JOURNAL OF BACTERIOLOGY, Mar. 1972, p. 1027-1033

Vol. 109, No. 3
Printed in U.S.A.

Cell Cycle Dependency of Sporulation in *Saccharomyces cerevisiae*[1]

JAMES E. HABER AND HARLYN O. HALVORSON
Rosenstiel Basic Medical Research Center, Brandeis University, Waltham, Massachusetts 02154

Received for publication 29 November 1971

The study of sporulation in *Saccharomyces cerevisiae* is complicated by the fact that not all cells in the population complete sporulation and that the kinetics of development of those which do are not synchronous. By separating vegetative cells by zonal rotor centrifugation into fractions of increasing cell volume and hence progressive stages of the vegetative cell cycle, it was possible to observe sporulation of more homogeneous, synchronous populations. The capacity of *S. cerevisiae* to complete sporulation is low for small single cells at the beginning of the cell cycle and is greatest for large budded cells about to divide. The capacity of a cell to complete sporulation thus appears to be directly related to the stage in the vegetative cell cycle from which it was taken. The use of synchronously sporulating cultures made it possible to examine very early decision events leading to the commitment of a cell to sporulation. In addition, differences in the capacity of a mother and daughter cell produced by cell scission were examined.

The study of sporulation of the yeast *Saccharomyces cerevisiae* has been restricted by the lack of precise information concerning the sequence of events during the process of differentiation. In large measure, the problem of studying specific morphological or biochemical changes has been caused by the lack of synchrony or sporulating cells, as reflected by both the kinetics and extent of sporulation. The broad time course of the completion of sporulation in a population and similarly broad periods of deoxyribonucleic acid (DNA) replication (2, 9) reflect asynchronous sporulation. In contrast to studies of the vegetative cell cycle of *S. cerevisiae* synchronous cultures, in which the synthesis of particular enzymes can be measured as discrete steps, the lack of a truly synchronous sporulation process has precluded a precise determination of the temporal order of sporulation events.

The study of sporulation is further complicated by the fact that usually only 50 to 70% of the population completes sporulation, as evidenced by the formation of refractile spores within an ascus. Thus the measurement of the appearance of particular enzymatic activities may be further obscured or distorted by the presence of cells which fail to complete sporulation, since these cells may still slowly continue growth or sporulation processes.

The need for a more homogeneous, synchronously sporulating culture prompted us to investigate the degree of asynchrony in commonly applied methods of sporulation. By using cell-separation techniques which have proved most successful in understanding cell cycle events during vegetative growth, it has been possible to determine the basis of asynchrony in sporulating cultures and to develop new methods for the study of more homogeneous, synchronously sporulating cells. This approach has permitted a study of events early in the sporulation process which may determine the ability of a cell to complete meiosis and sporulation.

MATERIALS AND METHODS

Yeast strains. Three diploid strains of *S. cerevisiae* were used in these studies: Y185, a heterothallic diploid derived from two haploid mutants unable to metabolize sucrose; D649, a heterothallic diploid; and Y55, a homothallic diploid.

Growth conditions. Vegetative growth was carried out in sterile flasks on a rotary shaker at 30 C. When glucose was the carbon source, YEP [2% glucose, 2% peptone (Difco) and 1% yeast extract (Difco)] was used. Vegetative growth with acetate as the carbon source was carried out by using the medium described by Roth and Halvorson (8).

[1] Work conducted in part at the Laboratory of Molecular Biology, University of Wisconsin, Madison, Wis.

Synchronization of yeast. Synchronous cultures of *S. cerevisiae* were obtained by zonal rotor separation of cell sizes. The procedure has been described in detail by Sebastian, Carter, and Halvorson (10). Fractionation was carried out by using either a 15 to 40% sucrose or 15 to 40% sorbitol gradient as a support. Particular size classes, corresponding to different stages in the vegetative cell cycle, were washed twice in sterile water and suspended either in fresh vegetative growth media at a cell density of 4×10^6 cells/ml or else directly into sporulation medium at a density of 2×10^7 cells/ml. Cell size of the zonal rotor fractions and during synchronous growth was monitored with a Channelyzer (Coulter Electronics, Inc.). Cell number was measured either with the Channelyzer or by counting under a microscope with a hemocytometer. Each cell, regardless of the presence of a bud, was counted as one cell.

