1* genes, it has been speculated (Pretorius et al. 1986c; Pardo et al. 1986, 1988) that they are under common control by the mating type locus and the *STA10* gene. Glucoamylase production by *STA* strains normally requires a recessive allele of *STA10*. This gene, which is present in most standard *S. cerevisiae* strains, prevents *STA* expression even when the cells contain multiple copies of functional *STA* genes (Polaina and Wiggs 1983; Yamashita and Fukui 1984). It has been reported that *SGA1*, like the other *STA* genes, may be expressed vegetatively when *STA10* is absent from the strain background, suggesting that *STA10* plays a role in regulating *SGA1* and perhaps other sporulation-specific genes.

All of the *STA* genes, including *SGA1*, are also regulated by the mating type locus (Yamashita et al. 1985b; Yamashita and Fukui 1983). *STA* genes are repressed in diploids, possibly by the *MAT*a1/*MAT*α2-dependent repressor as for other haploid-specific genes, e.g. *MAT*α1 (Siliciano and Tatchell 1986), *RME1* (Mitchell and Herskowitz 1986), Ty1 (Errede et al. 1980), and *HO* (Jensen et al. 1983). *SGA1*, by contrast, may depend only on the role of *MAT*a1/*MAT*α2 in promoting sporulation in diploids (Strathern et al. 1981), via a transcriptional coupling of *SGA* to sporulation events. Alternatively *SGA1* may be repressed by the

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MAT products, and relieved of this control at the time of meiosis I.

The aim of this work was to determine the extent to which regulatory features of the *STA* gene family have been conserved among its various members. To examine *STA*10 and mating type regulation of *SGA*1, we used strains in which the endogenous *SGA*1 gene had been inactivated by transplacement, so that expression of *SGA*1 and *STA*2 could be assayed independently under a variety of growth conditions. We show that *SGA*1 was not relieved from sporulation control in an *sta*10 background, although some expression from high copy number plasmids was detected, irrespective of the *STA*10 genotype or mating type of the cells.

*SGA*1 expression during sporulation in an *STA*10 background required the *MAT* products, but only indirectly: activity was absent from strains that were deficient at *MAT* but restored by mutations that relieve the requirement for *MAT* information for sporulation (Rine et al. 1981; Mitchell and Herskowitz 1986; Kassir and Simchen 1976; Hopper and Hall 1975). Further, *SGA*1 did not require the expression of the haploid cell type specific genes, since *SGA*1 expression occurred at wild-type levels in strains that could not haploidize their genomes but could form spores (Malone and Esposito 1981). We conclude that *SGA*1 expression occurs as a result of a meiosis-specific signal, rather than as a direct consequence of mating type gene expression or activity, and that *SGA*1 is regulated independently of *STA*2.

Materials and methods

Chemicals and enzymes. [α^{32}P]dCTP (600 or 3000 Ci/mmol) and [γ^{32}P]ATP (3000 Ci/mmol) were from ICN. Restriction enzymes were from Bethesda Research Laboratories (BRL) or United States Biochemicals (USB) and were used according to the manufacturers' recommendations. Reactions involving polynucleotide kinase (USB), T4 DNA ligase (New England Biolabs), and the Klenow fragment of DNA polymerase I (BRL) were performed as described in Maniatis et al. (1982). Nitrocellulose was from Schleicher and Schuell. General chemicals were from Sigma. Oligo-STA was a gift from J. Yarger and D. Leland, and was synthesized by the phosphoramidite protocol with an Applied Biosystems 380A DNA synthesizer.

Strains and genetic manipulations. Strains of *S. cerevisiae* and *S. cerevisiae* var. *diastaticus* used in this study are listed in Table 1. Strain constructions were carried out by standard genetic techniques (Sherman et al. 1981). The methylmethanesulfonate (MMS) sensitivity of *rad* strains was scored by plating the cells on YEPD medium containing 0.008% MMS (Quah et al. 1980; Borts et al. 1980).

Strains secreting glucoamylase were identified by their ability to form yellow halos on YPSB indicator plates containing 0.003% bromcresol purple (0.75% yeast extract, 0.5% peptone, 3.0% soluble starch, pH 6.8; Pretorius et al. 1986c). Chromosome assignments were made using orthogonal field alternation gel electrophoresis (OFAGE; Carle

