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Conditional Mutants of Meiosis in Yeast

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Three temperature-sensitive mutants, spo1-1, spo2-1, and spo3-1, were characterized with respect to their behavior in sporulation medium at a restrictive temperature. The time of expression of the functions defective in the mutants was determined by temperature-shift experiments during the sporulation process. In addition, each mutant was examined for the following: (i) its ability to undergo the nuclear divisions of meiosis; (ii) deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis; (iii) protein turnover; and (iv) colony-forming ability after exposure to sporulation medium. Mutant spo1-1 is defective in a function which confers a temperature-sensitive period which extends over 32% of the sporulation cycle. The temperature-sensitive period of mutant spo2-1 occupies 34% of the cycle, whereas the temperature-sensitive period of mutant spo3-1 extends over 2% of the sporulation cycle. Cytological evidence indicates that all three mutants initiate but do not complete the meiotic nuclear divisions. The DNA content of sporulation cultures of mutants spo1-1 and spo3-1 did not increase to the wild-type level; DNA synthesis in spo2-1 was normal. All three strains exhibit a loss of colony-forming ability during incubation in sporulation medium at the restrictive temperature. RNA and protein synthesis and protein turnover occur in the mutants.

Meiosis is a fundamental aspect of the life cycle of sexually reproducing eucaryotes. In yeast, meiosis occurs during sporulation of diploid cells and terminates in the production of an ascus containing four haploid ascospores. The changes in ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein cell content which accompany meiosis and sporulation of wild-type diploid *Saccharomyces cerevisiae* were previously described (3, 4, 6). Temperature-sensitive sporulation-deficient mutants of *S. cerevisiae* have been isolated to facilitate the study of meiosis; their common phenotype is growth by mitosis but no formation of asci at restrictive temperatures (5).

Our aim in the present experiments is to define the functions unique to meiosis in yeast and to determine the sequence of their expression during sporulation. The availability of a number of temperature-sensitive mutations of meiosis permits one to analyze the meiotic process by temperature-shift experiments. Shifting cultures from the nonpermissive to the permissive temperature during meiosis defines the time of expression of a temperature-sensitive function. The reverse experiment defines the end, and thus the duration, of the dependence on this gene function. In the present investigation, three sporulation-deficient mutants, spo1-1, spo2-1, and spo3-1, are characterized with respect to macromolecular synthesis and the nuclear divisions of meiosis. By taking advantage of the temperature sensitivity of the strains, the time of expression of the functions defective in the mutants was determined.

MATERIALS AND METHODS

Yeast strains. Five homothallic diploid strains of *Saccharomyces* were used; their genotypes are given below.

S41	a/α	D/D	arg 4-1/arg 4-1	acr 1-1/acr 1-1	
Z140-9A	a/α	D/D	lys 2-1/lys 2-1	met/met	
74-1A	a/α	D/D	arg 4-1/arg 4-1	acr 1-1/acr 1-1	spo 1-1/spo 1-1
79-1B	a/α	D/D	arg 4-1/arg 4-1	acr 1-1/acr 1-1	spo 2-1/spo 2-1
89-1D	a/α	D/D	arg 4-1/arg 4-1	acr 1-1/acr 1-1	met spo 3-1/met spo 3-1

Diploids S41 and Z140-9A are normal with respect to sporulation ability. Diploids 74-1A, 79-1B, and 89-1D are deficient in sporulation. The method of isolation of these mutants has been reported elsewhere (5). The symbols are as follows: a, α, mating-

type alleles; acr, resistance to actidione (cyclohexamide); arg, arginine auxotroph; D, diploidization gene conferring homothallism (8, 19); lys, lysine auxotroph; met, methionine auxotroph; and spo, sporulation-deficient.

Media. The amounts of the components specified below are those required for the preparation of one liter of medium. YEP contained 20 g of glucose, 20 g of peptone, and 10 g of yeast extract; sporulation medium II contained 20 g of potassium acetate and 10 to 75 mg of L-arginine hydrochloride; sporulation medium III contained 20 g of potassium acetate and 75 mg of L-lysine hydrochloride. In experiments in which incorporation of ^{14}C adenine and ^{32}P into RNA were studied, the sporulation media were supplemented with adenine and phosphate in the amounts specified in the text. Sporulation of arginine auxotrophs was performed in sporulation medium II and lysine auxotrophs were sporulated in sporulation medium III. All liquid media contained 20 mg of tetracycline per liter to prevent bacterial contamination.

Sporulation and counting procedure. The culture techniques used to prepare diploid cell populations for inoculation into sporulation medium were previously described in detail (6). The per cent asci in sporulated cultures was determined by hemocytometer counts as already reported (5, 6). Portions of sporulated cultures which could not be counted immediately were fixed in 4% Formalin and refrigerated.

