# Developmental Regulation of a Sporulation-Specific Enzyme Activity in *Saccharomyces cerevisiae*†

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An α-glucosidase activity (SAG) occurs in a/α *Saccharomyces cerevisiae* cells beginning at about 8 to 10 h after the initiation of sporulation. This enzyme is responsible for the rapid degradation of intracellular glycogen which follows the completion of meiosis in these cells. SAG differs from similar activities present in vegetative cells and appears to be a sporulation-specific enzyme. Cells arrested at various stages in sporulation (DNA replication, recombination, meiosis I, and meiosis II) were examined for SAG activity; the results show that SAG appearance depends on DNA synthesis and some recombination events but not on the meiotic divisions.

It is well documented that as cells undergo developmental processes the proteins synthesized change (1–3, 41). Although this phenomenon has been observed and analyzed in higher and lower eucaryotes and in procaryotes, the molecular basis is poorly understood. Sporulation in the yeast *Saccharomyces cerevisiae* is a useful system for studying development for several reasons. Initiation of sporulation is easily manipulated experimentally, and one can obtain relatively synchronous populations of cells in various stages of sporulation. During the process of sporulation, the cells undergo DNA synthesis, meiosis, and spore formation (6, 10, 30, 35), and conditional mutants which arrest development at particular stages under restrictive conditions are available (8). In addition, the genetic system of *S. cerevisiae* is well understood, making sophisticated genetic analysis possible.

Several reports of attempts to identify sporulation-specific gene products by two-dimensional gel analysis have appeared (21, 40). In general, they have revealed few proteins which are made only during sporulation, although a few such sporulation-specific proteins were observed in one study (I. Dawes, personal communication). Although two-dimensional gel analysis reveals when proteins are made, it does not provide information about their function in development or their regulation. An alternative approach, which is more difficult but could be more fruitful in the long run, is to look for proteins which mediate specific sporulation events and to analyze their regulation by bio-

chemical and genetic techniques. The sporulation amyloglucosidase (SAG) (first described in this laboratory [5]) is such a protein. It is easily detected and amenable to such analysis. The enzyme appears in sporulating cells at about 8 to 10 h after the shift to sporulation medium (SPM) and is responsible for the extensive glycogen degradation which occurs in the cells at the time of completion of meiosis (5). Nonsporulating cells, by contrast, accumulate large amounts of glycogen, but do not degrade it (5). SAG is distinct from glycogen phosphorylase (12) and α-glucosidase (maltase) (16, 28), which are found in vegetative cells; it may be a sporulation-specific enzyme. Understanding the regulation of SAG appearance in sporulating cultures may therefore provide insight into the way that other sporulation events are regulated.

As a first step toward understanding SAG regulation, we have analyzed its appearance in cells arrested at a series of stages in sporulation, either by the presence of inhibitors or because of a genetic constitution which renders them asporogenous (i.e., haploidy, mating type homozygosity, or temperature sensitivity in functions required for DNA replication and meiosis). The purpose of this analysis was to determine whether SAG appearance in sporulating cells depends on normal progress of the cells through DNA replication and meiosis or whether it is regulated in some other way independent of the meiotic process. The results demonstrate that premeiotic DNA replication and possibly some recombination events are necessary for the appearance of SAG but that completion of the meiotic divisions is not. We have also examined vegetative cells under conditions where glycogen catabolism occurs to determine whether SAG is

expressed. The absence of the activity in these cells suggests that SAG is a sporulation-specific enzyme.

## MATERIALS AND METHODS

**Yeast strains.** The standard yeast strains used in this study were AP1a/α, AP1α/α (20), AP3a/α, AP3a/a, AP3α/α, and X2180-1A which was obtained from The Yeast Genetic Stock Center in Berkeley, Calif. The AP3 strains were obtained from A. Hopper. *AP3a/α* was derived from a cross between A36A4 and α₃131-20. AP3a/a and AP3α/α were derived from UV irradiation of the diploid AP3. Temperature-sensitive diploid homozygous *spo* mutants (*spo1 spo3 spo7*) (8, 11) and their parent strain S41 were obtained from R. E. Esposito. A diploid homozygous for the temperature-sensitive cell division cycle mutation, *cdc4* (18), was obtained from Breck Byers. RD5, a diploid homozygous for the *rad52-1* (33) allele was constructed from haploid *rad52-1* strains obtained from R. Malone.

