SHORT COMMUNICATION

Germination and Outgrowth of *Schizosaccharomyces pombe* Spores Isolated by a Simple Batch Centrifugation Technique

By R. JOHNKE AND G. M. PADILLA

Department of Physiology, Duke University Medical Center,
Durham, North Carolina 27710, U.S.A.

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Spores of the fission yeast *Schizosaccharomyces pombe* have been separated from vegetative cells by a simple and rapid centrifugation (800 g for 20 min) through a 35% Hypaque solution to a purity > 95%. Approximately 35% of the spores were recovered. Regrowth in EMM2 plus glucose showed that over 97% of the spores germinated within the first 2 h and outgrowth continued between 5 and 10 h. Sucrose induced germination in > 95% of the spores with a 1 h delay and outgrowth in 50% of the spores with a 3 h delay. There was little protein synthesis during germination but the protein content increased linearly coincident with outgrowth. The RNA content increased slightly during germination, but increased linearly 1 h before the onset of outgrowth and protein synthesis. After 8 h of regrowth, coincident with the onset of DNA synthesis, the rate of RNA synthesis was accelerated. The DNA content had increased 1.7-fold after 10 h of regrowth from a haploid level of 1.36×10^{-8} $\mu\text{g spore}^{-1}$.

INTRODUCTION

Germination and outgrowth of yeast spores may be considered as a model of cellular differentiation since the transition from dormancy to vegetative growth involves a complex series of morphological and physiological changes. The budding yeast *Saccharomyces cerevisiae* has been used to study the processes underlying this transition (Rousseau & Halvorson, 1973a, b, c; Steele & Miller, 1974, 1977; Tingle *et al.*, 1974), but the fission yeast *Schizosaccharomyces pombe* would seem preferable for at least two reasons: the spores are spontaneously released from the ascus, and mating and conjugation do not occur prior to completion of the first round of cell division. Unfortunately, until recently (Padilla *et al.*, 1974, 1975; Nishi *et al.*, 1978), it was difficult to obtain spores free of vegetative cells. Density gradient centrifugation through sucrose (Padilla *et al.*, 1974) or Urografin (Nishi *et al.*, 1978) was employed in these studies, but the yield of spores was very low and the separation procedures were costly and time consuming. As *S. pombe* has been reported to possess sucrase activity (Mitchison & Creanor, 1969), exposure of the ascospores to sucrose may also lead to premature induction of germination.

We have developed a rapid, inexpensive and simple batch separation technique that yields spores essentially free of vegetative cells in sufficient quantities to allow an analysis of the time course of nucleic acid and protein synthesis during germination and outgrowth, as reported in this paper.

METHODS

Growth, sporulation and separation of spores. Cultures of the fission yeast *Schizosaccharomyces pombe* (Luepold's H90 strain, 972h⁻) were obtained from Professor J. M. Mitchison and grown in a rotary shaker bath at 32 °C in EMM2 medium (Mitchison, 1970). Sporulation was initiated by seeding a 2 l culture in a 4 l round-bottom flask with an early stationary culture to a cell density of about 2×10^8 cells ml⁻¹. The liquid sporulation medium devised by Egel (1971) will produce a suspension of 75 % spores and 25 % vegetative cells at a density of about 5×10^7 cells ml⁻¹ after 8 day growth at 28 °C with constant stirring. The cells were harvested by centrifuging for 10 min (800 g), washed twice with distilled water and stored at -20 °C.

Samples (10 ml) of the spore/cell suspension (resuspended to about 10^8 cells ml⁻¹) were layered on to 80 ml of a 35 % Hypaque solution (Winthrop Labs, Division of Sterling Drugs, New York, U.S.A.) and centrifuged for 20 min (800 g) at room temperature. The supernatant was discarded; the pellet was washed twice with distilled water, resuspended to a density of about 5×10^8 cells ml⁻¹ and stored in 1 ml quantities at -20 °C.

Cell counts, each on at least 200 cells, were done in quadruplicate with a haemocytometer. Germination and outgrowth indices were determined using phase contrast optics.

Chemical analyses and dry weight determinations. The protein content of *S. pombe* spores was determined on triplicate samples (each about 10^8 spores) washed twice with 2 % (w/v) trichloroacetic acid (TCA) by the Folin method using bovine serum albumin as the standard. RNA was assayed by a microspectrophotometric method based on the Schmidt-Thannhauser procedure (Blum & Padilla, 1962). The DNA content was determined on duplicate samples (each about 10^8 spores) by the diphenylamine procedure of Adelman *et al.* (1973) except that the volume of the DNA hydrolysate was only 1.25 ml. Dry weights were determined gravimetrically on triplicate samples of spores dried at 60 °C for 48 h on preweighed filter discs.

RESULTS

The results of five separations by batch centrifugation through 35 % Hypaque indicated that even though the original spore suspensions contained only 75 to 85 % spores, the pellet contained > 95 %. More importantly, we recovered about 35 % of the spores from the original suspension. Therefore, a 2 l culture yielded about 4×10^{10} spores. This quantity and yield was far greater than obtained by zonal centrifugation through sucrose (Padilla *et al.*, 1974) or Urografin gradients (Nishi *et al.*, 1978).