Colorimetric determination of deoxyribonucleic acid. DNA was assayed colorimetrically by using the diphenylamine reagent (1). Salmon sperm DNA from Mann Research Laboratories was employed as a standard. DNA was extracted from samples of approximately 10^9 cells, by the technique of Ogur and Rosen (11). Nucleic acid was extracted from each sample three times to assure total recovery of DNA. All determinations were performed in triplicate.

Isotope incorporation. Measurement of the incorporation of ^{14}C amino acids into protein and ^{14}C adenine or ^{32}P incorporation into RNA was performed by the method of Rodenberg et al. (14).

Staining of yeast nuclei. The nuclei of vegetative and sporulating cells were observed by a Giemsa-staining technique modified slightly from that described by Pontrefact and Miller (13). Cells to be stained were fixed in a 0.3% Formalin solution in 0.1 M Gurr phosphate buffer (*p*H 7). RNA was extracted by 1 hr of incubation at 30 C in 1 N NaOH, and the cells were washed twice in 0.1 M Gurr phosphate buffer (*p*H 7) and resuspended in the same buffer. The cells were transferred to gelatin-coated cover slips and stained for 3 hr in a 10% solution of Gurr Giemsa "R66" stain in 0.1 M phosphate buffer (*p*H 7). The cells were destained for 30 sec in 95% ethanol, dehydrated for 10 minutes in xylene, and mounted in Biomount.

Materials. ^{14}C-L-arginine (316 mCi/mmole), ^{14}C-L-phenylalanine (355 mCi/mmole), and ^{32}P-orthophosphate (carrier-free) were supplied by Schwartz Bio-Research Inc., Orangeburg, N.Y. Adenine-*8*-^{14}C sulfate (28.9 mCi/mmole) was obtained from Nuclear-Chicago Corp., Chicago, Ill. Improved Giemsa R66 stain and phosphate buffer (*p*H 7) manufactured by George T. Gurr Ltd., England, were obtained from ESBE Laboratory Supplies, Toronto, Canada.

RESULTS

Temperature-sensitive mutants. The sporulation ability of a wild-type diploid and three temperature-sensitive sporulation-deficient mutants at temperatures between 20 and 34 C is shown in Table 1. These data illustrate that 30 C is the optimal temperature for sporulation of the wild-type strain. The three mutants shown are recessive and complement one another. Ascus formation of these diploids is extensively inhibited at 34 C. At temperatures below 34 C, sporulation ability is restored in the mutant strains. Ascus production by the mutant strains at the lower temperature does not return to the full wild-type level. In the physiological characterization of the mutants, 25 C was used as the permissive temperature and 34 C was used as the restrictive temperature.

Meiotic nuclear division at the restrictive temperature. The sporulation-deficient mutants were recognized by their inability to form visible asci detectable by microscopic examination. Since ascus formation represents the completion of meiosis and spore development, these mutants might be expected to represent lesions throughout the process of sporulation. It was therefore of interest to inquire whether the developmental changes normally associated with sporulation are initiated at the restrictive temperature and to what points the mutants proceed. The two nuclear divisions which accompany meiosis in yeast can be detected by Giemsa staining of cells in sporulation medium. By this technique, one can discern

TABLE 1. *Per cent asci after 72 hr of sporulation*[a]

Strain	Temp			
	20 C	25 C	30 C	34 C
$\frac{+}{+}$	54.6	58.5	75.0	45.6
$\frac{spo1\text{-}1}{spo1\text{-}1}$	41.2	36.4	8.5	0.1
$\frac{spo2\text{-}1}{spo2\text{-}1}$	29.5	39.4	32.1	0.2
$\frac{spo3\text{-}1}{spo3\text{-}1}$	20.9	35.8	10.5	0.6

[a] Cultures were sporulated in medium II; values are the averages of four experiments.

mononucleate cells, binucleate cells in which the first division of meiosis has occurred, and tri- and tetranucleate cells in which the second nuclear division has been completed.

Cultures of the wild-type diploid, S41, and of the three mutant diploids were introduced into sporulation medium at 34 C. Samples were taken from these cultures at the times shown in Table 2 for fixation and Giemsa staining. Bi-, tri-, and tetranucleate cells were observed in the 12-hr samples of the wild-type strain. Their frequency declined as ascus formation proceeded to completion. Cultures of the three mutants at 36 hr contained some bi-, tri-, and tetranucleate cells but no asci. Mutant spo1-1 contained binucleate cells but no cells with three or four nuclei. Mutants spo2-1 and spo3-1 contain both binucleate and tri- and tetranucleate cells.

These results provide cytological evidence that meiosis is initiated at the restrictive temperature in the mutants. At least 7% of cells in cultures of spo1-1 proceed through the first division of meiosis. The absence of tri- and tetranucleate cells in cultures of this mutant indicate that the mutant cannot complete the second meiotic division. Giemsa-stained preparations of 36-hr cultures of mutants spo2-1 and spo3-1 indicate that these mutants permit 3 to 6% of the cells to proceed into the second meiotic nuclear division.