Table 1. Strains of *Saccharomyces cerevisiae*

Strain	Genotype	Source
SGA cloning		
DEX1354	*MAT*a *STA2 sta1*	G.G. Stewart and I. Russell
DK10a	*MAT*α *leu2 trp1 ade2 his3 ura3 sta*° *STA*10	D. Mannix
DK10c	*MAT*a *leu2 trp1 ade2 his3 ura3 sta*° *STA*10	D. Mannix
30c	*MAT*a *leu2 trp1 his3 ura3 sta*° *sta*10	This study
DK10a *sga:LEU2*	Same as DK10a with a *LEU2* insertion in *SGA*1	This study
30c *sga:LEU2*	Same as 30c with a *LEU2* insertion in *SGA*1	This study
SK1	*MAT*a/*MAT*α *HO/HO*	J. Game
W66-8a	*MAT*a/*MAT*α *HO/HO leu2/leu2 lys2/lys2 met4/met4 trp5/trp5 ade2/ade2 ura3/ura3 can1/can1*	R. Rothstein
*SGA*1 mapping		
TYP3b	*MAT*a *leu2 sga:LEU2 ura3 trp1 ade2*	This study
TYP4a	*MAT*α *leu2 sga:LEU2 ura3 trp1 ade2*	This study
MJC15a	*MAT*a *leu2 ura3 trp1 ade2 his5 lys11*	This study
A236-24c	*MAT*α *lys11 leu2 trp1 met4 aro7 his3 can1*	Cold Spring Harbor
A4840A	*MAT*α *his5 ade2*	Cold Spring Harbor
*SGA*1 sporulation dependence		
TYP1	*MAT*a/*MAT*α *spo3/+ rad50/+ leu2/leu2 ade2/ade2 lys1/+ lys2/+ ura3/+ aro7/+ his6/+ his1/+*	This study
TYP100	*MAT*a/*MAT*α *spo13/spo13 rad50/rad50 leu2/leu2 ade2/ade2 lys1/+ his1/+ ura3/+ aro7/+*	This study
TYP101	*MAT*a/*MAT*α same as TYP100	This study
K399-5c	*MAT*a *spo13 leu2 ade2 his6 lys1 lys2 ura3 aro7*	R. Elder
MAY7c	*MAT*α *rad50-1 leu2 ade2* His$^-$	M. Clancy
GK231	*MAT*a/*MAT*α *ho:HIS3/ho:HIS3 leu2/leu2 ura3/ura3 trp1/trp1*	G. Kao
GK25	*MAT*a *ho:HIS3 leu2 ura3 trp1*	G. Kao
GK31	*MAT*α *ho:HIS3 leu2 ura3 trp1*	G. Kao
GK232	*mata1:LEU2/MAT*α *ho:HIS3/ho:HIS3 leu2/leu2 ura3/ura3 trp1/trp1*	G. Kao
GK125-30	*mata1:LEU2/MAT ho:HIS3/ho:HIS3 leu2/leu2 ura3/ura3 trp1/trp1 RES1-1/RES1-1*	G. Kao

and Olson 1984); blots were kindly provided by B.B. Magee, and the chromosome IX marker (SUP17-14g) by J. Dutchik and M. Olson.

Cell growth and enzyme assays. S. cerevisiae strains were grown and sporulated using YEPD, YEPA, PSP and SPM media (Sherman et al. 1981) as described previously (Holaway et al. 1985). Studies of vegetative glucoamylase expression utilized YPGE (Pretorius et al. 1986b). Progress through sporulation was monitored using 4′, 6-diamidino-2-phenylindole (DAPI) as described by Williamson and Fennel (1975). Fluorescent nuclei were visualized using a Nikon Lab-Phot research microscope equipped with an IVEL vertical illuminator for UV epifluorescence. Yeast strains were transformed with plasmids using the method of Ito et al. (1983).

Sporulation glucoamylase (*SGA1*) and *STA2* glucoamylase were quantitated by determination of the rate of glucose release from glycogen using a coupled assay system employing glucose oxidase, horseradish peroxidase, and *o*-dianisidine as described previously (Colonna and Magee 1978; Clancy et al. 1982). Units of glycoamylase activity are expressed as nanomoles of glucose released per minute, and specific activity as units of glucoamylase per milligram of protein. Protein content was quantitated by the method of Lowry et al. (1951) or Bradford (1976).

Intracellular and extracellular glucoamylase were determined using 5.0 ml aliquots of sporulating or vegetatively growing cultures ($2–5 \times 10^7$ cells/ml). The cells were harvested by centrifugation at 5000 *g* for 10 min, washed once with 10 ml of 0.1 M sodium citrate pH 6.2, resuspended in 0.5 ml of breakage buffer [0.1 M sodium citrate pH 6.2, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF)], and transferred to 1.5 ml microfuge tubes. Cell suspensions were vortexed with glass beads until at least 80% of the cells were broken, as determined by light microscopy. The extract and one wash fraction (1.0 ml of breakage buffer) were pooled, dialyzed against 0.1 M sodium citrate buffer pH 6.2 containing 5% glycerol and assayed for glucoamylase activity in the presence of 0.3 mM *p*-chloromercuri-benzoate (PCMB).

Escherichia coli strains DH5 (BRL) and HB101 (Maniatis et al. 1982) were used for plasmid contructions and maintenance. *E. coli* cells were grown as described in Maniatis et al. (1982). Transformation was accomplished using the calcium chloride method (Maniatis et al. 1982) or the method of Hanahan (1983).

Preparation of nucleic acids. S. cerevisiae and *S. cerevisiae* var. *diastaticus* DNA was prepared from 5–40 ml cultures as in Sherman et al. (1981) or from 500 ml cultures according to Hereford et al. (1979). Total RNA was isolated by the guanidinium isothiocyanate method (Maniatis et al. 1982) as described by Kaback and Feldberg (1985). Plasmid DNA was prepared from small-scale (1.5–10 ml) *E. coli* cultures by the method of Holmes and Quigley (1981). Alternatively, purified plasmid DNA was obtained by centrifugation of lysed cell extracts in CsCl according to Davis et al. (1980), except that the lysis buffer contained 0.5% Triton X-100.

Construction of S. cerevisiae *var.* diastaticus *genomic library.* Approximately 500 µg of total genomic DNA from *S. cerevisiae* var. *diastaticus* was digested with *Sau*3A (0.3 U/µg DNA) until the majority of the DNA fragments were between 2 and 10 kb in length (30 s–2 min). The digested DNA was fractionated by centrifugation through a sucrose gradient (10%–40%, Maniatis et al. 1982) and fractions containing molecules of the desired size were retained. The DNA was recovered and ligated to the yeast/*E. coli* shuttle vector YEp24 (Botstein and Davis 1982) which had been treated with *Bam*HI and calf intestinal phosphatase (Boehringer-Mannheim). The DNA was used to transform *E. coli* strain HB101 to ampicillin resistance (Maniatis et al. 1982). Approximately 2.6×10^4 transformants were recovered, of which 90% were tetracycline sensitive. The average insert size was approximately 5.3 kb as determined by analysis of 105 Ap^r, Tc^s plasmid isolates.

