This is to certify that the

dissertation entitled

ISOLATION OF SPO17, AN ESSENTIAL EARLY SPORULATION GENE IN SACCHAROMYCES CEREVISIAE

presented by

ALLAN L. KENNEDY

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Microbiology and Public Health

P Meyer
Major professor

Date 5/11/89

ISOLATION OF *SPO17*, AN ESSENTIAL EARLY SPORULATION GENE IN *SACCHAROMYCES CEREVISIAE*

By

Allan L. Kennedy

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

1989

ABSTRACT

ISOLATION OF *SPO17*, AN ESSENTIAL EARLY SPORULATION GENE IN *SACCHAROMYCES CEREVISIAE*

By

Allan L. Kennedy

Sporulation in the yeast *Saccharomyces cerevisiae* involves the fundamental process of meiosis. Entry into the pathway is known to require the conditions of starvation and diploidy, but the lack of understanding of regulatory mechanisms is due, in part, to the dearth of characterized genes involved in the early events of sporulation. One such early sporulation gene has been isolated because it complements strains that are blocked early in meiosis by a mutation, *spo17*, first identified in the strain SL484. A *spo17* recipient strain, R92W, was constructed by crossing SL484 with the *ade2* strain W66-8A and screening the progeny for certain selectable markers and for heterozygosity at the *ADE2* locus. After transformation of R92W with a YCp50 yeast genomic library, transformants were screened for sporulation-competence by assaying for the production of red papillae on white colonies; in a homothallic *ADE2/ade2* background red papillae can be produced when cells sporulate and produce progeny that have undergone diploidization and are *ade2/ade2*. A complementing plasmid, p1912, was found to bear *SPO17* on a 5.8 kb yeast insert. When the

coding region (identified by deletion mapping) was disrupted by *in vitro* mutagenesis and transformed into wild-type strains, the cells lost sporulation-competence. Northern analysis demonstrated the presence of at least two bands, 2.7 and 2.1 kb, in wild-type cells under both sporulation and vegetative conditions, but these signals are replaced by smaller messages in sporulation-deficient disruptants. To determine the influence of *SPO17* on *RME1*, a gene that exerts its effects by repressing the meiotic inducer gene *IME1*, the *spo17/spo17 rme1/rme1* double mutant strain 221a3 was constructed. 221a3 is unable to sporulate under any conditions. Thus it was concluded that *SPO17* does not exercise its effect by regulating *RME1*. The activity of the *SPO17* product in the sporulation process may lie downstream of the activity of *RME1* or on a pathway independent of the *RME1-IME1* cascade.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Sporulation in the yeast *Saccharyomyces cerevisiae* is a fundamental process which involves meiosis. One way to study such basic cellular processes in microorganisms is by generating and characterizing mutant strains that can be used in genetic and molecular studies. Although many sporulation deficient mutants have been generated, few of the affected genes have been isolated. Moreover, most genes that have been characterized are involved in the later events of sporulation, and their products are not likely to be regulators of early sporulation events. Genes expressed early in the sporulation pathway are of particular interest because they are likely to be involved in the regulation of meiosis.

This work was undertaken to isolate and characterize genes involved in sporulation. Initially, it was part of a larger effort (reviewed in Appendix A) to develop a library of sporulation-specific genes, based on the differential expression of those genes during sporulation. Appendix B is a brief survey of data developed from work on *SPR2*, a gene from the sporulation-specific library. *SPR2*, although expressed at significantly higher levels under sporulation conditions than in vegetative media, is not essential for sporulation. *In vitro* mutagenesis of *SPR2* by both insertion and deletion methods, followed by transformation and diploidization of wild-type strains, did not seem to

affect sporulation. This result and the work of others suggested that genes isolated by methods that rely on differential expression are likely not to be essential for sporulation and meiosis. Though they are interesting, their functions are probably quite varied and may involve spore structural development or fine-tuning of the processes. Some workers have even suggested that sporulation may include a period of general deregulation wherein many messages, including those of genes not required for sporulation, are indiscriminately expressed (Gottlin-Ninfa and Kaback, 1986); the genes for such messages might easily be included in a screen based on differential expression.

The work of Smith (1985) attempted to address the problem by the more conventional approach of UV mutagenesis. She developed an assay for an enzyme, sporulation amyloglucosidase (SAG), which is expressed at high levels in sporulating cultures. After UV mutagenesis of haploid spores from the homothallic strain SCMS7-1, she screened surviving diploid colonies for SAG activity and identified two strains that are sporulation-deficient, SL484 and SL641. The mutations in these strains have been given the designations *spo17* and *spo18*, respectively. Her work can be reviewed in Appendix C. The work reported in this thesis is based upon and extends those results.

Because of the rising interest in the characterization of early sporulation genes, I sought to isolate the *SPO17* gene, which would complement strain SL484. This was done by introducing the *spo17* mutation into a red *ade2* strain which was made heterozygous at the *ADE2* locus by plating on adenine-deficient media and selecting a white revertant colony. The new *spo17/spo17 ADE2/ade2* strain was transformed with a yeast genomic library and the transformants were subjected to

sporulation conditions. Following transfer to rich growth media, colonies were screened for sporulation-competence by scoring for the appearance of multiple red papillae, and plasmids isolated from such strains were found to bear the complementing gene, *SPO17*. To prove that *SPO17* is essential to sporulation, several experiments were performed, including an *in vitro* mutagenesis analysis. Spores from transformed mutagenized strains segregated 2:2 for the ability to sporulate.

Though initial entry by cells into the sporulation pathway is not well understood, it has been shown to depend upon at least two influences, available nutrition and the mating type locus, *MAT*. Interactions between environmental conditions, the *MAT* locus, and the meiotic regulator genes *IME1* and *RME1* have been demonstrated by studies using double mutants. Work with the *rme1 ime1* strain D320 (Kassir *et al.* 1988) showed that *IME1*, which induces sporulation, is negatively regulated by *RME1* as well as by nutritional signals. The *rme1* mutation permits cells to enter the meiotic pathway in strains that ordinarily do not, such as **a**/**a** diploids, α/α diploids or haploids. Thus, *IME1* may represent a key point in the pathway where different influences on sporulation converge.

Can *SPO17* be positioned on the pathway? In an attempt to answer this question, the *spo17* mutation was introduced into an *rme1* strain, thereby creating a double mutant, and the sporulation-competence of the new strain was assessed. The results suggest that *SPO17* does not interact directly with *RME1*; the strain does not sporulate in spite of the *rme1* mutation. Furthermore, the *SPO17* transcript is expressed normally in the *rme1/rme1* strain. On the other hand, the expression of

RME1 does not seem to be affected in *spo17/spo17* cells. Thus, the position of the *SPO17* product in the sporulation pathway probably lies downstream of *RME1* or on a parallel pathway.

Chapter I of this thesis is a review of the current literature. Chapter II is a prepared manuscript that has been submitted to *Molecular and Cellular Biology*. This work was carried out by me under the direction of Dr. Magee and is being submitted under joint authorship. Chapter III is a larger discussion of the results reported in Chapter II and other unpublished data. Appendix A is the publication which resulted from the initial work done by Clancy *et al.* (1983) using the differential hybridization approach. My contribution to this work was screening the lambda library and identifying clones that contain sporulation-specific yeast DNA sequences. A brief overview of the work done on *SPR2* is contained in Appendix B. Appendix C is the work of Smith *et al.*, in which the *spo17* mutant SL484 was isolated. The acceptance of this work for publication was dependent upon the proof that the mutation in SL484 was different than that in SL641, a second-sporulation mutant strain also reported in the paper. This could not be done until I had isolated the plasmid complementing SL484 and showed that it could not complement SL641. Additional data produced during the isolation of *SPO17* but not included in the manuscript (Chapter II) are in Appendix D.

REVIEW OF LITERATURE

Because of their fundamental nature, eukaryotic developmental pathways have become the focus of many investigators. Sporulation in yeast has provided a major access to the study of basic processes such as genetic recombination and chromosome segregation - events involved in meiosis - because of the well-developed genetics and molecular biology in this organism.

The ascomycete *Saccharyomyces cerevisiae* has many distinctly eukaryotic features (for review, see Struhl 1983), including a well-defined nucleus and chromosomes which contain a centromere, two telomeres and multiple DNA replication sites. Transcription and translation characteristics include three distinct RNA polymerases, 5'capped-3' poly(A) mRNAs, translation beginning at the first AUG codon, lack of operons, and occasional splicing of mRNAs. The yeast cell cycle involves two haploid cell types, a and α, which will mate to form diploid zygotes; these zygotes will grow vegetatively as diploids. The latter undergo meiosis and sporulation under specific conditions. Cellular organelles are similar to those in higher eukaryotes: lysosymes, Golgi apparatus, mitochondria, 80s ribosomes and nuclear membranes are all present.

Although some have argued that *S. cerevisiae* is not truly representative of higher eukaryotes (Beggs et al. 1980; Langford et al. 1983; Kaufer et al. 1985), this yeast offers an extremely powerful approach to the study of eukaryotic gene expression, DNA replication,

recombination, transposition, chromosome segregation, chromatin structure, secretion, cell cycle and control of cell type (Struhl 1983). Many distinctly eukaryotic proteins, such as histones and tubulin, are present in yeast. Studies of the yeast *RAS* proteins and their effector system have contributed greatly to the development of a model for this important eukaryotic system (Field *et al.* 1988).

A variety of genetic manipulations are possible because of the yeast mating system. Mating is controlled by the *MAT* locus on chromosome III, where either of two genetic configurations, designated as mating type a or mating type α, can exist. The stability of these configurations is under the control of another locus, the *HO* gene, whose activity confers homothallism: germination of haploid spores of homothallic strains is followed by a mating type switch by certain daughter cells, so that a "self" mating between daughter cells is ensured, thus producing a new homozygous diploid strain. Heterothallic (*ho*) strains, on the other hand, are stable at *MAT*, and in the absence of haploid cells of the opposite mating type, switch their *MAT* locus, mate, and diploidize only rarely. These features of yeast genetics have permitted the development of techniques to study many biochemical systems. For example, heterozygous diploids in homothallic backgrounds can be sporulated and their tetrads dissected to isolate spores; after germination these strains diploidize, giving rise to homozygotes. In this way the effects of a recessive mutation can be evaluated. Other studies such as mapping, linkage, and cross-over frequency analysis are easily performed by mating various haploid mutants and analyzing phenotypes of colonies grown from their dissected spores.

Molecular techniques have further enhanced yeast genetics for

metabolic studies. The well-developed transformation system (Struhl 1983; Rothstein 1983) permits introduction of metabolic or regulatory genes in several ways; an intact or disrupted gene may be integrated at its normal chromosomal location (Shortle *et al.* 1982), it may be carried on a multicopy plasmid, or it may exist as a single copy on an artificially constructed chromosome. Gene disruptions effected in homothallic strains may, upon sporulation and tetrad dissection, be rendered homozygous at the mutant locus. Furthermore, haploids may be made temporarily homothallic by transformation with a plasmid-borne *HO* followed by curing. Mutant haploids may be generated either by dissection of spores from mutagenized heterothallic diploid strains or by direct mutagenesis.

In addition to their strongly eukaryotic features, yeast have many characteristics analogous to those of prokaryotes; these may also be exploited in the laboratory. Gene isolation, which is accomplished with relative ease by complementation, may be followed by studies on regulation and gene dosage effects. Other advantages include the generation time (only about 90 minutes), the ease of growing and culturing the organism under a variety of experimental conditions, and its non-pathogenicity. Thus, the ease of genetic, biochemical and molecular manipulations, as well as the extensive characterization of many of the biochemical and physiological pathways, make yeast an attractive system for eukaryotic studies.

The process of meiosis in yeast is an integral part of sporulation and is easily induced. Although cultures often sporulate at less than 75% under optimum conditions and sporulating cells may exhibit asynchrony, qualitative analysis of sporulation can be accomplished

with ease (for review, see Esposito and Klapholz 1981). Most studies of meiosis in the yeast system have been directed toward the morphology, biochemistry, and genetics of wild-type cells, and have involved altered nutritional conditions and the generation of sporulation mutants. More recently, molecular approaches have been incorporated, whereby the involved genes themselves have been isolated by complementation of mutants blocked in the process. Many sporulation-specific genes of unknown function have also been isolated and are now being analyzed for their contribution to this developmental pathway (Clancy *et al.* 1983; Percival-Smith and Segall 1984). Molecular approaches have identified several genes that have products essential to the successful completion of the pathway, including those that permit sporulation to occur under non-sporulation conditions (Hopper *et al.* 1975; Kassir and Simchen 1976; Mitchell and Herskowitz 1986).

Overview of the Early Events in Sporulation

Meiosis in yeast is closely related to mitosis, in that many of the mitotic processes are essential for meiosis, including spindle pole body duplication and segregation, DNA replication, and chromosome segregation. Almost all genes known to be involved in regulating the nuclear events of mitosis are required also for meiosis (Pringle and Hartwell 1981). General conditions for entry into the wild type meiotic pathway are the presence of both *MAT*a and *MAT*α information, nitrogen deprivation, and a nonfermentable carbon source such as acetate. The optimum temperature is approximately 30°C with ascus formation greatly reduced below 25°C and above 35°C. Glucose and some other sugars inhibit sporulation by a variety of mechanisms, including increased levels of cAMP (Matsumoto et al. 1983), repression of the TCA cycle enzymes which are required for acetate utilization, and repression (Miyake *et al.* 1971) of gluconeogenic enzymes (which are involved in the synthesis of storage carbohydrates). Nitrogen repression of developmental pathways in microorganisms is usually meiosis-related and is a phenomenon which has been generally observed though not well understood; ammonium ions, glutamine, and methylamine (an ammonium analog) have been shown to inhibit yeast ascus development. Ammonium ions have also been observed to block a variety of developmental processes in other organisms, including gametogenesis, heterocyst formation and sporulation (Heywood and Magee 1976; Esposito and Klapholz 1981). Sporulation requires aerobic conditions, and though entry into the pathway can be enhanced by pregrowth in acetate

medium before shifting to nitrogen starvation, acetate exposure is probably not continuously required. Maximum sporulation is achieved when cells are grown to about $5x10^7$ cells/ml in complete acetate medium, then transferred to acetate medium lacking a nitrogen source.

The events of sporulation proceed in a general temporal order as follows (Esposito and Esposito 1978):

1. Premeiotic DNA synthesis
2. Commitment to recombination
3. Polycomplex body formation
4. Synaptonemal complex formation
5. Commitment to sporulation
6. First spindle pole body duplication
7. Nuclear elongation and Meiosis I
8. a) Second SPB duplication
   b) Initiation of spore wall synthesis
9. a) Nuclear budding and Meiosis II
   b) Spore wall growth
10. a) Nuclear separation
    b) Spore wall closure
11. Ascospore maturation

Spore wall formation is dependent upon, and begins at about the same time as, the second spindle pole body duplication and appears to exist on a parallel pathway. These events were subsequently arranged and modeled (Esposito 1987) to reflect the existence of the separate pathways (Figure 1).

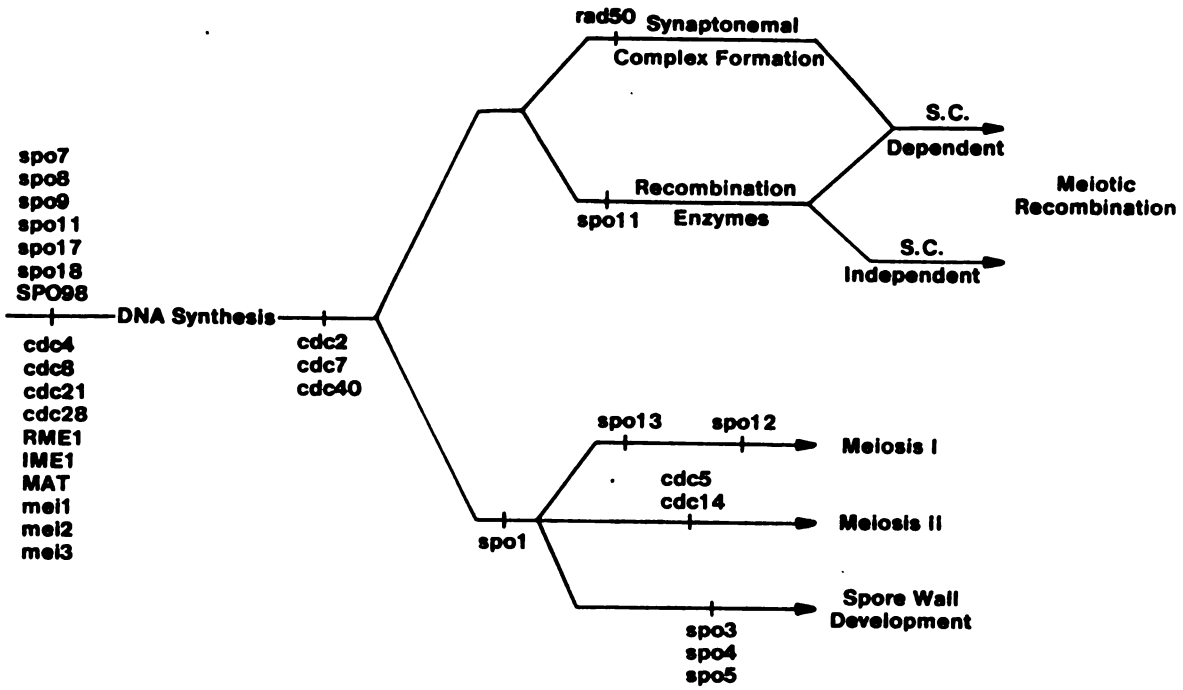


Figure 1. A general model of sporulation (Esposito 1987), modified to include *spo17* and *spo18*.

After introduction to starvation medium budded cells complete cell division, and are arrested in the G1 phase of the cell cycle. The fraction of budded cells falls from 30% to 2% within 48 hours. A cell failing to complete the cycle is thought to be unable to sporulate, remaining as a mother with an anucleate bud. Thus, cells enter meiosis from the G1 phase (Hartwell 1974).

After about 7 hours in sporulation medium, cells may be returned to growth medium and mitotic growth will resume; however, at about 5 hours commitment to both intragenic and intergenic recombination has

occurred. This feature permits a brief window for the analysis of recombination events and characterization of mutants, since cells committed to meiotic recombination are not committed to sporulation, and the return to rich medium permits cells to resume vegetative growth after meiotic events have occurred.

Landmark biochemical events that take place during sporulation are shown on a time course in Figure 2. Of these processes, glycogen degradation (Hopper *et al.* 1974; Colonna and Magee 1978) is of particular interest because of its utility as an assay for sporulation. The two sporulation mutants isolated by Smith (1988), SL484 (*spo17*) and SL641 (*spo18*) were isolated because of their inability to produce the glycogen degradation enzyme, sporulation amyloglucosidase (SAG), although the enzyme itself is not required for completion of sporulation (Yamashita and Fukui 1985).

From about 5 hours after introduction into sporulation medium until about 9 hours, the DNA content of the sporulating cell doubles (Simchen *et al.* 1972). Inhibitors of DNA synthesis such as hydroxyurea and 8-hydroxyquinoline when applied during the first 8 hours of sporulation prevent ascus formation, but the cells generally remain viable if plated on rich medium (Simchen *et al.* 1976).

RNA synthesis also increases during sporulation between 4 and 10 hours, with a peak at about 6 hours (Hopper *et al.* 1974), but declines by the time of spore formation due to an extensive degradative (RNase) activity which may be sporulation specific (Hopper *et al.* 1974). The appearance of sporulation-specific messages has permitted the construction of cDNA probes (Mills 1980); about 7% of the cDNA made

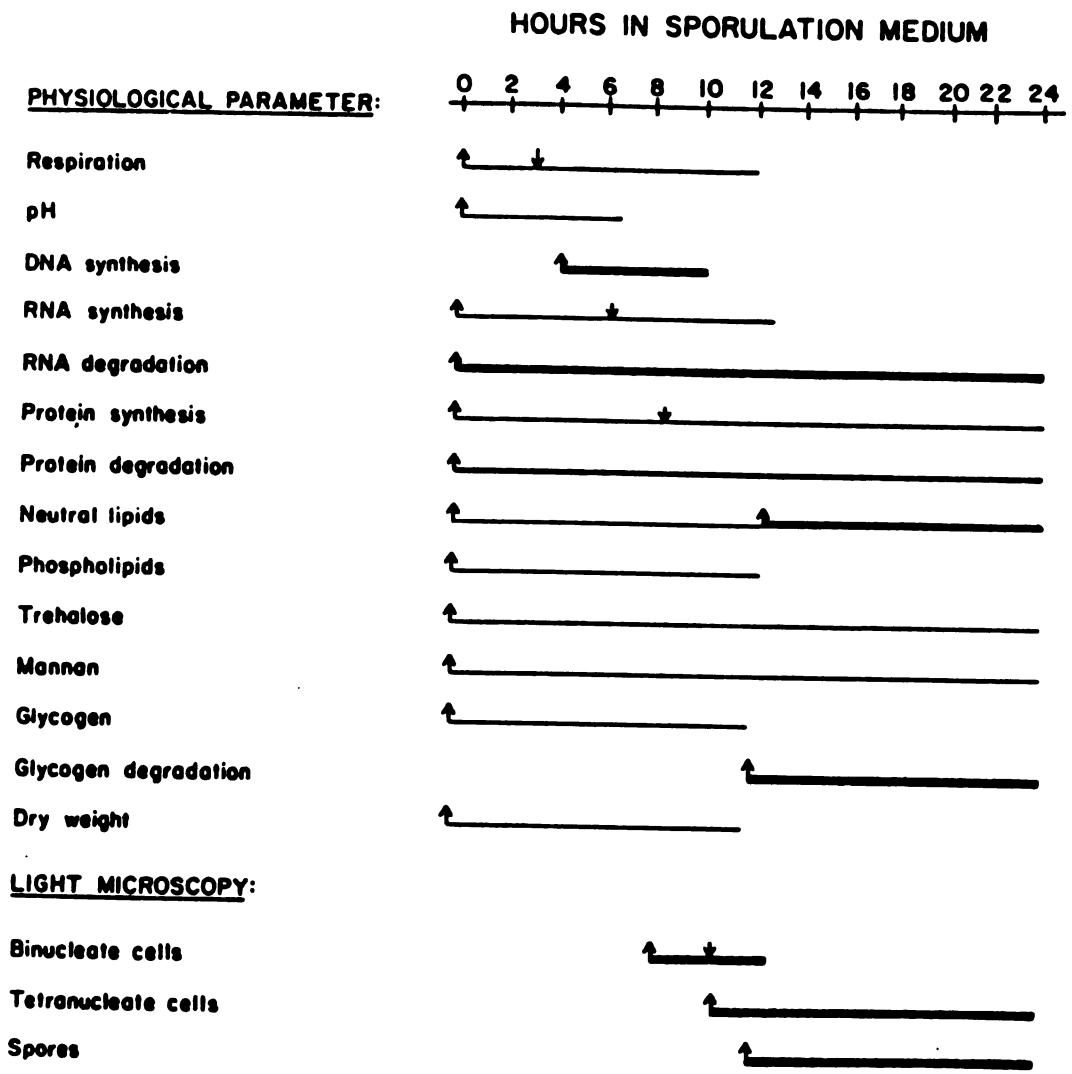


Figure 2. Biochemical events during sporulation (Esposito and Klapholz 1981).

from the messages of sporulating cells fails to hybridize to vegetative RNA. Sporulation-specific probes have been constructed and used to isolate sporulation-specific genes (Clancy *et al.* 1983; Percival-Smith and Segall 1984; Kaback and Feldberg 1985), but many of these genes, while exhibiting strong expression during sporulation, are not required for the completion of spore formation.