Sporulation conditions. Sporulation of *S. cerevisiae* was carried out in 1% potassium acetate, *p*H 7, with 10 μg of tetracycline per ml added to retard bacterial contamination. Cells which had grown vegetatively in YEP were harvested from the stationary phase for sporulation. When the cells were grown in acetate growth medium, however, the cells could be harvested from the exponential growth phase and suspended in sporulation medium. Sporulation of logarithmic-phase cells from acetate pregrowth made it possible to follow the sporulation of cells from different points in the cell cycle by removing samples of cultures growing synchronously in acetate medium, washing twice with sterile water, and placing the cells into sporulation medium. In 1% potassium-acetate sporulation medium, vegetative growth was almost totally absent.

DNA determination. DNA was determined by fluorescence measurement of DNA with diaminobenzoic acid according to a modification of the method of Wehr and Parks (12) suggested by L. W. Parks (*personal communication*). A 5-ml amount of a culture containing at least 5×10^6 cells/ml was precipitated with an equal volume of 10% trichloroacetic acid and then washed four times with 0.1 N potassium acetate in 95% ethanol. The precipitate was then twice suspended with 95% ethanol and heated at 60 C. The pellet was then evaporated to dryness and suspended in 0.2 ml of 1.0 N HCl containing 0.3 g of 3,5-diaminobenzoic acid dihydrochloride (Aldrich Chemical Co., Inc.) per ml and heated at 60 C for 30 min. A 1-ml amount of 0.6 N perchloric acid was then added, and the sample was centrifuged to remove debris. Samples were measured in an Aminco-Bowman spectrofluorometer with excitation at 408 nm and emission at 520 nm. As a standard, calf thymus DNA (Sigma Chemical Co.) was used.

RESULTS

Degree of asynchrony in sporulating cultures. Asynchrony in the sporulation of a random population of cells was tested by comparing the kinetics and extent of sporulation of homogeneous subpopulations obtained by zonal rotor centrifugation. Cells growing exponentially in an acetate growth medium can be sporulated without first being grown to stationary phase (8). In Fig. 1, the kinetics and extent of sporulation of the two extreme size classes, the smallest single cells and the large double cells about to divide, are compared with the unfractionated random population. Clearly, the random population does not sporulate in a synchronous fashion but in fact represents a summation of widely different rates and extents of sporulation of cells at different points in the cell cycle. In the small, single cell fraction, not only are the asci produced at a later time, but the asci produced also differ morphologically from those in the fraction containing large, budded cells. More than 65% of the asci formed in the small cell fraction contained only two spores, whereas more than 80% of the asci formed in the large cells contained three or four spores. In addition, the extent of sporulation in the small cell fraction did not continue to increase with subsequent incubation beyond 30% total sporulation.

The differences between newly formed, small single cells and large budded cells were not restricted either to the strain of *S. cerevisiae* chosen or to vegetative growth in an acetate medium. Cells grown in YEP to the early stationary phase and fractionated by zonal rotor centrifugation on a 15 to 40% sucrose gradient exhibited similar properties (Table 1). There are substantial differences in the capacity of the two extreme size classes to com-

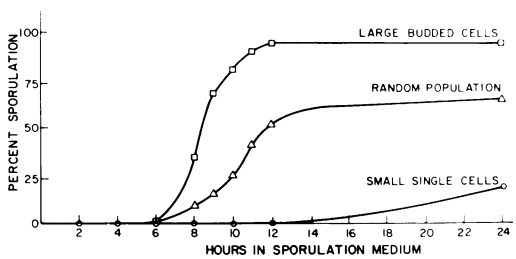

FIG. 1. *Effect of cell size on the kinetics of sporulation of Y-55. A culture of Y-55 was grown in KAc synthetic growth medium of Roth and Halvorson (8). The culture was harvested in logarithmic growth and fractionated according to size on a sucrose gradient in the zonal rotor. Small single cells (ca. 0.1 to 0.2 of cell cycle) and large budded cells (ca. 0.8 to 0.9 of cell cycle) were collected from the gradients. As a control, a fraction of the original population (random population) was suspended in a 15% sucrose solution for an equivalent period of time. The three samples were collected by centrifugation, washed twice in sterile water, suspended in KAc sporulation medium at a density of 2×10^7 cells/ml, and aerated at 30 C. All asci containing two or more spores were counted.*

