Protein synthesis during germination of heterothallic yeast ascospores

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Received 15 August 1991; accepted 7 April 1992

Abstract. Protein synthesis during ascospore germination of the heterothallic *Saccharomyces cerevisiae* strain AP-3 was investigated. Protein synthesis in the germinating ascospores appeared to begin approximately 20 min following glucose initiation. Since RNA synthesis did not start until approximately 70 min after the onset of germination, strain AP-3 ascospores must contain RNA which is ready for immediate translation. Both trehalase and glyceraldehyde-3-phosphate dehydrogenase activities were found to be affected by the onset of germination. Trehalase activity was found to increase severalfold following 60 min of spore germination but remained relatively constant over the subsequent 120 min examined. Dehydrogenase activity was not detectable in AP-3 ascospores but was measurable in germinating ascospores.

Key words. Germination; yeast; protein synthesis; trehalase; glyceraldehyde-3-phosphate dehydrogenase.

The developmental process germination in yeast represents the transition from an ascospore to a vegetative cell. It is an excellent model to study eukaryotic cell differentiation. The transition of an eukaryotic cell from a dormant metabolic state to an active one provides the means to learn what macromolecular and biochemical changes must be initiated to complete this change. An important macromolecular change that begins during the return to the vegetative state is the initiation of protein synthesis. Relatively few studies have closely examined protein synthesis during ascospore germination of the yeast *Saccharomyces cerevisiae*. Those studies that have been performed involved the use of homothallic strains [1–4]. From these studies, it was observed that the syntheses of the enzymes trehalase (EC 3.2.1.28) and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) were affected by ascospore germination [3, 5–7]. Unfortunately, the isolation of germination mutants using homothallic strains will not reveal much about the genetics controlling this developmental process. With respect to heterothallic yeast strains, it has been shown that ascospores of *S. cerevisiae* strain AP-3 mitochondrial mutants failed to complete germination [8]. This is likely related to respiration resumption during outgrowth of the wild-type ascospores [8]. It was also shown that DNA synthesis in AP-3 spores was initiated 2 h after glucose addition [8]. If germination mutants are to be isolated from heterothallic strains, it is vital to learn how protein synthesis is influenced by ascospore germination. In this report, protein synthesis of the heterothallic *S. cerevisiae* AP-3 ascospores is examined. In particular, the activities of trehalase and glyceraldehyde-3-phosphate dehydrogenase were investigated during early germination.

Materials and methods

S. cerevisiae strain AP-3 (a/α) was used in this study and maintained on YEPD agar. After the cells grown in acetate medium [3] reached a concentration of 2×10^7 cells/ml, they were collected by centrifugation. Following washing, the cells were suspended in sporulation medium for 24 h [3]. Ascospores were isolated after treatment with Lyticase (1000 units/ml) for 1 h using the conditions previously described [3]. Buffered synthetic succinic acid medium (SSM) was utilized in the ascospore germination experiments [9].

Uptake and incorporation studies. When measuring uptake, SSM (5–10 ml) cultures containing 1 μCi/ml [^{35}S] methionine or [^{14}C] adenine were prewarmed to 30 °C. The final ascospore concentration present in each culture was 1×10^8 cells/ml and 1% glucose was used to initiate germination. When added, the concentration of cycloheximide was 0.1 mg/ml. Medium (0.05 ml) was sampled at selected intervals during germination onto prewashed 0.45 μM nitrocellulose filters. Filters were washed with SSM containing an excess of the unlabeled compound being examined, to remove non-specifically bound labeled compound, and then counted.

Incorporation of labeled precursors into trichloroacetic acid (TCA) precipitable material was used to monitor macromolecular syntheses. To initiate germination, ascospores were added to SSM cultures containing 1 μCi/ml labeled compound. Samples (0.05 ml) of the medium were treated with 5 ml cold 5% TCA. Each treated sample remained in ice for 10 min and then was filtered onto Whatman GF/A filters. After washing with 5% TCA, the filters were counted.