Later events of the sporulation pathway make it hard to define other parameters. A gradual increase in the pH of the medium inhibits the uptake of labeled precursors. Spore wall formation hinders cell disruption. Assays for macromolecular synthesis are difficult because of the extensive RNA and protein degradation and recycling. Nonetheless, for studies of early meiotic events *S. cerevisiae* remains the system of choice. The later major temporal events lie outside the scope of this discussion, and have been reviewed (Esposito and Klapholz 1981).

Two general approaches have been employed in the characterization of sporulation genes. The first, which relies on the generation of *spo* mutants, followed by the isolation of the genes themselves, has led to the development of the general model (Esposito and Klapholz 1981; Esposito 1987). The second approach has relied on the differential hybridization screen (St. John and Davis 1975) to identify clones from a yeast genomic library that bear genes which hybridize specifically to cDNA probes made from RNA of sporulating cells and do not hybridize to probes from non-sporulating cells (Clancy *et al.* 1983; Percival-Smith and Segall 1984). Although a large number of genes has been isolated by the second method, they have added, as yet, little to the understanding of developmental regulation. Hybridization-screened

genes disrupted *in vitro* have given either no visible phenotypes (Kaback and Feldberg 1985; Percival-Smith and Segall 1986; Primerano et al. 1988b; Clancy, personal communication), or phenotypes which exhibit only a slight interference with successful spore formation (Law and Segall 1988; Primerano et al. 1988a). Only one gene isolated by this technique, the *SPS1* gene (Percival-Smith and Segall 1986), has been shown to be absolutely required for sporulation.

The apparent lack of requirement for many if not most of the genes isolated by the differential hybridization method has led to much speculation. Kaback and Feldberg (1985) have proposed that a generalized relaxation of transcriptional control during sporulation could result in expression of sequences that do not have specific functions in the process. While this may be true, there are two points to bear in mind. First, some important sporulation-required genes may have been missed by the screen because they are expressed in undetectable quantities or because they are also expressed during vegetative growth; their absence from the pool of characterized genes distorts the view we have of sporulation. Secondly, later evidence for two genes, *SPR1* and *SPS100* has shown that their mutant phenotypes are obvious only after spores have been subjected to a battery of experimental conditions. Thus many sporulation-specific genes seem dispensable for spore formation, but they may make subtle and highly regulated contributions to the process.

Four temporally distinct classes of genes have been observed in sporulation. The early, middle and late designations (Holaway *et al.* 1985) are based on the time of transcript appearance in the rapidly sporulating strain SK1. Transcripts of early genes appear soon after

transfer to sporulation medium. Those for the middle genes appear at about the time of DNA synthesis, and late gene transcripts make their appearance at about the time of meiosis I. A fourth class, the very late genes, appears even later (discussed below).

SPO13 (Klapholz and Esposito 1980a; Klapholz and Esposito 1980b) is a prototype early gene. Its expression, which begins shortly after transfer to sporulation medium, gives a product which functions in chromosome separation during meiosis I. The preceding events, DNA synthesis, genetic recombination, and spindle formation, proceed normally in *spo13* mutants, and after the failure of chromosome disjunction, cells proceed through meiosis II and give rise to two diploid spores. *SPO13* is developmentally regulated by *MAT*, but initial induction is also triggered by starvation, regardless of the *MAT* phenotype (Wang *et al.* 1987).

The late gene *SPR1* (Primerano *et al.* 1988a) has been shown to play a role in spore viability; spores produced from cells homozygous for the *spr1* mutation exhibit increased sensitivity to elevated temperatures. Moreover, the gene product of *SPR1* bears a hydrophobic leader sequence and is thought to accumulate in the spore wall (Primerano *et al.* 1988a). The late gene *SPR2* however, has no apparent essential function in sporulation (Primerano *et al.* 1988b).

Among a cluster of three genes, *SPS1*, *SPS2*, and *SPS3*, only one, *SPS1*, gives an asporogenous phenotype upon disruption. The 5′ region of *SPS2*, which is first expressed at about 15 hours after introduction into starvation medium, inhibits sporulation when introduced into cells on a high copy number plasmid, but disruption of *SPS2* itself does not inhibit sporulation (Percival-Smith and Segall 1986; Percival-Smith and

Segall 1987).

The very late genes, whose transcripts do not appear until 12 hours after transfer to sporulation medium and continue to be expressed steadily until 35 hours, are represented by *SPS100* and *SPS101* (Law and Segall 1988). *SPS100* is involved in spore wall development; resistance to ether (Dawes and Hardie 1974) is delayed in mutants by about 5 hours.

The interest in early genes has been enhanced by the characterization of the later genes. Whether attempts to identify a mutant phenotype were successful (*SPR1*, *SPS1*, and *SPS100*) or unsuccessful (*SPS2*, *SPS3*, *SPR2*), these genes have provided little information about meiosis or its regulation. Though they may eventually provide avenues for the isolation of other regulatory genes, their functional products may act primarily at the end of the pathway to spore formation and thus be representative of processes quite specific to spore-forming organisms. To date, attention continues to focus on the variety of early mutants as a means to isolate the early genes.

Several mutants that are blocked in premeiotic but not mitotic, DNA synthesis have been isolated; these include *spo7*, *8*, *9* and *11* (Esposito et al., 1972), *spo17* and *18* (Smith 1988), *mei1*, *mei2* and *mei3* (Roth 1973), and the dominant *SPO98* (Esposito et al, 1972). Two mutants, *cdc2* and *cdc7*, undergo premeiotic DNA replication, yet fail to undergo mitotic DNA synthesis (Esposito and Klapholz 1981) and some mutants, *cdc4*, *cdc8* and *cdc21*, are blocked in both processes (Simchen 1974; Game 1976). The relationship between mitosis and meiosis thus seems to be one of intricate and complex regulation.

Regulation of meiosis and spore formation

After the completion of DNA synthesis, the process of sporulation is separated into several distinctly regulated pathways, each of which involves a separate commitment. A similar observation has been made for bacterial sporulation (Mandelstam 1971). Commitments to recombination and chromosomal segregation, which have been shown to occur separately (Klapholz and Esposito 1980) in *spo1* mutants (see Figure 1), are followed by separate commitments to other downstream events. For example, cells that are unable to undergo Meiosis I (*spo12* or *spo13* mutants) can nonetheless be committed to and complete all other processes of sporulation.

In addition to the apparent separation of commitments, arrest of spore development and resumption of mitotic growth occurs more readily when cells are returned to rich rather than poor medium, suggesting that some stages of commitment are sensitive to the medium composition. Thus, single events in the pathway of sporulation appear to be dependent on a series of conditions, and commitment to those events is part of a complex multistep process (Esposito and Klapholz 1981).

Mutational analysis has provided a useful, though limited, understanding of the regulation of sporulation. Structural mutations are not easily distinguished from regulatory mutations, because little is known yet of the enzyme activities of the process. The discovery of the separation of the events, however, has permitted the construction of the model in Figure 1.

Regulation by the mating type locus is fundamental to DNA

replication (Roth and Lusnak 1970), genetic recombination (Friis and Roman 1968; Roth and Fogel 1971), nuclear division, and spore formation (Roman and Sands 1953). Although upon transfer to sporulation medium many of the initial responses of a/α strains resemble those of a/a and α/α diploids, only cells heterozygous at the mating type locus will undergo meiosis and sporulation - diploid homozygotes a/a and α/α do not carry out premeiotic DNA synthesis (Roth and Lusnak 1970). The products of *MAT*a1 and *MATα2* (reviewed by Herskowitz and Oshima 1981; Nasmyth 1982) are thought to form a negative regulator.

In sporulating wild type strains, heterozygosity at the *MAT* locus is brought about by mating. Products of the haploids *MAT*a (a-factor) and *MATα* (α-factor) are secreted and arrest cells of the opposite mating type in the G1 phase of the cell cycle so that mating may occur (see Thorner, 1981, for review). Strains which have a stable mating type are designated as heterothallic and cannot undergo a switch to the opposite type except at very low frequencies. Homothallic strains, however, undergo a switch that is catalyzed by the product of the *HO* gene: transposable information of the opposite mating type is inserted into an expression locus within the mating type region. In a cell line arising from a germinated spore, a mating type switch is engineered when an existing cassette is removed and replaced by a copy of the gene for the opposite mating type. This occurs in the original mother cell after the second division, and continues in generations thereafter. The transposable a or α information resides in silent loci which have been designated as HML (the usual locus of the gene for α) and HMR (usual a locus); these regions are normally repressed by the action of the *SIR* (*s*ilent *i*nformation *r*egulator) genes, of which there are four

complementation groups. Yet another large group that also exerts control over the process of mating, the *STE* genes, is thought to include the a and α pheromone regulatory genes and the genes involved in their processing and secretion (Pringle and Hartwell 1981; Thorner 1981; Herskowitz and Oshima 1981). Rare strains which are *HML*a and *HMR*α occur and can also diploidize.

The *MAT* locus, in addition to controlling mating and sporulation, is involved in the regulation of several related activities, and cells that are genetically identical in all aspects except at the *MAT* locus exhibit markedly different properties. Cell cycle arrest by the a and α pheromones (previously noted), a-α cell to cell agglutination, a-cell inactivation of α-factor, and budding patterns (a/a and α/α cells bud equatorially and a/α cells have polar budding), are some of these regulated functions. a/α cells, which do not produce or respond to pheromones, will undergo premeiotic DNA synthesis and sporulation while the homozygous diploids a/a and α/α will not; some processes are influenced neither by a nor α alone, but by an interaction between them. Thus, the function of the MAT locus has come to be viewed primarily as regulatory (MacKay and Manning 1974).

The discovery of the *RME1* locus (Hopper *et al.* 1975; Kassir and Simchen 1976), in which mutations permitted sporulation of a/a and α/α diploids, showed that *MAT* exerts some of its effects via this negative regulator. Homozygous *rme1* diploids sporulate regardless of their mating type, and haploids bearing *rme1* are able to undergo premeiotic DNA replication under sporulation conditions. Thus, *RME1* was proposed (Rine *et al.* 1981) to be an inhibitor of sporulation: the *RME1* gene product is not functional in a/α diploids, but a/a and α/α diploids

express *RME1* and cannot sporulate (Mitchell and Herskowitz 1986).

MAT products a1 and α2 are both required for sporulation in wild type strains and act by repressing *RME1* transcription (Mitchell and Herskowitz 1986). a haploids fail to repress because the *MATα2* product is absent, and α haploids lack the *MATa1* product; thus neither possesses sporulation capability. *rme1* haploids or haploids expressing *a1-α2* activity can enter the sporulation pathway and undergo DNA synthesis, recombination and some later meiotic events (Kassir and Simchen 1976; Hopper and Hall 1975; Roth and Fogel 1971; Wegstaff et al 1982), but cannot complete normal meiosis because they lack homologous chromosomes (Roth and Fogel 1971; Wegstaff et al. 1982). *RME1* activity thereby prevents haploid entry into what would be an aberrant meiosis.

Transcriptional repression of *RME1* in *a/α* diploids is not complete; only a 10-20 fold reduction is seen. In contrast, other genes under the control of *MAT* exhibit a 100-fold or greater decrease in transcription (Klar et al 1981; Nasmyth et al 1981; Jensen et al 1983). This incomplete repression is not understood, but the residual transcription may have no function, since the null mutation (Mitchell and Herskowitz 1986) displays no unpredicted phenotype.

RME1 responds to only one of the two known signals, starvation and diploidy, for successful sporulation. That is, cells with an inactivated *RME1* are relieved of *MAT* control, but remain under nutritional regulation. Reports of a single gene *PAT1* (*RAN1*) in the fission yeast *Schizosaccharyomyces pombe* (Iino and Yamamoto 1985a; Nurse 1985; Iino and Yamamoto 1985b) which, when lost, relieved cells of both mating type and nutritional control of sporulation suggested that such a gene might also exist in *S. cerevisiae*. The *PAT1* product

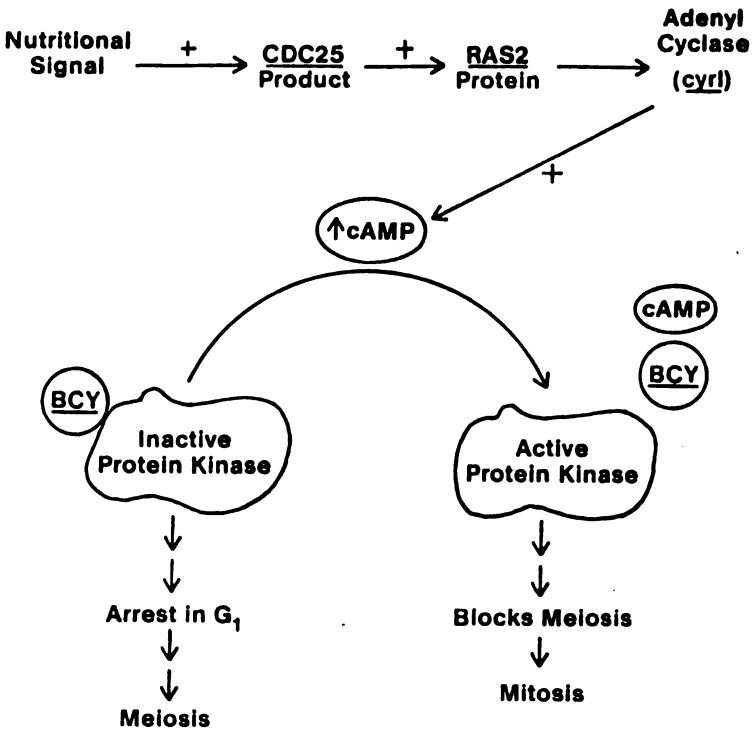


Figure 3. cAMP-dependent regulation of meiosis.

is a negatively regulating protein kinase that lies between two positive regulators *MEI2* and *MEI3* in that sporulation pathway. In *S. cerevisiae*, disruptions of the cAMP-dependent protein kinase system are known to affect sporulation. *CYR1*, the adenyl cyclase gene (Matsumoto *et al.* 1983) and its two known effectors *CDC25* (Broek *et al.* 1987; Robinson *et al.* 1987) and *RAS2* (Toda et al. 1985) maintain cellular levels of cAMP (Figure 3). When cAMP levels are lowered by loss of any of these components, starvation control of meiosis is lost and sporulation is no longer suppressed by nitrogen. The regulatory unit of the cAMP-dependent protein kinase is encoded by the gene *BCY1* (Matsumoto *et al.* 1983); homozygous *bcy1* cells do not undergo meiosis

and sporulation, and suppress mutations occurring earlier. Therefore, meiosis is negatively regulated by a protein kinase activity which is itself sensitive to environmental nutritional conditions.

The *IME1* gene in *S. cerevisiae*, a positive regulator of sporulation, is under the control of both *MAT* and starvation (Kassir *et al.* 1988), and was isolated from a yeast genomic library because it permitted sporulation when transformed on a high copy-number plasmid into a *MAT*-defective strain. The creation of an *rme1-ime1* double homozygote led to the simple model shown in Figure 4. Because this strain was unable to sporulate, the investigators concluded that *RME1* normally represses *IME1*. Further characterization of *IME1* revealed that it is also environmentally regulated: i) no transcripts are found in cells growing in glucose, ii) acetate growth is accompanied by *IME1*

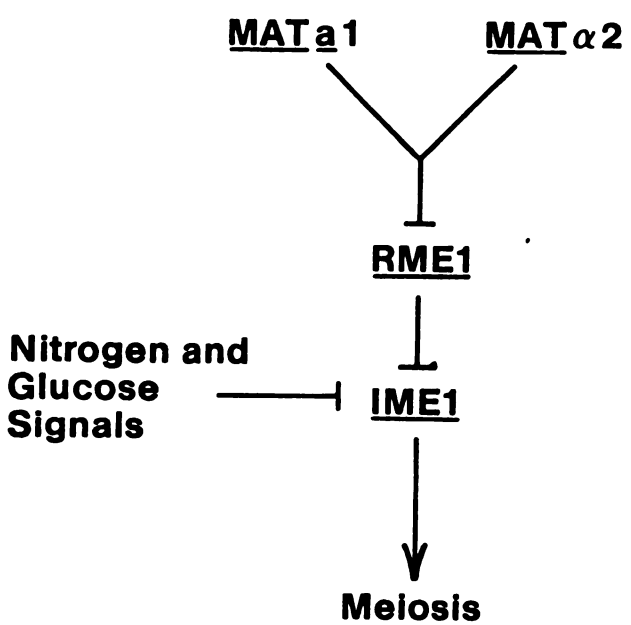


Figure 4. Regulation of meiosis by *IME1* (Kassir et al. 1988).

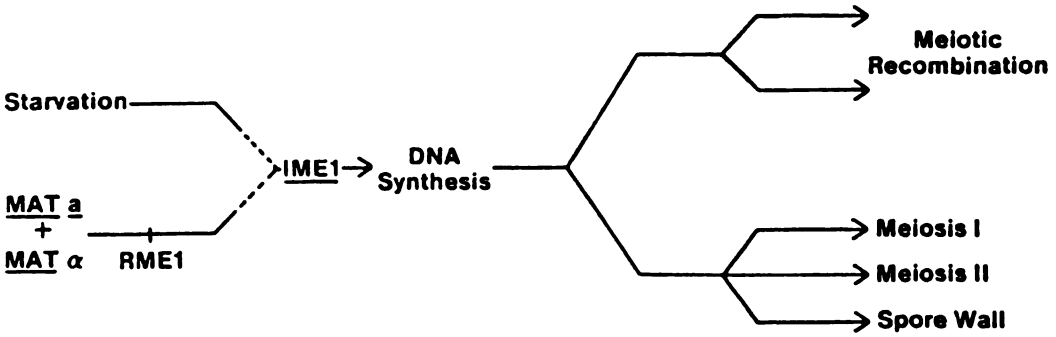


Figure 5. Position of IME1 on the sporulation pathway.

transcription, iii) under nitrogen starvation, *IME1* transcription increases sharply in sporulation-competent a/α diploids whereas none is seen in a/a or alpha/alpha strains, thus demonstrating an *IME1* response to *MAT*, iv) in vegetatively growing cells, neither *MAT* nor *RME1* seems to affect *IME1* transcription, and v) in rich medium, *IME1* multicopy plasmids promote sporulation. Figure 5 incorporates the models shown in Figures 1 and 4, reflecting the regulation upon *IME1* of both nutrition and *MAT*.

The regulation by starvation of at least two early genes, *IME1* and *SPO13*, poses some interesting questions. Are *IME1* and *SPO13* (and possibly others) regulated directly by the same product? Does this regulation result from DNA-specific binding, and if so, are there

analogous sequences located at or near each of the target genes? If the regulation is exercised by different mechanisms, are these then subject to a common regulator? The demonstration of the key role of *IME1* has opened new avenues for the investigation of meiosis. First, the regulation by starvation may now be addressed more specifically by monitoring transcriptional responses of *IME1* to various nutritional conditions and mutations. Second, newly isolated early sporulation genes may be positioned on the pathway by constructing double mutants with either *RME1* and *IME1* or both. We have tried such an approach with the early sporulation gene *SPO17* which was isolated by screening plasmids from a YCp50 yeast genomic library for the ability to complement the original *spo17* mutant (Smith *et al.* 1988). After isolation and characterization of the gene, the double mutant *spo17/spo17 rme1/rme1* was constructed and evaluated for the ability to sporulate.

The rudimentary model of sporulation that has been proposed (Figure 1) reflects a lack of understanding of early regulation. This problem, which exists because of the lack of isolated early genes, can be approached by generating early sporulation mutants; such mutants, which are likely to affect regulation, would be useful in the isolation and characterization of early genes. An understanding of the interaction between negative regulatory genes (mutations in these permit sporulation under non-sporulation conditions) and positive regulatory genes (mutations in these prevent spore formation) depends upon the characterization of early gene activity.

CHAPTER II

(ARTICLE)

ISOLATION OF *SPO17*, A YEAST EARLY SPORULATION GENE

By A.L. Kennedy and P.T. Magee

Submitted to *Molecular and Cellular Biology*

ISOLATION OF *SPO17*, A YEAST EARLY SPORULATION GENE

By

Allan L. Kennedy

College of Osteopathic Medicine

Michigan State University

East Lansing, Michigan 48824

and

P. T. Magee*

College of Biological Sciences

University of Minnesota

St. Paul, Minnesota 55108

* Author to whom inquiries should be directed

ABSTRACT

Diploids of the yeast *Saccharomyces cerevisiae* that bear the *spo17* mutation are blocked in sporulation before premeiotic DNA synthesis occurs. We have isolated a plasmid bearing the *SPO17* gene sequence by its complementation of a *spo17* mutant. An *ADE2/ade2 spo17/spo17* recipient strain was transformed with a YCp50 yeast genomic library and sporulation-competent transformants were identified by their production of red papillae from white colonies on rich media after exposure to sporulation conditions. The complementing plasmid, p1912, was found to bear *SPO17* on a 5.8 kb yeast insert. Northern analysis demonstrated the presence of at least two bands, 2.7 and 2.1 kb, in mRNAs from wild-type cells under both sporulation and vegetative conditions, but at significantly higher levels in sporulating cells. Because *SPO17* is an early sporulation gene, a *spo17/spo17 rme1/rme1* strain was constructed to see if its effects are exerted by regulation of the early gene *RME1*, a sporulation repressor. The double mutant is unable to sporulate under any conditions. Since the *rme1* mutation does not relieve the *spo17* sporulation block, we conclude that *SPO17* does not exercise its effect by the inhibition of *RME1*.