TABLE 1. *Sporulation capacity of Saccharomyces cerevisiae at the beginning and end of the vegetative cell cycle*[a]

Strain	Percentage of sporulation							
	Pregrowth on acetate (log phase)				Pregrowth on YEP (stationary phase)			
	Three or four spores/ascus		Total		Three or four spores/ascus		Total	
	Small	Large	Small	Large	Small	Large	Small	Large
Y55	6	88	25	95	6	62	34	65
Y185	11	65	42	70	9	58	46	67
D649	1	45	1	61	1	57	2	66

[a] *S. cerevisiae* was grown to exponential phase in potassium-acetate growth medium or to early stationary phase in YEP. Cells were harvested and separated on a 15 to 40% sucrose gradient with a zonal rotor, and the two extreme size classes corresponding to the small single cells formed by cell division and the large budded cells about to divide were then placed in sporulation medium for 48 hr.

plete sporulation. If one considers only asci containing three or four spores in an ascus, the differences in the ability of cells to complete sporulation becomes even more striking.

Relation of sporulation capacity to the vegetative cell cycle. The above results suggest that there is a significant change in the capacity of cells to complete sporulation about the time of cell scission. Large budded cells about to divide exhibit a very high frequency of sporulation, whereas newly formed single cells resulting from cell scission have a much reduced ability to complete sporulation. If the capacity of a cell to complete sporulation were directly dependent on its stage in the vegetative cell cycle, a periodic variation in the extent of sporulation would be expected if sporulation were monitored over the time of more than one synchronous cell cycle. The scheme for such an experiment is presented in Fig. 2. Cells grown in acetate growth medium were harvested and fractionated by zonal centrifugation to obtain the newly formed single cells. These cells were then reintroduced into acetate growth medium to begin synchronous growth. At intervals, samples were removed, washed twice in sterile water, and introduced at a constant cell number into sporulation medium. In this way, more than a single cell cycle could be followed, although growth in acetate medium has been found to be less well sustained than in media with glucose as the carbon source.

The results of this type of experiment indicate that the capacity of cells to complete sporulation does indeed depend on the stage of the vegetative cell cycle at which point sporulation was initiated (Fig. 3). At the time of cell scission, when the number of cells nearly doubles, the percentage of cells having the capacity to produce an ascus containing three or four spores decreases significantly. During the first half of a second, less synchronous genera-

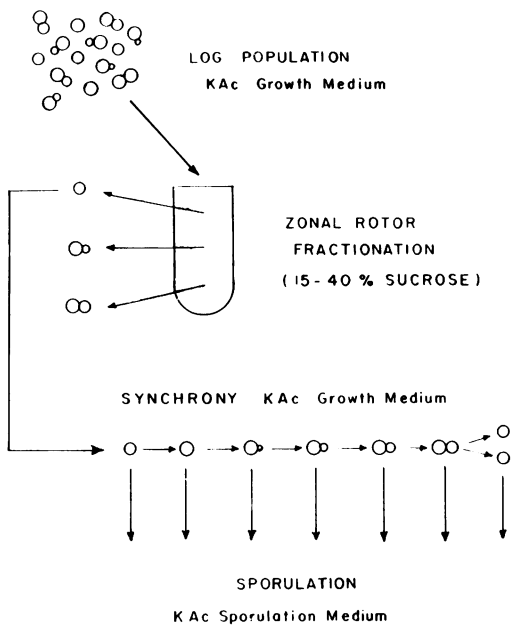

FIG. 2. *Experimental design to study sporulation capacity at different points in the cell cycle. Cells growing exponentially in potassium-acetate growth medium were harvested and separated according to cell volume on a 15 to 40% sucrose gradient by zonal centrifugation. The small cells corresponding to the beginning of a cell cycle were returned to acetate growth medium to begin synchronous growth. At intervals, samples were removed, washed, and introduced into sporulation medium. After 48 hr, the samples were counted to determine the extent of sporulation. In this way, the sporulation capacity can be studied over a period of more than one complete cell cycle.*