DNA synthesis during meiosis at the restrictive temperature. The nuclear-staining data presented above reveal that, after 36 hr in sporulation medium, from 78 to 93% of the cells of the three mutants remain mononucleate. It is of interest to inquire whether these cells have undergone the bulk DNA synthesis which precedes meiosis in yeast cells. To answer this question, portions were removed at intervals from cultures in sporulation medium at 34 C, and the DNA content of these samples was determined colorimetrically; results are shown in Table 3. The cultures of wild-type and mutant diploids used to obtain these values were the same as those which provided the material for nuclear staining (Table 2). The DNA content of wild-type cells increases 85% after 36 hr in sporulation medium (Table 3). The DNA content of spo2-1 rises to 83% of the initial value. The DNA increase of spo1-1 and spo3-1 at 36 hr is less than that of the wild type.

TABLE 2. *Nuclear division during sporulation at 34 C*[a]

Strain	Hr	Per cent cells			Per cent asci
		Mononucleate	Binucleate	Tri-, Tetranucleate	
$\frac{+}{+}$	0	99.5	0.5	0.0	0.0
	6	99.0	1.0	0.0	0.0
	12	86.0	6.0	6.0	2.0
	24	50.0	4.0	7.0	39.0
	30	38.0	3.0	1.0	58.0
	36	47.0	1.0	2.0	50.0
$\frac{spo1\text{-}1}{spo1\text{-}1}$	36	93.0	7.0	0.0	0.0
$\frac{spo2\text{-}1}{spo2\text{-}1}$	36	87.0	10.0	3.0	0.0
$\frac{spo3\text{-}1}{spo3\text{-}1}$	36	77.0	17.0	6.0	0.0

[a] Cultures were sporulated in medium II.

From the cytological data (Table 2) and DNA increases (Table 3), it is possible to calculate the distribution of 2C and 4C cells in the mononucleate class. This calculation is based on two assumptions: (i) that the per cent increase in DNA is proportional to the per cent of cells which have doubled their DNA (i.e., 4C), and (ii) that bi-, tri-, and tetranucleate cells and asci are 4C. The possibility that increases in DNA content during meiosis are attributed to selective mitochondrial synthesis was ruled out by the finding that the ratio of mitochondrial DNA to nuclear DNA remains constant during this period (Sena, *unpublished data*). One can therefore characterize the 36-hr cell population of the wild-type and mutant strains with respect to their DNA content. Table 4 summarizes the putative distribution of 2C and 4C nuclei in these cultures.

In the wild type, 85% of cells are 4C and 62% (53/85) of these proceed through both meiotic divisions. In cultures of mutant spo1-1, after 36 hr 49% of the cells are 4C; of these, 14% (7/49) enter meiosis I. In cultures of spo2-1, a larger percentage (83%) of the cells are 4C, and, of these, 16% (13/83) enter meiosis I. In the last mutant (spo3-1), 40% of the cells are 4C, and more than half of these [57% (23/40)] proceed into meiosis I or beyond.

The combined analysis of the nuclear staining data and DNA synthesis at the restrictive temperature demonstrate that the mutants spo1-1 and spo3-1 reduce both the probability that a cell will undergo DNA replication and the probability that a 4C cell will proceed through meiosis I. On the other hand DNA synthesis is normal in mutant spo2-1, but the probability that these cells will go beyond the first meiotic nuclear division is diminished.

Protein synthesis and turnover in wild-type and mutant strains. We have previously demonstrated that, during meiosis and sporulation of wild-type homothallic diploids of yeast at 30 C, protein syn-

TABLE 3. *DNA content during sporulation at 34 C*

Strain	Hr	DNA per 10^9 cells [a]				Per cent increase[b]
		I	II	III	Avg	
		μg	μg	μg	μg	
+ / +	0	53.9	46.7	43.8	48.1	
	12	62.5	74.4	76.2	71.1	48
	24	83.4	79.4	85.8	82.9	73
	36	83.5	93.1	89.5	88.7	85
spo1-1 / spo1-1	0	49.7	53.4	52.4	51.9	
	12	55.1	62.7	54.5	57.4	11
	24	70.7	67.0	61.0	66.2	28
	36	75.6	79.4	76.7	77.2	49
spo2-1 / spo2-1	0	58.5	58.5	57.5	58.2	
	12	75.2	74.7	85.4	74.4	35
	24	100.0	96.5	98.4	98.5	69
	36	114.1	98.8	106.0	106.3	83
spo3-1 / spo3-1	0	57.7	53.4	59.1	56.8	
	12	74.0	76.9	88.2	79.7	40
	24	88.6	81.7	90.6	87.0	35
	36	78.1	84.2	78.1	80.1	41

[a] Cultures were sporulated in medium II. Three samples (I, II, III) were removed from each culture at each time interval. DNA was determined by the diphenylamine method as described in the text.