**Growth and sporulation of cells.** Cells were ordinarily pregrown in the acetate-containing presporulation medium (PSP) of Roth and Halvorson (34) or in YEP (10 g of yeast extract and 10 g of peptone) + 10 g potassium acetate per liter of distilled water. Cell cultures (100 to 1,000 ml) were grown at 30 or 22°C (*cdc4*) with shaking until the cell density reached $1 \times 10^7$ to $2 \times 10^7$ cells per ml. The cells were harvested by centrifugation at $4,000 \times g$ for 5 min, washed twice by centrifugation in sterile distilled water, suspended in SPM (3 g of potassium acetate and 0.2 g of raffinose per liter of distilled water) to a concentration of $2 \times 10^7$ to $3 \times 10^7$ cells per ml, and incubated with shaking at the sporulation temperature (22, 30, or 36°C, depending on the experiment). Potassium chloride (4.5%) was added to the SPM in some experiments with the sporulation mutants, and adenine (40 μg/ml) or arginine (40 μg/ml) was included in the medium when the strains used contained these auxotrophic markers. In some experiments, cells were grown in YEPD (10 g of yeast extract, 10 g of peptone, and 20 g of dextrose per liter of distilled water).

**Preparation of cell extracts.** Washed cells were suspended to a density of $1 \times 10^9$ to $3 \times 10^9$ cells per ml in 0.1 M Na-citrate buffer (pH 6.2) containing the protease inhibitors phenylmethylsulfonyl fluoride (0.3 mg/ml, predissolved in 95% ethanol [32]) and aprotinin (Sigma Chemical Co.) (13). When $5 \times 10^9$ cells or more were to be broken, the Bronwill homogenizer was used. The cell suspension was transferred to a Bronwill flask containing 2 to 10 g of Glasperlen glass beads (0.45 mm diameter; B. Braun Melsungen AG, Germany), and the cells were cooled with compressed $CO_2$ during homogenization (90 to 120 s). When fewer than $5 \times 10^9$ cells were used, they were broken by blending in a Vortex mixer with glass beads by a method similar to that of Kraig and Haber (21). Breakage with either method was always greater than 90%. The broken cell suspension was immediately centrifuged at $12,000 \times g$ (10,000 rpm in a Sorvall SS34 rotor) for 20 min and then at 50,000 rpm for 2 h in a Beckman Type 65 rotor ($R_{max}$, $218,000 \times g$). The supernatant was dialyzed for 12 to 18 h against two changes of 0.1 M sodium citrate buffer at pH 6.2 and then either stored at −20°C or assayed immediately for SAG and protein. Control experiments demonstrated that SAG activity was very

low in particulate fractions in both nonsporulating cells and in cells undergoing sporulation.

**Nuclear staining.** Progress through meiosis and the percentage of sporulation were monitored by use of the fluorescent stain 4,6-diamidino-2-phenylindole (43). Epifluorescence of the cells was observed with a Zeiss fluorescence phase-contrast microscope, and cell types were counted with a phase-contrast hemocytometer. At least 300 cells were counted for each time point.

**Analytical methods.** SAG was assayed by measuring the rate of glucose release from glycogen using a coupled assay system containing glucose oxidase (EC 1.1.3.4), peroxidase (EC 1.11.1.7), and *o*-dianisidine, as described previously (5), except that 0.33 mM *p*-chloromercuribenzoate (PCMB) was included in the assay mixture in some of the experiments. D-Glucose was used as a standard. Specific activity was expressed in milliunits per milligram of protein, where one unit is defined as 1 μmol of glucose released per minute. Protein was determined by the method of Lowry et al. (26).

## RESULTS

**SAG appearance in sporulating cells.** Cycloheximide arrests sporulation if it is added to the cells at any time before ascus formation is complete (20). Glycogen degradation becomes insensitive to the drug only shortly before this event occurs (20), suggesting that it might be mediated by proteins which are synthesized at this time. To determine whether SAG appearance depended on continued protein synthesis, we examined AP1a/α cells incubated in SPM in the presence and absence of cycloheximide. The addition of cycloheximide (100 μg/ml) after 5 h of incubation in SPM completely prevented SAG appearance (Fig. 1). When the inhibitor was added at 9 h, when SAG specific activity is increasing, no further increase was observed and the level of SAG activity remained constant. In the control culture, SAG appeared normally. This experiment suggests that SAG may be synthesized de novo during sporulation, but it is also possible that its appearance depends on the synthesis of an activator or other proteins.