INTRODUCTION

Sporulation in *Saccharyomyces cerevisiae* is a well-established system for the study of meiosis, and is easily induced in diploid cells by starvation. The diploidy requirement is met by two alleles at the mating-type locus, *MAT***a** and *MAT*α. In haploids the pheromone products produced by these genes arrest cells of the opposite mating type in the G1 phase of the cell cycle so that mating may take place. Diploid cells bearing only *MAT***a** or *MAT*α are unable to sporulate because of active repression by the *RME1* gene product, a negative regulator of meiosis (17). The *RME1* product is thought to act by inhibiting the effects of the inducer gene *IME1*, which is sensitive also to nutritional status (14). The development of a more complete model of the early regulation of sporulation has been impeded by the dearth of well-characterized early genes.

The pathway to sporulation proceeds through meiotic DNA synthesis, an event that can be blocked independently of mitotic DNA synthesis, although both pathways may employ the same synthetic machinery (20). Several mutant series have been identified in which premeiotic DNA synthesis is blocked. These include the sporulation mutants *spo7*, *spo8* and *spo9* (5), the cell division cycle mutants *cdc4*, *cdc8* (26), *cdc21*

(8), *cdc28* (26), and the meiosis mutants *mei1*, *mei2*, and *mei3* (23). Smith *et al.* (27) have generated two such mutant strains and have given these mutations the designations *spo17* and *spo18*. Although many sporulation-specific genes have been isolated by the technique of differential hybridization (4, 9, 19), they are usually not absolutely required for successful sporulation, and thus are unlikely to be involved in the regulation of meiosis.

We have now isolated the *SPO17* gene by transforming a *spo17/spo17 ADE2/ade2* strain with a genomic yeast library, and screening the transformants for sporulation-competence. Such strains subjected to sporulation conditions, then replica plated to rich medium, can be evaluated for meiosis because successful sporulation generates progeny that have undergone meiotic chromosome segregation, are *ade2/ade2*, and appear as red papillae on white colonies. This method was used by Fonzi and Sypherd (7) to clone the ornithine decarboxylase gene of *Saccharyomyces cerevisiae*.

We have also constructed a *spo17/spo17 rme1/rme1* double mutant to evaluate the interaction between these two genes. The *spo17* phenotype is epistatic to *rme1*. We thus conclude that the *spo17* function lies either on a parallel pathway of sporulation events, or in the same pathway but downstream of the *RME1* function.

MATERIALS AND METHODS

Yeast strains. See Table 1.

Plasmids. See Figure 1 and Table 2.

Media and growth conditions. For culture maintenance and vegetative growth, cells were grown in YEPD (1% yeast extract, 1% Bact-peptone, and 3% glucose) liquid medium, or on YEPD plates (1.5% agar added). To increase the efficiency of sporulation, cells were first grown in presporulation acetate medium PSP2 (0.67% yeast nitrogen base without amino acids, 0.1% yeast extract, and 100 ml of 50mM potassium pthalate at pH 5.5 per liter; reference 22). After growth to 2-5 X 10^7 cells/ml, the cells were harvested, washed twice in sterile water, and transferred to sporulation medium SP2 (0.3% potassium acetate, 0.02% raffinose) and incubated in a rotary shaker. Cells were grown at either 30° (optimum) or 34°C, depending upon the desired conditions. Quantitative measurements of sporulation were carried out microscopically. At least 300 cells were counted for each point.

Isolation of plasmid DNA from *E. coli*. Plasmids were maintained in *E. coli* strains DH1 and DH5. Large scale isolations were prepared by the protocol of Maniatis et al. (16), but with some revisions (15). Plasmids were prepared by inoculating 600 ml of Luria broth

(1% Tryptone, 0.5% yeast extract, 1% NaCl, ampicillin 20ug/ml) with a 1.0 ml culture of the appropriate strain. After growth to stationary phase, cells were harvested and suspended in 8.0 ml (25% sucrose, 50mM Tris, pH 8.0). 0.6 ml of 10mg/ml lysozyme (freshly prepared in cold 0.25 M Tris pH8.0) was added. Cells were incubated on ice 10 min. After addition of 1.5 ml of 0.25M NaEDTA pH8.0 and 4.8 ml Brij-DOC (50mMTris, 60mM NaEDTA, 1% Brij-35, 0.4% Na deoxycholate pH 8.0) the prep was centrifuged at 45K for 30 min, the supernatant extracted with phenol/chloroform, and precipitated by adding 0.25M NaAcetate (to a final concentration of 0.025M) followed by adding 2 volumes of 95% ethanol. The precipitate was pelleted (12K, 15min) and resuspended in 5 ml low TE (10mM Trizma base, 0.1mM NaEDTA, pH8.0). RNA was digested with RNAse (50ul of 10 mg/ml) at 37° for 30 min. After precipitation, pelleting, and resuspension in 5 ml low TE, a solution of 5.0 M NaCl was added to a final concentration of 1.5 M. 30% PEG (polyethylene glycol 8000 in 1.5 M NaCl) was added to a final volume of 25% of the total, and the solution was incubated at 0° for 30 min. The precipitate was pelleted at 10K, 4 min at 4 C, washed gently with 70% ethanol and resuspended in 2.0 ml of low TE.

Transformation of yeast strains. Lithium acetate transformations were carried out by the method of Ito et al (1983) with some revisions. Yeast cells were grown in 100 ml YEPD to 2 X 10^7 cells/ml, pelleted and washed once in TE (10mM Tris-HCl pH 7.5, 1.0 mM NaEDTA). After resuspension in 5 ml LiAc/TE (0.1 M lithium acetate, in TE), the cells were incubated at 30°C for 60 min, vortexed gently and 0.1 ml aliquots were placed in 1.5 ml microfuge tubes. 5 ul (50ug) carrier (salmon

sperm) DNA and 1-15 ul (1-10 ug) of transforming DNA were added, and the preparation was incubated 30 min at 30°C. 0.7 ml PEG/LiAc/TE (40% w/v polyethylene glycol 4000 in LiAc/TE) was added to each tube, they were gently vortexed, then incubated 60 min at 30°C. After 5 min at 42°C, the preparations were washed twice with 0.5 ml TE, resuspended in 0.1 ml TE, then plated on the appropriate media and incubated 3-4 days at 30° C. When higher transformation yields were desired, the protocol of Sherman *et al* (24) was followed; DNA fragments bearing genes disrupted with selectable markers were transformed into appropriate yeast strains by the spheroplasting method.

Preparation of RNA. Cells were grown to a concentration of 3 X 10^7 to 5 X 10^7 cells/ml in PSP2 (1.0 liter), then washed and transferred to SP2 (5 X 10^7 cells/ml). After 4 hrs, the cells were harvested by centrifugation and suspended in 6 mls of cold extraction buffer (100 mM Tris-HCl, 100 mM LiCl, 20 mM DTT, pH 7.5). This suspension was then added to a Braun bottle containing 5.0 mls of phenol (pH 7.5), 5.0 mls of chloroform, and 1.0 ml of 10% sodium dodecyl sulfate (SDS). Glass beads were added to 2/3 volume and the mixture was subjected to 3 30 sec bursts in a Braun homogenizer. Calcium chloride was added to a final concentration of 0.01 M to precipitate the SDS, the lysate was transferred without the beads to a 30 ml Corex tube, and centrifuged for 30 min at 4°C, 11,500 RPM to remove other debris. The supernatant was ethanol precipitated, pelleted, and resuspended in 4.0 ml of DEPC treated water (made 0.1% diethylpyrocarbonate, incubated at 37°C overnight, then autoclaved). An equal volume of a 10% sarkosyl solution and 0.35 g CsCl were added, then this solution was layered

over 2.75 ml of 5.7 M CsCl, and centrifuged 16 hours at 15°C in an SW41 rotor at 25K RPM. The RNA pellet was resuspended in DEPC treated water, spectrophotometrically quantified, added to 2.5 volumes of 95% ethanol, and stored at -70°C.

Southern analysis. Genomic yeast DNA was prepared by the method of Hereford *et al.* (11), digested with the appropriate restriction enzyme, and subjected to agarose gel electrophoresis (16). Nitrocellulose paper was used for blotting (28). Preparation of DNA probes and hybridizations were carried out by the method of Maniatis et al (16). Desired fragments were isolated from agarose before being used for probe preparation.

Northern analysis. RNAs isolated from appropriate strains were separated on a formaldehyde agarose gel, transferred to nitrocellulose paper and hybridized to DNA probes as described by Thomas (29). Poly(A)+ RNA was prepared as described (10).

Enzymes and radioisotopes. Restriction enzymes, T4 DNA ligase and DNA polymerase I were purchased from either Bethesda Research Laboratories or New England Biolab. The suppliers' instructions were followed in their use. α-[^{32}P] dATP was from New England Nuclear.

OFAGE analysis. Orthogonal-field-alternation gel electrophoresis was performed by the method of Carle and Olson (3).

Expression of *RME1* in *spo17* mutants. *rme1::LacZ* strains used were

grown on YEPD plates. 100 ul of X-gal (2%) was spread on each plate prior to inoculation. Plates were incubated for 2 days at 30°C, then at ambient temperature for 5-7 days. The rme1::LacZ colonies turned blue when *RME1* was expressed.

RESULTS

STRATEGY FOR CLONING THE *SPO17* GENE

The temperature sensitive sporulation mutant SL484 was first isolated in our laboratory by Smith *et al.* (27). This mutation, which maps to chromosome VII, is of particular interest because cells which carry it are blocked in pre-meiotic, but not mitotic, DNA synthesis. We wanted to isolate the *spo17* gene by complementation. Because most available yeast genomic libraries are on either *LEU2* or *URA3* vectors, we constructed a *spo17/spo17* strain which was *leu2/leu2 ura3/ura3* and also *ADE2/ade2*. After transformation with a genomic library, such a strain could be assessed for sporulation by first plating on sporulation medium, incubating, then replica plating to a rich medium. The appearance of red papillae at high frequency should take place only in colonies where sporulation competence was conferred by the transforming plasmid. To construct the strain, we sporulated SL484 at 30°C, the permissive temperature, dissected the tetrads, and mated the spores with W66-8A, an *ade2* strain that bears other useful markers. Products of this mating were then sporulated and their tetrads dissected. Because both parents are homothallic (*HO*) any strains selected after these manipulations would be diploid, and homozygous at all loci (except *MAT*).

Construction and characterization of the recipient strain R92W. Of several hundred red (*ade2/ade2*) colonies from the mating between SL484

and W66-8A, twenty were found to be *TRP5 LEU1 leu2*. Since *SPO17* is linked to *TRP5* (27), we expected that the *TRP5* progeny would be *spo17*. One of these, R9, was also found to be *ura3*, and its *spo17* phenotype was verified. To establish heterozygosity at the *ADE2* locus, R9 was plated on minimal medium with all requirements added except adenine. At the end of one week, four white colonies had arisen from the initial plating; of these, one (R92W) was selected and shown to produce red papillae after sporulation on plates at the permissive temperature, followed by replica-plating to YEPD.

To determine if the ADE^+ phenotype of R92W were due to a reversion or suppression event, we subjected the strain to further tests. Spores gave a 2:2 segregation of reds and whites; one of the white colonies (R92WH) was then sporulated and the spores were mated with an *ADE2* haploid strain BB4. We reasoned that if suppression of *ade2* were responsible for the ADE^+ phenotype of R92W, some progeny of the mating between R92WH and BB4 should be red, because R92WH would carry the *ade2* mutation. After mating and sporulation, seventeen tetrads from the cross were dissected and all spores gave ADE^+ white colonies. We thus concluded that the ADE^+ phenotype of R92W was due to a reversion in the *ade2* gene. (It could, of course, also be due to a very tightly linked suppressor.)

Isolation of *SPO17*. R92W was transformed with a YCp50 (*CEN4 ARS1 URA3*) yeast genomic library obtained from Mark Rose (21). The average insert size of this library is 15-20 kb; thus we calculated a screen of 4,500 colonies was needed to cover 99% of the yeast genome, assuming a yeast genome size of 17 million base pairs. Transformants were subcultured to duplicate sporulation plates and incubated at permissive temperature

(30°C) and non-permissive temperature (34°C). After five days, these plates were replica plated to YEPD plates, incubated for three days at 30°C, then scored for production of red papillae.

Initally, 14 positive transformants were isolated in a screen of only 2700 colonies. A second screen reduced the number of positives to eight. The plasmid from each strain was isolated and used to transform (13) the *E.coli* strain DH1. Three plasmids, p98, p1912 and p1940, were again transformed into the recipient strain R92W. These transformants were also able to sporulate at both permissive and nonpermissive temperatures. Restriction map comparisons of the plasmids gave a common pattern (data not shown), and we inferred that all contained identical or overlapping DNA inserts.

To prove that sporulation competence was due to the plasmid, clones containing p98 and p1912 were plated on media containing 5-fluoro-orotic acid (2). Cells that produce uracil in the presence of FOA are unable to survive, so any cells that survive no longer contain the *URA3* plasmid. Three FOA survivors (R98FOA1, R98FOA2, R12FOA1) were tested and all three failed to generate red papillae at the nonpermissive temperature. This result, as well as the data in Table 3, clearly demonstrate the loss of sporulation ability.

Plasmid p1912 was selected for further study because the size of its insert is only about 6 kb. Deletion of portions of this plasmid followed by transformation into R92W identified a region which contains *SPO17* (Figure 1 and Table 4). Plasmid p12δS, which has a deletion between the two *Sal*I sites, complements R92W. The failure of plasmids p12δH (containing a *Hin*dIII - *Hin*dIII deletion) and p12δSH (which has both deletions) to complement, however, suggests that sequences

essential to the function of *SPO17* span the *Hin*dIII site. Moreover, the plasmids pEMBB and YCpBB, which have the 2.4 kb *BamH*I - *Bgl*II fragment subcloned into the multicopy plasmid pEMBLY30 (Baldari and Cesareni 1985) and into the single copy plasmid YCp50, respectively, fail to complement. These data further suggest that *SPO17* and/or its regulatory region spans the *Bgl*II site.

MAPPING OF *SPO17*

The cloned *SPO17* sequence is on chromosome VII. Figure 2B is a Southern blot of yeast chromosomes which have been separated by orthogonal-field-alternation gel electrophoresis. After the stained gel pictured in Figure 2A was blotted, the blot was divided into three strips. The strip pictured in lane a in Figure 2B was probed with the sporulation-regulated gene *SPR2*, which we have previously shown to be on chromosome XV (not published). Probes made from both the insert of p1912 (lane b) and *TRP5* (lane c) give signals that demonstrate hybridization to the band identified as chromosome VII. We conclude that the sequence in p1912 is located on chromosome VII.

The cloned *SPO17* sequence is linked to *TRP5*. *spo17* was previously shown to be linked to *TRP5* on on chromosome VII and the map distance was calculated to be 8.8 m.u. (27). To confirm that the sequence isolated was identical with *SPO17*, plasmid pYi12 (Figure 1) was constructed by inserting a 4.8 kb DNA fragment containing *SPO17* (*BamH*I-*Eco*RI) into the yeast integrating vector YIp5, which was then cleaved with *Bgl*II and used to transform (25) the *ura3* haploid M1417-8B. Of 93

transformants, three were rescreened, and one, M8B-T1, was mated to spores of the *ura3 trp5* strain W66-8A. Two of the four spores successfully mated with the transformed haploid and produced the diploids T1X1A and T1X1C, which were capable both of growth on nonsupplemented medium and of sporulation.

We then asked whether *URA3* from the integrated plasmid was linked to *TRP5*, as would be expected if the sequence directing integration were *SPO17*. 31 tetrads from T1X1A and T1X1C were analyzed for cosegregation of *URA3* and *TRP5*. Of 27 tetrads showing a 2+:2- segregation for each of the two genes, 23 gave the parental ditype and 4 gave the tetra-type. The formula of Mortimer and Schild (18):

$$cM = \frac{100}{2}\frac{(T+6NPD)}{PD+NPD+T}$$

gives a linkage distance of 7.4 cM. This is in close agreement with the previously reported data of Smith *et al.* (27).

THE FUNCTION OF *SPO17*

SPO17 is not RAD6. We selected two *ura3 spo17* progeny of the cross to test for complementation by the *RAD6* gene, which is also located on chromosome VII (6), and is involved in sporulation. Data in Table 5 show that the *URA3 RAD6* plasmid pR67, which we obtained from L. Prakash, did not complement cells bearing the *spo17* mutation (strains PT4a67, PT4c67). Moreover, *spo17* strains did not exhibit an increased sensitivity to UV irradiation (data not shown).

In vitro mutagenesis of *SPO17* confers a tight *spo* phenotype upon transformed strains. A *LEU2* disruptant (plasmid pLB174) was constructed by inserting the 3.0 kb *LEU2* marker from YEp13 into the *Bgl*II site of pYi12 (Figure 1). After complete digestion with *Bam*HI and partial digestion with *Eco*RI, the 7.8 kb fragment was isolated and transformed into the wild type diploid SCMS7-1 and the haploid BB4. Southern analysis was carried out by probing *Bam*HI-*Eco*RI digested genomic DNA from the transformants with the radiolabeled 4.8 kb *Bam*HI-*Eco*RI fragment from the p1912 insert which contains *SPO17*. Results are shown in Figure 3. The transformants SLB0 and BLB1 gave two bands because of the *Eco*RI site in *LEU2*. The sum of the sizes of the two bands, 4.4 kb and 3.6 kb, is approximately equal to the sum of the size of the wild-type band plus the size of the *LEU2* insert, 4.8 kb and 3.0 kb). Transformants SLB1, SLB2, and SLB3 did not give the predicted bands, and were not analyzed further. Strain SLB0 gave bands identical to those of the haploid transformant BLB1. In SLB0, however, the disrupted genes were probably integrated in both homologs; it is unable to sporulate at either 30°C or 34°C.

Northern analysis of the disruptants. Northern analysis suggests that the DNA included in the p1912 insert produces at least two messages, of approximately 2.7 and 2.1 kb (Figure 4). These messages are also expressed in vegetatively growing cells, though to a lesser extent (lane a), and expression seems to occur normally in an *rme1/rme1* diploid background (lane d). SLB0 has lost both messages but gives one or possibly two smaller signals about 1.2 kb in length (lane c). The blot was also probed with DNA containing the *URA3* gene (1.1 kb band); the intensity of this signal indicates that approximately the same

amount of RNA was loaded in each lane with the possible exception of SLB0, where the signal is somewhat fainter.

Interaction between *SPO17* and *RME1*. Kassir *et al.* (14) demonstrated the interaction between *RME1* and *IME1* by constructing diploids homozygous for the two mutations *ime1* and *rme1*. We obtained an **a** *rme1*$^-$ haploid (strain 2209) and an α *rme1*$^-$ haploid (strain 2210) from Mitchell (17), and made each *spo17* by transformation with DNA bearing the disrupted gene (the *Bam*HI-*Eco*RI fragment from plasmid pLB174). Because the *rme1* disruptions were made by inserting *LacZ* (in-frame) into the coding region, cells attempting to express *RME1* will form colonies that turn blue on plates containing X-gal. *RME1*, a repressor of meiosis, is expressed in haploids; both strains 2209 and 2210 were blue in the presence of X-gal. Strains 2209-a and 2210-3, the *spo17*$^-$ disruptants, also turned blue. When the two *spo17* haploids were mated, the resulting diploid (strain 221a3) was unable to sporulate and remained white on X-gal plates (Table 6).

To prove that the strain we had derived was in fact the diploid product of a mating, we transformed it with the plasmid p1912 (our original *SPO17*$^+$ plasmid). Three transformants, 312-1, 312-2 and 312-3 were examined and found to be sporulation competent.

DISCUSSION

Using a yeast genomic library, we have isolated a plasmid that complements the sporulation deficient strain SL484. We know that the gene involved, which we have called *SPO17*, is absolutely required for sporulation because wild-type diploids that have been rendered mutant at both *SPO17* loci are no longer able to sporulate. As we have previously reported, *SPO17* is among the earliest genes identified in the sporulation pathway, and presents a restriction map that is different from other characterized sporulation genes.

Strains which have mutations in the *RAD6* gene, which has been mapped to chromosome VII, also are unable to sporulate. The *RAD6* plasmid pR67, however, does not complement *spo17* strains, nor do *spo17* strains exhibit increased sensitivity to UV irradiation as do *rad6* mutants. We conclude that *SPO17* is not *RAD6*. As we reported previously (27) *spo17* defines a complementation group different from those of *spo1*, *spo3* or *spo7*. Thus, *SPO17* appears to differ from all other characterized early sporulation genes.

A variety of mutant genes give asporogenous phenotypes when their strains are sporulated. Thus, a *ts* *Spo*$^-$ phenotype can be due to a leaky allele of some gene which is in fact required for mitotic growth. The fact that both haploid and homozygous diploid disruptants of *SPO17* grow normally on several media (min, PSP2, and YEPD) at 24°, 30° and 34° suggests that if *SPO17* does have a mitotic phenotype it is a

relatively subtle one. The simplest explanation is that *SPO17* is a true sporulation gene. The low level of vegetatively produced message, on the other hand, might suggest a vegetative function for the gene.

SPO17 appears to be an early acting gene, since the mutant is blocked before premeiotic DNA synthesis. One possible role is that of a regulator of *RME1*. If *SPO17* were involved in turning off *RME1* in diploids, the loss of its function would block sporulation by failing to relieve *RME1* inhibition of *IME1*. If this model were correct, a *spo17/spo17 rme1/rme1* strain should sporulate; however, the *spo17* phenotype is epistatic to the *rme1* phenotype. Furthermore, the *rme1::LacZ* fusion is not expressed in *spo17* diploids, demonstrating that the normal repression is operating. We conclude that *spo17* functions either after *RME1* or on a parallel pathway to regulate sporulation.

The assay system we have used offers a useful tool for isolating cells which are sporulation-competent after transformation with a genomic library. Development of a recipient strain that is heterozygous at the *ADE2* locus was accomplished by crossing the mutant SL484 with the *ade2 trp5* strain W66-8A. This was possible because SL484 is a temperature sensitive *spo* mutant, and we could easily obtain haploid spores at the permissive temperature. Furthermore, selection of progeny from this cross was facilitated by the tight linkage of *spo17* to *TRP5*, which permitted a rapid screen. We generated heterozygosity at the *ADE2* locus by simply plating red *ade2/ade2 spo17/spo17* products on medium lacking adenine. An *ADE2* heterozygote that could be transformed and screened by the method of Fonzi and Sypherd (7) was among the revertants.

The *in vitro* mutagenesis performed by inserting *LEU2* into the coding region of *SPO17* resulted in the loss of two major mRNA bands (Figure 4, lane c), and the appearance of smaller molecules. The wild-type bands could represent different transcripts from *SPO17*, or they could be from two different genes; both DNA strands might bear genes in the *SPO17* region. A simpler explanation would be that transcripts from a single gene in the region begin at two or more start sites, or if they have introns, that there are alternate splice sites.