[b] Calculated from the average DNA rises over the zero-hour samples.

thesis is continuous and occurs before and during ascus production (6). Sporulation of yeast is sensitive to cyclohexamide, an effective inhibitor of protein synthesis in yeast, and accompanied by extensive turnover of protein. One would therefore expect that a gene mutation conferring a deficiency in either protein synthesis or turnover under conditions of sporulation would have the phenotype of a sporulation-deficient mutant. To determine whether any of the three mutants was deficient in protein synthesis, the rate of protein synthesis at the restrictive temperature (34 C) was measured by pulse-labeling with the optimal concentration (10 μg/ml) of ^{14}C-arginine during sporulation of the wild type and three mutants. The results of this experiment for the wild-type strain S41, and the mutants are summarized in Fig. 1. At both temperatures, the wild type displays several periods of ^{14}C-arginine incorporation. At 25 C, the maximal rate of incorporation occurs at 8 hr and a secondary shoulder at 15 hr. At 34 C, the major peak occurs earlier (7 hr) and the shoulder from 11 to 15 hr. None of the mutants are deficient in protein synthesis at 34 C. The profile of changes in the rate of ^{14}C-arginine incorporation into protein of spo3-1 is most similar to that of the wild type. The incorporation rate profile of mutants spo1-1 and spo2-1 depart from the wild-type pattern. At 34 C, spo1-1 displays a prominent peak of incorporation at 15 hr, which is present as a shoulder on the curve of the wild type at this time. Incorporation of exogenous ^{14}C-arginine into spo2-1 at 6 hr is 1.5 times greater than the maximal rate observed in the wild-type strain at both temperatures, and maintains a higher rate of incorporation than normal through 36 hr when both experiments were terminated.

The bulk protein content of sporulating yeast cells at first increases and then declines as meiosis proceeds (3, 6). The decline in overall protein content is probably due to the extensive turnover of proteins which is observed when diploid cells pregrown in medium containing radioactive amino acids are transferred to unlabeled sporulation medium (6). Direct measurements of intact yeast cells reveal that the proteolytic activity is enhanced on transfer from growth medium to sporulation medium (2). A sporulation-deficient mutant which fails to demonstrate increased proteolytic activity in sporulation cultures has been described (2), indicating that this activity may be necessary for sporulation. Protein degradation during intracellular differentiation in *Bacillus subtilis* were previously observed, and asporogenous mutants defective in protease were also previously described (16).

To determine whether the strains employed in the present investigation were defective in protein

TABLE 4. *Putative per cent distribution of 2C and 4C nuclei in meiotic cells*[a]

Strain	Mononucleate[b]		Binucleate (4C)	Tri- and tetra-nucleate (4C)	Asci (4C)
	2C	4C			
+ / +	15	32	1	2	50
spo1-1 / spo1-1	51	42	7	0	0
spo2-1 / spo2-1	17	70	10	3	0
spo3-1 / spo3-1	59	18	17	6	0

[a] Data from Tables 2 and 3 after 36 hr of sporulation.

[b] Per cent of 4C mononucleate cells equals per cent of increase DNA minus (per cent of multinucleate cells plus per cent of asci); per cent of 2C mononucleate cells equals per cent of mononucleate cells minus per cent of 4C mononucleate cells.

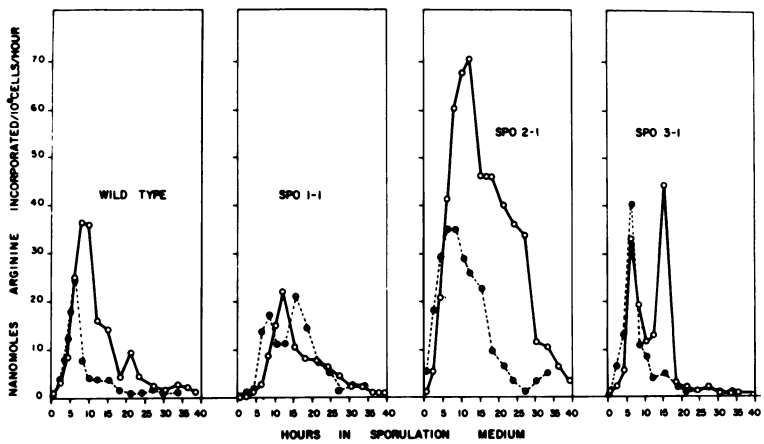

FIG. 1. *Rate of incorporation of ^{14}C-arginine into protein during sporulation. Wild type (S41) and spo1-1, spo2-1, and spo3-1 were sporulated in medium II containing 10 μg of arginine per ml. One culture was incubated at 25 C and the other at 34 C. At intervals, two 1-ml portions of cells were removed from each flask, and 0.2 μCi of ^{14}C-arginine was added to each portion. Cold trichloroacetic acid, 10% final concentration, was added to one at zero time, and the other was incubated for 1 hr at either 25 or 34 C and then precipitated with trichloroacetic acid. Based on the specific activity, the nanomoles of ^{14}C-arginine incorporated per 10^8 cells per hour (corrected for zero-time adsorption) was calculated and plotted as a function of time in hours during sporulation at 25 C (solid line) or 34 C (broken line).*

turnover at the restrictive temperature the wild-type strain Z140-9A, and the three mutants were pregrown in YEP medium containing 0.25 μCi of ^{14}C-phenylalanine per ml and transferred to unlabeled sporulation medium at 34 C. There is an initial slight incorporation of ^{14}C-phenylalanine (from the soluble pool) into proteins in the wild type and mutant strains (Fig. 2). After the incorporation, the ratio of ^{14}C-phenylalanine at any time to time of sporulation steadily declines in all strains. Thus the turnover of proteins characteristic of sporulation is normal in the mutants.

