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GENES CONTROLLING MEIOSIS AND SPORE FORMATION IN YEAST[1]

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SPORULATION of diploids of *Saccharomyces cerevisiae* involves meiosis and subsequent ascospore formation. The genetic and physiological control of this process is of interest as a model system for the control of meiosis and gametogenesis and has been the subject of several reviews (FOWELL 1969; HABER and HALVORSON 1972; TINGLE *et al.* 1973). Study of meiosis and gamete formation at the biochemical, genetic, and cytological levels in several organisms has yielded a wealth of information. However, we still have relatively little knowledge of the nature and number of independent gene functions which control the events we observe and integrate them into a successful developmental sequence. Our primary objective has been to dissect the genetic control of meiosis and sporulation in yeast through the study of variants, mutant in gene functions indispensible for the completion of sporulation but which are capable of growth by mitosis and budding. We have thus attempted to restrict our attention to the properties of mutants defective in functions specific to meiosis and ascospore development. Both recessive (*spo*) and dominant (*SPO*) temperature-sensitive sporulation-deficient mutants have been isolated and eleven loci have been identified among recessive *spo* mutants (ESPOSITO and ESPOSITO 1969; and ESPOSITO *et al.* 1972). Complementation studies suggest that approximately 50 loci coding functions indispensible for sporulation may be recovered (ESPOSITO *et al.* 1972).

In this report we wish to describe recent progress in the characterization of *spo* mutants of ten loci and three dominant mutants. These studies provide information with respect to the stages of sporulation where development may terminate or become abnormal among mutants which fail to form typical ascospores at a restrictive temperature. Sporulation-deficient mutants of three loci (*spo1*, *spo2*, and *spo3*) have been examined for both recombinational ability (ESPOSITO and ESPOSITO 1974) and fine structural development at the level of electron microscopy (MOENS, ESPOSITO and ESPOSITO 1974). These mutants demonstrate features of the integration and coordinate control of certain landmark events of meiosis and spore formation in yeast.

MATERIALS AND METHODS

Yeast strains: Homothallic diploid strains of Saccharomyces were employed in this study. Z190-8B served as the control sporulation-proficient strain and has the following genotype: *a/α*, *D/D*, *ade2/ade2*, *ade6/ade6*, *his7/his7*, *leu1/leu1*, *lys2–2/lys2–2*, and *trp5–R/trp5–R*. Homothallic

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diploids of both recessive and dominant temperature-sensitive sporulation-deficient mutants previously described (Esposito *et al.* 1972) were studied.

Media: The amounts indicated are those required for the preparation of one liter of medium. Yeast Extract Peptone Dextrose (YPD)—20g dextrose, 20g peptone and 10g yeast extract. Yeast Extract Peptone Acetate (YPA)-10g potassium acetate, 20g peptone and 10g yeast extract. Sporulation medium-20g potassium acetate, pH 7. YPD, YPA and sporulation medium were supplemented with 50mg/l of each of the following nutrilites: adenine sulfate, L-arginine hydrochloride, L-histidine hydrochloride, L-leucine, L-lysine hydrochloride, L-tryptophan, L-tyrosine and uracil.

Procedure of sporulation: Cells grown in YPD medium were harvested at early stationary phase (ca. 2×10^8 cells/ml), washed twice with sterile water and resuspended in prewarmed sporulation medium at ca. 5×10^7 cells/ml. Cells grown in YPA medium were harvested during the exponential phase of growth (at ca. 1×10^7 cells/ml), washed twice with sterile water and resuspended in sporulation medium at 1×10^7 cells/ml.

Staining of yeast nuclei: The nuclei of vegetative and sporulated cells were observed by Giemsa staining as previously described (Esposito *et al.* 1970).

Colorimetric measurement of cellular DNA content: DNA was assayed colorimetrically with the Diphenylamine reagent (Ceriotti 1955) as previously reported (Esposito *et al.* 1969).

Labeling of DNA with ^{14}C-uracil during growth and sporulation: Cells were grown in YPA medium containing 2μc/ml uracil-2-^{14}C (New England Nuclear) and transferred to sporulation medium containing 2μc/ml uracil-2-^{14}C. To monitor incorporation of isotope into DNA, 1 ml of 2 N NaOH was added to 1 ml of culture (1×10^7 cells) and the mixture was incubated for 24 hours at room temperature to hydrolyze RNA. Two ml of chilled 50% trichloracetic acid were then added to each sample and filtered precipitates were prepared for scintillation counting.