Hybridizations. Unless specified otherwise, DNA restriction fragments were resolved by electrophoresis through 0.7% agarose gels using TRIS-acetate buffer (Maniatis et al. 1982). They were transferred to nitrocellulose (Southern 1975) and hybridized to nick-translated (Rigby et al. 1977) probes prepared as in Maniatis et al. (1982) or oligonucleotides which had been end-labeled with [γ^{32}P]ATP using polynucleotide kinase. For nick-translated probes, hybridization was carried out as described (Clancy et al. 1983); for oligonucleotides, hybridization was as detailed in Woods et al. (1982). Total yeast RNA was resolved by formaldehyde agarose gel electrophoresis and hybridized as described by Yarger et al. (1986). The RNA was transferred to nitrocellulose as in Thomas (1983). Northern blots were hybridized to labeled oligonucleotides as in Woods et al. (1982), except that the hybridization solution contained 10% dextran sulfate. Colony hybridization was performed by a modification of the procedure of Maniatis et al. (1982).

Results

Detection of STA*10 and construction of cloning strain*

It has been reported that most laboratory strains of *S. cerevisiae* contain dominant alleles of the *STA*10 gene; that is, they repress glucoamylase expression even if presented with excess copies of the wild-type gene. To verify that this was so for our strains and to identify a suitable recipient for cloning *STA*2 and *SGA*1, we crossed our standard *S. cerevisiae* haploid strain, DK10a, with DEX1354 (kindly provided by Drs. G.G. Stewart and Inge Russell), a glucoamylase-secreting *S. cerevisiae* var. *diastaticus* strain (*STA*2 *sta*10).

Analysis of the segregants of this cross verified that DK10a contained the *STA*10 regulator but no active *STA* structural gene. Segregants were first analyzed for glucoamylase production by a plate assay (Materials and methods). The pattern of glucoamylase production was as expected if two genes affecting this phenotype were segregating. That is, individual tetrads gave rise to two (PD; 5 tetrads), one (TT; 19 tetrads) or no (NPD; 6 tetrads) glucoamylase-positive cultures, depending on the patterns of segregation of *STA* and *STA*10. If DK10a had not contained the modifier, glucoamylase production would have segregated 2:2 in all tetrads.

To detect segregants that contained neither the modifier nor the *STA*2 gene, we hybridized genomic DNA to an oligonucleotide that could detect *STA*2 as well as *SGA*1 sequences (oligo-STA). The oligonucleotide was homolo-

gous to a conserved region within the catalytic domain of *STA1*, and was therefore expected to detect all members of the *STA* gene family (Yamashita et al. 1985c). Figure 1 shows that the parental strains differed as expected with respect to the genomic bands detected by the *STA* oligonucleotide probe. A 4.0 kb *Bam*HI fragment containing the *SGA1* gene was present in both strains, whereas DEX1354 also contained a 4.4 kb *Bam*HI fragment, lacking in *S. cerevisiae*, which represents *STA2*.

The 4.4 kb band segregated 2:2 in all tetrads examined (Fig. 1 and not shown). Analysis of segregants from 0:4 tetrads showed that two spores from each contained the *STA2* band; these isolates were inferred to be *STA2 STA10*, since they did not produce glucoamylase. The remaining two were *sta10*, and were presumed to be capable of producing glucoamylase if provided with a wild-type *STA2* gene. The identities of two such segregants (30c and 62d) were confirmed by genetic analysis; crosses of these strains with STA^+ segregants yielded 2:2 segregation for glucoamylase production in ten complete tetrads (data not shown). The *STA2* band cosegregated with glucoamylase production in the four tetrads examined (Fig. 1 and not shown).