The nutritional sensitivity of *IME1* expression shown by Kassir *et al* (14) has identified at least one mechanism by which nutrition regulates entry into meiosis. The preliminary experiments we have done do not suggest such tight nutritional regulation of *SPO17* expression. However, our initial data indicate that transcripts from the *SPO17* region are expressed at a significantly higher level in sporulating cells than in vegetatively growing cells, and we conclude that *SPO17* expression must be influenced by some other factors.

If the *SPO17* gene product is a structural protein, it is required only for meiosis and not at all for mitosis. Such a protein could be involved in special aspects of premeiotic DNA synthesis or in the earlier sporulation-specific nuclear changes. Alternatively, its early expression could suggest a role in regulation.

It is interesting to note that *SPO17* might not have passed a screen based on the differential hybridization of sporulation versus vegetative mRNAs, because its message is expressed at significant levels in vegetative as well as in sporulating cells.

ACKNOWLEDGEMENTS

We thank Dr. Linda Smith for sharing some unpublished data, Dr. Mark Rose for providing the YCp50 genomic library, Dr. Louise Prakash for the *RAD6* plasmid, and Dr. Aaron Mitchell for the *rme::Lacz* strains. We also thank Dr. Don Primerano, Dr. Erik Rikkerink and especially Dr. Anita Hopper for helpful discussions.

This work was supported by Grant No.
to PTM. ALK was supported by the Michigan State University College of Osteopathic Medicine.

REFERENCES

1. Baldari, C. and G. Cesareni. 1985. Plasmids pEMBlY: new single-stranded shuttle vectors for the recovery and analysis of yeast DNA sequences. Gene *35*:27-32.

2. Boecke, J.D., F. LaCroute, and G.R. Fink. 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. *197*: 345-346.

3. Carle, G., and M. Olson. 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA 82:3756-3760.

4. Clancy, M.J., B. Buten-Magee, D.J. Straight, A.L. Kennedy, R.M. Partridge, and P.T. Magee. 1983. Isolation of genes expressed preferentially during sporulation in the yeast *Saccharyomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *80*:3000-3004.

5. Esposito, R.E., N. Frink, P. Bernstein, and M.S. Esposito. 1972. The genetic control of sporulation in *Saccharyomyces*. II. Dominance and complementation of mutants of meiosis and spore formation. Mol. Gen. Genet. *114*:241-248.

6. Esposito, R.E., and S. Klapholz. 1981. Meiosis and ascospore

development. p. 211-287. *In* J.N. Strathern, E.W. Jones, and J.R. Broach (ed.), The molecular biology of the yeast *Saccharyomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

7. Fonzi, W. and P. Sypherd. 1985. Expression of the gene for ornithine decarboxylase of *Saccharyomyces cerevisiae* in *Escherichia coli*. Mol. Cell. Biol. *5*:161-166.

8. Game, J. 1976. Yeast cell-cycle mutant *cdc21* is a temperature sensitive thymidylate auxotroph. Mol. Gen. Genet. *146*:313-315.

9. Gottlin-Ninfa, E. and D.B. Kaback. 1986. Isolation and functional analysis of sporulation-induced transcribed sequences from *Saccharyomyces cerevisiae*. Mol. Cell. Biol. *6*:2185-2197.

10. Harper, J.F., M.J. Clancy, and P.T. Magee. 1980. Properties of polyadenylate-associated ribonucleic acid from *Saccharyomyces cerevisiae* ascospores. J. Bacteriol. *143*:958-965.

11. Hereford, L.M., K. Fahrner, J. Woolford, Jr., M. Rosbash, and D. Kaback. 1979. Isolation of yeast histone genes H2A and H2B. Cell *18*:1261-1271.

12. Hoffman, C.S., and F. Winston. 1987. A ten minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene *57*:267-272.

13. Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*:163-168.

14. Kassir, Y., D. Granot, and G. Simchen. 1988. *IME1*, *a* positive regulator gene of meiosis in *S. cerevisiae*. Cell *52*:853-862.

15. Krieg, P. and D. Melton. 1985. Plasmid preparation. Promega Biotec 85/86 catalogue and applications guide, p. DB3.

16. Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Molecular cloning: a laboratory manual. (Cold Spring Harbor Press, New York.)

17. Mitchell, A. and I. Herskowitz. 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature (London) *319*:738-742.

18. Mortimer, R.K., and D. Schild. 1981. Genetic mapping in *Saccharomyces cerevisiae*, pp. 11-26. *In* J.N. Strathern, E.W. Jones, and J.R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

19. Percival-Smith, A. and J. Segall. 1986. Characterization and mutational analysis of a cluster of three genes expressed preferentially during sporulation of *Saccharyomyces cerevisiae*. Mol.

Cell. Biol. *6*:2443-2451.

20. Pringle, J.R. and L.H. Hartwell. 1981. The *Saccharyomyces cerevisiae* cell cycle. p.97-142. In J.N. Strathern, E.W. Jones J.R. Broach (ed.), The molecular biology of the yeast *Saccharyomyces cerevisiae*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

21. Rose, M. and G.R. Fink. 1987. *KAR1*, a gene required for function of both intranuclear and extranuclear microtubules in yeast. Cell *48*:1047-1060.

22. Roth, R. and H.O. Halvorson. 1969. Sporulation of yeast harvested during logarithmic growth. J. Bacteriol. *98*:831-832.

23. Roth, R. 1973. Chromosome replication during meiosis: identification of gene function required for premeiotic DNA synthesis. Proc. Natl. Acad. Sci. USA *70*:3087-3091.

24. Sherman, F., G.R. Fink, and J.B. Hicks. 1981. Methods in yeast genetics. Cold Spring Harbor Press, New York.

25. Shortle, D., J.E. Haber, and D. Botstein. 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. Science *217*:371-373.

26. Simchen, G. 1974. Are mitotic functions required in meiosis?

Genetics *76*:745-753.

27. Smith, L., L. Robbins, A. Kennedy and P.T. Magee. 1988. Identification and characterization of mutations affecting sporulation in *Saccharyomyces cerevisiae*. Genetics *120*:899-907.

28. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. *98*:503-517.

29. Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA *77*(9):5201-5205.

Table 1. Yeast Strains

Strain	Genotype	Source
SCMS7	*MATa HO ade his4 leu2* *MATα HO ade his4 leu2*	Smith, 1985
SL484	*MATa HO ade his4 leu2 spo17* *MATα HO ade his4 leu2 spo17*	Smith, 1985
W66-8A	*MATa HO ade2 leu1 lys2 met4 trp5 ura3 can1* *MATα HO ade2 leu1 lys2 met4 trp5 ura3 can1*	Rothstein
R9	*MATa HO ade2 leu2 ura3 spo17* *MATα HO ade2 leu2 ura3 spo17*	This work
R92W	*MATa HO + leu2 ura3 spo17* *MATα HO ade2 leu2 ura3 spo17*	This work
R92WH	*MATa HO leu2 ura3 spo17* *MATα HO leu2 ura3 spo17*	This work
BB4	*MATa ho leu2 arg*	This laboratory
M1417-8B	*MATa ho ura3 met8*	This laboratory
M8B-T1	*MATa ho ura3 met8 SPO17::URA3*	This work
T1X1A	*MATa HO + + + + + ura3 +* *MATα ho ade2 leu1 lys1 met4 trp5 ura3 spo17::URA3*	This work
T1X1C	same as T1X1A	This work
SLB0	*MATa HO ade his4 leu2 spo17::LEU2* *MATα HO ade his4 leu2 spo17::LEU2*	This work
BLB1	*MATa ho leu2 his4 arg spo17::LEU2*	This work
PT4a	*MATa HO ura3 spo17* *MATα HO ura3 spo17*	This work
PT4b	*MATa HO ura3* *MATα HO ura3*	This work
PT4c	Same as PT4a	This work
PT4d	Same as PT4b	This work
2209	*MATa rme1::LacZ his4-519 ura3 leu2 trp1 can1*	A. Mitchell
2210	*MATα rme1::LacZ his4-519 ura3 leu2 trp1 can1*	A. Mitchell
2211	2209 x 2210	A. Mitchell
2209-a	*MATa rme1::LacZ his4-519 ura3 leu2 trp1 can1 spo17::LEU2*	This work
2210-3	*MATα rme1::LacZ his4-519 ura3 leu2 trp1 can1 spo17::LEU2*	This work
221a3	2209-a x 2210-3	This work
312-1	*MATa rme1::LacZ his4-519 ura3 leu2 trp1 can1 spo17::LEU2* *MATα rme1::LacZ his4-519 ura3 leu2 trp1 can1 spo17::LEU2*	This work
312-2	Same as 312-1	This work
312-3	Same as 312-1	This work

Table 2. Plasmids used and their sources.

Plasmid	Markers	Source
YCp50 library	*CEN4 ARS1 URA3 BLA*	M. Rose
p98	*CEN4 ARS1 URA3 BLA SPO17*	YCp50 library
p1912	*CEN4 ARS1 URA3 BLA SPO17*	YCp50 library
p1940	*CEN4 ARS1 URA3 BLA SPO17*	YCp50 library
p12δH	*CEN4 ARS1 URA3 BLA spo17*	This work
p12δS	*CEN4 ARS1 URA3 BLA SPO17*	This work
p12δSH	*CEN4 ARS1 URA3 BLA spo17*	This work
pYi12	*URA3 BLA SPO17*	This work
pLB174	*URA3 BLA spo17::LEU2*	This work
pR67	*CEN4 ARS1 URA3 BLA RAD6*	L. Prakash
YCpBB	*CEN4 ARS1 URA3 spo17*	This work
pEMBB	*LEU2 BLA spo17*	This work

Table 3. Complementation of *spo17* mutants by plasmids.

Strain	Transforming DNA	Markers	Sporulation 30°	Sporulation 34°
W66-8A			85.0%	49.3%
SL484			18.3	1.0
R92W			20.7	0.0
R92W	p98	*SPO17 URA3*	52.0	13.7
R92W	p1912	*SPO17 URA3*	61.0	9.0
R92W	p1940	*SPO17 URA3*	79.0	6.0
R98FOA1			+	-
R98FOA2			+	-
R12FOA1			+	-
PT4c	YRp12	*TRP1 URA3*	+	-

Table 4. Determination of the extent of the *SPO17* gene.

Strain	Transforming DNA	% Sporulation 30°C	34°C
R92W	p12δH	8.3	0.0
R92W	p12δS	23.3	7.3
R92W	p12δSH	11.7	0.0

Table 5. *RAD6* does not complement *spo17* mutations.

Strain	Transforming DNA	Markers	% Sporulation 30°C	34°C
PT4a			0.0	0.0
PT4b			31.0	30.0
PT4c			0.0	0.0
PT4d			25.0	21.0
PT4a67	pR67	*RAD6 URA3*	0.0	0.0
PT4c67	pR67	*RAD6 URA3*	4.0	0.0
PT4c	YRp12	*TRP1 URA3*	0.0	0.0

Table 6. Sporulation of the *rme1::LacZ spo17::LEU2* double mutants.

Strain	Genotype	Color on X-gal	Sporulation
2209	**a** *rme1::LacZ*	Blue	-
2210	**α** *rme1::LacZ*	Blue	-
2211	2209 x 2210	White	+
2209-a	**a** *rme1::LacZ spo17::LEU2*	Blue	-
2210-3	**α** *rme1::LacZ spo17::LEU2*	Blue	-
221a3	2209-a x 2210-3	White	-
312-1	221a3 + p1912	White	+
312-2	221a3 + p1912	White	+
312-3	221a3 + p1912	White	+

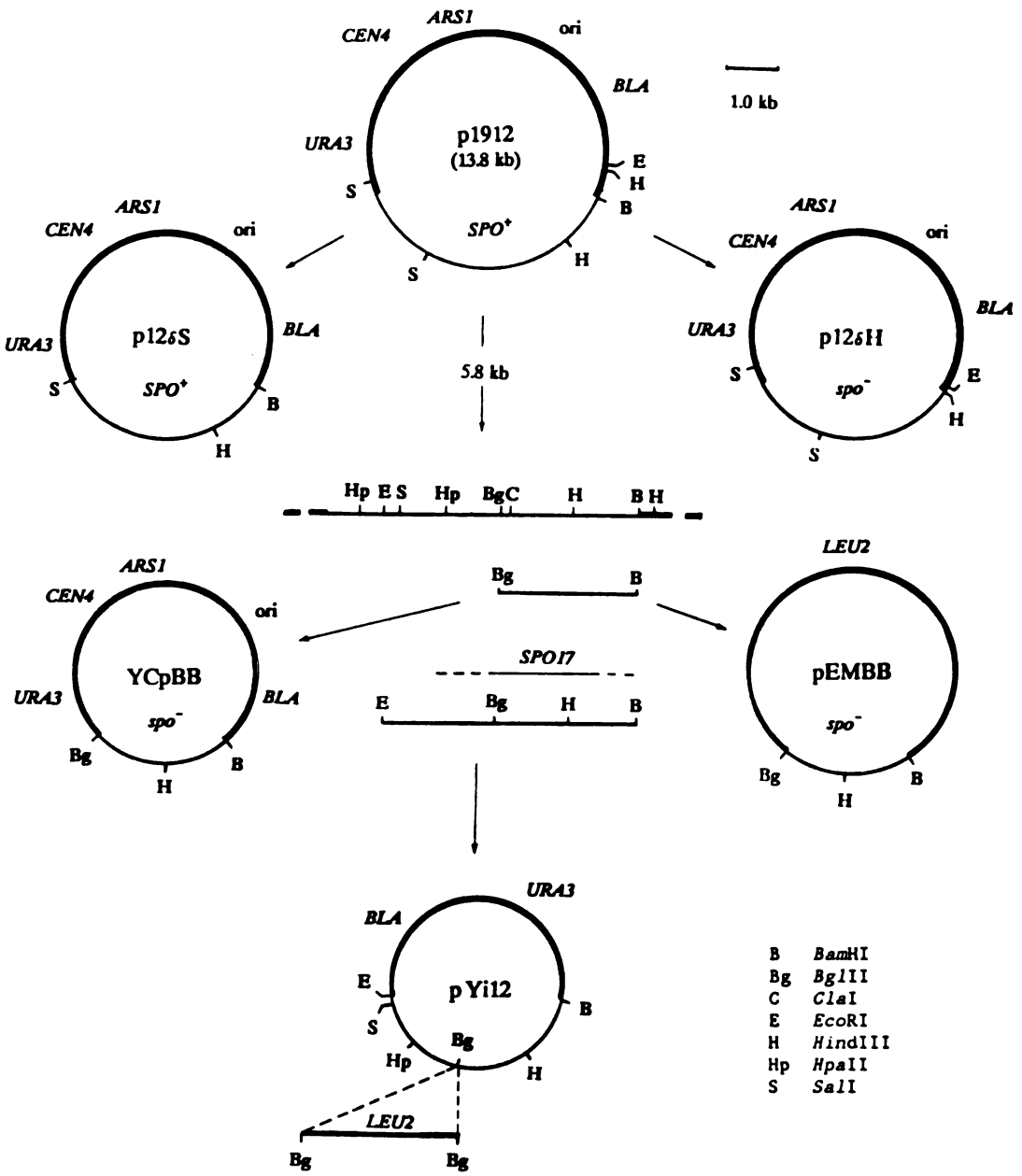


Figure 1. Plasmid constructs.

Figure 1. Plasmid constructs. The heavy line indicates the YCp50 sequences; the light line is the insert carrying the *SPO17* gene. Plasmids p12δS and p12δH were derived from p1912 by deleting the *Sal*I and *Hin*dIII fragments, respectively. YCpBB was made by inserting the *Bgl*II-*Bam*HI fragment into YCp50. The same fragment was inserted into the EMBLY vector pYE30 to give pEMBB. pYi12 was constructed by inserting the *Eco*RI-*BamH*I fragment into YIP5. *SPO17* was disrupted by inserting a copy of *LEU2* into the *Bgl*II site; the resulting plasmid pLB174, is not pictured.

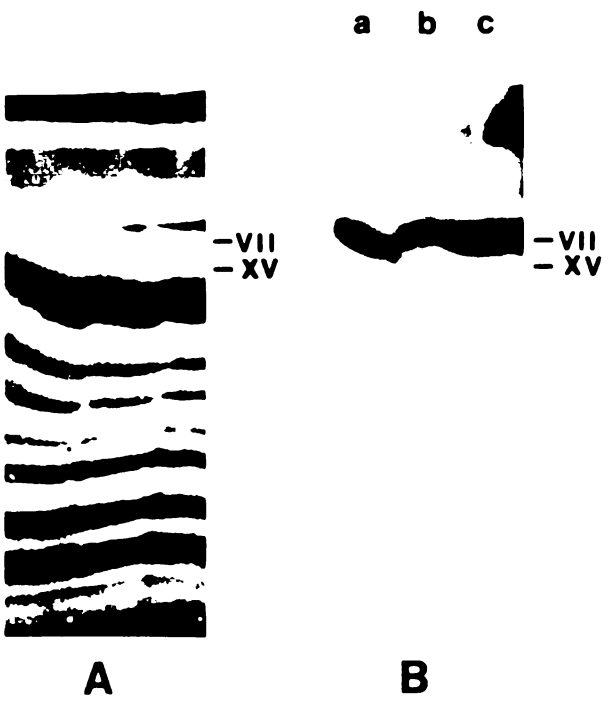


Figure 2. OFAGE analysis.

A. Genomic DNA separated by OFAGE and stained with ethidium bromide. B. Southern blot analysis of the DNA shown in A probed with nick translated DNA from one of the following genes: Lanes *a*, *SPR2*; *b*, *SPO17*; *c*, *TRP5*.

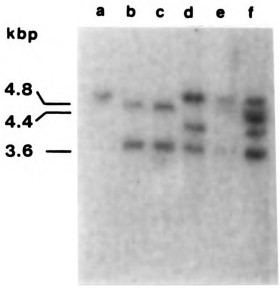


Figure 3. Southern analysis.

DNA from each strain was digested with EcoRI and 20 ug of digested DNA were loaded into each lane of an agarose gel and separated by electrophoresis. A Southern blot was prepared and probed with the radiolabeled *Hin*dIII-*BglII* fragment from the *SPO17* sequence. Lanes *a*, BB4; *b*, BLB1; *c*, SLB0; *d*, SLB1; *e*, SLB2; *f*, SLB3.

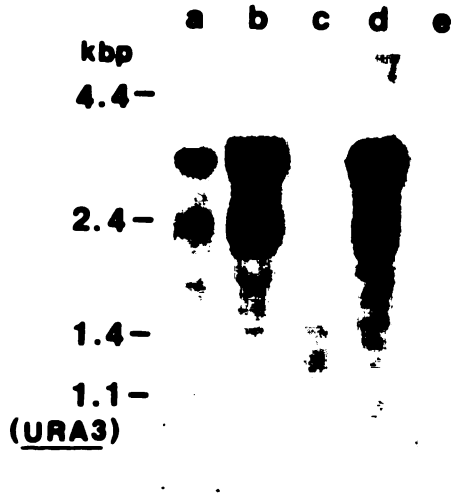


Figure 4. Northern analysis. 20 ug of poly (A+) RNA was loaded in in each lane of a 1.2% agarose denaturing gel and electrophoresed. A Northern blot from the gel was probed with the radiolabeled *Hin*dIII-*BglII* fragment (1.2 kb) from p1912 and autoradiographed. The blot was later probed with the 1.1 kb *URA3* gene. Sources of RNA were as follows: Lanes *a*, SCMS7-1 (*SPO17/SPO17*) vegetative cells; *b*, SCMS7-1 cells after 4 hrs in sporulation medium; *c*, SLBO (*spo17::LEU2/spo17::LEU2*) cells after 4 hrs in sporulation medium; *d*, 2211 (*spo17/spo17 rme1::LacZ/rme1::LacZ*) cells after 4 hours in sporulation medium; *e*, tRNA (control).

CHAPTER III

DISCUSSION

DISCUSSION

The isolation of *SPO17* was accomplished with relative ease because the donditional nature of the original *spo17* mutant, SL484, allowed the mutation to be introduced into a new genetic background from which the gene could be identified. After its isolation, *SPO17* was disrupted by *in vitro* mutagenesis and transformed into several wild type strains. Diploidized mutants generated by this method are unable to sporulate at any temperature, and it was concluded that the *SPO17* product is absolutely required for sporulation. The fact that mRNAs of nonsporulating disruptants differ markedly from wild type *SPO17* messages also supports this conclusion.

Does *SPO17* have a mitotic function?

A possible role for *SPO17* in the mitotic cell cycle is suggested by the presence of *SPO17* mRNAs in vegetative cells, even though all *spo17* mutants seem to grow normally. An alternative explanation for the vegetative expression is that *SPO17* is not tightly regulated by the cell. This is in contrast to the early gene *IME1*, which is so tightly regulated that its messages do not appear in glucose-grown cells (Kassir et al. 1988). Of course, *IME1* is regulated by at least two mechanisms, starvation and repression by *RME1*; thus, tight control of its expression might be expected.

Expression of the repressor gene *RME1* does not exhibit such tight

regulation. Although *RME1* is freely expressed in haploids to prevent entry into sporulation, there remains a residual level of expression in **a**/α cells (a 10 to 20-fold reduction is seen). *RME1* is negatively regulated by the **a**1-α2 product, which has been shown to repress two other genes, *MATα1* and *HO*; interestingly, the reduction of these transcript levels is greater than 100-fold.

Mitchell and Herskowitz (1986) were unable to find any functional significance for the residual *RME1* transcription in **a**/α cells. It seems curious that the *MAT* locus would tightly regulate the expression of some genes, yet permit residual expression of others. Although *SPO17* is probably not regulated by the same mechanism as *RME1*, its residual expression in vegetative cells is of interest. The extent of its induction during sporulation has yet to be determined.

There is another hint of a possible mitotic role for *SPO17*. Figures 1 and 2 (Appendix D) are photographs of plates which contain colonies that have been exposed to permissive and nonpermissive temperatures under sporulation conditions. Several points of interest can be noted.

During the initial screen of transformants, a difference in plate growth was noted. This can be seen in the colonies at positions 15, 16 and 17. These are strains that have lost the *SPO17* plasmid after growth on 5-FOA. As expected, no red papillae are produced on either plate. However, growth of the colonies that have been subjected to sporulation conditions at 34°C is much poorer than those subjected to 30°C. During the screen this difference was so striking that growth alone was sufficient to identify clones maintaining a *SPO17* plasmid. This difference was not seen, however, in SL484 (the original *spo17*

mutant). It may be that this feature is a characteristic of the new genetic background of R92W; a modifier gene may have been introduced or a mutation may have occurred during or after the cross with W66-8A. It should be noted that colonies from strain R92W do not exhibit the growth differential on these plates although usually the difference was quite marked.