**Sporulation specificity of SAG.** Many yeast strains grown under the appropriate condition contain enzymes which are capable of releasing glucose from α-1,4-glucosides and α-1,6-glucosides of various lengths. These include maltase, which can comprise up to 2% of the soluble protein in some strains (28), isomaltase (α-methylglucosidase) (22), and glucoamylase (19). Release of glucose from glycogen could also be due to an amylase in combination with maltase or to glycogen phosphorylase and a phosphatase. Although little or no soluble α-1,4-glucosidase activity is present in AP1a/α cells during pregrowth in PSP (Table 1), it was possible that SAG might be present in some vegetative cells but that this activity had been attributed to one
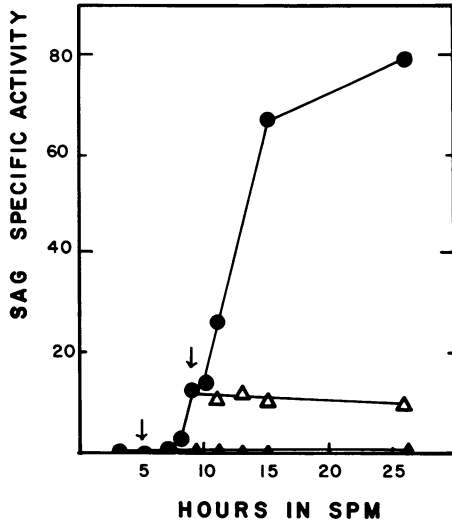
FIG. 1. Inhibition of the appearance of glycogeno-lytic activity by cycloheximide. A vegetatively grow-ing PSP culture of AP1a/α (~3 × 10⁷ cells per ml) was shifted into SPM and incubated at 30°C (●). At 5 (▲) and 9 h (△), cycloheximide (100 μg/ml) was added to portions of the culture as indicated by the arrows. At the indicated times, 50-ml samples were harvested from the three cultures, the cells were stored at −20°C until crude extracts were made by blending in a Vortex mixer with glass beads as described in Materials and Methods. SAG activity was assayed in the presence of 0.33 mM PCMB by measuring the release of glucose from glycogen with glucose oxidase, as described above.

of these other enzymes. SAG activity can be distinguished from other activities observed in crude extracts by sensitivity to inhibitors, par-ticularly the sulfhydryl reagent, PCMB (0.33 mM) (M. J. Clancy and P. T. Magee, manuscript in preparation). We therefore assayed amyloglu-cosidase activity in the presence and absence of this inhibitor in extracts from AP1a/α cells har-vested in the exponential phase of growth in YEP acetate, PSP, and YEPD, in stationary phase in YEPD, and in stationary-phase cells shifted to fresh YEPD for the presence of an enzyme insensitive to this inhibitor. Significant activity against glycogen was observed in ex-tracts from YEP-acetate-grown cells and in those from stationary-phase cells shifted to fresh YEPD, but these activities were about 85 to 90% inhibited by 0.33 mM PCMB (Table 1). The activity in extracts from sporulating cells was not inhibited at this concentration (Table 1), showing that the sporulation activity was dis-tinct from those present in vegetative cells, and that if SAG is present in the vegetative cultures examined, its level must be at least 20 to 100 times lower than in sporulating cells.