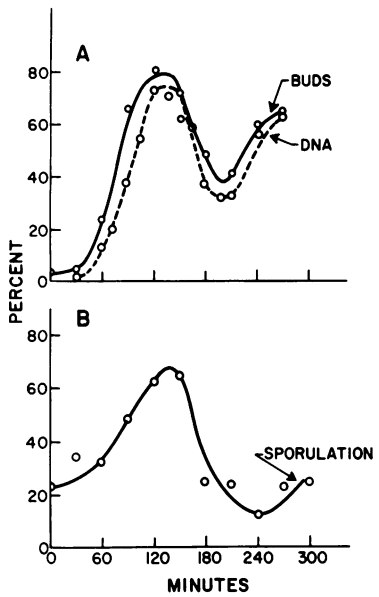

FIG. 3. *Percentage increase in DNA content and buds during synchronous growth. Y-55 was grown in KAc synthetic growth medium, harvested in logarithmic phase [optical density at 600 nm (OD_{600}) = 1.2], and the small single cells were collected from a zonal rotor fractionation with a sucrose gradient. These small cells were suspended in KAc synthetic growth medium at an OD_{600} of 0.4 and incubated with aeration at 30 C. At intervals, samples were removed for (A) DNA determination and microscopic counting of bud formation or (B) the ability to sporulate in KAc sporulation medium. DNA per cell is shown as the percentage increase over the zero time concentration. For sporulation, samples were removed, washed twice with sterile water by centrifugation, and suspended in KAc sporulation medium to 10^7 cells/ml and incubated at 30 C for 48 hr. Only three- and four-spored asci were counted.*

tion, the capacity to sporulate again rises. Analogous results were obtained with all three strains used, although in the case of D649 the extent of sporulation was considerably lower throughout the cell cycle (Table 1).

As noted earlier, in the two strains (Y-55 and Y-185) which grow most readily in acetate growth medium small single cells give rise to a large number of asci containing only two spores. When the production of two-spored asci is measured over the cell cycle, a periodic fluctuation is observed for two-spored asci as with the production of three- and four-spored asci (Fig. 4). The appearance of two-spored asci is, however, out of phase with the periodic rise of three- and four-spored asci.

Use of homogeneous cell fractions to study sporulation. With well-defined, homogeneous populations, it was possible to examine the sporulation process in greater detail. One central question was whether the cells which actually complete sporulation appear to be distinct from those which fail to sporulate. This problem was approached by comparing the size distribution of cells which actually sporulated at each point in the cell cycle with the size distribution of each population as introduced into sporulation medium 48 hr previously. The results of this analysis for Y-55 are summarized in Table 2 and in Fig. 5. There is a striking difference in the size distribution, as measured by the size of the bud, between cells which actually sporulated and those which did not. Cells which completed sporulation exhibited the same size distribution as did the initial populations before sporulation. Thus, no further bud enlargement, a measure of further vegetative growth, occurred in cells which had completed sporulation. On the other hand, cells which did not produce refractile spores exhibited a substantial amount of vegetative growth as monitored by bud formation and bud size. It would appear, therefore, that some decision to begin sporulation events or rather to continue vegetative growth occurs at a very early point in the sporulation process. The cessation of vegetative growth in asporogenous cells is most likely due to the lack of a nitrogen source in the sporulation medium, so that vegetative starvation results.

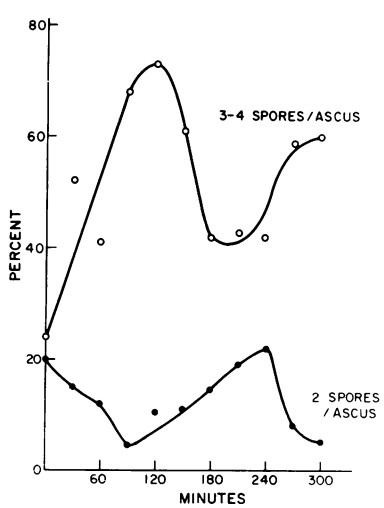

FIG. 4. *Differences in the pattern of sporulation capacity during synchronous division. A synchronous culture of Y-55 was sporulated at intervals during the cell cycle as described in Fig. 2. Maximum bud formation of the first cycle occurred at 105 min, and cell division was 80% complete by 180 min. Both two-spored asci as well as three- to four-spored asci were determined after 48 hr in sporulation medium.*