Extract preparation and enzyme assays. To prepare the crude extract for assaying trehalase or glyceraldehyde-3-phosphate dehydrogenase activity, 400 and 800 mg wet AP-3 ascospores, respectively, were allowed to germinate at 30 °C in 100 ml SSM containing 1% Tween 80 and 1% glucose with aeration on a rotary shaker (200 rpm). If cycloheximide was included in this medium, its final concentration was 0.1 mg/ml. For the trehalase or dehydrogenase assays, sample volumes corresponding to 100 and 200 mg wet ascospores, respectively, were removed from the untreated cultures at 0, 2, 5, 30, 60, 120 and 180 min

or from the cultures containing cycloheximide at 60 min after glucose addition. The spores were collected by centrifugation at 4000 × g for 10 min. For the trehalase assay[7,10], the spores were suspended in 5 mM potassium phosphate buffer, pH 7.5 (1.2 ml). The spores were suspended in 50 mM potassium phosphate buffer, pH 7.4 containing 2 mM 2-mercaptoethanol and 2 mM EDTA (1.2 ml) for subsequent assaying of dehydrogenase activity. After 2.5 g glass beads were added to the suspension in a tube, the spores were broken by high speed agitation using a vortex mixer (3 × 30 s) where the tube was kept in ice between disruptions. For the trehalase assay, the crude extract was centrifuged for 5 min at 10,000 × g and dialyzed overnight against 5 mM potassium phosphate buffer, pH 7.5[7,10]. For the dehydrogenase assay, the extract was centrifuged at 20,000 × g for 10 min and the supernatant was assayed immediately.

Trehalase activity was assayed at 30 °C as previously described using a spectrophotometric methodology[10]. A unit of trehalase activity is expressed as nmol glucose liberated/min. Glyceraldehyde-3-phosphate dehydrogenase was assayed at 25 °C by a prior method that follows the conversion of NADH to NAD at 340 nm[11]. A unit of dehydrogenase activity is given as nmol glyceraldehyde-3-phosphate utilized/min. Protein was determined by the procedure of Bradford[12] using lysozyme as the standard.

Results and discussion

Protein synthesis in germinating *S. cerevisiae* AP-3 ascospores was investigated. To measure protein synthesis during germination, it is necessary to use a labeled amino acid that is transported into ascospores early during germination[3,4]. It was found that [^{35}S] methionine is transported into the germinating ascospores immediately after glucose initiation (fig. 1 A). Uptake of the radiolabel by the germinating spores was relatively linear during the first 120 min following their initiation (fig. 1 A). With methionine being transported into the spores, it was possible to examine its incorporation into protein. From figure 1 B, it can be seen that methionine incorporation into protein had begun by 20 min after germination initiation. Methionine incorporation into protein was observed to be nearly linear over the next 100 min (fig. 1 B). If the eukaryotic protein synthesis inhibitor cycloheximide was added to the culture medium, methionine incorporation into protein failed to occur in the germinating spores. This indicated that incorporation is truly the result of protein synthesis. In studying protein synthesis during AP-3 ascospore germination, it was not known whether this synthesis was due to messenger RNA already present in the spores or whether RNA synthesis had resumed immediately upon initiation of ascospore germination. It has been shown in prior studies that RNA synthesis can be monitored by adenine incorporation[3]. Uptake of [^{14}C]adenine by germinating ascospores is shown in figure 2 A. Rapid adenine uptake

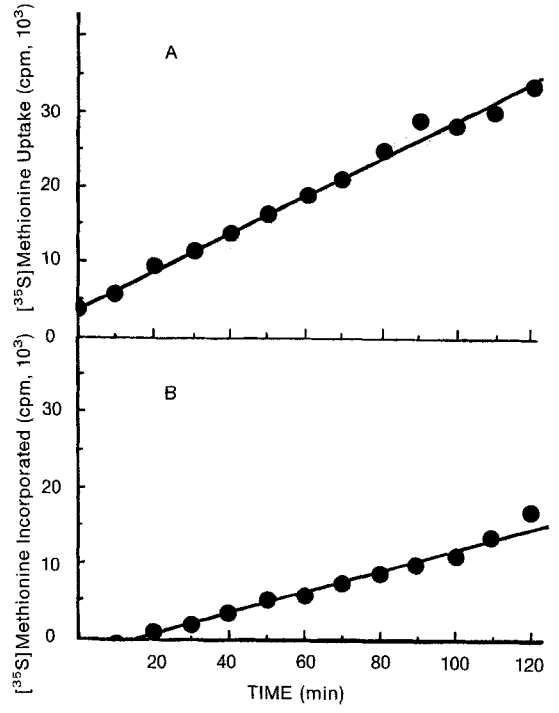

Figure 1. Uptake (A) and incorporation (B) of methionine by strain AP-3 germinating ascospores. Performed as described in 'Materials and methods'.