RNA synthesis in mutant and wild-type diploids. During meiosis and sporulation of diploids at 30 C, the RNA content per cell increases to a maximal level before the appearance of asci and declines thereafter (6). In this study, we examined the RNA synthetic activity of meiotic cultures by pulse labeling at intervals with ^{14}C-adenine. The pattern of RNA synthesis of sporulation cultures of the wild-type and mutant strains at 34 C are shown in Fig. 3. The results obtained with the wild-type strain reveal two periods of maximum RNA synthesis, one at 10 hr and the other at 20 to 25 hr of sporulation. The pattern of labeling in the mutant strains shows only slight variations from that observed in the wild-type strain. Similar kinetics in all four strains were observed when $^{32}PO_4^{3-}$ or ^{14}C-uracil was used instead of adenine to measure the rate of RNA synthesis. From these results, it is clear that these mutant strains are not deficient in RNA synthesis.

Functional mapping by temperature shift experiments. The previous experiments do not indicate the time during the sporulation cycle when the functions defective in the mutants participate in the meiosis and sporulation process. We determined these periods by taking advantage of the temperature sensitivity of the mutants.

Two types of temperature-shift experiments were performed. In the first experiment, sporulation cultures of mutant strains were incubated at the permissive temperature (25 C); at intervals, samples were shifted to the restrictive temperature (34 C). At 72 hr, all cultures were examined for the presence of asci. The rationale of this type of experiment is that the formation of asci will first be observed in samples shifted to the restrictive temperature when a fraction of the cells no longer requires the function specified by the temperature-sensitive mutation. The second type of temperature-shift experiment is designed to determine the time at which the temperature-sensitive function is first required. Sporulation cultures of mutants at the restrictive temperature are shifted to the permissive temperature at intervals, and the percentage of asci in the cultures was determined at 72 hr. The point at which the shifted cultures decline in their ability to produce asci at the permissive temperature marks the time when the cells begin to require the mutant function. With increasing time of incubation at 34 C, the ability of cells shifted to 25 C to sporulate progressively declines.

Both types of shift experiments were performed for the spo mutants (Fig. 4). The kinetics of the

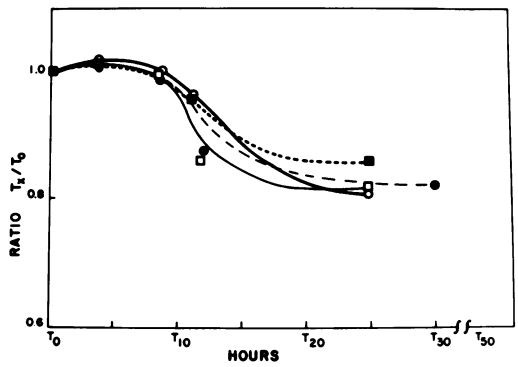

FIG. 2. *Protein turnover during sporulation. Wild type (Z140-9A) and temperature-sensitive sporulation mutants (spo1-1, spo2-1, and spo3-1) were grown in YEP medium containing 0.25 μCi of ^{14}C-phenylalanine per ml at 30 C, harvested at the end of growth, and transferred to unlabeled sporulation medium. The ratio of radioactivity in protein at any time (T_x) to radioactivity in protein at the onset of sporulation (T_0) is plotted as a function of time during sporulation for the wild type Z140-9A (○), and mutants spo1-1 (■), spo2-1 (●), and spo3-1 (□).*