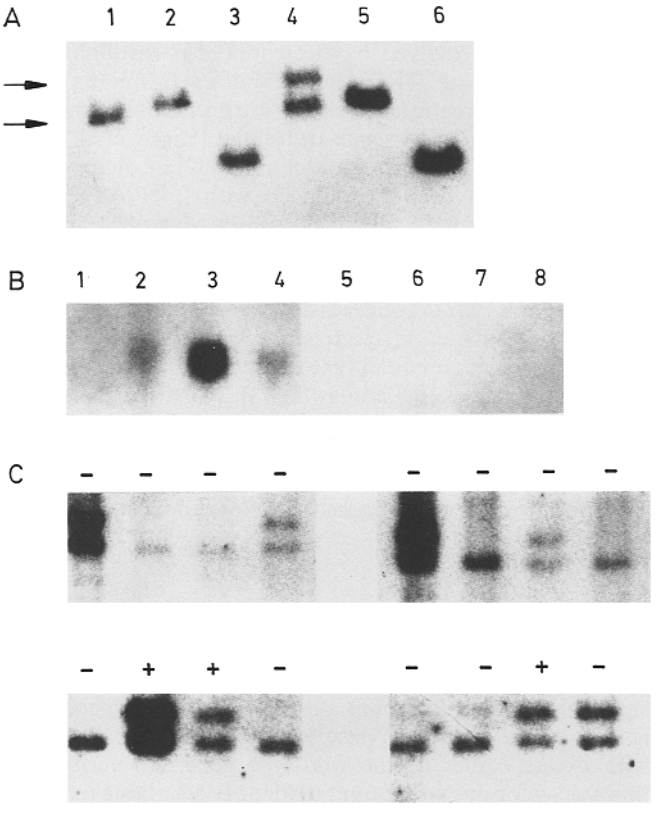

Fig. 1. A Approximately 10 µg of genomic DNA from *Saccharomyces cerevisiae* strain W66-8a (lanes 1, 2, 3) and *S. cerevisiae* var. *diastaticus* DEX1354 (lanes 4, 5, 6) was digested with *Bam*HI (lanes 1, 4), *Eco*RI (lanes 2, 5) or the two enzymes in combination (lanes 3, 6), and fractionated by electrophoresis on a 0.7% agarose gel. The fractionated DNAs were transferred to nitrocellulose and hybridized to a 5′ labeled oligonucleotide (5′ AAGCTATAGTTACTGGG 3′) complementary to the common portion of the *STA* and *SGA* genes. *Arrows* indicate the positions of the *Bam*HI fragment (4.0 kb) containing the *SGA1* genes of both organisms and the larger (4.4 kb) *Bam*HI fragment characteristic of *STA*. **B** The oligonucleotide used in **A** was hybridized to total RNA which had been extracted at intervals from sporulating SK1 cells (lanes 1–4) and an asporogenous DK10a control (lanes 5–8). Samples (10 µg of RNA per lane) were taken at the time of shift to sporulation medium (lanes 1 and 5), and at 4 h (lanes 2, 6), 6 h (lanes 3, 7) and 8 h (4, 8) afterward. Meiosis began in SK1 by 4 h, as determined by 4′,6-diamidino-2-phenylindole(DAPI) staining (data not shown). **C** Genomic DNA was extracted from segregants from a cross of DK10a and DEX1354, digested with *Bam*HI and hybridized as in **A** to the *STA* oligonucleotide to detect *STA2* and *SGA1* sequences. The Sta (+ or −) phenotype of each isolate is shown for complete tetrads

Cloning STA*2 and* SGA*1*. *STA2* was cloned from an *S. cerevisiae* var. *diastaticus* genomic library in YEp24 by complementation of the glucoamylase-negative phenotype of segregant 30c. Two glucoamylase-sereting colonies were detected among approximately 10^4 uracil prototrophs patched onto YPSB indicator medium. The plasmids were rescued from the yeast and characterized. Both plasmids hybridized to the oligonucleotide described above, and to the genomic 4.0 and 4.4 kb *SGA1* and *STA2* bands (not shown). Further, their restriction maps (Fig. 2) are comparable to published maps for *STA1* and *STA2* (Yamashita et al. 1985a; Pretorius et al. 1986b; Meaden et al. 1985; Erratt and Nasim 1986b; Pardo et al. 1986).

SGA1 was cloned from an *S. cerevisiae* library in YEp13 (Nasmyth and Tatchell 1980, kindly provided by A. Kennedy and D. Primerano) by hybridization of *E. coli* transformants to a 1.2 kb *Sal*I fragment derived from p*STA2*-1. The fragment (underlined in Fig. 2) spans the conserved portion of the glucoamylase coding region and includes the sequence which hybridizes to the oligonucleotide. Four consistently positive hybridization signals were obtained in a screen of approximately 7000 transformants.

Restriction mapping and hybridization analysis confirmed the similarity of the identified plasmids, p*SGA1*-1 and p*SGA1*-4, to *STA2*. The putative *SGA1* clones hybridized to the oligonucleotide used above, as well as to 4.0 and 4.4 kb genomic *Bam*HI fragments.

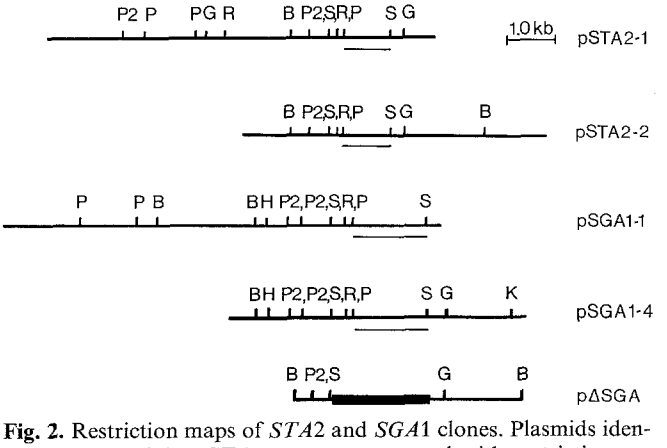

Fig. 2. Restriction maps of *STA2* and *SGA1* clones. Plasmids identified as containing *STA* genes were mapped with restriction enzymes. Enzymes used were *Bam*HI (B), *Bgl*II (G), *Pvu*II (P2), *Pst*I (P), *Sal*I (S), *Eco*RI (R), *Hin*dIII (H) and *Kpn*I (K). Fragments homologous to the oligonucleotide used for hybridization are indicated by *bars* below the diagrams. p*ΔSGA* is an *SGA1* null allele. The 4.0 kb *Bam*HI fragment containing *SGA1* was transferred to a YCp50 derivative from which the *Sal*I site had been deleted. The 1.2 kb *Sal*I fragment internal to the *SGA1* coding region was replaced by a 1.1 kb *Sal*I-*Xho*I fragment (indicated by *bold line*) containing the *LEU2* gene