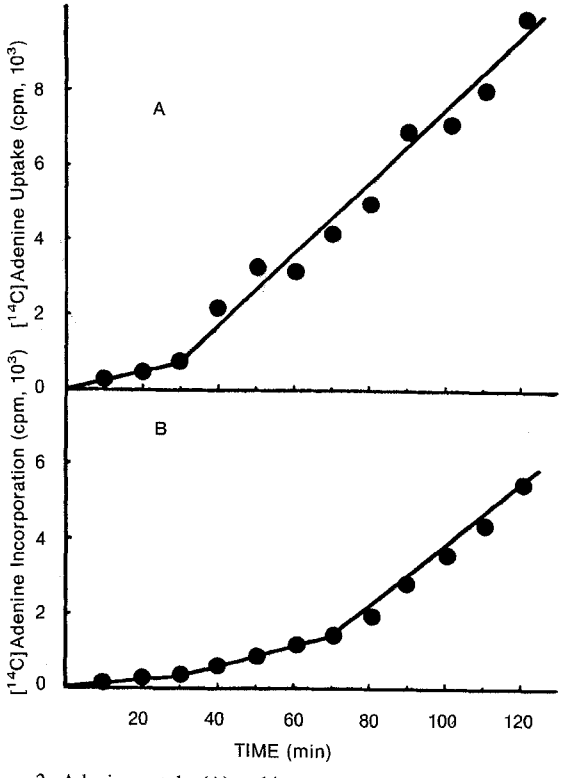

Figure 2. Adenine uptake (A) and incorporation (B) by strain AP-3 germinating ascospores. Performed as stated in 'Materials and methods'.

appears to be occurring at about 30 min following the start of spore germination (fig. 2A). If RNA synthesis resumes upon initiation of spore germination then rapid incorporation should also occur at 30 min. This was not found to occur. Instead, RNA synthesis appears to begin at about 70 min when a sharp increase in adenine incorporation can be noted (fig. 2B). Since RNA synthesis does not begin once germination has been initiated, the ascospores must contain RNA that is available for immediate translation. Polyadenylate-associated RNA, which can be rapidly translated, has been isolated from yeast ascospores[13,14]. Proteins necessary for the transition from spore to vegetative cell are likely synthesized from this stored RNA. An immediate transition in yeast ascospore protein synthesis has been observed after the first 15 min of germination[4]. In prior investigations, it has been shown that the synthesis of 2 enzymes, namely trehalase and glyceraldehyde-3-phosphate dehydrogenase, was influenced by the onset of germination[3,5–7]. The activities of these enzymes were determined during the first 3 h following the initiation of spore germination (table). It was observed that trehalase activity was present in strain AP-3 ascospores. Once spore germination had begun, its activity increased slightly during the first 5 min, doubled after 30 min and was more than 6-fold higher by the end of the first 60 min (table). Trehalase activity remained relatively constant over the subsequent 120 min (table). Similarly, trehalase activity in a homothallic *S. cerevisiae* strain has been noted to increase 10-fold during early germination[5–7]. As germination proceeds, this enzyme activity slowly diminished[5–7]. In this same strain, a significant decrease in the trehalose concentration of yeast ascospores has been witnessed during the first 90 min of germination[15]. In contrast to trehalase activity, dehydrogenase activity was not detectable in strain AP-3 ascospores but was measurable in the germinating ascospores when assayed after 2 min (table). During the initial 60 min of germination, dehydrogenase activity steadily rose (table). The start of glycolysis within the germinating spores by glucose addition is likely related to this change in activity. Over the remaining 120 min, this glycolytic enzyme activity was found to steadily decrease (table). In a prior study, the dehydrogenase has been shown to be synthesized in ascospores that had been undergoing germination for 100 min[3]. Trehalase or dehydrogenase activity was found to be 14.2 ± 0.50 (mean ± SEM) or 0.47 ± 0.07 units/mg protein, respectively, in spores that had been incubated for 60 min in culture medium containing the protein synthesis inhibitor cycloheximide. The above results indicate that the increases in both enzyme activities are not solely due to the synthesis of new protein. Post-translational regulation, which has been previously found to control neutral trehalase activity[16,17], appears to be a factor in the observed rise of each enzyme activity during germination. In conclusion, protein synthesis in germinating AP-3 ascospores has been shown to resume prior to RNA synthesis indicating the spores must contain RNA which can be immediately translated. This is also confirmed by the finding that novel syntheses of trehalase and glyceraldehyde-3-phosphate dehydrogenase seem to have occurred by 60 min after germination initiation. Novel syntheses of these enzymes did not account for the overall elevation of their activities since post-translational regulation of these enzymes was also evident.