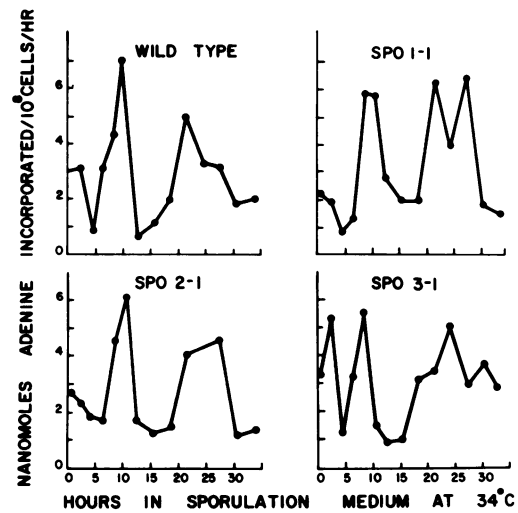

FIG. 3. *Rate of incorporation of ^{14}C-adenine into RNA during sporulation. Sporulation was carried out at 34 C in medium II containing 50 μg of adenine per ml. At intervals, 1-ml portions were removed, and 0.2 μCi adenine-8-^{14}C was added; 0.5 ml of the preparation was immediately removed and chilled in an ice bath, and 10% cold trichloroacetic acid was added. The remainder of the sample was incubated for 1 hr at 34 C and then treated as above. Radioactivity in the RNA fraction was measured as previously described (14). The nanomoles of adenine incorporated per 10^8 cells per hour (corrected for zero-time adsorption) was calculated and plotted as a function of time in hours during sporulation. The wild type in these experiments was S41.*

appearance of asci in cultures of the spo mutants incubated at 25 C is shown with the temperature-shift data. The temperature-sensitive period of a mutant begins at the point in the 34 to 25 C shift during which cells begin to lose sporulation ability. The first indication of the end of the temperature-sensitive period is the beginning of release from temperature sensitivity observed in the 25 to 34 C shift experiments.

The mutants differ from one another both in the time of initiation of the temperature-sensitive period and its duration. Mutant spo2-1 is of particular interest, since there are two temperature-sensitive periods which occur consecutively in

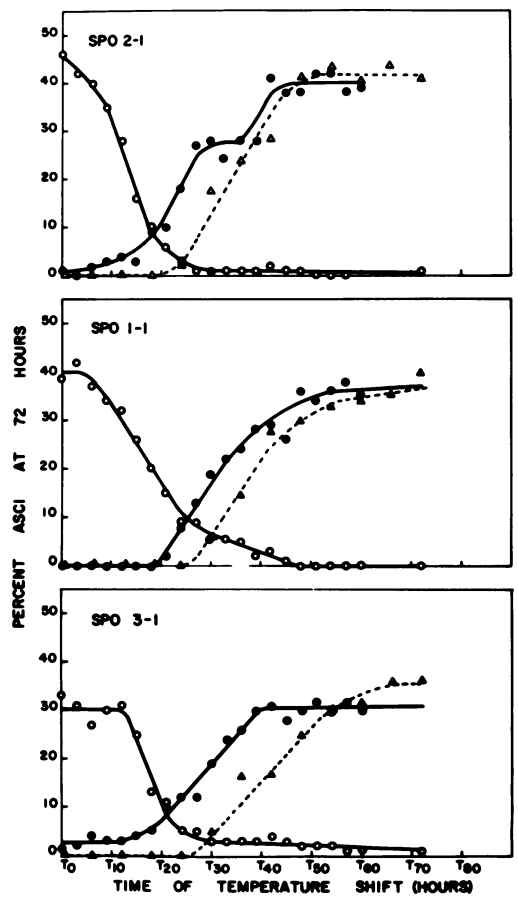

FIG. 4. *Effect of temperature shifts on sporulation. Cultures of spo1-1, spo2-1, and spo3-1 were sporulated at 25 and 34 C. At intervals, samples were removed and shifted from 25 to 34 C (●) or from 34 to 25 C (○). The final per cent of asci in each sample was counted at 72 hr. The sporulation kinetics of the cultures at the permissive temperature (25 C) is shown (△) by the dotted line; there were no asci in cultures of the mutants held at the restrictive temperature for 72 hr.*

time. These are distinguishable from one another by a change in the rate of the loss of sporulation ability in the 34 to 25 C shift and a change in the rate of release from temperature sensitivity in the 25 to 34 C shift.

The three mutant strains differ slightly from one another in the time at which asci are first seen at the permissive temperature in unshifted cultures. This complicates comparisons of the time of initiation and length of the temperature-sensitive periods of different mutants. This difficulty can be overcome by correcting the rate of sporulation at 34 C to 25 C and normalizing the data from the temperature-shift experiments by the following equations:

$$f_i = t_i\sigma/t_a$$
$$f_e = t_e/t_a$$

where f_i and f_e are the fractions of the sporulation cycle for the initiation and the end of the temperature-sensitive period, respectively; t_i is the time at which 90% of the sporulation potential is observed in the 34 to 25 C shift; t_e is the time at which 10% of the sporulation potential is observed in the 25 to 34 C shift; t_a is the time (at 25 C) of appearance of 10% of the final yield of asci in each culture and σ is the ratio of $t_{a\ 25\ C}/t_{a\ 34\ C}$, of the wild type. In the present medium, $\sigma = 1.35$ which reflects the fact that sporulation at 34 C begins earlier than at 25 C in the wild type (Esposito, *unpublished data*).

Both the initiation and end of the temperature-sensitive periods are influenced by the degree of asynchrony of sporulation cultures. The value of calculating the time of onset and end of the temperature-sensitive periods by the method described is that it directly applies to the cells that first form asci at the permissive temperature and expresses the length of the temperature-sensitive period in terms of the time required by a single cell to form an ascus.