Insertional disruption of SGA*1 in* sta*10 and* STA*10 genetic backgrounds*

To provide genetic backgrounds in which *STA2* and *SGA1* regulation could be monitored in the absence of endogenous *SGA1* activity, we inactivated the resident *SGA1* genes in the *sta*10 and *STA*10 strains 30c and DK10a by transplacement (Orr-Weaver et al. 1981; Rothstein 1983). The construct used is shown in Fig. 2. *SGA1*-1 was transferred as a *Bam*HI fragment into a derivative of the yeast/*E. coli* shuttle vector, YCp50 (Rose 1987), from which the *Sal*I site had been deleted. The 1.2 kb *Sal*I fragment internal to the *SGA1* gene was replaced with a *Sal*I-*Xho*I fragment containing the *S. cerevisiae LEU2* gene (Rothstein 1983). This construct was transferred to the genomes of 30c and DK10a by transformation of these strains with the 4.9 kb *Bam*HI fragment containing the inactive *sga1* allele and the *LEU2* selectable marker. Southern blots confirmed the expected disruption events (data not shown). Strains containing the disrupted alleles (30c *sga:LEU2* and DK10a *sga: LEU2*) were retained as haploids or diploidized by transformation with *HO* (Russell et al. 1986) for studies of glucoamylase expression.

As expected, strains which carried null *sga:LEU2* alleles did not produce detectable glucoamylase activity when exposed to sporulation medium (below and not shown). *SGA1*-directed activity could be restored to such strains, however, when intact alleles were provided by transformation with the corresponding wild type described above. As has been described previously (Yamashita and Fukui 1985), diploid strains which lacked *SGA1* activity were capable of normal sporulation and the production of viable ascospores.

Inhibition of STA*2 and* SGA*1 expression by* STA*10 and* MAT

To evaluate the effects of *STA*10 and diploidy on *SGA1* and *STA2* expression, we introduced these genes into strains (described above) that lack any active glucoamylase gene. *SGA1* was transferred to yeast/*E. coli* shuttle vectors which were expected to be maintained in single (YCp50) or multiple copies per cell (YEp24). The former contains a yeast centromere and the latter utilizes the origin of replication and copy control mechanism of the two micron plasmid which is endogenous to the yeast (Broach et al. 1979). *SGA1* was cloned as a 4.1 kb *Bgl*II fragment into the unique *Bam*HI sites of YCp50 and YEp24, respectively. For *STA2*, we used the original p*STA2*-1 plasmid isolate, and a derivative of this plasmid to which a yeast centromere had been introduced at the unique *Sma*I site of the YEp24 vector.

These constructions were regulated in the same manner as the chromosomal gene. Neither *STA*10 nor *sta*10 strains expressed *SGA1* at detectable levels during vegetative growth in YEPD or YEPA, regardless of whether *SGA1* was present on a plasmid or in the chromosome (below and not shown). Similarly, only diploids expressed *SGA1* during sporulation.

Table 2. Inhibition of *SGA1* and *STA2* expression in vegetative cells by *STA*10 and *MAT*

Strain and relevant genotype		Plasmid	Enzymatic activity		Cells retaining plasmid (%)[b]
			Intracellular (specific activity)[a]	Culture supernatant (units/ml)	
Haploid strains					
30c	*sta*10, *sga:LEU2*	None	–	–	
		YCp*SGA1*	<0.1	<0.1	102
		YEp*SGA1*	1.9	<0.1	39
		YCp*STA2*	18.3	4.0	77
		YEp*STA2*	100.4	268.0	43
DK10a	*STA*10, *sga:LEU2*	None	–	–	–
		YCp*SGA1*	<0.1	<0.1	88
		YEp*SGA1*	2.0	<0.1	48
		YCp*STA2*	1.9	<0.1	59
		YEp*STA2*	12.0	14.3	45
Diploid strains					
30c	*MAT*a/*MAT*α, *sta*10, *sga:LEU2*	None	–	–	–
		YCp*SGA1*	<0.1	<0.1	92
		YEp*SGA1*	1.2	<0.1	71
		YCp*STA2*	5.0	1.2	71
		YEp*STA2*	74.5	78.5	42
DK10a	*MAT*a/*MAT*α, *STA*10, *sga:LEU2*	None	–	–	–
		YCp*SGA1*	<0.1	<0.1	96
		YEp*SGA1*	4.9	<0.1	68
		YCp*STA2*	0.1	<0.1	111
		YEp*STA2*	5.4	0.4	62

[a] Specific activity was defined as nanomoles glucose released from glycogen per minute per milligram protein at 30° C, under the conditions described in Materials and methods. One units was defined as 1 nmol released per minute. Results from several experiments were combined

[b] The fraction of plasmid-bearing cells was determined by comparing the number of colony-forming units per milliliter of culture when aliquots were plated on minimal medium with and without a uracil supplement

Table 2 shows the levels of plasmid-directed *SGA1* and *STA2* glucoamylase activities produced by *STA10* and *sta10* strains 30c *sga: LEU2* and DK10a *sga: Leu2* and their isogenic diploid derivatives. The strains were grown to stationary phase (approx. 2×10^8 cells/ml) in a medium (YPGE) which had been shown previously to support maximal levels of *STA2* glucoamylase activity. *SGA1* activity was not detected in haploid or diploid vegetative cells of either strain when the gene was maintained in single copy in the cells. Activity from the high copy number construct was readily detectable, but the levels were approximately equal in the *STA10* and *sta10* strains in haploids as well as diploids. This suggests that the low levels of activity were the result of an increase in basal expression of the gene or of titration of regulators other than *STA10*. We conclude that *SGA1* is not regulated by *STA10*, at least under these conditions. In all cases, the activity was intracellular, as no activity could be detected in the culture fluid from cells containing either YCp50-*SGA1* or YEp24-*SGA1*.