Labelling of RNA with ^{14}C-uracil and protein with ^{3}H-tyrosine during sporulation: Cells grown in YPA medium were transferred to sporulation medium containing .5μc/ml uracil-2-^{14}C (New England Nuclear) and .2μc/ml of L-tyrosine-3,5-^{3}H (Amersham). One half ml of chilled 20% trichloroacetic acid was added to half ml samples of the culture (5×10^6 Cells) and filtered precipitates were prepared for scintillation counting.

RESULTS AND DISCUSSION

Sporulation in the wild type

Yeast diploids can be induced to sporulate by transferring cells from growth medium to sporulation medium which contains acetate as a carbon source and in which nitrogen sources are absent or present in reduced amount. Sporulation is optimal if cultures are established employing cells already adapted to respiration and which possess sufficient cellular reserves to support macromolecular synthesis under conditions of nitrogen deprivation (Croes 1967). Cells harvested during the exponential phase of growth in acetate nutrient medium (YPA) or the early stationary phase of growth in glucose nutrient medium (YPD) were employed in the studies to be described. Insofar as we are able to ascertain, the basic features of meiosis and sporulation are identical employing these regimes though sporulation is more synchronous and more extensive in the case of acetate grown cells (Roth and Halvorson 1969; Fast 1973).

The temporal sequence of landmark events of sporulation relevant to the study described are summarized in Figure 1. When diploid cells are introduced into sporulation medium growth by budding ceases. Buds increase in size, mitotic nuclear segregation occurs and buds become separable from mother cells. At 4 hours premeiotic DNA synthesis is initiated (Croes 1966), cells acquire en-

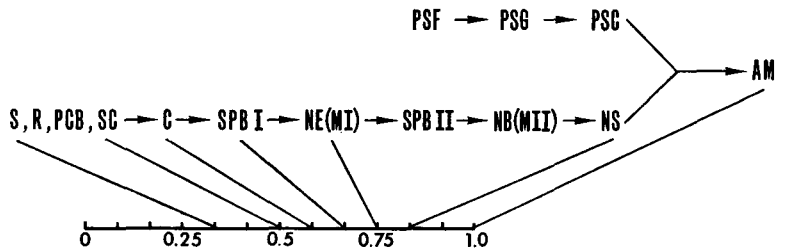

FIGURE 1.—Temporal order of events of meiosis and sporulation in yeast. The arrows indicate the temporal sequence: S, premeiotic DNA synthesis begins; R, commitment to recombination begins; PCB, polycomplex body formation, SC, synaptonemal complex formation; C, commitment to sporulation; SPBI, first spindle pole body duplication; NE(MI), nuclear elongation and meiosis I; SPBII, second spindle pole body duplication; NB(MII), nuclear budding and meiosis II; NS, nuclear separation; PSF, prospore wall formation; PSG, prospore wall growth; PSC, prospore wall closure; AM, ascospore maturation. The approximate time of occurrence of individual events is shown in an 0–1 time scale, where 1 is the time of appearance of asci (at 30°C mature asci are first seen at 12 hr, i.e., 1 = 12 hr).

hanced recombinational ability (SHERMAN and ROMAN 1963; ROTH 1972; ESPOSITO and ESPOSITO 1974) and synaptonemal complex elements become visible within the yeast nucleus (ENGELS and CROES 1968; MOENS and RAPPORT 1971). At approximately 8 hours the spindle pole body duplicates. A spindle is established, the nucleus elongates and chromatin segregation occurs (meiosis I). The spindle pole bodies duplicate again; spindles for the second meiotic nuclear segregation are established and the nuclear material now buds toward the four poles. During the nuclear budding process, prospore walls form at the surface of the nuclear membrane at the site of the spindle pole bodies of the second meiotic nuclear division. Each nuclear bud is engulfed by a growing prospore wall; at 10 hours the prospore wall closes and nuclear separation occurs. At 12 hours the ascospores become spherical and refractile (MOENS and RAPPORT 1971; MOENS 1971).