Table 5 summarizes the normalized data from two temperature-shift experiments. In both cases, mutant spo2-1 displays a biphasic pattern in the temperature-shift profiles; for our purposes, we considered these as one period of temperature-sensitivity. To determine whether the release of the temperature-sensitive period proceeds to completion, the maximal percentage of asci observed in the shift experiment was compared to the per cent of asci in control cultures incubated at the permissive temperature (25 C).

The information in Table 5 is illustrated as a physiological map in Fig. 5. The normal allele of spo3-1 specifies a function which is needed near the midpoint of the cycle for a short period of time. The function defective in spo1-1 is required when approximately 47% of the time before ascus completion has elapsed and confers a temperature-sensitive period which occupies 32% of the cycle. The function defective in spo2-1 is needed earlier in the sporulation cycle (22%), and the temperature-sensitive period occupies 34% of the cycle.

Changes in viability in sporulation cultures at the restrictive temperature. The spo mutants are capable of growth by budding at 34 C but do not sporulate. The shift experiments described above indicate that with increasing time of incubation at 34 C mutants lose their capacity to sporulate at the permissive temperature. The onset of this loss of sporulation ability is different in the three mutant strains, occurring earliest in spo2-1 and later in spo1-1 and spo3-1. To determine whether the loss of sporulative ability at 34 C is associated with a loss in colony-forming ability, sporulation cultures of the wild type and the three mutants at 25 C and 34 C were sampled at intervals, and the cells were plated on solid YEP medium and incubated at 34 C. The results of this experiment are shown in Fig. 6. A progressive loss of colony-forming ability was observed in the 34 C sporulation cultures of all the mutants. The loss of colony-forming ability by spo1-1 and spo2-1 occurs after the beginning of their respective temperature-sensitive periods. The behavior of spo3-1 is different in this respect. Loss of colony-forming ability by spo3-1 precedes its temperature-sensitive period.

DISCUSSION

The genetic manipulability of *Saccharomyces* allows the isolation of thermosensitive mutants which are capable of mitotic division but unable to form asci. Since meiosis precedes ascospore formation, such mutants might be expected to have defects in functions required for the meiotic divisions as well as subsequent spore development. These variants thus provide a method of identifying genes controlling meiosis. The purpose of this study was to characterize three sporulation-deficient mutants (spo1-1, spo2-1, spo3-1) and to ascertain whether they represent mutational blocks in meiosis or in subsequent stages of sporulation.

Sporulation cultures of mutants defective in meiosis would be expected to accumulate mononucleate and binucleate cells; mutants affecting the development of spores after the meiotic divisions would be expected to contain primarily tetranucleate cells. In the three mutants which we studied, the mononucleate cells were the most

TABLE 5. *Normalized TSP*[a]

Strain	Expt[b]	f_i	f_e	TSP, $f_e - f_i$	Release in 25 to 34 C shift[c]
					%
spo1-1	1	0.567	0.800	0.233	100
spo1-1	2	0.379	0.754	0.398	100
	Avg	0.473	0.777	0.316	100
spo2-1	1	0.205	0.523	0.316	100
spo2-1	2	0.243	0.600	0.357	95
	Avg	0.224	0.561	0.337	98
spo3-1	1	0.635	0.647	0.012	85
spo3-1	2	0.639	0.666	0.027	86
	Avg	0.637	0.657	0.020	86

[a] Abbreviations: TSP, temperature-sensitive period; f_i and f_e, fractions of the sporulation cycle for the initiation and the end of the TSP.

[b] For data in experiment 1, see Fig. 4.

[c] Per cent of release equals maximum per cent of asci in 25 to 34 C shift to maximum per cent of asci in 25 C unshifted culture.

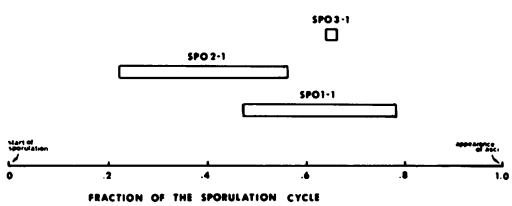

FIG. 5. *Physiological map of the temperature-sensitive periods of mutants spo1-1, spo2-1, and spo3-1. The duration and position of the temperature-sensitive period(s) of each mutant in the sporulation cycle are shown by horizontal bars. The method of calculating the temperature-sensitive period is given in the text.*

frequent class (Table 2). These data indicate that the block in these mutants occurs before the completion of the meiotic divisions. Sporulation cultures of all three mutants from the restrictive temperature, however, contained some multinucleate cells. The multinucleate cells in these populations do not represent all of the cells which initiated processes associated with meiosis. Sporulation cultures of the three mutants all show increases in DNA content at the restrictive temperature. The per cent increase in DNA content of these cultures was greater than the fraction of multinucleate cells present (Tables 3 and 4), indicating DNA synthesis by cells which did not proceed into the meiotic nuclear divisions.