STA2, by contrast, responded strongly to the *STA10* genotype of DK10a. The intracellular enzyme levels were eight- to tenfold lower in *STA10* vs the *sta10* haploid and at least tenfold lower in the diploid, even when the gene was carried on the high copy vector. The decline in extracellular activity was even more striking. *STA2* glucoamylase levels were approximately 20- and 200-fold lower in the *STA10* haploid and diploid strains, respectively, as compared with the *sta10* controls.

The effect of diploidy on *STA2* expression was less pronounced. Intra- and extracellular glucoamylase levels in the *sta10* strain were repressed approximately threefold in the diploid as compared with the isogenic haploid in strains carrying *STA2* on the single copy vector. This was also true for the *STA10* strain. For *STA2* genes carried on the high copy number vector, repression varied from two- to threefold. By contrast, diploidy had little or no effect on *SGA1* activity in either the *STA10* or *sta10* background, as intracellular glucoamylase activity produced by strains which carried the gene in multiple copies was not repressed detectably in diploid vs isogenic haploid strains. We conclude that diploidy regulates *STA2* but not *SGA1* in vegetative cells.

Sporulation regulation of SGA1 *and* STA2

To examine the effects of sporulation conditions on *SGA1* and *STA2* expression, we determined glucoamylase activities directed by plasmid-borne copies of these genes in *STA10* and *sta10* backgrounds, using isogenic haploid and diploid strains. Neither *sta10* nor *STA10* haploids expressed *SGA1* at detectable levels under sporulation conditions, when *SGA1* was carried on the single copy *CEN4* plasmid, YCp50 (Table 3). Glucoamylase activity was readily detected in both haploid strains, however, when a high copy number vector was used (3.9 vs 4.3 specific activity). By contrast, only the *sta10* haploid strain expressed detectable levels of glucoamylase when this activity depended on the *STA2* gene, irrespective of whether the vector was present

Table 3. *SGA1* and *STA2* expression during sporulation in *STA10* and *sta10* cells

Strains			Enzymatic activity		Sporulation (%)[b]	Cells retaining plasmid (%)[c]
			Intracellular (specific activity)[a]	Culture supernatant (unit/ml)		
Haploid strains						
30c	*sta10, sga: LEU2*	No plasmid	<0.3	<0.1	0.0	–
		YCp*SGA1*	<0.3	<0.1	0.0	54
		YEp*SGA1*	3.9	<0.1	0.0	52
		YCp*STA2*	12.8	0.6	0.0	74
		YEp*STA2*	19.4	8.1	0.0	48
DK10a	*STA10, sga: LEU2*	No plasmid	<0.3	<0.1	0.0	–
		YCp*SGA1*	<0.3	<0.1	0.0	86
		YEp*SGA1*	4.3	<0.1	0.0	94
		YCp*STA2*	<0.3	<0.1	0.0	49
		YEp*STA2*	0.6	<0.1	0.0	84
Diploid strains						
30c	*sta10, sga: LEU2*	No plasmid	<0.3	<0.1	49	–
		YCp*SGA1*	21.3	<0.1	34	85
		YEp*SGA1*	106.1	0.6	54	59
		YCp*STA2*	25.1	0.4	35	90
		YEp*STA2*	26.1	6.4	40	84
DK10a	*STA10, sga: LEU2*	No plasmid	<0.3	<0.1	17	–
		YCp*SGA1*	2.8	<0.1	28	81
		YEp*SGA1*	71.1	<0.1	24	90
		YCp*STA2*	0.3	<0.1	28	43
		YEp*STA2*	<0.3	<0.1	28	93

[a] Intracellular specific activity was as defined in the legend to Table 2. Values shown are for one typical experiment. Cell were assayed after 24 h incubation in SPM

[b] The percentage of sporulated cells was determined by light microscopy

[c] This was determined as described in the legend to Table 2

in single or multiple copies. This is in accord with the results presented above for vegetatively growing cells; *SGA*1 loses some aspect of regulation which *STA*2 retains when present in high copy number in the cells.

This was corroborated when the same experiment was performed using diploid cells (Table 3). *SGA*1 was expressed during sporulation at relatively high levels in both *sta*10 and *STA*10 backgrounds, whereas *STA*2-directed activity was detected only in *sta*10 strains. This suggests that *STA*10 regulates only *STA*2 and that *SGA*1 activity is expressed independently of the *STA*10 gene.

Independence of SGA*1 expression from haploidization*

One possible mechanism for the expression of *SGA*1 during sporulation is that haploidization during meiosis is directly responsible for this and other late gene expression. If so, both *SGA*1 and *STA*2 could utilize this mechanism of control.

To examine this possibility, we constructed diploid strains wich do not haploidize, due to a lack of *SPO*13 and *RAD*50 activities. The double mutant strain produces viable diploid progeny of a meiosis lacking recombination in which the *MAT*a and *MAT*α alleles of the mating type locus are not segregated to separate haploid cytoplasms. The *spo*13 defect results in an aborted meiosis I division; strains carrying null mutations in this gene proceed to a mitotic-like meiosis II division and form two viable diploid spores from each sporulating cell (Klapholz and Esposito 1980a, b; Wagstaff et al. 1982; Wang et al. 1987). The genetic composition of these diploids is like that of the mother cell, except where recombination has led to segregation of parental alleles at meiosis II. The result is that most of the progeny retain their original *MAT*a/*MAT*α genetic configuration, while approximately 10% become either a/a or α/α due to recombination.