**Appearance of SAG in sporulating and non-sporulating cells.** Cells which are either haploid or diploid and homozygous at the mating type locus (α/α or a/a) undergo the physiological changes associated with starvation which are presumably involved in the initiation of meiosis in a/α cells, but fail to undergo premeiotic S or meiosis (20). To determine whether SAG ap-pearance depended on entry into meiosis or resulted merely from prolonged starvation in SPM, we shifted cultures of the sporulation-proficient AP1a/α and the asporogenous diploid AP1α/α and haploid X2180-1A to SPM and ex-amined them for SAG activity and sporulation at 0, 24, 48, and 72 h after the shift. The specific activity of SAG after 24 h of incubation in SPM was about 200-fold higher in the AP1a/α culture than in the AP1α/α culture and 400-fold higher than in the haploid X2180-1A (Table 2). The SAG activity in the sporulated culture declined after the completion of sporulation, whereas the slight activity observed in the α/α and haploid cultures increased marginally. At 72 h, when the activity in the AP1a/α culture had declined to 50% of the value obtained at 24 h, the activity in the α/α culture was still at least eightfold lower than in the a/α cells. The extent of sporulation was 48% in the a/α culture, and no asci were observed in the other cultures. A similar experi-ment was performed with the diploid strains AP3a/α, AP3a/a, and AP3α/α (Table 2). As in experiment 1, glycogen-degrading activity was low in the nonsporulating cells (a/a and α/α) but high in the sporulating a/α culture. These results demonstrate that the physiological adaptations to starvation occurring in asporogenous (α/α, a/a, and haploid) cells are not sufficient for SAG expression and suggest that events specific to a/α cells may also be necessary.

**Appearance of SAG in cells unable to com-plete DNA synthesis and recombination.** To determine whether premeiotic DNA synthesis is required for SAG appearance, we measured the

TABLE 1. SAG specific activity in AP1a/α cells with glycogen as substrate

| Medium | Sp act | |
|---|---|---|
| | −PCMB | +PCMB |
| YEP acetate | 2.25 | 0.221 |
| YEPD | | |
| Exponential growth | 0.0381 | ND[a] |
| Stationary phase | 0.261 | ND |
| Fresh YEPD[b] | 7.66 | 1.08 |
| PSP | 0.309 | 0.0387 |
| Sporulating cells | 23.3 | 23.7 |

[a] ND, Not determined.
[b] Cells were grown to stationary phase in YEPD then shifted to fresh YEPD and incubated at 30°C for 30 min.

TABLE 2. SAG specific activity in strains of *S. cerevisiae*[a]

| Strain | Sp act at following time in sporulation medium (h): | | | |
|--------|------|------|------|------|
| | 0 | 24 | 48 | 72 |
| AP1a/α | 0.202 | 16.2[b] (ND)[c] | 12.5[b] (48)[d] | 8.45[b] (43) |
| AP1α/α | 0[b] | 0.104[b] (0) | 0.697[b] (0) | 1.67[b] (0) |
| X2180-1A | 0.0487 | 0.0382[b] (0) | 0.540[b] (0) | 1.24[b] (0) |
| AP3a/α[e] | 0.681 | 9.77 (58.0) | 11.3 (ND) | 8.07 (78.0) |
| AP3a/a[e] | 0.418 | 0.369 (0) | 0.282 (0) | 0.124 (0) |
| AP3α/α[e] | 0.234 | 0.562 (0) | 1.19 (0) | 0.446 (0) |

[a] Unless otherwise noted, all extracts were made from cells incubated at 30°C in SPM after pregrowth in PSP, and SAG activity was assayed in the presence of 0.33 mM PCMB.

[b] Assayed in the absence of 0.33 mM PCMB.

[c] ND, Not determined.

[d] Numbers in parentheses indicate the percentage of sporulation.

[e] YEP acetate preculture.

specific activity of SAG in AP1a/α cells incubated in SPM in the presence of DNA synthesis inhibitors and in a strain containing a temperature-sensitive mutation affecting DNA synthesis (*cdc4* [39]). Table 3 shows the effect of hydroxyurea (6 mg/ml) (38) and sulfanilamide (12 mg/ml) (4) on the level of SAG in AP1a/α cells incubated for 24 h in SPM after pregrowth in PSP. The specific activity of SAG was about 65-fold lower in the hydroxyurea-treated culture and 220-fold lower in the sulfanilamide-treated culture than in the untreated AP1a/α cells. Cells treated with these inhibitors failed to sporulate and remained mononucleate.