Premeiotic DNA synthesis

Sporulation-deficient mutants have been characterized with respect to premeiotic DNA synthesis, RNA and protein synthesis, and completion of mitotic cell division in sporulation medium. Premeiotic DNA synthesis was monitored in a control strain (Z190-8B) and sporulation-deficient mutants to determine whether any of the mutants fail to exhibit such synthesis at the restrictive temperature (34°). For these experiments, control and mutant diploids were grown at 34° in YPA medium containing uracil-2^{14}C to label DNA. Cells were harvested during the exponential phase of growth and transferred to sporulation medium at 34° containing the isotope at the same specific activity. ^{14}C incorporated into DNA was measured in TCA-precipitated material which had been previously incubated in NaOH to hydrolyze RNA (cf. MATERIALS AND METHODS). Samples were removed at zero hours and at intervals thereafter to monitor further incorporation of ^{14}C into DNA in sporulation medium (Figure 2). The values for each diploid are shown relative to the value at zero hours. In the control there is an

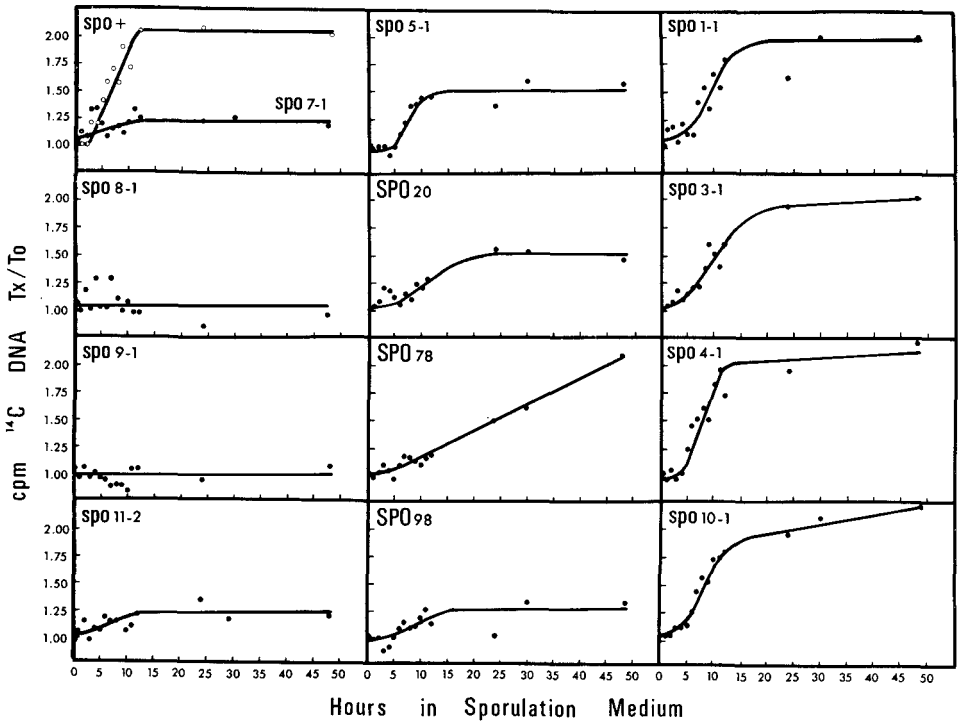

FIGURE 2.—Premeiotic DNA synthesis in sporulation medium at 34° by sporulation-deficient diploids (*spo* and *SPO*) and a control strain (+). The ratio of ^{14}C in DNA at various times (T_x) is shown relative to the value at zero hours (T_0).

approximate doubling in the level of ^{14}C incorporated into DNA. Colorimetric determinations of DNA synthesis by sporulating cultures of the control at 34° also indicate a doubling in DNA content (ESPOSITO *et al.* 1970). Diploids of the recessive mutants, *spo7*, *spo8*, *spo9* and *spo11* and the dominant mutant *SPO98* fail to undergo premeiotic DNA synthesis. The remainder of the mutants, including *spo2* reported elsewhere (ESPOSITO *et al.* 1970) exhibit premeiotic DNA synthesis at the restrictive temperature.

These results demonstrate that mutants selected on the basis of inability to form normal ascospores include variants blocked before as well as after premeiotic DNA synthesis. It remains to be determined whether the failure of premeiotic DNA synthesis exhibited by *spo* mutants is due to defects in gene products directly involved in premeiotic DNA synthesis or the pleiotropic effect of gene mutations which prevent cells from arriving at the point in sporulation where premeiotic DNA synthesis is initiated.