Table 4. Dependence of *SGA*1 expression on sporulation events

Strain	Relevant genotype	SGA specific activity[a]	Sporulation (%)[b]
TYP1	*MAT*a/*MAT*α *spo*13/+ *rad*50/+	25.1	56
TYP100	*MAT*a/*MAT*α *spo*13/*spo*13 *rad*50/*rad*50	16.3	11
TYP101	*MAT*a/*MAT*α *spo*13/*spo*13 *rad*50/*rad*50	25.3	26
K399-5c	*MAT*a *spo*13	<0.5	<0.1
GK231	*MAT*a/*MAT*α +/+	10.8	73.9
GK25	*MAT*a +	0.08	<0.2
GK31	*MAT*α +	0.04	<0.2
GK232	*mata*1/*MAT*α +/+	0.05	<0.2
GK125-30	*mata*/*MAT*α *RES*1-1/*RES*1-1	0.66	2.7

[a] *SGA*1 specific activity is defined as nonomoles glucose produced per minute per milligram protein, under the conditions described in Materials and Methods. Cells were extracted and assayed after 24 h of incubation in sporulation medium

[b] The percentage of asci was determined by light microscopy

This level of mating type homozygosity can be almost completely abolished by also including a *rad*50 defect in the strain background (Malone and Esposito 1981). Strains lacking *RAD*50 do not recombine and in the presence of the wild-type *SPO*13 function, yield four inviable progeny spores per meiotic event. This presumably results from the failure of homologous chromosomes to assort correctly at meiosis I, due to the inability of *rad*50 strains to achieve and maintain normal synapsis (Game et al. 1980; Borts et al. 1980). The *spo*13 *rad*50 double mutant strain allows viable progeny spores to be produced without recombination, because the lethal event (meiosis I segregation) is bypassed. The result of meiosis in such strains is generation of progeny spores in which the configuration of parental markers is completely maintained.

Table 4 shows the results of an experiment in which *SGA*1 activity was monitored in sporulating cultures of *rad*50 *spo*13 cells. Two independent diploids produced glucoamylase during sporulation, at levels comparable to a control strain which was heterozygous for both mutations (15–25 units, in all cases). As expected, singly mutant strains also expressed *SGA*1 (data not shown) whereas haploids did not (Table 4). These results indicate that *SGA*1 expression does not depend on either recombination or the segregation of chromosomes; consequently the formation of compartmentalized haploid cytoplasms is dispensable as well. We conclude that *SGA*1 expression occurs independently of the mating type configuration of the developing ascospore.

The above results and others suggest that the role of the mating type locus in *SGA*1 expression is indirect; that is, *SGA*1 expression depends on the role of the two *MAT* alleles in initiating sporulation in diploids, but not on these gene products themselves. We examined this possibility by monitoring *SGA*1 expression in diploids whose sporulation ability is independent of the mating type of the cell. We utilized a mutation, *RES*1-1, recently isolated in this laboratory (G. Kao and M. Clancy, in preparation), which allows sporulation to occur at low levels in MAT-insufficient diploids.

Table 4 shows the levels of spore formation and *SGA*1 activity attained after 24 h of incubation of wild-type and *MAT* mutant cells in the presence and absence of the *RES*1-1 mutation. As expected, a diploid strain (GK232) which was isogenic to a wild-type control (GK231) except for a null mutation at *MAT*a1 did not sporulate or express *SGA*1 at detectable levels. No asci were detected out of several thousand cells examined, and glucoamylase activity was extremely low (approx. 0.04 units as compared with 10.8 units for the wild-type control). Isogenic haploids (GK31, GK25) also did not sporulate or express *SGA*1.

The sporulation defect and the lack of *SGA*1 expression in the *mat*a1 mutant strain were simultaneously suppressed by the dominant *RES*1-1 mutation (GK125-30). The restored levels of both were low (2.7% for sporulation and 0.66 units for *SGA*1) but substantially above the mutant controls. This result indicates that *SGA*1 depends on the sporulation process, and not on the *MAT* functions themselves.

Mapping of SGA1

We hybridized the cloned *SGA*1 gene to a preparation of *S. cerevisiae* chromosomes which had been resolved by

Table 5. Mapping *sga:LEU2* with respect to chromosome IX markers

Cross	PD[a]	NPD	TT	Map distance (cM)[b]
TPY3b × A236-24c	56	0	0	*sga:LEU2-lys11*, (<0.4)
TPY4a × K399-5c	13	3	30	*sga:LEU2-his6* loosely linked, (39.3)
	6	3	31	*sga:LEU2-trp1*, loosely CEN linked, (>50)
	12	11	16	his6-*trp1*, CEN linked, (20.5)
TPY4b × MJC15a	7	0	6	*sga:LEU2-his5*, (23)
	7	0	6	lys11 − *his5*, (23)

[a] PD, NPD and TT refer to parental ditype, nonparental ditype and tetratype asci, respectively

[b] cM is centimorgans, or average number of crossovers per chromatid times 100. Distances were calculated using Perkin's formula (reviewed in Mortimer and Schild 1981), or, for CEN linkage, 1/2 the percentage second division segregation frequency (SDS) where SDS is regarded as equivalent to the frequency of tetratype asci

OFAGE. In accord with results in other laboratories (Pretorius and Marmur 1988), the *SGA1* probe hybridized to the single band corresponding to Chromosome IX. Hybridization of the same blot with a Chromosome IX marker (SUP17-14g) confirmed this assignment (data not shown).