To further examine these growth characteristics, strain SLB1, which is a heterozygous (*SPO17/spo17*) transformant, was sporulated and growth of the progeny was evaluated in rich liquid medium and in liquid acetate medium. No significant differences in the rate of growth between the *spo17/spo17* or the *SPO17/SPO17* strains could be seen, nor was there any apparent loss of viability in rich, acetate, or sporulation media (data not shown). *spo17/spo17* cells did appear to be slightly smaller, but a quantitative analysis was not carried out. Strain SLB1 was later found not to have exactly the predicted bands after its disruption (Chapter II, Figure 3, lane d), but the *spo17* disruption is evident, and its progeny segregated 2:2 for sporulation-competence. The growth phenotype of strain R92W remains a mystery and awaits further investigation.

Consistency of the generation of red papillae.

Production of red papillae in most of the transformants (colonies at positions 7 through 14 and 18 through 29) can be noted on both plates. Some transformants occasionally fail to produce red papillae (eg., colony No. 13) after exposure to either temperature. This could be due to the variation in sporulation efficiency that is sometimes seen in cultures, even when sporulated in liquid medium. Another

explanation is that the *SPO17* plasmids borne by these strains are different. This is known to be true because most of the restriction enzymes used in mapping gave different restriction patterns in different plasmids. Plasmid p1912 (strain 1912, position 13) was selected for further study because of the small size of its insert. In fact, most plasmids which passed the screen had inserts which were much larger. The variation in the plasmids could account for the variability seen in the production of the red papillae.

The five strains at positions 25 through 29 were transformed with the multicopy plasmid pRB367, which contains most of the insert (*BamH*I-*Eco*RI fragment) from plasmid p1912. These strains consistently gave good growth and sporulation. Whether this vigor is due to the small size of the insert or because of the multicopy effect of the plasmid is not known.

Is *SPO17* also subject to multiple regulation?

Transcription of both *SPO13* and *IME1* begins very soon after introduction into sporulation medium, and both genes are known to be regulated by two mechanisms, nutrition and the *MAT* locus. It seems possible that the *SPO17* product is involved in the cell's response to the available nutrition. Could its expression also be regulated by nutritional status? The masking effects of *MAT* regulation on *SPO17*, if it exists, could be removed by analyzing *SPO17* expression in **a**/**a** and α/α diploids after their introduction into starvation medium.

What is the relationship between *IME1* and *SPO17*?

It seemed reasonable to ask how the effects of *SPO17* might be

related to the established model of sporulation, and this question was addressed by creating a *spo17/spo17 rme1/rme1* strain and evaluating its ability to sporulate. The results did not suggest a role for *SPO17* in the regulation of *RME1*. It would be very interesting to determine the level of *SPO17* expression in *ime1* mutants after sporulation induction. Conversely, the level of *IME1* expression in *spo17* mutants could be determined. If no interaction between *IME1* and *SPO17* exists, *SPO17* may prove to be very interesting, indeed.

APPENDIX A

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3000–3004, May 1983
Genetics

Isolation of genes expressed preferentially during sporulation in the yeast *Saccharomyces cerevisiae*

(microbial development/cDNA probe/differential plaque hybridization/meiosis and ascosporogenesis)

MARY J. CLANCY*, BEATRICE BUTEN-MAGEE, DAVID J. STRAIGHT, ALLAN L. KENNEDY, ROGER M. PARTRIDGE, AND P. T. MAGEE

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824-1101

Communicated by Adrian M. Srb, January 31, 1983

ABSTRACT A library of *Saccharomyces cerevisiae* DNA in the vector λ Charon 28 was probed for sequences complementary to cDNA made from poly(A)$^+$ RNA isolated from the well-sporulating yeast strain AP1 a/α. The RNA was isolated from cells that had been incubated 7, 9, 11, and 13 hr in sporulation medium. DNA complementary to poly(A)$^+$ RNA from α/α (nonsporulating) AP1 was used as a control, and 46 bacteriophage that gave a stronger response with a/α cDNA than with α/α cDNA were obtained in a screening of three yeast genomes worth of DNA. Two of the bacteriophage appeared to contain a/α-specific genes, in that they hybridized to cDNA from vegetative a/α RNA. The rest appeared to correspond to a/α genes expressed preferentially during sporulation. Restriction endonuclease analysis of four of the cloned sequences revealed a single major region of transcription in each; these regions ranged in size from 2.5 to 4.0 kilobases. RNA blot analysis showed that, in three of the four cases, transcripts of two different sizes were homologous to the cloned sequence. In all four cases, the homologous transcripts appeared at about 7 hr and were decreasing in amount by 13 hr. These results provide evidence for transcriptional control of genes expressed during sporulation and for at least one group of genes that is turned on at about the time of meiosis I in sporulation.

The ascomycete *Saccharomyces cerevisiae* has a relatively complex life cycle that includes a morphologically defined cell cycle, mating and zygote formation, and meiosis and ascosporogenesis. The combined techniques of genetics and molecular biology have been used to elucidate several aspects of this life cycle and have led to a number of advances in our understanding of the cell cycle (1) and the control of mating type (2–6). The study of meiosis and sporulation (7), however, has heretofore lagged, partly because of the difficulty of isolating and characterizing mutations in genes expressed only in diploids (8).

Meiosis and ascosporogenesis is a complex process involving macromolecular synthesis (9, 10), recombination (11), and turnover of preexisting cellular components (12). It occurs in cells expressing both a and α mating type alleles on a shift to medium lacking ammonia and glucose but containing a respirable carbon source. Cells expressing only one *MAT* allele (haploid a or α, diploid α/α or a/a cells) do not undergo the process; such cells fail to undergo premeiotic DNA synthesis (13, 14) or any of the subsequent events that have been monitored. Meiosis and sporulation are therefore under the control of the mating type locus, although nutritional signals must also play a role in the initiation of the process.

The product of the developmental program, an ascus containing (usually) four haploid spores, is morphologically distinct from vegetative cells. In a typical (well-sporulating) diploid, 60–80% of the cells achieve this differentiated state in a relatively synchronous process that lasts 16–24 hr under the conditions we use (15). Physiological studies have shown that premeiotic S phase occupies the period from 3 to 7 hr (in the culture as a whole, the S period of individual cells appears to last about 1 hr), recombination occurs over roughly the same period, beginning and ending about half an hour later than S phase, and meiosis I takes about 120 min, from 8 to 10 hr (9, 12). Meiosis II follows immediately and is completed by 12 hr. Immature asci are detectable at 10 hr in some cells, and ≈80% of the final level of asci is found in the culture by 16 hr, with the remainder forming over the next 8 hr.

Several attempts to identify specific gene products involved in this process have failed, despite the existence of numerous mutations affecting gene products needed in sporulation but not for vegetative growth (8). One- and two-dimensional gel analyses of proteins synthesized during a pulse label of sporulating cells have identified few (16) or no (12, 17, 18) proteins specific to sporulation. This may be because the abundance of these gene products is simply too low to be detected on gels.

Recently, workers in this laboratory have identified an enzyme, sporulation amyloglucosidase (SAG), that occurs only in a/α cells in sporulation medium and whose appearance is dependent on the progression of the cells well into the pachytene stage of meiotic prophase (19, 20). It seemed worthwhile to ask whether a group of genes was expressed during the time that this enzyme activity appears, how large this hypothetical group might be, and whether these genes share common functional (e.g., duration of transcription) and structural properties.

We have therefore screened a *Saccharomyces cerevisiae*–λ Charon 28 (21) library for bacteriophage containing sequences specifically expressed from 7 to 13 hr in sporulation medium. Although we have not yet identified the *SAG* gene, we have found 46 hybrid phage that contain sequences more highly expressed in sporulating MATa/MATα cells than in otherwise isogeneic MATα/MATα cells. The characterization of six of these sequences is reported here.

MATERIALS AND METHODS

Radioisotopes and Enzymes. [^{32}P]dCTP (>400 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear. Restriction enzymes and oligo(dT) primer were from Bethesda Research Laboratories and were used as recommended by the supplier. DNA polymerase and avian myeloblastosis virus reverse transcriptase were from Boehringer Mannheim and Life Sciences, respectively. Nitrocellulose sheets and filter discs were from Schleicher

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SAG, sporulation amyloglucosidase; kb, kilobase pairs.

* Present address: Dept. of Microbiology, University of Notre Dame, Notre Dame, IN 46556.

& Schuell. Formamide for hybridization (Aldrich) was deionized with Amberlite (Aldrich) before use. *Drosophila* cytoplasmic RNA was a gift from Jerry Dodgson.

Growth Conditions and Preparation of RNA. The *Escherichia coli* strain used for growth of the bacteriophage was KH802, a derivative of K-12. *S. cerevisiae* strains used were AP1 a/α and its isogeneic asporogenous derivative AP1 α/α (12). The cells were ordinarily grown and sporulated as described (15) in acetate pregrowth medium. Vegetative or sporulating yeast cells were harvested by centrifugation, washed in ice-cold sterile distilled water, and broken by mixing in a Vortex with glass beads (18) or in a Bronwill homogenizer (22). Poly(A)$^+$ RNA was prepared as described (22).

Library Construction. A *S. cerevisiae* library was constructed in λ Charon 28. DNA from *S. cerevisiae* strain AH22 was extracted by the method of Hereford *et al.* (23) and digested with *Mbo* I to yield fragments of ≈15 kilobase pairs (kb). Fifteen- to 20-kb fragments were isolated by sucrose gradient centrifugation, and these were ligated to Charon 28 arms prepared by digestion with *Bam*HI (24). The ligated DNA was packaged *in vitro* (25), plated, and amplified. Minipreparations of λ DNA were used for all experiments, essentially as in Maniatis *et al.* (24).

Differential Plaque Hybridization. The method used was essentially that of St. John and Davis (26). Aliquots of the library were plated onto *E. coli* KH802 on NZCYM (24) and grown to yield ≈300 plaques per plate. Isolated plaques were picked using sterile toothpicks into an ordered array on freshly poured lawns of *E. coli* KH802 (≈50 μl of an overnight culture) and allowed to grow for 8–12 hr. This amplification was necessary to allow detection of the hybridization signals from a majority of the plaques; only 10–20% of the plaques yielded signals when filters were made directly from the original plaque plates whereas >90% of the clones gave detectable signals with both probes when patch plates were used (26). Duplicate nitrocellulose filter replicas were prepared from the plates essentially by the method of Benton and Davis (27). Hybridization was carried out as in Engel and Dodgson (28).

Preparation of Hybridization Probes. ^{32}P-Labeled cDNA was prepared from poly(A)$^+$ RNA by the method of St. John and Davis (26), except that 40 mM sodium pyrophosphate was included in the reaction mixture. The reaction was started by the addition of reverse transcriptase, allowed to proceed at 40°C for 60–90 min, and terminated by the addition of base. Hydrolysis of RNA and separation of the cDNA from the unincorporated nucleotides was done as in St. John and Davis (26). Probes were used at a concentration of 1 to 2 ng/ml. Nick-translation of cloned DNA was accomplished by the method of Rigby *et al.* (29).

Agarose Gel Electrophoresis and Nitrocellulose Filter Hybridization. Agarose (Bethesda Research Laboratories) gels were run submerged in 40 mM Tris·HCl/20 mM NaOAc/1 mM EDTA, pH 7.5, at 60–100 mA and stained with ethidium bromide (1 μg/ml). The gels were 0.4%, 0.7%, or 1.2% agarose, depending on the experiment.

DNA from agarose gels was transferred to nitrocellulose by the method of Southern (30) and hybridized to cDNA prepared as described by St. John and Davis (26). For RNA blots, ≈5 μg of poly(A)$^+$ RNA per lane was denatured with glyoxal (31) and loaded onto 1.5-ml agarose gels (30) and electrophoresed. Then, the RNA was transferred to nitrocellulose by the method of Thomas (32) and hybridized to nick-translated DNA as described above. Dot blots were prepared by spotting 5-μl aliquots of the λ phage stocks onto nitrocellulose filters. The DNA was denatured and neutralized. The filters were baked as described for differential plaque hybridization and then hybridized to cDNA probes prepared as described above.

RESULTS

Differential Plaque Hybridization as a Screen for Sporulation-Specific Genes. Differential plaque hybridization, a modification of the plaque filter hybridization method of Benton and Davis (27), allows the isolation of genes whose RNAs are present at increased concentration in one population of cells compared with another. This method involves the preparation of two [^{32}P]cDNA probes, one against each of the RNA preparations to be compared, and hybridization of these probes to duplicate nitrocellulose filter replicas of a collection of λ clones on a Petri plate. Clones that hybridize strongly to one probe but not to the other contain genes that are expressed at different levels in the two populations. This method has been used successfully in yeast to isolate galactose-inducible genes (26) and genes expressed at high levels in low-phosphate medium (33).

We have used differential plaque hybridization to screen a λ Charon 28–*S. cerevisiae* library for sequences expressed preferentially during sporulation. A [^{32}P]cDNA probe was prepared against RNA isolated from MATa/MATα cells after 6–13 hr of incubation in sporulation medium (Fig. 1) and, as a comparison, a similar probe was made from an otherwise virtually isogeneic MATα/MATα strain that is incapable of sporulation. The RNA preparation from the sporulating MATa/MATα cells should contain sequences transcribed specifically during sporulation that the MATα/MATα cells would lack. These might include those coding for products required for premeiotic DNA

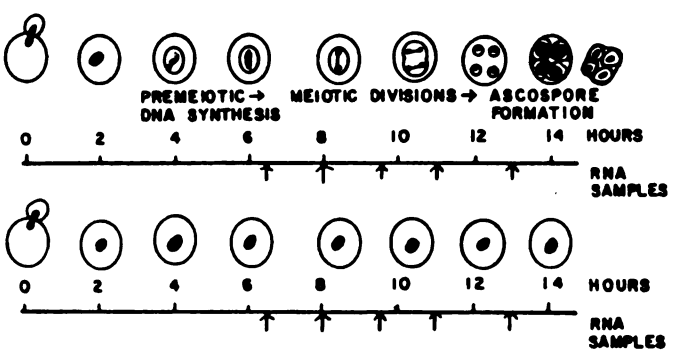


FIG. 1. Preparation of hybridization probes. One-liter cultures of the sporulation-proficient strain AP1 a/α (*Upper*) and the asporogenous AP1 α/α strain (*Lower*) were grown to 2 × 10^7 cells/ml and shifted to sporulation medium. The cells were allowed to progress into sporulation and 500-ml samples were taken at the indicated times for the preparation of RNA. The samples from each culture were pooled for the preparation of poly(A)$^+$ RNA, and [α-^{32}P]cDNA probes were prepared against these RNAs and used for differential plaque hybridization.

synthesis, recombination, the two meiotic divisions, and spore formation, as well as RNAs that are stored in the spore in preparation for dormancy and germination. The RNA sequences common to both probes would include those homologous to housekeeping genes or to genes induced by starvation. That these latter are the predominant sequences is suggested by the fact that two-dimensional gel electrophoresis of the proteins made in sporulation medium by sporulating-proficient and sporulation-deficient cells gives similar or identical patterns (16–18).

Isolation of Sporulation-Specific Genes. Aliquots of the library were plated in top agar onto *E. coli* KH802, individual clones were picked and amplified by patching into an ordered array onto freshly poured lawns of KH802 and duplicate nitrocellulose filters were prepared and hybridized. Clones that gave stronger signals with the MATa/MATα probe were picked and retested. The second test was necessary to purify the phage and also served to eliminate false positives, which represented >50% of the clones chosen on the first test, whether the same cDNA preparation was used for both the first and the second test or the probes were different. A sample autoradiograph from such an experiment is shown in Fig. 2. Plaques 1 and 2 gave stronger signals with the a/α probe than with the corresponding α/α probe on the original plate. On retesting, however, only clone 2 isolates hybridized preferentially with the a/α probe. Such clones were retained and phage stocks were prepared.

We screened ≈3,700 plaques and retained a total of 46 clones that appeared to contain differentially expressed genes. These clones varied at least 10-fold in the intensities of the hybridization signals obtained as well as in the intensity differences between the a/α and α/α probes. The dot blots (Fig. 3 *A* and *B*) show that some clones (e.g., nos. 1, 11, 16, 34, and 42) gave extremely intense signals with the a/α cDNA but weak signals with the corresponding α/α probe while others (e.g., nos. 2, 4, and 7) showed only slight qualitative differences. The 46 clones ought to represent some 15 genes, assuming that each is represented only once per haploid genome and that no highly expressed genes that are not sporulation specific are located nearby.

It is possible that the differentially expressed genes isolated in this screen were actually MATa/MATα specific, rather than sporulation specific, because the only criterion for selection was that the clones hybridize more strongly to the probe made from MATa/MATα cells in sporulation medium than to the corresponding MATα/MATα probe. That this is not the case is shown in Fig. 3 *C* and *D*. [^{32}P]cDNA probes were prepared to poly(A)$^+$ RNA from vegetatively growing MATa/MATα and MATα/MATα cells and the signals obtained when the 46 clones were hybridized to the two probes were compared. Most clones hybridized equally well to the two vegetative probes and more weakly to both than to the cDNA from sporulating cells. Clones 7 and 69 were exceptions to this since they hybridized strongly to both MATa/MATα probes and weakly to both MATα/MATα probes. These two clones, therefore, contain a/α rather than sporulation-specific genes. Clone 16 was surprising, since it hybridized more strongly to the MATα/MATα probe prepared from vegetative cells than to a comparable probe from a/α cells. Subsequent analysis showed that the apparent α/α-specific sequence was located ≈5 kb from the sporulation-specific sequence within the same clone (see below).

Restriction Maps and Time of Appearance of the Sporulation-Specific Transcripts. We wanted to determine whether each clone contained a single sporulation-specific gene or several and that time during sporulation at which the corresponding transcripts appeared. Six clones, nos. 1, 11, 16, 32, 34, and 42 were chosen for this analysis, because they gave particularly clear differential signals. Restriction maps were constructed and the locations of the differentially transcribed portion in each was determined by Southern blotting (30) using [^{32}P]cDNA from sporulating MATa/MATα cells as the probe. As shown in Fig.

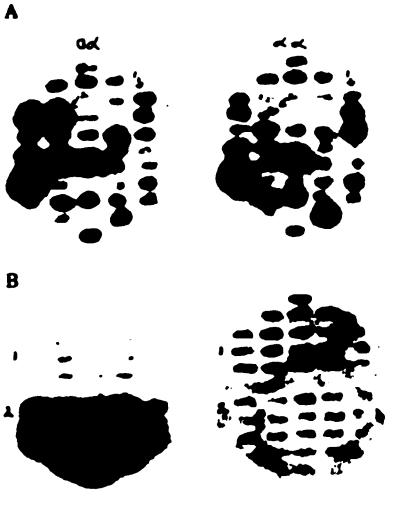


FIG. 2. Differential plaque filter hybridization as a screen for sporulation-specific genes. Aliquots of the λ library were patched onto *E. coli* KH802, and duplicate nitrocellulose filter replicas were prepared and hybridized to [^{32}P]cDNA probes prepared against RNA from sporulating MATa/MATα cells and nonsporulating MATα/MATα cells. The hybridized blots were washed and exposed to x-ray film and the signals obtained with the two probes were compared. Clones 1° and 2° (these numbers refer to the order of testing and are not related to clone numbers referred to in the text) appeared to contain sporulation-induced genes on the first test (*A*) but only clone 2 appeared positive on the second test (*B*).

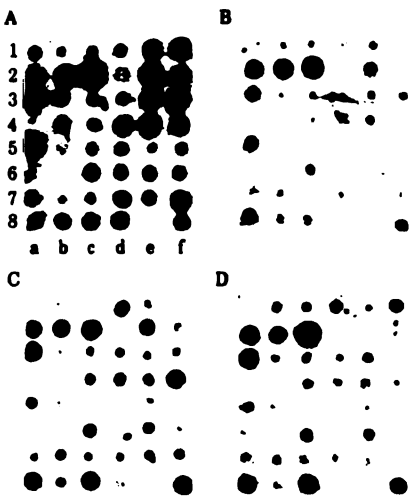


FIG. 3. Dot blot analysis of the 46 clones. Dot blots were prepared from isolated phage that appeared to contain sporulation-inducible genes and hybridized to [^{32}P]cDNA probes prepared against poly(A)$^+$ RNA from MATa/MATα cells undergoing sporulation (*A*), MATα/MATα cells incubated in sporulation medium (*B*), MATa/MATα cells growing vegetatively (*C*), and MATα/MATα growing vegetatively (*D*). The clones referred to in the text are 1, position 1a; 2, 1b; 4, 1c; 7, 1d; 11, 1e; 16, 1f; 32, 2c; 34, 2f; 42, 3f; and 69, 4f. Equal amounts of YEP13 DNA were included as controls (position 8f).

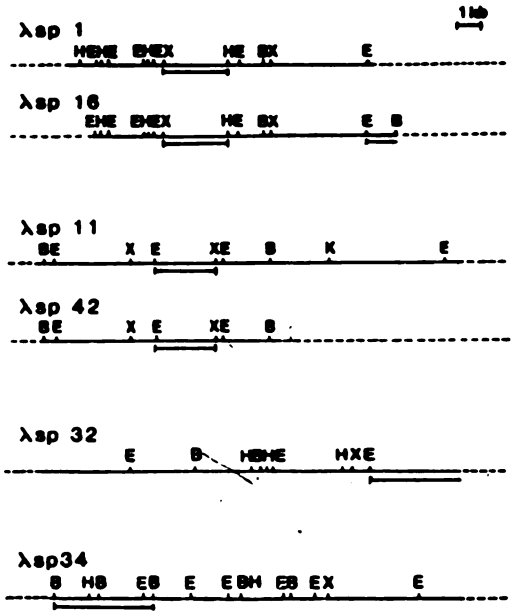


FIG. 4. Restriction enzyme analysis of selected clones. Single and double restriction enzyme digests of the purified λ DNAs were analyzed on 1.2% and 0.4% horizontal agarose gels. Size standards were restriction enzyme-digested λ and YEP13 DNAs. Enzymes used were E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; X, *Xba* I; K, *Kpn* I. The dotted lines represent the λ Charon 28 arms and the heavy lines represent yeast DNA. The thick lines shown below the maps represent the approximate locations of the sporulation-specific sequences within each phage, as determined by Southern blotting of the gels followed by hybridization to the ^{32}P-labeled MATa/MATα and MATα/MATα probes used to isolate the clones originally. Clones 11 and 42 contained *Hin*dIII sites but these are not shown on the maps.

4, clones 11 and 42 apparently contain the same sporulation-specific gene, as do clones 1 and 16. Clones 32 and 34 differ from each other as well as from the other clones. The maps of the two pairs of clones (nos. 1 and 16; nos. 11 and 42) are similar to each other, although not identical. In each case, the sporulation-specific gene could be located on a single restriction fragment <3 kb long, as shown by the bars in Fig. 4. The small size suggests that each clone contains only one, or possibly two, closely linked sporulation-specific genes.