TABLE 2. *Effect of transfer to sporulation medium on the formation and growth of buds in a synchronous culture*[a]

Time of removal from synchronous growth (min)	Percentage of sporulation[b] after 48 hr	Percentage of bud types					
		Initial		48 Hr in sporulation medium			
				Cells containing 3 to 4 spores		Cells which did not sporulate	
		A	B	A	B	A	B
0	11.8	0.5	0	0	0	9	1
30	36.3	1	0	1	1	13	1
60	37.3	3	0	6	1	72	2
90	47.6	43	4	41	4	58	11
120	64.4	53	34	56	36	50	34
150	70.2	28	62	24	65	17	68
180	53.3	11	53	14	49	18	30
210	25.5	19	16	10	18	26	10

[a] An exponential culture of Y55 was synchronized and tested for sporulation capacity. The distribution of buds at each point was considered for (A) buds just forming up to the size of a bud nearly the size of the mother cell and (B) full-sized doubles (where the bud is the same size as the mother cell). Cell division was 80% complete by 210 min.

[b] Three to four spores per ascus.

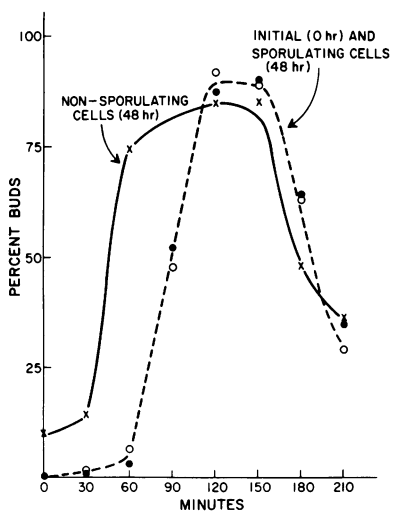

FIG. 5. *Vegetative growth of sporulated and non-sporulated cells. Y-55 was synchronized and assayed for sporulation capacity as described in Fig. 2 and Table 2. After 48 hr in sporulation medium, each sample was examined to determine the percentage of cells which were budded. Separate determinations were made for cells which had produced asci containing three or four spores and those which had failed to sporulate. Cells which had sporulated (O) exhibited the same distribution of bud incidence as the samples had contained just at the time of introduction into sporulation medium 48 hr earlier (●). Cells which had failed to complete sporulation, however, continued vegetative growth, as evidenced by an increase in bud incidence in the early part of the cell cycle and a decrease in budded cells toward the end of the cell cycle (×).*

A second measurement can be made from homogeneous sporulating cultures to determine whether the mother and daughter cells are equivalently competent to sporulate. In a sample containing a high proportion of cells in which the daughter had achieved full size just prior to cell scission (Table 2, 150 min), the sporulation capacity of both halves of a double cell can be examined. Of these cells which sporulated, only one of the two equal-sized halves completed sporulation in 84% of the cases examined. When both halves contained spores (16%), the second ascus contained only two spores in approximately 30% of the sample. Thus, in the majority of cases, only one of the two cells about to divide (presumably the mother cell) completed sporulation. Since the size distribution of cells which complete sporulation remains the same as that initially introduced, it seems unlikely that cells in which both mother and daughter sporulated had then separated into two cells.

DISCUSSION

The study of sporulation in *S. cerevisiae* has been limited by a lack of precise information on the sequence of morphological and biochemical events accompanying meiosis and sporulation. In the past several years, several new techniques have appeared which now make it possible to begin to catalog systematically different stages of development. The refinement of electron microscopy (E. Guth et al., Bacteriol. Proc., p. 61, 1971) has made it

possible to visualize a sequence of events associated with meiosis. At the same time, the isolation of a large number of temperature-sensitive sporulation mutants in *S. cerevisiae* (4) and in *Schizosaccharomyces pombe* (1) provides another powerful tool for studying processes essential for sporulation. By mapping the period of temperature sensitivity of these mutants (5) and by relating the point of arrest to morphological markers, it should now be possible to subdivide the sporulation process into a large number of discrete steps.