We then took advantage of the disrupted allele of *SGA1* constructed above to map the gene with respect to other markers on Chromosome IX using the *LEU2* marker to score *SGA1* (Table 5). The gene mapped to the left arm of Chromosome IX, extremely close to *lys11* (no recombinants out of 56 tetrads). The *sga:LEU2* mutation was also mapped with respect to the other Chromosome IX markers, *his6* and *his5*, and to the centromere (Table 5). These results confirmed the location of *SGA1* to be very close to *lys11*, approximately 23 cM from *his5* on the left arm of Chromosome IX. The order of *lys11* and *SGA1* relative to *his5* could not be determined, as there were no recombinants between *lys11* and *SGA1*.

These results suggested that *LYS11* might be close enough to *SGA1* to be present on the same recombinant plasmid. We therefore transformed two *leu2 lys11* strains (19b and 31b) with p*SGA1*-2 and p*SGA1*-4, to determine whether these cloned DNAs could complement a *lys11* mutation. Neither plasmid supported growth of the auxotrophic strains in the absence of exogenous lysine. We conclude that these plasmids did not contain *LYS11* and that this gene must be more than 2 kb away from *SGA1*.

Discussion

The results presented above demonstrate that the factors regulating the expression of *SGA1* are distinct from those modulating *STA2* and, presumably, the other members of the *STA* gene family. *SGA1* activity was insensitive to *STA10*, the primary determinant of *STA2*-directed glucoamylase levels. The *STA10* allele present in common laboratory strains of *S. cerevisiae* inhibits *STA2* very dramatically, whether the cells are growing vegetatively or have been induced to sporulate. Inhibition of *STA2* was released when the cells carried the recessive *sta10* allele. *SGA1* expression, by contrast, was not released under these conditions; even when *SGA1* was carried on a high copy vector derived from the endogenous two micron circle plasmid, *SGA1* levels were indistinguishable in *STA10* vs *sta10* backgrounds.

These results are in marked contrast to those reported in several earlier studies, in which *SGA1* expression was monitored at the level of mRNA abundance or enzymatic activity (Pretorius et al. 1986c; Pardo et al. 1986, 1988). This may be accounted for in part by methodological differences between our own and earlier work. We examined glucoamylase activity under conditions which inhibited other competing activities in the cell (i.e. in the presence of PCMB). In addition, we looked exclusively at plasmid-directed enzymatic activity in strain backgrounds which lacked functional chromosomal alleles of either *STA2* or *SGA1*. We are thus quite confident that the activities we observed actually reflected *SGA1* or *STA2* expression.

Unlike earlier work, however, our study of glucoamylase gene expression required that the corresponding mRNA be translated, and possibly that the protein be transported through the secretion machinery of the cell to be detected. It is thus possible that our failure to detect *SGA1* in *sta10* cells might reflect the failure of a post-transcriptional step in the expression process, rather than a block at the transcriptional level. We do not consider this possibility to be very likely, because we have previously detected high levels of *SGA1* activity in vegetative cells when transcription was driven by the *GAL1* upstream activation site (UAS) (Pugh et al. 1989). In addition, *SGA1* activity was readily detected when the gene was present in multiple copies in vegetative cells. It is possible, however, that we did not detect *SGA1* because the enzymatic assay is less sensitive than a blot analysis might be.

One possible problem in interpreting our experiments arises from the unknown role of *STA10* in regulating *STA2* and the undefined nature of the *STA10* and *sta10* alleles used. Our genetic analysis showed that glucoamylase inhibition segregated as a single gene in the strains we examined, but whether the gene we detected is actually the same as that examined in other laboratories is not at all clear. Consequently, we interpret the release of *STA2* but not *SGA1* expression in *sta10* strains to mean that some aspect of regulation of these two genes is different but not necessarily the same as has been examined previously. Thus, *SGA1* and *STA2* could share a common response to a different *STA10*-like inhibitor or activator function. It would be useful to examine potential allelism among the genes reported by various laboratories.

We also examined the role of the mating type locus in *SGA1* expression. We found that *SGA1* is not regulated directly by *MAT*, but depends on the role of the mating type products in supporting sporulation in diploids. *SGA1* activity was not produced in cells in which the *MATa1* gene was inactive, but was restored to low levels when the cells also carried a mutation (*RES1-1*) which rendered sporulation partially independent of the mating type products.

It would have been preferable to use the well-characterized *rme1:LEU2* mutation (Mitchell and Herskowitz 1986) rather than the previously undescribed *RES1-1* for these experiments. Our attempts to do so were frustrated by the low and extremely variable amounts of sporulation supported by *rme1* in our strain background. We were not able to detect levels of *SGA1* activity which were convincingly above background in such experiments. The *RES1-1*

mutation, whose phenotype is very much like that of *rme*1, gave reproducibly higher levels of sporulation than did the latter; this very likely led to levels of *SGA*1 expression which were sufficient to be detected.

Similarly, haploidization during meiosis was not required for *SGA*1 expression, even though the activity is normally highest during the time when haploid ascospore development is occurring. This is in accord with our earlier studies, which showed that *SGA*1 expression required DNA replication and entry into meiosis, but not functions needed for recombination or spore formation.

Work presented in a recent report has also examined the role of the mating type locus in regulating *STA* gene expression. Dranginis (1989) has observed that *STA*1 and *SGA*1 expression are uncoupled in *sta*10 strains when the background also contains a deletion in *MAT*a1. The lack of functional *MAT*a1 releases *STA*1 from diploid control, as would be expected if, like the other haploid-specific genes, *STA*1 were regulated by the a1/α2 repressor. Conversely, such strains do not express *SGA*1 when incubated in sporulation medium. These results complement those presented above, and are consistent with the view that *STA*1 (or *STA*2) and *SGA*1 are regulated by *MAT* by different mechanisms. The first involves a direct interaction with the *MAT* products and the latter requires their functions in promoting sporulation.

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