A culture of the homozygous diploid strain *cdc4* (temperature sensitive for vegetative and premeiotic DNA synthesis) was pregrown in YEP acetate at 22°C and shifted to SPM at 34°C (the restrictive temperature) or at 22°C. In this experiment and in those experiments with *spo* mutants (to be described below) the cells were precultured in YEP acetate because these strains grew poorly in PSP. As a result, sporula-

TABLE 3. SAG specific activity in a/α diploid strain AP1 arrested at premeiotic DNA synthesis[a]

| Inhibitor | % Sporulation | Sp act |
|-----------|---------------|--------|
| None | 70% | 37.6 |
| Hydroxyurea | <0.5% | 0.578 |
| Sulfanilamide | <0.3% | 0.171 |

[a] A 100-ml culture of AP1a/α was grown in PSP to a concentration of $3.7 \times 10^7$ cells per ml and shifted to 150 ml of SPM. This culture was divided immediately into three subcultures, and hydroxyurea (6 mg/ml) or sulfanilamide (12 mg/ml) was added to two subcultures. The third culture contained no inhibitor (control). After 24 h of incubation in SPM, cells were harvested by centrifugation and broken by blending in a Vortex mixer with glass beads (18). Extracts were prepared and assayed for SAG in the presence of 0.33 mM PCMB as described in Materials and Methods.

tion was slower than that observed with PSP-grown cells. Samples were removed at 0, 24, 48, and 72 h after the shift, and the cells were monitored for SAG appearance and progress through meiosis. *cdc-4* cells at 22°C progressed normally through meiosis and spore formation, as indicated by a high percentage of bi- and tetranucleate cells (data not shown) and by the amount of sporulation at 48 and 72 h (46.7 and 50.3%, respectively). The 34°C cells, however, were generally arrested at the mononucleate stage, and the specific activity of SAG was at least 10 times lower than in the 22°C cells (Table 4). We conclude from these experiments that premeiotic DNA synthesis is necessary for SAG expression in sporulating cells. Further experiments were then performed to determine whether later meiotic events (i.e., recombination and meiotic divisions) were also required for SAG expression.

AP1a/α cells harbor a mutation, *pac1*, which prevents completion of meiosis at 36°C (7); these cells undergo DNA synthesis, though slightly later than do those at 30°C, form synaptonemal complexes, and become committed to recombination at high levels, but they fail to complete recombination or to undergo the meiotic division. They complete recombination normally if returned to vegetative medium at either 30 or 36°C (unpublished data). To determine whether SAG appears in AP1a/α cells arrested in pachytene at 36°C, extracts were prepared from AP1a/α cells sampled at intervals during incubation in SPM at 30 and 36°C and assayed for this activity. Only the 30°C (control) cells showed a high specific activity of SAG, although some activity was present in 36°C cells at later times (Fig. 2A). This may be due to a slight leakiness of the 36°C block, since a few cells (~5%) were able to progress through meiosis to form asci in some experiments (not shown). The low level of activity observed in the 36°C cells cannot be
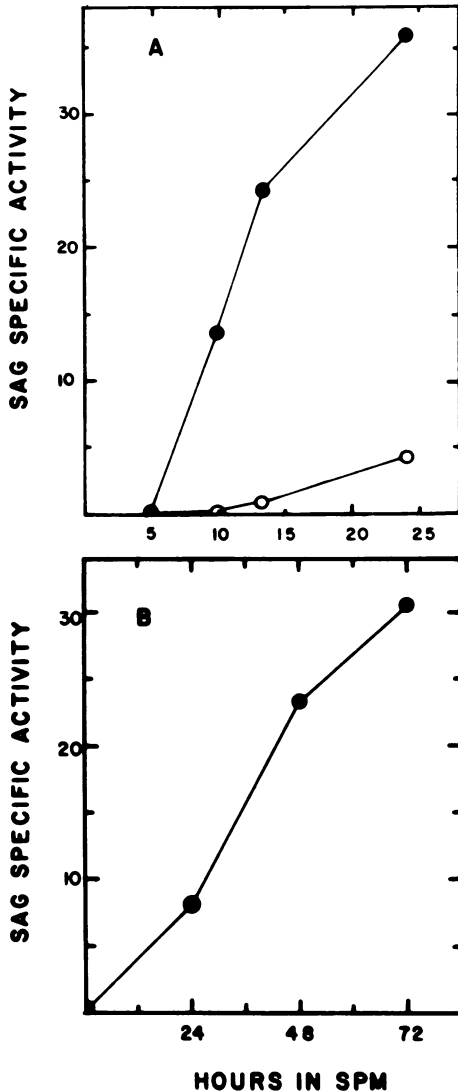
FIG. 2. SAG activity in cells blocked in pachytene or in cells unable to complete recombination. PSP cultures were shifted into SPM at a concentration of $2.5 \times 10^7$ cells per ml. (A) AP1$a/\alpha$ incubated at either 30°C (●) or 36°C (○). At various times, 50-ml portions were harvested, broken by blending in a Vortex mixer with glass beads, and assayed as in Fig. 1. Ascus formation was 63 and 0% at 24 h in the 30 and 36°C cultures, respectively. (B) PSP preculture of RD-5 was shifted to SPM at $2 \times 10^7$ cells per ml and incubated at 30°C. At the indicated times, 250-ml portions were harvested, broken by Bronwell homogenation and assayed as in Fig. 1.