Synchronously sporulating cultures are required for examination of the time and stage of arrest in mutants during the sporulation cycle and especially for characterization of the associated sensitive biochemical events during the sporulation cycle. Such an approach has proven valuable with vegetative cultures to demonstrate the order of synthesis of a large number of enzymes (6). To determine whether the same order of enzyme synthesis observed in the vegetative cell cycle is preserved in the interval preceding meiotic DNA replication requires a similar degree of synchronization.

The requirement for well-defined sporulating cultures is emphasized by the results presented above. Not all cells in a population are capable of sporulation, so that the measurement of the appearance of a particular enzyme may be obscured by the presence of cells which do not express sporulation functions but rather attempt to continue vegetative growth.

By the use of synchronized populations, we have found that the capacity of a cell to complete sporulation is highly dependent on its stage in the vegetative cell cycle. In the early part of the cell cycle, up to the time of bud initiation and DNA replication, the intrinsic capacity of a cell to complete sporulation is low. Later in the cell cycle, however, the ability to complete sporulation rises significantly. The control of such periodic fluctuation of sporulation capacity is not known. It is possible that sporulation is limited by the availability of one or more essential compounds which either fluctuate periodically throughout a cell cycle or are present in greater amounts in cells of greater volume (i.e., later in the cell cycle). These results might also be explained by the existence of a control mechanism for the expression of sporulation-specific functions which would be inducible only during a limited period of the vegetative cell cycle.

Certainly a substantial portion of the decrease in sporulation capacity after cell scission can be attributed to a sharp difference in the sporulation capacity of newly formed daughter cells from that of mother cells. The observation that sporulation occurs 85% of the time in only one of the halves of a double cell about to divide is in close agreement with the conclusions reached by Yanagita et al. (13) in studying sporulation of mother and daughter cells from late stationary phase. Nevertheless, a significant dependency of sporulation activity on the cell cycle, for both mother and daughter cells, is still indicated by the extent of variation in sporulation capacity from the beginning to the end of cell cycle. This dependency can be seen in two ways. First, if the decrease in sporulation following cell scission were fully attributable to the generation of one-half of the population which could not sporulate (daughter cells), the subsequent rise in successful sporulation would be attributed to the acquisition of competence later in the cell cycle. Thus, it seems that newly formed daughter cells require some period of growth, apparently at least up to the point of bud initiation and the onset of DNA synthesis, before sporulation is possible. A second indication of direct cell cycle dependency of sporulation is that the variation in sporulation exceeds a factor of two attributable to a total difference in the sporogenic activity of mother and daughter cells. With D649 (Table 1) sporulation ranges from less than 1% at the onset of the cell cycle to 60%. With Y-55 and Y-185, the variation is less extreme and is further complicated by the appearance of two-spored asci. Whether two-spored asci are the product of normal, but incomplete, sporulation, limited by available cellular reserves, or whether they are in some way different is currently under investigation. In terms of asci containing three or four spores per ascus, the variation in sporulation capacity ranges from approximately 10 to 75%. Thus, in addition to the development of competence by daughter cells after a period of growth following cell division, the sporulation capacity of mother cells must also increase significantly in the later part of the cell cycle.

Some indication of the nature of the control of sporogenic activity can be found in the studies of the fate of cells which do or do not sporulate throughout the cell cycle. If the capacity of cells to complete sporulation, as measured by the appearance of refractile spores within an ascus, were limited primarily by the total concentration of biosynthetic reserves, then it is likely that cells which did not complete sporulation would be arrested at some intermediate point. An examination of cells which did not sporulate revealed that

these cells were apparently not arrested at an intermediate step of sporulation but rather had not entered sporulation. Cells which did not sporulate exhibited substantial vegetative growth, as measured by bud initiation and enlargement. On the other hand, cells which did complete sporulation apparently ceased all vegetative growth very soon after transfer to sporulation medium. The initial decision to begin sporulation thus appears to occur very soon after transfer to sporulation medium. The clear distinction between sporulated and nonsporulated cells indicates that the control of sporulation capacity is determined by mechanisms more specific than the concentrations of biosynthetic reserves. In terms of the cell cycle, this shift toward sporulation is most likely to occur after bud initiation and the beginning of DNA replication. The possibility that, during DNA replication, certain masked regions of one or more chromosomes become more available for transcription can be regarded as a possible interpretation of these data.