The restriction fragments flanking those containing the sporulation-specific gene also hybridized to the a/α probe but at a greatly diminished intensity. It was not possible to determine whether this was the result of low levels of transcription from a flanking gene or whether the transcribed portion of the sporulation-specific gene extended past the fragments shown. In all cases except clone 34, the indicated fragment also hybridized to the α/α probe to some extent. This may reflect a low level of transcription of the "sporulation gene" by MATα/MATα cells or the presence of an adjacent transcription unit that is expressed in common between MATa/MATα and MATα/MATα cells.

We have used RNA blot analysis (34, 32) to determine the time of appearance of the transcripts corresponding to these genes and their sizes. AP1 a/α cells growing vegetatively were shifted to sporulation medium and aliquots were removed from the culture at intervals during sporulation for the preparation of poly(A)$^+$ RNA. This RNA was denatured with glyoxal, electrophoresed on 1.5% agarose gels (31), and blotted to nitrocellulose. The blots were hybridized to λ clone DNA that had been labeled *in vitro* with [^{32}P]dCTP by nick-translation (28).

FIG. 5. RNA blot analysis of clones 16 (*A*), 34 (*B*), and 42 (*C*). Poly(A)$^+$ RNA was prepared from MATa/MATα cells incubated in sporulation medium for 1, 3, 5, 7, 9, 11, and 13 hr. Five-microgram samples of these RNAs were denatured with glyoxal, electrophoresed on 1.5% agarose gels, blotted to nitrocellulose, and hybridized to cloned DNAs labeled *in vitro* with [α-^{32}P]dCTP by nick-translation. Autoradiography was done at −70°C for 3–8 days with one intensifier screen. Numbers on the left of the gels indicate sizes (kb) of the developmentally regulated transcripts.

As shown in Fig. 5, clones 16 and 34 each hybridized to two developmentally regulated transcripts rather than one, and clone 32 also hybridized to two distinct transcripts (data not shown). Clone 42 hybridized to one major transcript, but other faint bands were also present. We do not know whether the multiple bands represent transcription of the same gene from different promoters, processing of the larger transcript, or hybridization of the cloned DNA to messages from two closely linked developmentally regulated genes. It is also possible that the second transcripts are from different but related genes elsewhere in the genome. None of these transcripts was detected in blots made to RNA from α/α cells, although such transcripts may have escaped detection because they were present at low levels.

DISCUSSION

Although a considerable amount is known about the physiology of the developmental pathway leading to recombination, meiosis, and ascosporogenesis in *S. cerevisiae* and extensive genetic analysis has been done (8), molecular approaches have been somewhat unproductive. The genetics has provided part of the explanation for this lack of progress in that it has been calculated that a relatively small number of genes are sporulation specific—i.e., required for the process but otherwise dispensable (8). If the proteins that are the products of these genes are present in low abundance, the failure to see specific proteins on two-dimensional gels is easily explained. We sought to identify the genes involved in sporulation directly, by differential plaque hybridization, with the hope that this method would be sensitive enough to detect sporulation-specific sequences. Although there is no doubt that our screen would fail to detect certain classes of genes, it has found a number of sequences that are preferentially expressed in a/α cells in sporulation medium.

There are two major groups of genes that will have escaped our screen. One of these is the class that comprises genes located close to a gene actively transcribed in α/α cells in sporulation medium. On the average, 15% of our clones gave a signal with α/α cDNA so strong that we would not have been able to detect an a/α difference. Thus, we may have missed 5–10 clones. The more important class contains genes whose transcripts are present in very low abundance or unstable to iso-

lation and cDNA synthetic procedure. We estimate that we can detect genes whose transcripts are present in 5–10 copies per cell; since >90% of all clones gave detectable signals in the initial screen, we must be detecting most of the middle abundance sequences and possibly lower abundance sequences as well. Many of the less highly expressed transcripts may have escaped our screen, however. In addition, of course, those genes whose transcripts are not polyadenylylated will not be represented among the sequences in our probe.

The four sequences we have studied have several characteristics in common. Most interestingly, transcripts of all of them appear between 5–7 hr after the shift to sporulation medium and decrease in abundance by 11–13 hr. The fact that our probe was prepared from mRNA isolated from cells at various stages of sporulation, the earliest of which was at 7 hr, may account for this coincidence. On the other hand, it may be that a significant number of sporulation-specific genes actually begin to be transcribed at this time, and our group is a representative sample. The fact that the *SAG* gene product appears at about this time, as shown by methods independent of the hybridization screen used here, is interesting in this context.

A second significant characteristic of three of these sequences is the presence of two coordinately controlled transcripts. A number of possible explanations exist for these transcripts. One is that we are seeing either processing events or a failure to terminate transcription part of the time. Another possibility is that there are two promoters for each gene, both of which are developmentally regulated. Finally, it is possible that each clone contains two clustered genes, both of which are sporulation specific. The last possibility might be examined by R-looping experiments. Whatever the explanation, it seems likely that the phenomenon may have some developmental significance; while dual transcripts from a single gene are not unknown in yeast (35, 36), they are rare and it seems improbable that three separate genes would exhibit this behavior by chance.

The role of these differentially expressed genes in the sporulation process is not known. It is possible that they represent genes whose products are required for the meiotic divisions or spore formation; they may also represent transcripts that are stored in the spore in preparation for germination. It is known that the spore contains a large number of transcripts (22) and that these include histone RNAs as well as those for glycolytic enzyme (D. Kaback, personal communication). Some of the clones we have identified may correspond to these RNAs.

The success of this screening method in identifying developmentally controlled genes whose transcripts are present in relatively low abundance is somewhat surprising. Although analogous methods have been used to identify conidiation-specific genes in *Aspergillus* (37), in that system the probe was enriched by "cascade hybridization" for specific sequences (38). By amplifying the "receptor" DNA on the filter by simple patching, we have been able to bypass the enrichment step. We expect that analysis of the structure and transcriptional control of genes described here may give important insights into sporulation in particular and eukaryotic development in general.

We gratefully acknowledge the aid of Dr. E. F. Fritsch in the construction of the library. We thank Jerry Dodgson and Ronald Patterson for advice and many helpful discussions, Larry Snyder for critically reading the manuscript, and the departmental clerical staff for help in preparing it. This work was supported by National Science Foundation Grant PCM 78-12581-04. A.L.K. and D.J.S. were supported by a grant from the National Foundation–March of Dimes to the Michigan State University College of Human Medicine. This is journal article no. 10,448 from the Michigan Agricultural Experiment Station.

1. Hartwell, L. H. (1974) *Bacteriol. Rev.* **38**, 164–198.
2. Haber, J. E. & George, J. P. (1979) *Genetics* **93**, 13–35.
3. Hicks, J., Strathern, J. M. & Klar, A. J. S. (1979) *Nature (London)* **82**, 478–483.
4. Nasmyth, K. A. & Tatchell, S. K. (1980) *Cell* **19**, 753–764.
5. Oshima, T. & Takano, I. (1980) *Genetics* **94**, 859–870.
6. Rine, J., Spragne, G. F., Jr., & Herskowitz, I. (1981) *Mol. Cell. Biol.* **1**, 958–960.
7. Croes, A. F. (1967) *Planta* **76**, 209–226.
8. Esposito, M. S. & Esposito, R. E. (1974) *Genetics* **78**, 215–225.
9. Esposito, M. S., Esposito, R. E., Arnaud, M. & Halvorson, H. O. (1969) *J. Bacteriol.* **100**, 180–186.
10. Magee, P. T. & Hopper, A. K. (1974) *J. Bacteriol.* **119**, 952–960.
11. Game, J. C., Zamb, T. J., Braun, R. J., Resnick, M. & Roth, R. M. (1980) *Genetics* **94**, 51–68.
12. Hopper, A. K., Magee, P. T., Welch, S. K., Friedman, M. & Hall, B. D. (1974) *J. Bacteriol.* **119**, 619–629.
13. Roth, R. & Lusbak, K. (1970) *Science* **168**, 493–494.
14. Esposito, R. E. & Klapholz, S. (1981) in *The Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance*, eds. Strathern, J., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 211–287.
15. Roth, R. & Halvorson, H. O. (1969) *J. Bacteriol.* **98**, 831–832.
16. Wright, J. F., Ajam, N. & Dawes, I. W. (1981) *Mol. Cell. Biol.* **1**, 910–918.
17. Trew, B. J., Frieson, J. & Moens, P. (1979) *J. Bacteriol.* **138**, 60–69.
18. Kraig, E. & Haber, J. E. (1980) *J. Bacteriol.* **144**, 1098–1112.
19. Colonna, W. J. & Magee, P. T. (1978) *J. Bacteriol.* **134**, 844–853.
20. Clancy, M. J., Smith, L. M. & Magee, P. T. (1982) *Mol. Cell. Biol.* **2**, 171–178.
21. Rimm, D. L., Horness, D., Kucera, J. & Blattner, F. R. (1980) *Gene* **12**, 301–309.
22. Harper, J. F., Clancy, M. J. & Magee, P. T. (1980) *J. Bacteriol.* **143**, 958–965.
23. Hereford, L. M., Fahrner, K., Woolford, J., Jr., Rosbach, M. & Kaback, D. (1979) *Cell* **18**, 1261–1271.
24. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1981) *A Manual in Genetic Engineering: Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
25. Hohn, B. & Murray, K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3254–3263.
26. St. John, T. P. & Davis, R. W. (1979) *Cell* **16**, 443–452.
27. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
28. Engel, J. D. & Dodgson, J. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2856–2860.
29. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
30. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
31. McMaster, G. K. & Carmichael, G. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4838.
32. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
33. Kramer, R. A. & Anderson, N. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6541–6545.
34. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **75**, 5350–5354.
35. Carlson, M. & Botstein, D. (1982) *Cell* **28**, 145–154.
36. Perleman, D. & Halvorson, H. O. (1981) *Cell* **25**, 525–536.
37. Zimmerman, C. R., Orr, W., Leclerc, R., Barnard, E. & Timberlake, W. (1980) *Cell* **21**, 709–715.
38. Timberlake, W. E. (1980) *Dev. Biol.* **78**, 497–510.

APPENDIX B

APPENDIX B

DISRUPTION OF THE SPORULATION-SPECIFIC GENE *SPR2*

A lambda clone bearing a DNA fragment which contains the sporulation-specific gene *SPR2* was among those isolated by the differential hybridization method (Appendix A). Because of its strong signal during the screen it was selected for further study and subcloned into an *E.coli* plasmid. A large (5.7 kb) fragment was deleted from the original DNA insert and replaced with a selectable marker (*URA3*) to see if disruption would affect sporulation. The disrupted fragment was transformed into a homothallic wild type strain that was then sporulated and the asci were harvested. Upon tetrad dissection, progeny were found to segregate 2:2 for sporulation-competence. Northern analysis revealed the presence of two transcripts from the region, and the genes were designated *SPR2* (SPorulation-Regulated) and *HKP2* (HouseKeePing). *HKP2* is expressed significantly during vegetative growth as well as during sporulation.

A restriction map of the isolated DNA fragment is pictured at the top of Figure 1. Beneath it are wavy lines that represent the expressed messages and their transcriptional orientation. Shown at the bottom of Figure 1 is the 5.7 kb *Hin*dIII-*Hin*dIII deletion that was thought, initially, to be responsible for loss of sporulation capability. Four *LEU2* disruptions are also pictured.

Surprisingly, none of the *LEU2* disruptions interfered with

diploidized. This result led to a further search of the region for other genes, and the *sterile* gene *STE4* was discovered. The location of *STE4* is shown in Figure 2.

The asporogenous phenotype of the strains that are homozygous for the 5.7 kb deletion is due to the disruption of *STE4*. Without a functional *STE4*, these strains are sterile (cannot mate) and remain in the haploid state, unable to sporulate.

The functions of *SPR2* and *HKP2* remain unknown.

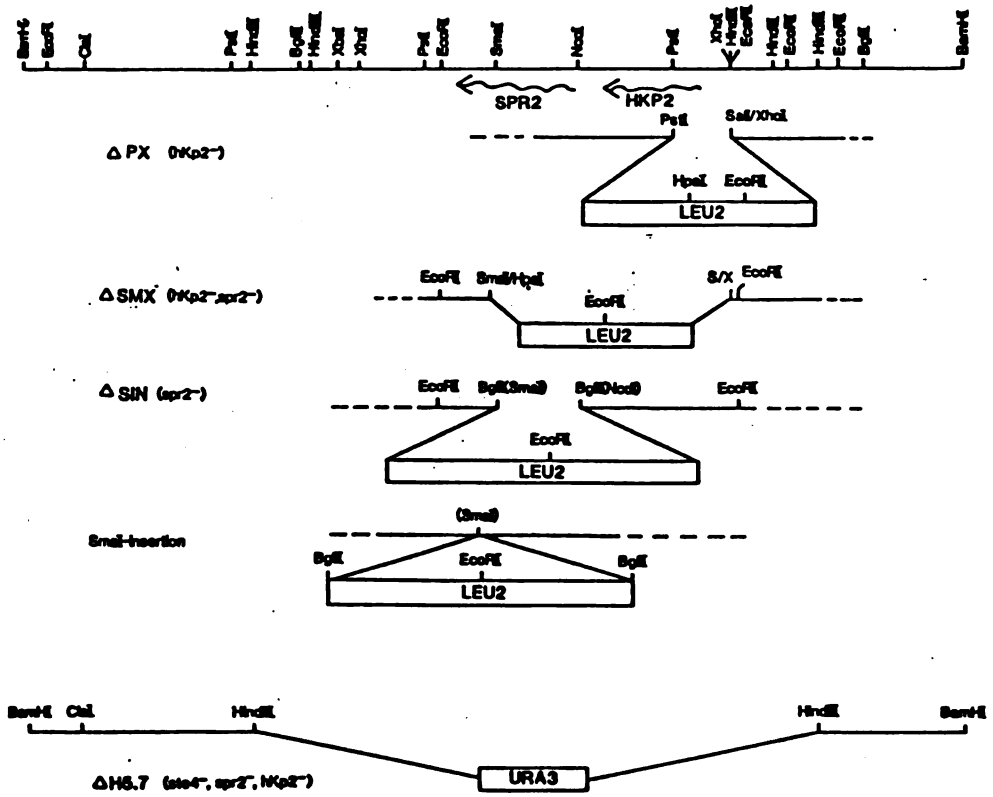


Figure 1. Gene disruption at the *SPR2* locus.

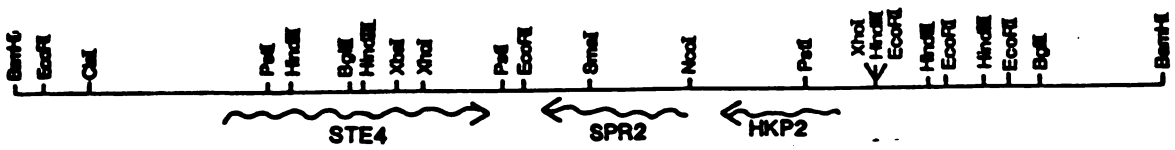


Figure 2. Location of the *STE4* transcript.

APPENDIX C

Identification and Characterization of Mutations Affecting Sporulation in *Saccharomyces cerevisiae*

Linda M. Smith,* L. G. Robbins,† A. Kennedy* and P. T. Magee*[1]

* *Department of Microbiology and* †*Department of Zoology, Michigan State University, East Lansing, Michigan 48824*

Manuscript received May 4, 1988
Revised copy accepted August 18, 1988

ABSTRACT

Mutations affecting the synthesis of the sporulation amyloglucosidase were isolated in a homothallic strain of *Saccharomyces cerevisiae*, SCMS7-1. Two were found, both of which were deficient in sporulation at 34°. One, SL484, sporulated to 50% normal levels at 30° but less than 5% at 34° or 22°. The other, SL641, failed to sporulate at any temperature. Both mutants were blocked before premeiotic DNA synthesis, and both complemented *spo1*, *spo3*, and *spo7*. Genetic analysis of the mutation in SL484 indicated linkage to *TRP5* and placed the gene 10 map units from *TRP5* on chromosome *VII*. A plasmid containing an insert which complements the mutation in SL484 fails to complement SL641. We therefore conclude that these two mutations are in separate genes and we propose to call these genes *SPO17* and *SPO18*. These two genes are (with *SPO7*, *SPO8*, and *SPO9*) among the earliest identified in the sporulation pathway and may interact directly with the positive and negative regulators *RME* and *IME*.

SACCHAROMYCES cerevisiae normally can grow mitotically in either the haploid or diploid state. Mating type is conferred by two alleles, the a allele and the α allele, at the mating-type locus, *MAT*. *MAT*a and *MAT*α haploid strains produce mating pheromones, a-factor and α-factor respectively, and can mate to form diploid cells of the genotype *MAT*a/*MAT*α which do not produce pheromones or mate. When incubated in a medium that lacks nitrogen and contains a carbon source that can be respired, such as acetate, *MAT*a/*MAT*α diploids will divide meiotically to give rise to four haploid ascospores. A number of biochemical processes have been observed in sporulating cells, including DNA synthesis, RNA and protein synthesis and degradation (ESPOSITO and KLAPHOLZ 1981; RESNICK *et al.* 1984; WEIR-THOMPSON and DAWES 1984; WILLIAMSON *et al.* 1983), alterations in the synthesis or activity of particular proteins (DAWES *et al.* 1983; ESPOSITO and KLAPHOLZ 1981; OTA 1982; RESNICK *et al.* 1984), modification of histones (MARIAN and WINTERSBERGER 1982), synthesis of storage molecules such as lipids and carbohydrates (KANE and ROTH 1974; HOPPER *et al.* 1974) and the eventual degradation of glycogen as mature ascospores appear (HOPPER *et al.* 1974). Some of the biochemical events seen in sporulating cells are also observed in asporogenous cells incubated in sporulation medium and are thought to be a response to the starvation conditions that induce sporulation. Other events are specific to sporulation; these include DNA synthesis (ROTH and LUSNAK 1970; RESNICK *et al.* 1984), RNA degradation (HOPPER *et al.* 1974), synthesis of neutral lipids after about 12 hr of incubation (ESPOSITO and KLAPHOLZ 1981), synthesis of a spore-surface-specific antigen (DAWES *et al.* 1983), and glycogen degradation (HOPPER *et al.* 1974). There are also reports of meiosis-specific increases in the activity of DNA polymerases I and II, two different deoxyribonucleases (RESNICK *et al.* 1984), and 1,3-β-glucanases (DEL RAY *et al.* 1980; HIEN and FLEET 1983), as well as a change in the properties of RNA polymerase II (MAGEE 1974), and a loss of mitochondrial circular RNAs (SCHROEDER, BREITENBACH and SCHWEYEN 1983).

This complex series of events has recently been shown to depend on two central regulatory genes, *RME* and *IME*. *RME* is a negative regulator of meiosis; mutations in this gene allow haploids or diploids homozygous at the mating type locus to sporulate (MITCHELL and HERSKOWITZ 1986). *IME* is a positive regulator which leads to sporulation when present in a high copy number plasmid even when *RME* is present and active (MARGOLSKEE *et al.* 1986). Both these genes act before premeiotic DNA synthesis. Mutations in three *SPO* genes have been shown to block cells before premeiotic S; these mutants are *spo7*; *spo8* and *spo9* (ESPOSITO and KLAPHOLZ 1981). The molecular function of these genes is unknown.

COLONNA and MAGEE (1978) first described the enzyme responsible for the extensive degradation of glycogen specific to sporulating cells. The glycogen-degrading enzyme, sporulation amyloglucosidase (SAG), appears after 8–10 hr of incubation in sporu-

[1] Present address: Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108; to whom correspondence should be addressed.

TABLE 1

Genotypes of *S. cerevisiae* strains used in this paper

Strain	Genotype	Source
SCMS7-1	a/a (*HO leu*^{2}-112 *his*^{4} *ade*)	This laboratory
SL484	Same as SCMS7-1 except spo$^-$	This study
SL641	Same as SCMS7-1 except spo$^-$	This study
6-3A	a/α (HO *ura*^{3} *lys*^{2} *met*^{4} *adex* (*spo17*)	This study
XMB4-12b	α *sst1* Killer + *arg9 ilv3 ura1*	I. Herskowitz
RC757	α *sst2-1 met1 his6 can1 cyh2*	I. Herskowitz
W66-8A	a/α (*HO ade2-1 leu1 trp5-2 ura3-1 met4-1 lys2-1*)	R. Rothstein
74-1A	a/α (*HO arg4-1 spo1-1*)	R. E. Esposito
89-1D	a/α (*HO arg4-1 met4 spo3-1*)	R. E. Esposito
C52-4B	a/α (*HO arg4-1 spo7-1*)	R. E. Esposito

lation medium. Its appearance coincides with the onset of glycogen degradation and with the first appearance of mature spores. The analysis of sporulation mutants (R. E. Esposito, personal communication) suggests that the sporulation pathway branches after the initial event of premeiotic DNA synthesis. The event(s) critical for SAG appearance occurs in the pachytene stage of meiosis (Clancy, Smith and Magee 1982), and is therefore very close to this decision point. The enzyme, however, is not required for cells to complete sporulation (Yamashita and Fukui 1985).

In this study, we have capitalized on SAG's position in the sporulation pathway to identify mutations blocked early in sporulation. Two mutants were isolated: SL484, which is temperature-sensitive for SAG production and for sporulation, and SL641, which fails to produce SAG and sporulate under any conditions. Spheroplast fusion was used to analyze SL641.

MATERIALS AND METHODS

Yeast strains: The strains of *S. cerevisiae* used in these experiments and their genotypes are given in Table 1.

Media, growth and sporulation of cells: Standard yeast media were essentially as described by Sherman, Fink and Hicks (1981). For some sporulation experiments, the presporulation (PSP) medium of Roth and Halvorson (1969) was used. In other experiments, YEPacetate [10 g yeast extract, 10 g peptone, 10 g potassium acetate and 100 ml 0.1 M phthalate buffer (pH 5.2) per liter] was used to adapt cells to growth on acetate. Cells were sporulated in liquid SPM (Colonna and Magee 1978) at the indicated temperature after pregrowth in either PSP or YEPacetate as described previously (Clancy, Smith and Magee 1982). To sporulate cells on solid medium, patches of cells were grown overnight on YEPD, then replica-plated onto PSP and incubated 24–48 hr. The patches were then transferred to SPM using toothpicks, and incubated for three days.