due to loss of viability, since these cells had normal levels of SAG if they were returned to 30°C (not shown).

The failure of cells blocked in DNA synthesis or pachytene to express SAG suggests that the appearance of this activity may require completion of recombination. To examine this possibility, the appearance of SAG was monitored in RD-5, a diploid strain homozygous for the *rad52-1* allele, which increases X-ray sensitivity and reduces sporulation (14, 33). The *RAD52* gene product may be involved in generalized recombination, since mutations in the *RAD52* gene reduce both meiotic (15, 31) and mitotic (27) recombination. RD-5 cells incubated in SPM at 30°C were sampled at 0, 24, 48, and 72 h and examined for progress through meiosis and for SAG activity. Although mature asci were not observed, the appearance of bi- and tetranucleate cells indicated that entry into meiosis had occurred (data not shown). SAG activity was present in these cells at essentially normal levels (Fig. 2B). This shows that successful completion of recombination is not necessary for the appearance of SAG.

**SAG appearance in cells unable to complete meiosis.** The specific activity of SAG was also determined in sporulation mutants which are blocked at meiosis I (*spo1*) or in nuclear migration after meiosis II (*spo3*) (8). Cultures of *spo1* and *spo3* were grown in YEP acetate at 30°C and shifted to SPM at 22 and 34°C. Samples were taken at intervals for determination of the percentage of sporulation and the specific activity of SAG. In both mutants SAG occurred at roughly the same specific activity in the 22°C cells as in the 34°C cells (Table 4). The specific activity in *spo1* (Table 4) at both temperatures, however, was considerably lower than in *spo3* or its parent strain S41 (Table 4). This was probably due to a failure of many of the *spo1* cells to initiate sporulation even at 22°C, as suggested by the low percentage of asci (9.2%).

We conclude from the experiments with mutants and inhibitors that SAG appearance is a developmental event which depends on DNA synthesis and some pachytene steps but not on completion of the meiotic divisions.

## DISCUSSION

The evidence presented here indicates that the amyloglucosidase activity which appears in sporulating *S. cerevisiae* cells at the time of completion of meiosis is developmentally regulated and is probably unique to sporulating cells. The temporal regulation of this activity may be achieved by coordination with the meiotic process. SAG falls into the class of functions which are under the control of mating type, since it is not expressed in a cells or $\alpha/\alpha$ and a/a cells in sporulation medium. Neither does it occur in a/$\alpha$ cells under vegetative conditions in which accumulation or degradation of glycogen occurs (23). The low level of activity seen in the nonsporulat-

TABLE 4. SAG specific activity in mutants of *S. cerevisiae*[a]

| Strain | Stage of arrest | Sp act at following time in sporulation medium (h): | | | | |
|--------|-----------------|---------|-----------|-------------|-------------|------------|
| | | 0 | 24 | 48 | 72 | 96 |
| S41 | | ND[b] 22°C | 2.18 (10.5)[c] | 78.4 (48) | 64.9 (54) | ND |
| | | 34°C | 3.00 (4.4) | 53.8 (34.0) | 51.8 (29) | ND |
| *cdc4* | DNA synthesis | 0.154 22°C | 3.63 (18.0) | 23.0 (46.7) | 24.6 (50.3) | ND |
| | | 34°C | 1.69 (0) | 1.18 (0) | 2.82 (0) | ND |
| *spo1*[d] | Meiosis[d] | 0.421 22°C | ND | 5.54 (7.1) | 9.80 (9.2) | 12.5 (9.2) |
| | | 34°C | ND | 9.47 (0) | 9.82 (0) | 10.5 (0) |
| *spo3*[d] | Spore formation | 0.151 22°C | 1.37 (0) | 13.2 (ND) | ND | 61.6 (51.0) |
| | | 34°C | 3.19 (0) | 55.7 (ND) | ND | 95.5 (9.5) |