In yeast (3, 6) and higher plants (9, 10, 18), synthesis of RNA and protein is observed during meiosis. Inhibition of protein synthesis during meiosis prevents further development (6, 12). The isotope incorporation experiments described here demonstrate that the meiotic arrest of the mutants is not due to an inability to synthesize RNA or protein nor to an inability to turnover protein.

The utility of mutations which perturb meiosis is that they reveal aspects of its control which are not evident in studies of normal individuals. The analysis of the temperature-sensitive periods of the mutants demonstrates that there are gene functions which are required at different times and for intervals of different lengths (Table 5 and Fig. 5). A thermosensitive period may reflect a thermosensitive mutant alteration in the transcription, translation, or activity of a gene product involved in sporulation. Although the temperature-shift experiments do not discriminate among these alternatives, one can study the interdependence of the physiological processes which accompany meiosis by examining the phenotypes of the spo mutants and the position of their thermosensitive periods. For example, of the mutants examined spo2-1 confers the earliest

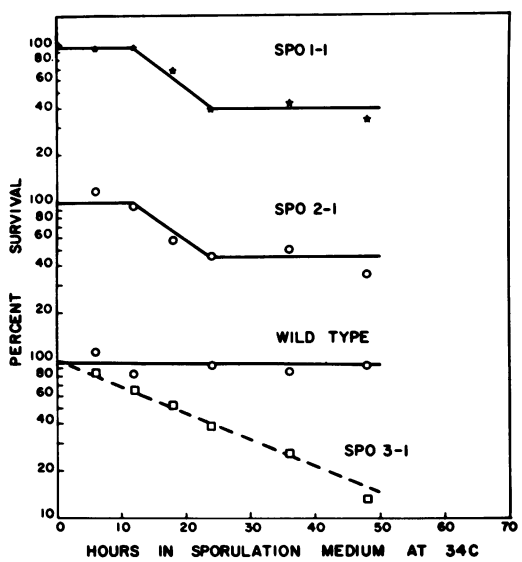

FIG. 6. *The loss of colony-forming ability in sporulation medium. The wild type (S41) and mutants spo1-1, spo2-1, and spo3-1 were placed in sporulation medium at 25 and 34 C. Samples of each strain were plated on YEP, and the plates were incubated at 34 C. Approximately 1,500 cells (0.3 ml) were plated for each determination of viability. The loss of colony-forming ability due to incubation of cells in sporulation medium at the restrictive temperature (34 C) is illustrated, as calculated by the following: per cent survival = (number of colonies from the 34 C sporulation culture/0.3 ml)/(number of colonies from the 25 C sporulation culture/0.3 ml) × 100.*

temperature-sensitive period (Table 5 and Fig. 5). Since macromolecular synthesis occurs during the temperature-sensitive period, the normal spo2 gene function is not essential for the continued synthesis of DNA, RNA, or protein.

Meiosis in yeast is a process of intracellular differentiation wherein cells stop growth by budding and mitosis and enter meiosis. Wild-type diploid cells in sporulation medium arrive at a point of commitment to meiosis which may be observed by returning portions of sporulation culture to glucose nutrient medium in which cells do not ordinarily sporulate (7). Cells returned to nutrient medium early in sporulation revert to mitotic division; later in sporulation, cells become committed to meiosis and give rise to asci in nutrient medium. The fraction of committed cells increases with time and is highly correlated with the percentage of cells at or beyond the binucleate condition (15). These results indicate that the point of commitment to meioisis coincides closely with the onset of the reductional division.

One would expect that a meiotic mutant which does not prevent commitment would lose colony-forming ability during sporulation and subsequent incubation in nutrient medium at 34 C as cells become committed to meiosis and can neither revert to mitosis nor complete the meiotic process. Each of the three mutants we have studied exhibited a decline in viability during sporulation and incubation in nutrient medium at 34 C.

If inviability is due to commitment and commitment occurs during the reductional division, the per cent of inviability should equal the per cent of multinucleate cells in the culture. In the case of all three spo mutants, the amount of inviability exceeded the fraction of multinucleate cells at 36 hr (Table 2, Fig. 6). These results may be interpreted in two ways. (i) Inviability is due to factors other than commitment, or (ii) commitment precedes the reductional division. In mutants spo1-1 and spo2-1, the decline in viability occurs within the temperature-sensitive period(s) and could be due to the expression of the spo mutation itself with respect to functions required for meiosis and spore formation. However, lethality in spo3-1 precedes the temperature-sensitive period and cannot be due to events occurring in the temperature-sensitive period. It remains to be determined whether the loss in viability is due to commitment or a pleiotropic effect of the spo mutants. If it is due to commitment, commitment to meiosis may actually precede the reductional division, as in *Lilium*, in which commitment to meiosis occurs in the late G2 of the premeiotic interphase (17).

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