Preparation of cell extracts: Washed cells were suspended at a cell density of 1×10^7 to 3×10^7 cells per ml in 0.1 M sodium citrate buffer (pH 6.2), hereafter called citrate buffer, that contained 0.3 mg per ml of the protease inhibitor phenylmethylsulfonylfluoride (PMSF) and the protease inhibitor aprotinin (Sigma Chemical Co.) at a concentration of 200 KU (kallikreinin units) per ml. Cells were broken by blending in a vortex with glass beads (0.45 mm diameter; B. Braun Melsugen AG, Germany). The cells were alternately vortexed for 15 sec and cooled on ice for 15 sec until they had been vortexed a total of 3–5 min. Breakage was always greater than 90%. The broken cell suspension was immediately centrifuged at 12,000 × *g* for 20 min and then at 45,000 rpm for 2 hr in a Beckman type 65 rotor. The supernatant was dialyzed for 16–24 hr at 4° against two changes of citrate buffer. The extract was generally assayed for SAG immediately after dialysis.

SAG and protein assays: Qualitative and quantitative assays for SAG were done using a coupled assay system which has been described previously (Clancy, Smith and Magee 1982; Colonna and Magee 1978). For qualitative analyses, a whole cell assay was employed. In these assays, 0.2 ml of citrate buffer containing 0.33 mM *p*-chloromercuribenzoate (PCMB) was added to cells permeabilized by air drying on Whatman 3MM filter paper (Mowshowitz 1976). Duplicate 2.1 cm filters were made in each experiment. To one filter, 0.4 ml of a 1% solution of glycogen (Sigma, type II from oyster) in citrate buffer was added. To the second filter, 0.4 ml of citrate buffer was added. The final volume of the reaction mixture for each filter was 0.6 ml. The filters were incubated at 30° or 34° for 18–24 hr and then 1.2 ml of glucose oxidase reagent was added. After 30–60 min of incubation at 30°, 0.8 ml of concentrated HCl was added and the reaction mix vortexed immediately. The duplicate filters were compared, and SAG activity was indicated by formation of greater color by the cells incubated with glycogen.

For quantitative determinations, aliquots of a cell extract (0.05–0.1 ml per 0.6 ml total reaction volume) were incubated at 22°, 30°, or 34° in an assay mixture containing citrate buffer, 0.66% glycogen and 0.33 mM PCMB. At various times, 0.6 ml samples were removed and boiled for 10 min. The samples were centrifuged and the amount of glucose present in the supernatant was determined using glucose oxidase reagent (Colonna and Magee 1978). D-Glucose was used as a standard. Specific activity was expressed in milliunits per mg of protein, where one unit is defined as 1 mmol of glucose released per minute. Protein was determined by the method of Lowry *et al.* (1951).

Isolation and mutagenesis of spores: A 300-ml culture was sporulated and the asci harvested by centrifugation. The asci were resuspended in 10 ml of 24 mM phosphate buffer (pH 7.0) containing 20 μl of 2-mercaptoethanol and 15 mg Zymolyase 60,000 (Kirin Brewery, Japan). The digestion mixture was incubated at 30° with gentle shaking for 1 hr. The spores were harvested by centrifugation and washed three times with sterile distilled water. Aggregates of spores were disrupted by sonicating the spore suspension for three 30-sec bursts. The spores were washed two times in a sterile 1% solution of Tween 80 in distilled water, then finally washed two times in sterile distilled water and plated onto YEPD at a concentration of 1000–2000 spores per plate. The spores were mutagenized by UV irradiation for 60 sec (10% survival), and incubated in the dark at 34°.

The surviving ascospore colonies that were to be screened for SAG activity were inoculated onto solid YEPD, incubated overnight, then replicated twice to premoistened 3MM Whatman filter paper lying atop plates of PSP containing histidine, leucine and adenine. After two days of incubation, the ascospore colonies growing on the filters were shifted to SPM by simply transferring the filter to leucine-supplemented SPM plates. The colonies were incubated in SPM for 3 days, then the filters were removed and air dried for 4–5 hr at room temperature. The individual

colonies were separated and assayed using the whole cell assay for SAG.

Test for pheromone production: The ability to produce mating pheromones was determined using the procedure of SPRAGUE and HERSKOWITZ (1981). Patches of cells to be tested were replica-plated onto BBMB plates spread with either XMB4-12b or RC757.

Nuclear staining and measurement of DNA synthesis: Progress through meiosis was monitored cytologically with the fluorescent dye 4,6-diamidino-2-phenylindole (DAPI) as described previously (CLANCY, SMITH and MAGEE 1982). DNA synthesis was measured by collecting triplicate, one ml samples of cells incubated in SPM at the indicated times. The cells were frozen and stored until DNA content was assayed using the fluorescent compound 3,5-diaminobenzoic acid (DABA) (HESSE, LINDNER and KREBS 1975). Calf thymus DNA was used as a standard.

Spheroplast fusion: A modification of the procedure used by KAKAR and MAGEE (1982) was used. A 10-ml culture of cells was grown overnight in YEPD at 30°, then diluted with 15 ml fresh YEPD and incubated for 1.5–3 hr to allow the cells to reenter log phase growth. Approximately 1×10^9 to 3×10^9 cells were harvested, washed once in distilled water, once in sorbitol-phosphate [1 M sorbitol, 0.1 M potassium phosphate (pH 7.5)] and then resuspended in sorbitol-phosphate containing 5 mg Zymolyase 5,000 (Kirin Brewery, Japan) and 12.5 μl 2-mercaptoethanol. The cells were incubated at room temperature in the spheroplasting mix until greater than 90% of the cells were converted to spheroplasts. This was determined by checking the degree of lysis in either 5% sodium dodecyl sulfate or distilled water. The spheroplasts were washed five times with sorbitol phosphate by centrifugation for 10 min at approximately $700 \times g$. All subsequent centrifugations were done in this manner. Spheroplasts to be fused were mixed in a 1:1 ratio. Generally, between 1×10^8 and 5×10^8 spheroplasts of each strain were used. The mixed spheroplasts were incubated for 20 min at room temperature, then centrifuged and resuspended in five ml of fusion mix [30% (wt/vol) polyethylene glycol 4,000, 10 mM $CaCl_2$, and 10 mM Tris (pH 7.5)]. The spheroplasts were incubated in the fusion mix for 1 hr at room temperature and then stored overnight at 4°. Prior to being plated, the spheroplasts were centrifuged, resuspended and incubated for 20 min at room temperature in 5 ml SOS, a recovery broth (SHERMAN, FINK and HICKS 1981). They were then centrifuged, resuspended in 1 ml of sorbitol-phosphate and either 0.01 or 0.1 ml of spheroplasts was mixed with 5 ml molten minimal agar medium containing 1 M sorbitol (MIN-sorbitol) held at 50°, then poured immediately onto MIN-sorbitol plates. The plates were incubated at 30° for 4–10 days. Prototrophic colonies were streaked onto MIN plates and in some cases transferred to YEPD plates where they were stored at 4° until needed.

Segregation analysis of mutants: Strains heterozygous for the sporulation defect were constructed by either spore-to-spore matings or spheroplast fusions. The heterozygotes were sporulated and the tetrads dissected by micromanipulation. Nutritional requirements were determined by replica-plating from YEPD master plates to various nutrient drop-out plates. The SAG phenotype and the ability to sporulate were determined in most experiments by incubating 10-ml cultures at either 30° or 34° in SPM after pregrowth in YEPacetate. Cultures were examined microscopically to ascertain the percent sporulation and SA was assayed using the whole cell assay by spotting 50 μl of cell suspension on 3MM Whatman filter paper circles (2.3 cm) and air drying for 2–4 hr. In some experiments, only the percent sporulation was determined after cells were sporulated on solid SPM.

Complementation tests: Strains heterozygous for two different sporulation defects were constructed by either spore-to-spore matings or spheroplast fusions. These strains were sporulated in 10-ml cultures at 25°, 30° or 34°. The ability to sporulate and the percent sporulation was determined. SAG was assayed using the whole cell assay after spotting cells onto 3MM Whatman filter paper circles and air drying as described above.

Transformation: Yeast transformation was carried out as described by HINNEN, HICKS and FINK (1978).

RESULTS

Mutagenesis: Homothallic strains can be used to isolate recessive and dominant mutations in genes that function only in diploids (ESPOSITO and ESPOSITO 1969). Through the action of the homothallism allele, *HO*, any mutation generated by mutagenizing the haploid ascospores of the homothallic strain will be present in a homozygous condition in the diploid colony that arises from each spore.

Ascospores from the homothallic strain SCMS7-1 were mutagenized by UV irradiation. The 2528 surviving ascospore colonies were prescreened in two ways. First, the ability of the survivors to grow in MIN medium that contained histidine, leucine, and adenine was tested, to eliminate nutritional defects other than those present in the parent strain. Second, the colonies were tested for their ability to grow on YPG, a medium that is commonly used to determine respiratory competence (SHERMAN, FINK and HICKS 1981), since cells must be able to respire in order to sporulate. A total of 2168 colonies were identified by these two criteria for screening for SAG activity.

Screen for SAG activity: Duplicates of each colony were sporulated on 3MM Whatman filter paper, then air dried and assayed for SAG. Included were *MATa/MATα* (positive) and *MATă/MATα* (negative) controls. For each ascospore colony tested, one of the duplicates was assayed in a reaction mixture containing glycogen, the substrate for SAG, and the other was assayed in a reaction mixture lacking glycogen. If a visual comparison showed that the duplicate assayed with glycogen produced more color than the duplicate assayed without glycogen, the colony was scored as positive for SAG. If the duplicates had an equal amount of color, the colony was scored as negative for SAG. Using this screening procedure, 296 putative SAG-minus colonies were identified. One hundred forty-one of these were retested for SAG activity in liquid culture at 34° using the whole cell assay. Twenty-one colonies were deficient for SAG and were characterized further.

Pheromone production: The screening procedure used in this study asked only whether or not the cells produced SAG. Thus, in addition to the expected identification of defects in early sporulation functions and in the structural and regulatory sequences for

TABLE 2

Specific activity of SAG in mutant strains and in their parent

Strain	Specific activity following time in sporulation medium (hr): 0		24	48	72
SL484	0.170	34°	1.13	2.52	8.34
		30°	3.22	19.9	35.1
		22°	0.35	0.56	0.81
SL641	0.138	34°	1.06	1.10	1.99
		30°	0.157[a]	1.04[a]	1.81[a]
		22°	0.531[a]	0.267[a]	0.312[a]
SCMS7-1	0.415	34°	48.6	47.8	46.2
		30°	28.0	28.6	27.4
		22°	54.8[a]	88.2[a]	111[a]

Cells were incubated in SPM at the temperature indicated after pregrowth in PSP.

[a] Results of a single experiment.

SAG, mutants defective in *HO*, *MAT*, and mating functions might also be isolated. Such nonsporulation-specific mutants can be differentiated from the sporulation mutants based on the production of mating pheromones and on ploidy.

Of the 21 SAG-minus colonies identified after retesting for SAG activity in liquid cultures, two, SL484 and SL641, did not produce either mating pheromone and were characterized further. The ploidy of these strains will be considered later.

Specific activity of SAG in mutant strains: SL484, SL641 and their parent were incubated at 22°, 30° or 34° in SPM. At the indicated times, samples of cells were harvested and used to determine the specific activity of SAG (Table 2). Unless otherwise indicated, the results of two or more experiments are expressed as the mean specific activity. Most of the extracts were assayed at all three temperatures. No significant difference was found between the different assay temperatures and only the results obtained when the extracts were assayed at 30° are presented.

When incubated in SPM at 22° and 34°, SL484 had, at the most, one-fifth the specific activity of the parent strain, SCM7-1. However, at 30°, and after 72 hr in SPM, SL484 exhibited wild type levels of SAG. The only difference between SL484 and SCMS7-1 at 30° was that SCMS7-1 attained full activity by 24 hr and changed little thereafter. SL484, on the other hand, did not reach maximal activity until after approximately 48 hours of incubation. Thus, SL484 is temperature sensitive for SAG production, with 30° being the permissive temperature. SL641 exhibited approximately the same activity at all temperatures and at all incubation times. Its specific activity varied from 1/15 to 1/200 of wild-type activity. Thus, SL641 is not temperature sensitive for SAG production.

Sporulation and meiotic behavior of the mutants: The ability of the mutant strains to sporulate is shown in Table 3. The results presented are the average of at least two determinations. SL641 failed to sporulate at all three temperatures. SL484 sporulated very poorly at 22° and 34°, but, paralleling the SAG activity, it sporulated at nearly wild-type levels at 30°.

TABLE 3

Sporulation ability of mutants and their parent

Strain	Temperature	Percent sporulation at following time in SPM (hr): 24	48	72
SL484	34°	0.3	1.3	2.3
	30°	3.9	21.5	38.7
	22°	0[a]	0.6	1.3
SL641	34°	0	0	0
	30°	0	0	0
	22°	0	0	0
SCMS7-1	34°	35.1	47.1	46.8
	30°	38.2	67.9	69.4
	22°	44.4[a]	72.0[a]	73.6[a]

Cells were incubated in SPM at the indicated temperature after pregrowth in PSP. Unless otherwise noted, the values presented are the average of at least two determinations.

[a] Results of a single experiment.

TABLE 4

Progress of the mutants through meiosis

Hours in SPM	Percent cells	Strain SL484	SL641	SCMS7-1
24	Mono	99.0	99.0	21.0
	Bi	1.0	0.3	20.3
	Tetra	0	0.7	25.2
	Asci	0	0	33.4
48	Mono	99.3	97.3	33.9
	Bi	0.7	2.0	4.6
	Tetra	0	0.7	4.3
	Asci	0	0	57.2
72	Mono	100	89.7	20.6
	Bi	0	7.9	6.0
	Tetra	0	2.3	12.3
	Asci	0	0	61.1

Cells were incubated in SPM at 34° after pregrowth in PSP at 30°.

The progression of the mutants along the meiotic pathway was also examined. The nuclei of the cells were stained with the fluorescent stain DAPI which binds DNA and renders the nuclei visible when the cells are observed by fluorescence microscopy. The results of one experiment are shown in Table 4. For both mutants, most cells remained mononucleate. For SL641, the small percentage of cells (8%) that appears to progress through one or both nuclear divisions should be regarded with caution. Mitochondrial DNA is also stained by DAPI and we have observed that when cells are incubated in SPM but are unable to sporulate, large vacuoles are formed and the mito-

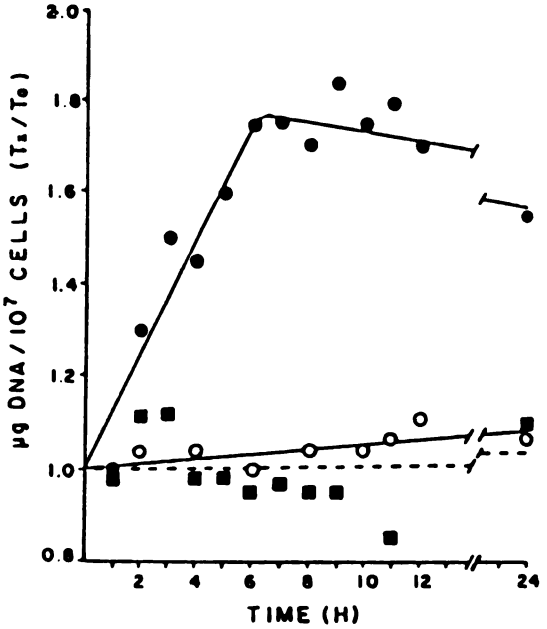


FIGURE 1.—Premeiotic DNA synthesis in mutant strains and their parent. Cells were pregrown in PSP then incubated at 34° in SPM. At the indicated times, 1-ml samples of cells were removed and frozen until assayed for DNA content. DNA content is expressed as the ratio of DNA at each time point (Tx) to DNA at time zero (To). Premeiotic DNA synthesis in the parent SCMS7-1 (●——●), and the mutants SL484 (○——○) and SL641 (■——■).

chondria are concentrated in a small area in the cell. Under such conditions, it is sometimes difficult to distinguish a group of mitochondria from the nucleus. Thus, these results are best interpreted as an indication that neither of the mutants progressed through the first meiotic division.

Premeiotic DNA synthesis: The occurrence of premeiotic synthesis in SL484 and SL641 was determined. Cells incubated in SPM at 34° were sampled after various times of incubation, and the DNA content of the cells measured using the fluorescent compound DABA. The results are shown in Figure 1. The parent strain began synthesizing DNA almost immediately, and completed the synthesis within six hours of incubation. During that time, the DNA content almost doubled. The failure to double completely the DNA content reflects the fraction of cells that failed to enter the meiotic pathway. In contrast, neither SL484 nor SL641 synthesized DNA. The DNA content of the strains at zero time was 0.301, 0.446, and 0.286 g of DNA per 1×10^7 cells for SL484, SL641, and SCMS7-1, respectively. These values are within 72–112% of the values reported for diploid *S. cerevisiae* strains, using the same and other methods of measurement (LAUER, ROBERTS AND KLOTZ 1977; MARAZ, KISS and FERENCZY 1978).

Segregation of the SL484 defect: Strains heterozygous for the SL484 defect were constructed by spore-to-spore matings of SL484 and the wild type strain W66-8A. The diploids produced by such matings are heterozygous at a number of loci. All ascospores produced by SL484/W66-8a diploids contain the *HO* allele and diploidize. Therefore, each ascospore colony can be tested for its ability to sporulate and produce SAG. SL484/W66-8a diploids were sporulated and the tetrads characterized for nutritional genetic markers and for their ability so sporulate after incubation on SPM plates for three days at 34°. SAG activity was not assayed. All phenotypes, including the ability to sporulate segregated 2+:2−. These results indicate that the sporulation deficiency in SL484 is the result of a defect in a single gene.

TABLE 5

Linkage analysis of SL484 defect

Linkage to:	PD[a]	NPD[b]	TT[c]	Map distance[d]
HIS4	4	3	8	Unlinked
URA3	8	7	10	Unlinked
MET4	2	7	15	Unlinked
LYS2	4	4	14	Unlinked
TRP5	20	0	5	10 m.u.

[a] PD is parental ditype ascus.
[b] NPD is nonparental ditype ascus.
[c] TT is tetratype ascus.
[d] Map distance is the recombination frequency times 100.

Linkage of the SL484 defect to *HIS4*, *URA3*, *MET4*, *LYS2* and *TRP5* was tested. The requirements for leucine and adenine were not studied, since two genes were segregating for each phenotype and these genes are not directly distinguishable in the progeny. The numbers of parental ditype, nonparental ditype, and tetratype asci are shown in Table 5. Only tetrads showing 2+:2− segregation for each gene were considered. For the *HIS4*, *URA3*, *MET4* and *LYS2* genes, the ratio of parental ditypes to nonparental ditypes was approximately 1:1 (by χ^2 analyses) indicating that these genes are not linked to the SL484 defect. However, with the *TRP5* gene, most of the tetrads were parental ditypes suggesting that the two genes are linked and 10 map units apart on chromosome *VII*. We call the gene affected by mutation in SL484 *SPO17*.

Ploidy of the mutants: The DNA contents of SL484 and SL641 suggest that both are diploids. The genetic analyses of the mutants also support this conclusion. SL484 sporulated at 30°, and its spores mated to give rise to diploids that segregated various genetic markers in a normal diploid fashion. Since SL641 was unable to sporulate, genetic analysis was undertaken by fusing spheroplasts of SL641 and W66-8A (SMITH 1985). The normal tetraploid segregation observed for the SAG-deficient phenotype in the SL641/W66-8a derived fusant is consistent with the conclusion that

SL641 is diploid. The viabilities of spores produced by the SL641 fusant also support this conclusion. If SL641 were haploid, fusion with W66-8A would have created a triploid strain. Sporulation of triploid yeast strains generally yields less than 15% viable spores (PARRY and COX 1970) while tetraploids exhibit good spore viability. If SL641 were diploid, then the fusion product would be tetraploid. The SL641 derived-fusant exhibited greater than 50% spore viability. The segregation of sporulation ability from the SL641/W66-8a fusant suggested that the SL641 defect was due to a single mutation. When compared in pairs with each of three markers from W66-8a, *lys2*, *ura3*, and *his4*, the sporulation defect segregated as a single gene, in that the numbers of the type I, II, and III asci were not significantly different from the expected values (data not shown) (ROMAN, PHILLIPS and SANDS 1955). Furthermore, *trp5* segregated aberrantly in the fusion examined, suggesting that the fusion was aneuploid for chromosome *VII*. Since the defect segregated normally, while the chromosome in which *spo17* is located did not, it seems highly unlikely that the two mutations affect the same gene (see below). We are therefore calling the affected gene *SPO18*.

Complementation tests: Three strains, C52-4B, 74-1A and 89-1D, which are defective in premeiotic DNA synthesis (*spo7*), the first meiotic division (*spo1*), and spore formation (*spo3*), respectively, were available in this laboratory for complementation tests. It was previously determined that at 34°, only *spo7* mutants do not exhibit SAG activity (CLANCY, SMITH and MAGEE 1982) (data not shown). Thus, though *spo1* and *spo3* mutants fail to sporulate, they progress far enough along the sporulation pathway to produce SAG. We asked then, whether the *spo* mutants would complement the SAG and/or sporulation deficient phenotype of SL484 and SL641. *trans*-Heterozygotes of *spo7* and SL484, and *spo3* and SL484 were constructed by spore-to-spore matings. A tetraploid heterozygous for both the *spo1* and the SL484 defects was constructed by fusing spheroplasts of the two strains. *trans*-Heterozygotes of SL641 and the three sporulation mutants were also constructed by spheroplast fusion. Ten-milliliter cultures of the heterozygotes were incubated at 34° and at the permissive temperature, either 25° or 30°, in SPM and examined for SAG activity using the whole cell assay for SAG. The results are shown in Table 6. All six heterozygotes produced SAG and sporulated to the same magnitude at both the restrictive and permissive temperatures. Although the percent sporulation was low for the SL484/*spo1*, SL641/*spo7* and SL641/*spo1* fusants, none of the parents sporulate to this degree at 34°. It is important to note that the *spo7* strain, C52-4B, used in this study is unconditionally asporogenous in our heads. This may explain the low level of sporulation in the heterozygotes. These results indicate that the SL484 and the SL641 mutations are probably not in the *SPO1*, *SPO3* and *SPO7* genes, although we cannot rule out intragenic complementation.

TABLE 6

Complementation tests: SL484 and SL641 vs. *spo1*, *spo3*, and *spo7* strains

Cross[a]	Sporulation defect	% Sporulation	SAG Activity
SL484 × *spo7*[b]	DNA synthesis	35.1	+
SL641 × *spo7*		16.3	+
SL484 × *spo1*	Meiosis I	17.0	+[c]
SL641 × *spo1*		4.2[c]	+[c]
SL484 × *spo3*[b]	Spore formation	50.5	+
SL641 × *spo3*		55.1	+

[a] Crosses of SL484 were done as spore-spore matings. Crosses of SL641 were done as spheroplast fusions. Unless otherwise noted, a culture of cells growing in YEPacetate was shifted into SPM and half of the culture was incubated at 34° and the other half was incubated at the permissive temperature, either 25° or 30°. After 48 h of incubation, the cultures were examined for sporulation and SAG was assayed using the whole cell assay described in MATERIALS AND METHODS. All cultures sporulated and produced SAG at the permissive temperature.