The very early shift of the cell along a pathway to sporulation precedes the irreversible point of commitment to sporulation. As defined by Sherman and Roman (11), commitment is reached when cells are unable to resume vegetative growth without first completing sporulation. The interval just after transfer to sporulation medium appears to be a critical period which merits further study.

The ability of *S. cerevisiae* to undergo sporulation is predetermined by the stage in the vegetative cell cycle from which the cells were derived. In many respects, this phenomenon is similar to the observation that *Bacillus subtilis* sporulation appears to be initiated only during the period of the cell cycle during which DNA replication occurs (3). In *S. cerevisiae*, Hartwell (7) demonstrated that a number of mutants affecting different stages of the vegetative cell cycle are expressed much earlier in the cell cycle than the time of execution. In the case of sporulation, execution of sporulation events also appears to depend on the expression of activities prior to the exposure of the cells to the direct stimuli for sporulation.

The development of homogeneous, synchronously sporulating cultures has made it possible to begin to examine the stage of sporulation in detail. Experiments are currently in progress in our laboratory to identify which aspects of the normal vegetative cell cycle are maintained during sporulation and to examine in greater detail the early regulatory events controlling the transition of the vegetative cell to sporulation.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-1459 from the National Institute of Allergy and Infectious Diseases, National Science Foundation grant B-1750, and a National Science Foundation Postdoctoral Fellowship held by one of us (J.E.H.). The technical assistance of Maria de los Angeles Argomaniz Lizondo was greatly appreciated.

LITERATURE CITED

1. Bresch, C., G. Müller, and R. Egel. 1968. Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. **102**:301-306.
2. Croes, A. F. 1967. Induction of meiosis in yeast. I. Timing of cytological and biochemical events. Planta **76**:209-226.
3. Dawes, I. W., D. Kay, and J. Mandelstam. 1971. Determining effect of growth medium on the shape and position of daughter chromosomes and on sporulation in *Bacillus subtilis*. Nature (London) **230**:567-569.
4. Esposito, M. S., and R. E. Esposito. 1969. The genetic control of sporulation in *Saccharomyces*. I. The isolation of temperature-sensitive sporulation-deficient mutants. Genetics **61**:79-89.
5. Esposito, M. S., R. E. Esposito, M. Arnaud, and H. O. Halvorson. 1970. Conditional mutants of meiosis in yeast. J. Bacteriol. **104**:202-210.
6. Halvorson, H. O., B. L. A. Carter, and P. Tauro. 1971. Use of synchronous cultures of yeast to study gene position. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 21. Academic Press, Inc., N.Y.
7. Hartwell, L. H., J. Culotti, and B. Reid. 1970. Genetic control of the division cycle in yeast. I. Detection of mutants. Proc. Nat. Acad. Sci. U.S.A. **66**:352-359.
8. Roth, R., and H. O. Halvorson. 1969. Sporulation of yeast harvested during logarithmic growth. J. Bacteriol. **98**:831-832.
9. Roth, R., and K. Lusnak. 1970. DNA synthesis during yeast sporulation: genetic control of an early developmental event. Science **168**:493-494.
10. Sebastian, J., B. L. A. Carter, and H. O. Halvorson. 1971. Use of yeast populations fractionated by zonal centrifugation to study the cell cycle. J. Bacteriol. **108**:1045-1050.
11. Sherman, F., and H. Roman. 1963. Evidence for two types of allelic recombination in yeast. Genetics **48**: 255-261.
12. Wehr, C. T., and L. W. Parks. 1969. Macromolecular synthesis in *Saccharomyces cerevisiae* in different growth media. J. Bacteriol. **98**:458-466.
13. Yanagita, T., M. Yagisawa, S. Oishi, N. Sando, and T. Suto. 1970. Sporogenic activities of mother and daughter cells in *Saccharomyces cerevisiae*. J. Gen. Appl. Microbiol. **16**:347-350.