RNA and protein synthesis

When sporulation-proficient diploids are introduced into sporulation medium, RNA and protein synthesis proceed. Inhibition of protein synthesis at any time before spores are formed arrests sporulation (ESPOSITO *et al.* 1969). Sporulation-

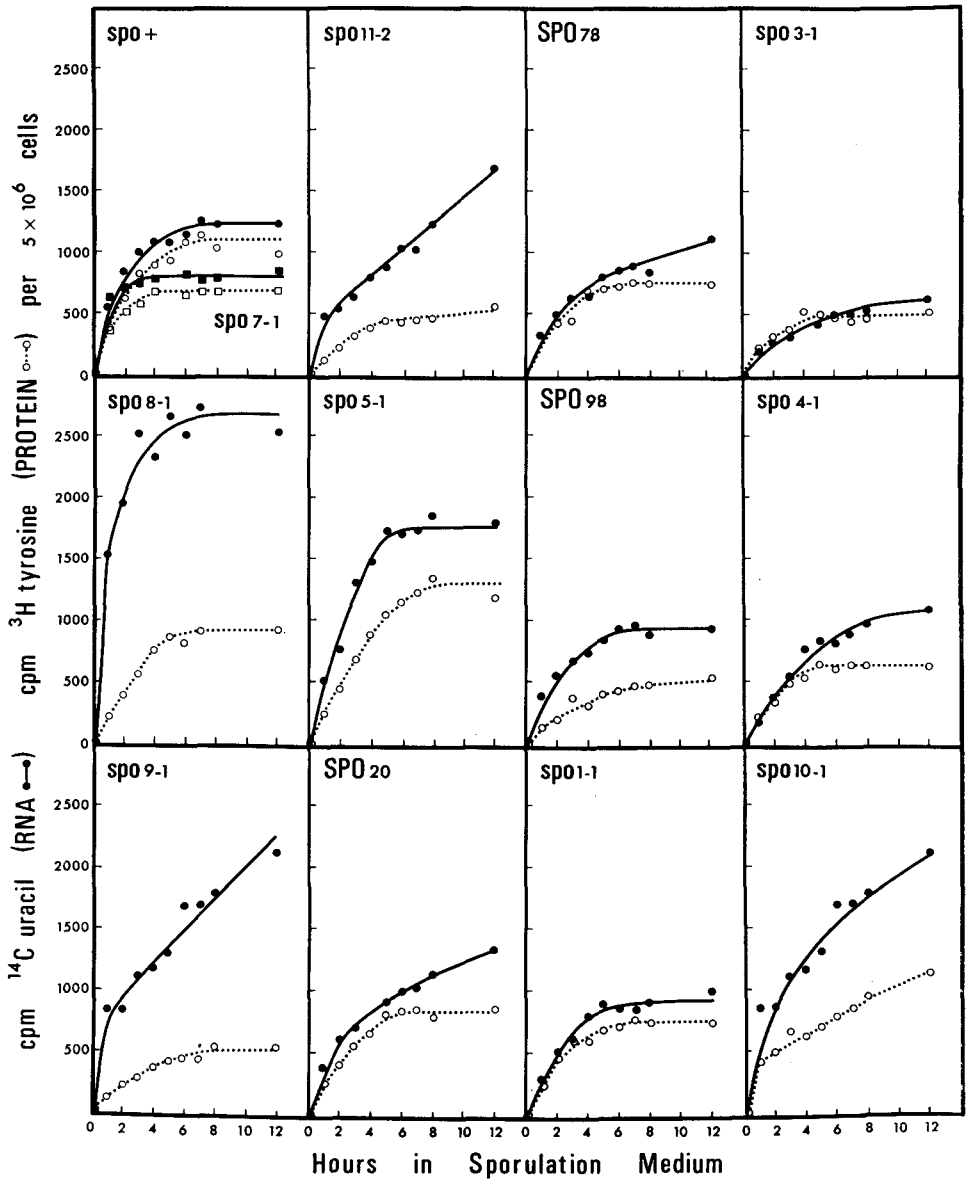

FIGURE 3.—RNA and protein synthesis in sporulation medium at 34° by sporulation-deficient diploids (*spo* and *SPO*) and a control strain (+).