Figure 1(a) summarizes the time course of germination and outgrowth. Germination in EMM2 plus glucose, which was marked by the loss of refractility (darkening) of the spores, began within the first 30 min. Germination was essentially completed by 2 h to a level of about 97 %. Between 4 and 5 h the spores began to exhibit polar growth and became markedly elliptical and elongated (outgrowth). Outgrowth was completed by 10 h when the cell plate maximum (about 10 %) was observed. With sucrose as the carbon substrate, germination was delayed by approximately 1 h, but as with glucose, over 95 % of the spores completed germination. Outgrowth was not only delayed, but also it proceeded at a reduced rate, so that even by 10 h only about 50 % of the spores had outgrown. Other carbon substrates such as fructose and ribose failed to induce germination and outgrowth, as did the absence of any substrate. It thus appears that the spores respond to the presence of sucrose and undergo germination but are not able to utilize this substrate to permit rapid outgrowth. The upward break in the curve at 10 h suggests that utilization of the substrate was enhanced.

We observed no difference in the lack of viability between the ungerminated spores in the original suspension and those obtained by this separation procedure. It was less than 3 % in both instances as determined by exclusion of the dye Janus Green B. It thus appears that practically all the Hypaque-isolated spores germinated and continued growing.

Figure 1(b) shows the time course of the increase in protein, RNA and DNA content following resuspension in EMM2. The protein content varied slightly from the dormant spore level [mean 4.7 (S.E. ± 0.7) $\times 10^{-6}$ μ g spore⁻¹, $n = 7$] during the first 4 h of regrowth but then began to increase linearly. By 10 h the protein content had more than doubled

Fig. 1. Germination, outgrowth and macromolecular synthesis in *S. pombe* spores in EMM2 medium. (a) Germination in the presence of glucose (●) or sucrose (○) and outgrowth in the presence of glucose (▲) or sucrose (△). (b) Synthesis of RNA (□), protein (○) and DNA (■) with glucose as substrate; results are expressed relative to the initial contents (at $t = 0$).

$[10.7 (\pm 0.5) \times 10^{-6} \mu\text{g spore}^{-1}]$. RNA synthesis began to increase from the dormant spore level $[0.4 (\pm 0.03) \times 10^{-6} \mu\text{g spore}^{-1}$, $n = 5$] at 3 h and was biphasic, with a much more rapid increase at 8 h. There was more than a 4-fold increase in RNA content by 10 h $[1.82 (\pm 0.24) \times 10^{-7} \mu\text{g spore}^{-1}]$. The 1.7-fold increase in the DNA content of the spores was limited to the last 2 h of outgrowth. At 8 h the DNA content had not varied from the dormant spore level $[1.36 (\pm 0.03) \times 10^{-8} \mu\text{g spore}^{-1}$, $n = 4$], but had increased to $2.42 (\pm 0.14) \times 10^{-8} \mu\text{g spore}^{-1}$ by 10 h. Note that the onset of DNA synthesis was coincident with an upward shift in the rate of RNA synthesis at 8 h.

DISCUSSION

The present investigation demonstrates that a simple batch separation technique effectively frees *S. pombe* spores from vegetative cells rapidly and in good yield. It also emphasizes that spores are induced to germinate and outgrow by sucrose, so that this compound should be avoided as a density gradient material. As for the synthetic patterns of macromolecular synthesis, it is apparent that changes in the rates of RNA and protein accumulation are closely linked to cytological events, especially those marking the onset of outgrowth. The order of synthesis of these two components is the same as with many other fungal systems (Allen, 1965; Van Etten, 1969; Bainbridge, 1971) where RNA synthesis increased before protein synthesis. The pattern in *S. pombe* differs from the pattern displayed in the budding yeast *S. cerevisiae* during regrowth. In *S. cerevisiae* an increase in protein synthesis occurred prior to an RNA increase (Rousseau & Halvorson, 1973c). We found little synthesis of these two components during the early hours of regrowth in contrast to Padilla *et al.* (1974) who reported substantial RNA and protein synthesis within the first hour of regrowth. In these earlier studies, synthesis was measured by the uptake of labelled precursors into TCA-precipitable material in spores isolated through sucrose density gradients. In view of the finding that sucrose does induce germination, the earlier findings may not be comparable to

those obtained in the present study. Also, the use of isotopes in the earlier work may reflect turnover occurring with no net synthesis.

In agreement with previous investigations, we found that the spores have a haploid complement of DNA, about 1.36×10^{-8} μg spore⁻¹ (Nishi *et al.*, 1978; Bostock, 1970). More importantly, and in analogy to the cell cycle of eukaryotic cells, *S. pombe* spores have a protracted 'G₁' period during which there is a well-defined sequence of events to achieve the transition to vegetative growth and cell division. DNA was synthesized only in the last 2 h of regrowth. This system, therefore, offers an opportunity to investigate the relationship between cytological and biochemical events so as to gain an understanding of the control mechanisms involved in this differentiation process.

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