[a] All strains were preincubated in YEP acetate at 22°C (*cdc4*) or 30°C (S41 and *spo* mutants), SAG-specific activity was determined in the presence of 0.33 mM PCMB after incubation in SPM at 22°C (permissive temperature) and 34°C (restrictive temperature) as described in Materials and Methods.
[b] ND, Not determined.
[c] Numbers in parentheses indicate the percentage of sporulation.
[d] Incubation in SPM was done in the presence of 4.5% KCl.

ing cells may be attributed to another enzyme, since it is sensitive to the addition of PCMB, a compound to which SAG is relatively insensitive. It could also be due to glycogen phosphorylase in combination with a phosphatase. It would thus seem that SAG is a true sporulation-specific enzyme, the only one so far identified, although numerous sporulation-specific activities (such as nucleases and DNA repair enzymes) have been postulated to exist (40). Developmentally specific proteins have also been observed in other systems, particularly in *Dictyostelium discoideum*, in which a series of stage-specific enzyme activities has been detected (24, 25, 36).

The programmed appearance of a substantial enzyme activity and its apparent restriction to sporulating cells is in apparent contradiction to the results of Trew et al. (40) and Kraig and Haber (21), who have looked for sporulation-specific proteins on two-dimensional gels with no success. Several possible explanations for this discrepancy may be considered. For example, the SAG protein might be a very small fraction of the protein synthesis at any one time, it might have properties which make it difficult to detect on a gel (i.e., be very basic), or it might be synthesized in vegetative cells and activated during sporulation by a proteolytic modification. Kraig and Haber have estimated that only the most prominent 10% of proteins synthesized by sporulating cells are observed by two-dimensional analysis; if SAG is not a member of this prominent group of proteins, it could easily have been undetected on the gels (21). These possibilities can be resolved by immunoprecipitation of labeled extracts. Our preliminary experiments with antibody against purified SAG suggest that SAG is, in fact, synthesized de novo during

sporulation (Clancy and Magee, unpublished data). The requirement for protein synthesis until the time of SAG appearance (Fig. 1) also supports this notion.

Genetic analysis of mutants defective in the cell cycle (*cdc*) (17, 31, 37, 39) and sporulation (*spo*) (8, 9) and similar mutants in other developmental systems (*D. discoideum* [25], *Polysphondylium violaceum* [42], and *Caulobacter crescentus* [29]) has led to models for development in which the order of events (i.e. in this case, DNA replication, nuclear division, etc.) is fixed; as a consequence particular events fail to occur when early events are blocked. The existence of these developmental mutants in *S. cerevisiae* has enabled us to ask whether SAG appearance is coordinated with the meiotic process and to characterize relatively precisely the stage in sporulation upon which it is dependent. Our results demonstrate that SAG activity fails to appear in asporogenous cells and in cells in which sporulation is arrested before or during premeiotic DNA synthesis (by the *cdc4* mutation or the inhibitors hydroxyurea or sulfanilamide). When meiosis is arrested at the first division (*spo1*) or after the second division (*spo3*), however, SAG appearance occurs normally. This indicates that SAG appearance in sporulating cells depends on premeiotic DNA synthesis or later steps, but not on completion of the meiotic divisions. The results obtained when SAG appearance was measured in cells arrested during recombination in *pac1* or *rad52* cells indicate that SAG expression depends on some recombination steps but not on the successful completion of recombination. These experiments demonstrate that SAG appearance depends on progress through meiosis and that SAG

is a developmentally regulated enzyme. An understanding of the molecular basis of this regulation will require isolation and a detailed characterization of the SAG gene and its product; these experiments are currently in progress.

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