deficient diploids were examined for their ability to synthesize RNA and protein at the restrictive temperature to determine whether any of the mutants are deficient in synthesis of these macromolecules. Cells grown in acetate nutrient medium 34° were transferred to sporulation medium at 34° containing ^{14}C-uracil to label RNA and ^{3}H-tyrosine to label protein. The specific activities of the two isotopes were adjusted to yield nearly equal corrected values of incorporation of

^{14}C-uracil into RNA and ^{3}H-tyrosine into protein for the control strain. The results are shown in Figure 3. None of the mutants is deficient in RNA or protein synthesis. The patterns of isotope incorporation of certain mutants, however, are different from the control. Diploids of *spo8*, *spo9*, *spo10* and *spo11* exhibit increased labelling of RNA relative to protein. The increased incorporation of ^{14}C into RNA in these strains could represent increased synthesis or diminished turnover of RNA made during sporulation. Studies in progress suggest that the mutants are not deficient in RNA turnover.

Previous studies have demonstrated that vegetatively growing yeast cells exhibit stringent control of the rates of synthesis of RNA and protein during nutritional shifts up and shifts down (WEHR and PARKS 1969). Since the transfer to sporulation medium is a nutritional shift down, mutants which exhibit a high rate of RNA synthesis relative to protein synthesis may be defective in components of the regulatory system which results in stringent control. In future studies we propose to determine which classes of RNA (ribosomal, transfer, etc.) are present at a higher than normal levels in these mutants and whether their phenotypes in this regard extend to nutritional shifts down involving growth medium as well.

Completion of mitotic cell division before sporulation

When sporulation cultures are established employing either early stationary phase cells from glucose nutrient medium (YPD) or cells harvested during the exponential phase of growth in acetate nutrient medium (YPA) the populations consist of cells at different stages of the cell cycle and contain budded as well as unbudded cells. We have observed that the budded cells complete cell division Buds enlarge, mitotic nuclear segregation occurs and buds become separable from the mother cell.

Mitotic nuclear segregation, premeiotic DNA synthesis and ascus formation were observed in a sporulation culture of the control incubated at the optimum temperature for sporulation (30°). Glucose-grown cells were employed. Mitotic nuclear segregation was monitored by Giemsa staining of cells at the time of introduction into sporulation medium and when sporulation was completed (48 hrs.). The fraction of unbudded cells, cells with small anucleate buds, and cells with buds containing a nucleus were determined at each time. The DNA content of an aliquot of the culture was determined initially and at 48 hrs employing the Diphenylamine reaction (cf. MATERIALS AND METHODS). These data are shown in Figure 4.

At the time of introduction into sporulation medium, the population contained 30% of cells with buds that did not contain a nucleus. At 48 hrs the population contained only 2% of cells with buds lacking a nucleus. Thus, most cells with anucleate buds are committed to the completion of mitotic nuclear segregation in sporulation medium.

Sporulation cultures established from cells harvested during the exponential phase of growth in acetate nutrient medium provide favorable material in which to examine mutants for the ability of budded cells to complete the mitotic cell cycle in sporulation medium at 34° because of the large fraction of budded cells

Hours of Sporulation	Frequency of Cell Types							μg DNA / 10^8 cells	Percent Asci
0	.63	.30	.07					2.7	0
48	.10	.02	.02	.49	.00	.27	.10	4.5	66.3

FIGURE 4.—Mitotic nuclear segregation and premeiotic DNA synthesis.

initially present. Diploids grown at 34° were surveyed for this property by enumerating the percentage of unbudded cells in populations following a brief dose of sonication, which separates buds and mother cells which have completed cell division (Figure 5). In the wild type the population initially consisted of 40% of unbudded cells, the remainder were chiefly doublets in which the buds ranged in size from barely detectable to those nearly equal in size to the mother cell. Samples were sonicated initially and at intervals after suspension of cells in sporulation medium and the fraction of unbudded cells was determined following sonication. Approximately 90% of cells of the wild type were unbudded following this treatment at five hours. Diploids of three mutants (*spo8*, *spo9*, and *spo10*) failed to exhibit completion of cell division in sporulation medium. This observation raises several interesting questions. Do these mutations reflect the existence of functions required for cell division only in sporulation medium or do they reflect alterations of gene products always required for cell division but which have been altered in a manner which prevents their functioning during sporulation? The latter possibility may be seriously entertained since it has been shown that certain enzymes involved in bacterial sporulation are enzymes present in the vegetative cell which have been modified by limited proteolysis (SADOFF, CELIKKOL and ENGELBRECHT 1970). One may imagine that certain amino acid substitutions might result in loss of function only after the protein is subjected to proteolytic modification or a changed intracellular milieu. Evidence in favor of the idea that certain *spo* mutants encode functions involved in mitosis could be obtained by testing them for allelism to the cell division cycle mutants of yeast described by HARTWELL and co-workers (HARTWELL, CULOTTI and REID (1970).