Trehalase and glyceraldehyde-3-phosphate dehydrogenase activities in ascospores and germinating ascospores

Time (min)	Specific activity Trehalase	Glyceraldehyde-3-phosphate dehydrogenase
0	6.9 ± 0.95	<0.01
2	6.5 ± 0.94	0.58 ± 0.05
5	7.5 ± 0.96	0.73 ± 0.11
30	14.1 ± 1.18	1.04 ± 0.07
60	42.3 ± 2.22	2.48 ± 0.20
120	44.7 ± 5.60	2.36 ± 0.10
180	44.7 ± 7.62	1.79 ± 0.15

Activity determinations were performed as described in 'Materials and methods'. Each specific activity (mean ± SEM) is presented as units/mg protein.

Acknowledgments. We wish to thank A. Hopper for providing us with strain AP-3 and wish to thank Beth Hamer for her expert technical assistance. This work was supported by NIH grant GM41115 and by the South Dakota Agricultural Experiment Station (Paper 2593, Journal Series).

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1 Rousseau, P., and Halvorson, H. O., J. Bact. *113* (1973) 1289.
2 Steele, S. D., and Miller, J. J., Can. J. Microbiol. *23* (1977) 407.
3 Armstrong, R. L., West, T. P., and Magee, P. T., Can. J. Microbiol. *30* (1984) 345.
4 West, T. P., Armstrong, R. L., and Magee, P. T., FEMS Microbiol. Lett. *28* (1985) 255.
5 Thevelein, J. M., den Hollander, J. A., and Shulman, R. G., Proc. natl Acad. Sci. USA *79* (1982) 3503.
6 Thevelein, J. M., and Jones, K.-A., Eur. J. Biochem. *136* (1983) 583.
7 Thevelein, J. M., Archs Microbiol. *138* (1984) 64.
8 Hartig, A., Schroeder, R., Mucke, E., and Breitenbach, M., Curr. Genet. *4* (1981) 29.
9 Sebastian, J., Carter, B. L. A., and Halvorson, H. O., J. Bact. *108* (1971) 1045.
10 Van Assche, J. A., Carlier, A. R., and Dekeersmaeker, H. I., Planta *103* (1972) 327.
11 Maitra, P. K., and Lobo, Z., J. biol. Chem. *246* (1971) 475.
12 Bradford, M. M., Analyt. Biochem. *72* (1976) 248.
13 Harper, J. F., Clancy, M. J., and Magee, P. T., J. Bact. *143* (1980) 958.
14 Kurtz, S., and Lindquist, S., Cell *45* (1986) 771.
15 Rousseau, P., Halvorson, H. O., Bulla, L. A. Jr., and St. Julian, G., J. Bact. *109* (1972) 1232.
16 Thevelein, J. M., Exp. Mycol. *12* (1988) 1.
17 App, H., and Holzer, H., J. biol. Chem. *264* (1989) 17583.

0014-4754/92/080786-03$1.50 + 0.20/0