The results described above demonstrate that several *spo* mutants exhibit pleiotropic effects with respect to premeiotic DNA synthesis, patterns of RNA and protein synthesis, and completion of mitotic cell division in sporulation medium. Diploids of *spo8* and *spo9* fail to exhibit premeiotic DNA synthesis, demonstrate altered patterns of RNA and protein synthesis and do not complete cell division in sporulation medium. Diploids of *spo11* fail to exhibit premeiotic DNA synthesis, demonstrate altered patterns of RNA and protein synthesis but complete cell division in sporulation medium. Diploids of *spo10* undergo premeiotic DNA syn-

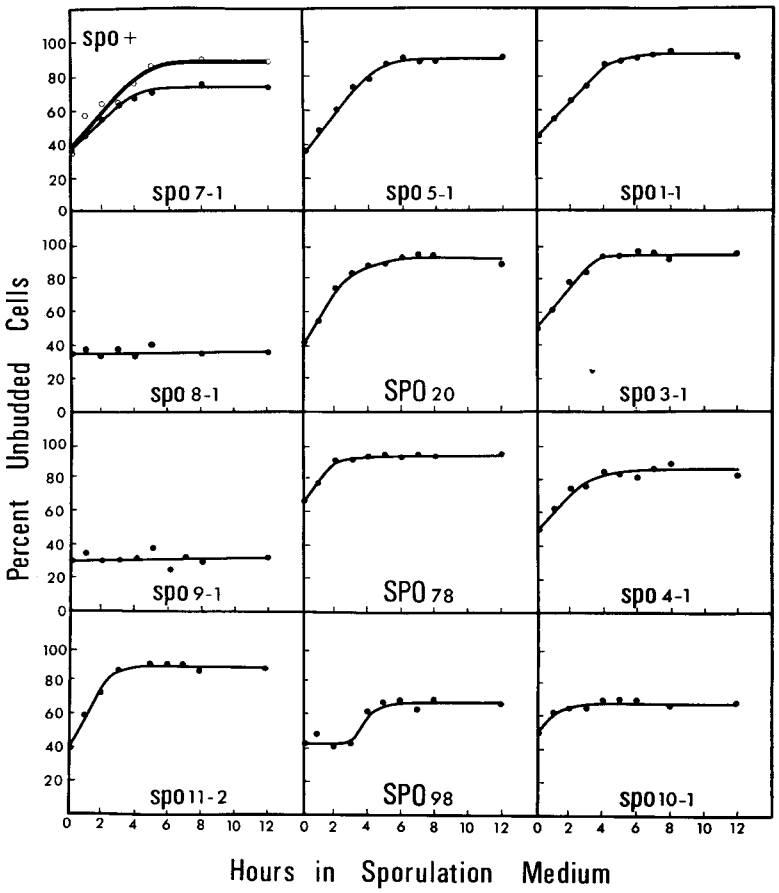

FIGURE 5.—Completion of cell division in sporulation medium at 34° by sporulation-deficient diploids (*spo* and *SPO*) and a control strain (+).

thesis; however, they exhibit altered patterns of RNA and protein synthesis, and fail to complete cell division in sporulation medium.

The behavior of *spo10* diploids demonstrates that the ability of cells to arrive at a point in the cell cycle where mother cells and buds are separate is not required for premeiotic DNA synthesis to occur. Cytological studies have shown that mitotic nuclear segregation occurs in budded cells of *spo10* diploids at the restrictive temperature indicating that the block in cell separation occurs after nuclear segregation.

Commitment to recombination

Following exposure of genetically marked diploid cells to sporulation medium, enhanced intragenic recombination (SHERMAN and ROMAN 1963) and intergenic recombination (ESPOSITO, FRINK and ESPOSITO 1971; ESPOSITO and ESPOSITO 1974) can be detected before cells become committed to meiosis. The recombinants are recovered as diploids which have returned to mitosis following plating

on medium selective for the growth of recombinants. They are recovered shortly after the onset of premieotic DNA synthesis (ROTH 1972). Intergenic recombination in certain intervals among cells uncommitted to meiosis has been found to be equal to standard meiotic map values suggesting that recombination in certain intervals may be completed before cells become committed to meiosis (ESPOSITO and ESPOSITO 1974). The recovery of genetic recombinants from sporulation cultures among cells which have returned to mitosis may reflect commitment to recombination or meiotic recombination itself since it is not known with certainty whether the genetic recombination event occurs in the sporulation medium or after plating.

Genetically marked diploid strains of three *spo* mutants which exhibit premeiotic DNA synthesis (*spo1*, *spo2*, and *spo3*) have been examined to determine whether they exhibit recombination ability. Diploids of the mutants exhibited enhanced recombination and the properties of both the intragenic and intergenic recombination events were similar to that observed in a control sporulation-proficient strain (ESPOSITO and ESPOSITO 1974). Thus the defects which result in failure to form asci in diploids of these mutants do not prevent acquisition of enhanced recombinational ability. Fine structural studies of these mutants at the level of electron microscopy (MOENS, ESPOSITO and ESPOSITO 1974), however, have shown that they differ with respect to the point during sporulation when cytological development terminates or becomes abnormal.

Cytological properties of sporulation-deficient mutants

Electron microscopic studies of yeast sporulation have shown that the meiotic divisions occur without breakdown of the nuclear envelope (MOENS 1971; MOENS and RAPPORT 1971). These studies have shown that sporulation involves spindle pole body duplication (SPBI), spindle formation and nuclear elongation at the first meiotic division (NE, MI), spindle pole body duplication for the second meiotic division (SPBII), formation of spindles for the second meiotic division and nuclear budding at the second meiotic division (NB, MII), formation of a prospore wall at the four spindle pole bodies of the second meiotic division (PSF), growth of prospore walls around each presumptive haploid nucleus (PSG), closure of the prospore wall pouch (PSC), nuclear membrane separation (NS), and ascospore maturation (AM) yielding spherical refractile spores.

Diploids homozygous at *spo1* terminate cytological development at the restrictive temperature with unduplicated spindle pole bodies (MOENS, ESPOSITO and ESPOSITO 1974). In *spo2/spo2* diploids sporulation is normal until the first division of meiosis. Nuclear elongation occurs and the nucleus then separates into two. This behavoir is repeated at the second meiotic division resulting in the formation of an ascogenous cell containing four separate nuclei. Prospore wall formation, growth and closure proceed but prospores rarely include any of the nuclei. The closed prospores remain immature, their walls do not thicken and become refractile and are not visible by light microscopy. In *spo3/spo3* diploids sporulation is morphologically normal until the second meiotic division. At this

time nuclear budding lags relative to closure of the prospore walls and anucleate immature ascospores and ascospores containing bits of nucleoplasm are formed.

The behavior of *spo2* and *spo3* diploids illustrate that prospore wall formation, growth and closure do not depend upon migration of nuclear material into the prospore wall pouch nor that the nuclear membrane remain intact. The fact that *spo1* diploids exhibit commitment to recombiantion indicates that this event does not require duplication of the spindle pole bodies for the first meiotic division.

Studies of the properties of sporulation-deficient mutants thus far allow certain conclusions to be made regarding the coordinate control and integration of the events of meiosis and ascospore formation in yeast. (1) The ability of cells to complete the cell cycle in sporulation medium and premeiotic DNA synthesis are coordinately controlled. Mutants defective in both processes due to a single gene mutation have been recovered. (2) Commitment to genetic recombination (at meiotic levels in certain intervals) does not commit cells to the reductional division of meiosis and does not require duplication of the spindle bodies for the first division of meiosis. (3) Prospore wall formation, growth and closure do not require entry of nuclear material into the growing prospore nor the integrity of the nuclear membrane. (4) Fully refractile, mature ascospores which are anucleate have not been observed, suggesting that ascospore maturation depends upon the presence of some nuclear material in the closed prospore. Further isolation and characterization of *spo* mutants will hopefully provide additional tools with which to understand the control of meiosis and gametogenesis in yeast.

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