[b] Cells were pregrown in PSP then shifted into SPM and incubated as above.

[c] Results after 24 hr of incubation.

SL484 and SL641 contain different mutations: Given their similar phenotypes, it was important to show that SL484 and SL641 contained different mutations. Tetraploid segregation data from SL641/W66-8a fusants (not shown) suggested that the genes had different map positions, but explanations for these results, such as chromosome loss upon fusion, could not be ruled out. Since SL641 will not sporulate under any tested conditions, we used the technique of spheroplast fusion to test for complementation between the two mutants. If the two mutations were in the same gene, the fusants would be sporulation-defective at temperatures nonpermissive for SL484, and sporulation at an SL484-permissive temperature should yield tetrads containing only SPO^- spores.

A derivative of the cross SL484 × W66-8A, strain 6-3A, which was *ura3 lys2 met4 adeX spo17 HO*, was fused with SL641 as described in Materials and Methods. The yield was low; only three prototrophs were obtained. Each of these failed to sporulate at 34° suggesting absence of complementation, but the fusants were aberrant; DNA measurements showed that each population was highly heterogeneous in DNA content, with value ranging from triploid to hexaploid. This variability was not diminished by continued recloning of the strains. The fusants did sporulate at permissive temperature, and 7 asci were dissected. Only one of these gave 4 viable spores. The results for all are shown in Table 7. Although neither the parents of the fusant nor the fusant were able to sporulate at 34°, many of the progeny were SPO^+ at

TABLE 7

Sporulation of meiotic progeny from a fusant of SL641 × 6-3A

Tetrad	Sporulation 34°	Sporulation 30°
1a	+	+
b	+	+
2a	+	+
b	−	+
c	+	+
3a	−	+
b	−	−
c	+ (all 2 spored)	+
4a	−	−
b	−	+
c	+	+
5a	+	+
b	−	−
6a	+	+
b	+	+
7a	−	−
b	−	−
c	−	−
d	−	−

The fusant was sporulated at 30° and 7 tetrads were dissected. All spores were tested for sporulation (but not SAG production) at 30° and 34°. Only tetrad 7 gave 4 viable spores.

TABLE 8

Sporulation of *spo7* and *spo18* cells transformed with plasmids containing a putative *SPO17* gene

Strain	Temperature	Time in sporulation medium 24 hr	Time in sporulation medium 48 h	No. of experiments
SL 484	30°	61°	59	3
	34°	11	9	
SL 641	30°	0	0	4
	34°	0	0	

° Figures are % sporulation. Strains were transformed and sporulated as described in MATERIALS AND METHODS. The fragment containing the putative *spo17* gene was carried as a 2μ plasmid containing *LEU2* as a selectable yeast marker.

both 30° and 34°. We interpret this to mean that the mutations in SL641 and SL484 are not allelic, although we cannot explain the behavior of the original fusants. Auxotrophic marker segregation was normal, that is followed expectations for segregation for a tetraploid, in the spores. We have recently cloned *SPO17* (A. KENNEDY and P. T. MAGEE, unpublished data) and have shown that a plasmid containing an insert which fully complements SL484 does not complement SL641 (Table 8). We therefore conclude that SL641 contains a mutation in a gene different from *SPO17*.

DISCUSSION

We have described the isolation and characterization of two SAG-deficient mutants of *S. cerevisiae*, SL484 and SL641. SL484 and SL641 had similar phenotypes. Neither synthesized DNA, completed meiosis I, or formed asci when incubated in sporulation medium at 34°. SL484 differed from SL641 by being temperature-sensitive for both sporulation and SAG production.

The 2+:2− segregation pattern of the sporulation-defective phenotype in cells heterozygous for the SL484 defect shows that the phenotype is caused by a mutation in a single gene. We call this gene *SPO17*. The mutation lies 10 map units from the *TRP5* gene on chromosome *VII*. SL641 is a nonconditional mutant so genetic analyses have been done using tetraploid strains constructed by fusing spheroplasts of SL641 with spheroplasts of other homothallic diploid strains. Preliminary results indicate that the SL641 phenotype is also the result of a defect in a single gene and that this gene is different from *SPO17*; hence we are calling this gene *SPO18*. We cannot rule out at this time the possibility that the mutation in SL641 is in *SPO7* and that the sporulation of the heterozygote was due to intragenic complementation.

Spheroplast fusions between a spore from the SL484/W66-8a cross and SL641 gave ambiguous results. A fusant heterozygous for both the *spo17* and *spo18* defects failed to sporulate at 34°; however, interpretation of negative results in such a complementation test is not straightforward. It has been our experience that tetraploids constructed by fusing two *MATa*/*MATα* diploids are often aneuploid (SMITH 1985). Thus, even if the two mutations were in different complementation groups, the fusant derived in this study could be trisomic for one of the *spo*-bearing chromosomes, for example *spo17*/*spo17*/+, and *spo18*/*spo18*/+/+ for the other mutation. Then the heterozygote might fail to sporulate at 34° because of dosage effects at the *SPO17* locus. If such a heterozygote sporulated at 30° (as does the *spo17* parent), then it would segregate progeny that were able to sporulate at the nonpermissive temperature. We observed that the SL484/SL641-derived fusant was able to sporulate at 30° and that it segregated progeny that sporulated at 34°. The fact that only 1/7 asci yielded 4 viable spores is consistent with the aneuploid hypothesis.

Another explanation for the behavior of the *SPO17*/SL641 fusant is that the two mutations are in fact different alleles of the same gene and that the sporulation-competent segregants were produced by intragenic recombination or complementation which restored the wild type gene. We feel that this is not the case, however, for two reasons. The first is that the frequency of sporulation-competent segregants

was too high. The second is that a study on the segregation of chromosomes in a fusant produced by SL641 and W66-8A showed that chromosome *VII* (the *SPO17*-bearing chromosome) missegregated while the SL641 defect segregated normally. This suggests that the SL641 mutation is not located on chromosome *VII* (SMITH 1985). Finally, complementation tests with the cloned *SPO17* gene indicate that the SL641 defect is not in *SPO17*.

The SAG gene has recently been cloned on the basis of its homology to the starch-degrading amyloglucosidase of *S. diastaticus*. It has been shown to be transcriptionally controlled during sporulation. However, it is dispensable for sporulation in that diploids homozygous for a disruption in the gene still sporulate normally (YAMASHITA and FUKUI 1985). In this it resembles some other genes which are transcribed exclusively or mainly during sporulation but which are dispensable for the developmental process (D. PRIMERANO, A. KENNEDY and P. T. MAGEE, unpublished data; M. J. CLANCY personal communication; GOTTLIN-NINFA and KABACK 1986; PERCIVAL-SMITH and SEGALL 1986). Since neither mutant discussed here sporulates at 34° and since both are blocked early, it seems unlikely that their defects are in the structural gene for SAG. Furthermore, both grow normally at the temperatures tested; therefore, they are not defective in cell-cycle genes. It is probable then that both are defective in some early sporulation function, probably one which functions before DNA synthesis. This would lead, of course, to a failure in recombination-competence, and the ability to form various meiotic structures such as spindle pole bodies and synaptonemal complexes (DAVIDOW and BYERS 1984; ESPOSITO and KLAPHOLZ 1981). With the exception of *spo7*, which was complemented by both SL484 and SL641, the relationship to other early functioning genes has not been examined.

Strains carrying mutations in *SPO8* and *SPO9* are not available at this time, so complementation tests could not be performed to establish the relationship between SL484 and SL641 and these genes. Since the mutation in SL484 maps on chromosome *VII*, while *IME* was found to be located on chromosome *X* (MARGOLSKEE *et al.* 1986), these 2 genes are not identical. We cannot rule out the identity of the gene altered in SL641 with *IME*.

Characterization of *SPO17* and *SPO18* is likely to help to sort out the early steps in the transition from mitotic to meiotic growth in a/α *S. cerevisiae*. However, the functions of the gene products defined by the mutations in SL484 and SL641 are not clear to us at this time. Both mutations block cells before DNA synthesis; thus, along with *spo7*, *spo8*, and *spo9*, they are among the earliest *spo* mutations isolated. The early part of the pathway to meiosis and sporulation has not been well studied, but since these mutations (or at least these alleles of them) do not affect vegetative growth, one might assume that they are after *RME* and *IME* or that SPO18 is allelic with the latter. If they are farther down the pathway, they may encode protein factors that are either involved in premeiotic DNA synthesis or that regulate the entry of cells into that part of the developmental pathway.

An alternative role for one or both of these genes is in the regulatory pathway that senses the state of starvation of the cells. No genes analogous to *RME1* or *IME* have been identified for this pathway, but the fact that *rme1* mutations do not bypass nutritional control suggests that there is a separate regulatory network operating. Tests of epistasis with mutations known to overide this control [*ras2*-val (KATAOKA, *et al.* 1984), and *cyr1* (MATSUMOTO, UNO and ISHIKAWA 1983)] will give some insight into whether *SPO17* and *SPO18* are a part of that pathway.

This work was supported by U.S. Public Health Service Grant GM33305. We are grateful to Professor Mary Clancy for many helpful discussions. This is Journal Article No. 12122 from the Michigan Agricultural Experiment Station.

LITERATURE CITED

CLANCY, M. J., L. M. SMITH and P. T. MAGEE, 1982 Developmental regulation of a sporulation-specific enzyme activity in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **2**: 171–178.

COLONNA, W. J., and P. T. MAGEE, 1978 Glycogenolytic enzymes in sporulating yeast. J. Bacteriol. **134**: 844–853.

DAVIDOW, L. S., and B. BYERS, 1984 Enhanced gene conversion and postmeiotic segregation in pachytene-arrested *Saccharomyces cerevisiae*. Genetics **106**: 165–183.

DAWES, I. W., S. DONALDSON, R. EDWARDS and J. DAWES, 1983 Synthesis of a spore-specific surface antigen during sporulation of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **129**: 1103–1108.

DEL RAY, F., T. SANTOS, I. GARCIA-ACHA and C. NOMBELA, 1980 Synthesis of β-glucanases during sporulation in *Saccharomyces cerevisiae*: Formation of a new sporulation-specific 1,3-β-glucanase. J. Bacteriol. **143**: 621–627.

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.

ESPOSITO, R. E., and S. KLAPHOLZ, 1981 Meiosis and ascospore development. pp. 211–287. In: *The Molecular Biology of the Yeast Saccharomyces. Life Cycle and Inheritance*. Edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

GOTTLIN-NINFA, E., and D. KABACK, 1986 Isolation and functional analysis of sporulation-induced transcribed sequences from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**: 2185–2197.

HESSE, G., R. LINDNER and KREBS, 1975 Schnelle fluorotometrische deoxribosespezifische DNS-bestimmung an nicht desintegrierten mikroorgismen mit 3,5-diaminobenzoesaure (DABA). I. Chemischanalytische methodik und anwendung auf hefepopulationen. Z. Allg. Mikrobiol. **15**: 9–18.

HEIN, N. H., and G. H. FLEET, 1983 Variation of (1,3)-β-glucanases in *Saccaromyces cerevisiae* during vegetative growth, conjugation, and sporulation. J. Bacteriol. **156**: 1214–1220.

HINNEN, A., J. B. HICKS and G. FINK, 1978 Transformation of yeast. Proc. Natl. Acad. Sci. USA **75**: 1929–1933.

HOPPER, A. K., P. T. MAGEE, S. K. WELCH, M. FRIEDMAN and B. D. HALL, 1974 Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. J. Bacteriol. 119: 619–629.

KAKAR, S. N., and P. T. MAGEE, 1982 Genetic analysis of *Candida albicans*: identification of different isoleucine-valine, methionine, and arginine alleles by complementation. J. Bacteriol. 151: 1247–1252.

KANE, S., and R. ROTH, 1974 Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. 118: 1–8

KATAOKA, T., S. POWERS, S. CAMERON, O. FOSANO, M. GOLDFARB, J. BROACH and M. WIGLER, 1984 Genetic analysis of yeast *RAS*1 and *RAS*2 genes. Cell. 37: 437–445.

LAUER, G. D., T. M. ROBERTS and L. C. KLOTZ, 1977 Determination of the nuclear DNA content of *Saccharomyces cerevisiae* and implications for the organization of DNA in yeast chromosomes. J. Mol. Biol. 114: 507–526.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

MAGEE, P. T., 1974 Changes in DNA-dependent RNA polymerase in sporulating yeast. Mol. Biol. Rep. 1: 275–281.

MARAZ, A., M. KISS and L. FERENCZY, 1978 Protoplast fusion in *Saccharomyces cerevisiae* strains of identical and opposite mating types. FEMS Microbiol. Letts. 3: 319–322.

MARGOLSKEE, J. P., Y. KASSIR, D. GRANOT and G. SIMCHEN, 1986 The genetic pathway by which *MAT* controls meiosis involves both positive and negative regulators. Abstr. 13th Int. Conf. Yeast Genetics and Molec. Biol. S229.

MARIAN, B., and U. WINTERSBERGER, 1982 Modification of histones during the mitotic and meiotic cycle of yeast. FEBS Lett. 139: 72–76.

MATSUMOTO, K., I. UNO and T. ISHIKAWA, 1983 Initiation of meiosis in yeast mutants defective in adenylate cyclase and cyclic AMP-dependent protein kinase. Cell 32: 417–425.

MITCHELL, A. P., and I. HERSKOWITZ, 1986 Activation of meiosis and sporulation by repression of the RME mutation in yeast. Nature 319: 738–742.

MOWSHOWITZ, D. B., 1976 Permeabilization of yeast for enzyme assays: an extremely simple method for small samples. Anal. Biochem. 70: 94–99.

OTA, A., 1982 Enzyme activities during early ascosporulation in *Saccharomyces cerevisiae*. Int. J. Biochem. 14: 111–118.

PARRY, E. M., and B. S. COX, 1970Δ. The tolerance of aneuploidy in yeast. Genet. Res. 16: 333–339.

PERCIVAL-SMITH, A., and J. SEGALL, 1986 Characterization and mutational analysis of three genes expressed preferentially during sporulation of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 6: 2443–2451.

RESNICK, M. A., A. SUGINO, J. NITISS and T. CHOW, 1984 DNA polymerases, deoxyribonucleases and recombination during meiosis of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 4: 2811–2817.

ROMAN, H., M. M. PHILLIPS and S. M. SANDS, 1955 Studies of polyploid *Saccharomyces*. I. Tetraploid segregation. Genetics 40: 546–561.

ROTH, R., and H. O. HALVORSON, 1969 Sporulation of yeast harvested during logarithmic growth. J. Bacteriol. 98: 831–832.

ROTH, R., and K. LUSNAK, 1970 DNA synthesis during yeast sporulation: genetic control of an early developmental event. Science 168: 493–494.

SCHROEDER, R., M. BREITENBACH and R. J. SCHWEYEN, 1983 Mitochondrial circular RNAs are absent in sporulating cells of *Saccharomyces cerevisiae*. Nucleic Acids Res. 11: 1735–1746.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1981 *Methods in Yeast Genetics*. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

SIPICZKI, M., and L. FERENCZY, 1977 Fusion of *Rhodosporidium* (*Rhodotorula*) protoplasts. FEMS Microbiol. Lett. 2: 203–205.

SMITH, L. M., 1985 Ph.D. thesis, Michigan State University, East Lansing.

SPRAGUE, G. F., JR., and I. HERSKOWITZ, 1981 Control of yeast cell type by the mating type locus. I. Identification and control of expression of the *a*-specific gene, *BAR*1. J. Mol. Biol. 153: 305–321.

WEIR-THOMPSON, E. M., AND I. W. DAWES, 1984 Developmental changes in translatable RNA species associated with meiosis and spore formation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 4: 695–702.

WILLIAMSON, D. H., L. H. JOHNSTON, D. J. FENNELL and G. SIMCHEN, 1983 The timing of the S phase and other nuclear events in yeast meiosis. Exp. Cell. Res. 145: 209–217.

YAMASHITA, I., and S. FUKUI, 1985 Transcriptional control of the sporulation-specific glucoamylase gene in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. 5: 3069–3073.

Communicating editor: D. BOTSTEIN

APPENDIX D

APPENDIX D

SPO17, ADDITIONAL DATA

I. Production of red papillae in sporulating transformants.

Figures 1 and 2 are photographs of plates that were inoculated with the various strains used in this work and subjected to identical sporulation conditions, but at different temperatures, as described in Chapter II. Several observations can be made about the plate experiments:

a) Strain R92W consistently failed to produce red papillae at both the permissive and nonpermissive temperatures. It did, however, sporulate in liquid cultures at the permissive temperature. The parent strain SL484 is a rather poor sporulator at the permissive temperature. Thus, R92W, without an intact copy of *SPO17*, may be incapable of sporulating on plates.

b) In the initial screen, transformants producing red papillae consistently grew better after exposure to sporulation conditions at 34°C than those that failed to produce red papillae. This suggested that *SPO17* might be involved in nutritional regulation. The difference in growth is demonstrated quite clearly in 98FOA-1, 2, and 3, strains that were generated by plating transformant 98, which carried a *SPO17* plasmid, on media containing 5-fluoro-orotic acid. The surviving

strains, having lost the *URA3* plasmid, were sporulation-deficient, failed to produce red papillae, and exhibited poor growth on rich media after exposure to sporulation at 34°C (Figure 2, colonies 15, 16 and 17).

c) Strain SL484 consistently gave good vegetative growth after exposure to sporulation conditions at both 30°and 34°C.

d) R92W usually failed to grow well after exposure to sporulation conditions at 34°C, but it appears to have grown well in the particular experiment pictured in Figures 1 and 2.

e) Some strains (eg., transformants 98 and 208) were not consistent in the production of red papillae in the plate experiments. This is probably due to a variation in sporulation efficiency among experiments, as is frequently observed even in carefully controlled liquid experiments.

f) The colonies at positions 25-29 are transformants containing the multicopy plasmid pEMBB. These strains were exceptionally consistent in growth and in the production of red papillae.

II. <u>*LacZ* expression in *rme1 spo17* double mutants</u>. *RME1* expression was evaluated in *spo17* mutants and the results are shown in Figure 3. The *rme1* strains (obtained from Aaron Mitchell) were disrupted by inserting the *LacZ* gene (in-frame) into the *RME1* gene. The haploid strain 2210 (α *rme1::LacZ*) turns blue when grown on media containing X-gal (not

pictured). Haploid strain 2210-3 (*α rme1::LacZ spo17::LEU2*) is pictured on the left in Figure 3. Even though this strain is *spo17*, the colonies remain blue on X-gal medium. Thus, *RME1* is expressed normally in *spo17* haploids. Strain 221a3 is the product of a mating between two *rme1::LacZ spo17::LEU2* haploids, and is pictured in Figure 3. These colonies remain white on X-gal medium, thus implying that normal repression of *RME1* occurs in *spo17* diploids.

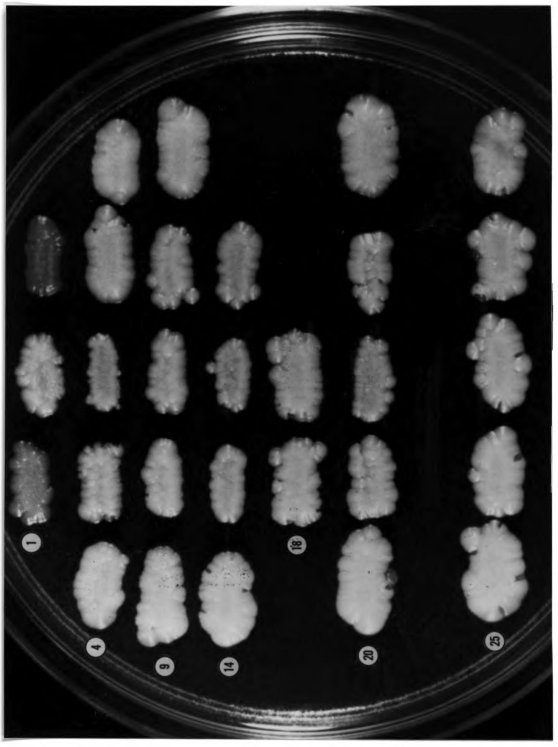


Figure 1

Figure 1. Growth of various experimental strains after exposure to sporulation conditions at 30°C. Colonies were subcultured with sterile toothpicks on to SP2 plates and incubated for 5 days at 30°C. The cultures were then replica-plated to YEPD, grown for 48 hours at 30°C, then refrigerated for 3 to 5 days or until red pigmentation appeared.

1. W66-8A
2. SCMS7-1
3. R9
4. R92W
5. SL484
6. SL641
7. 6
8. 98
9. 48
10. 440
11. 57
12. 731
13. 1940
14. 1912
15. 98FOA-1
16. 98FOA-2
17. 98FOA-3
18. 1912-T1
19. 1940-T2
20. 204
21. 208
22. 2636
23. 2930
24. 469

25-29. R92W/pEMBB

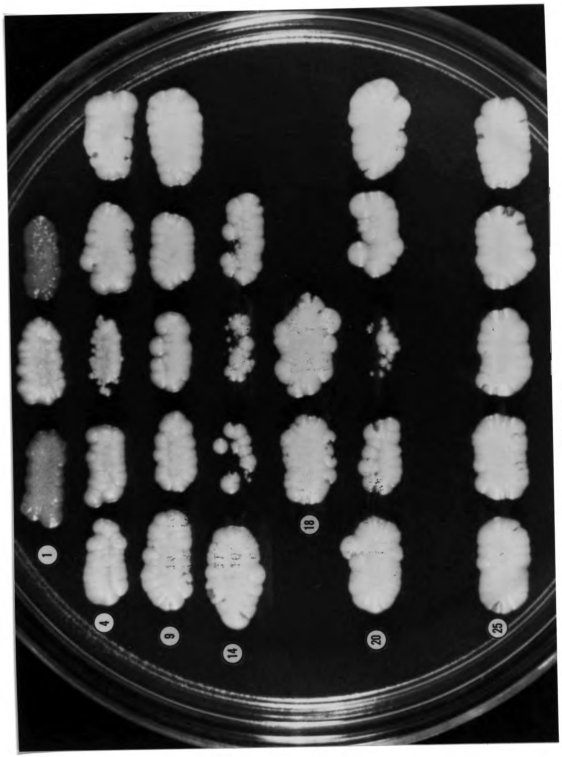
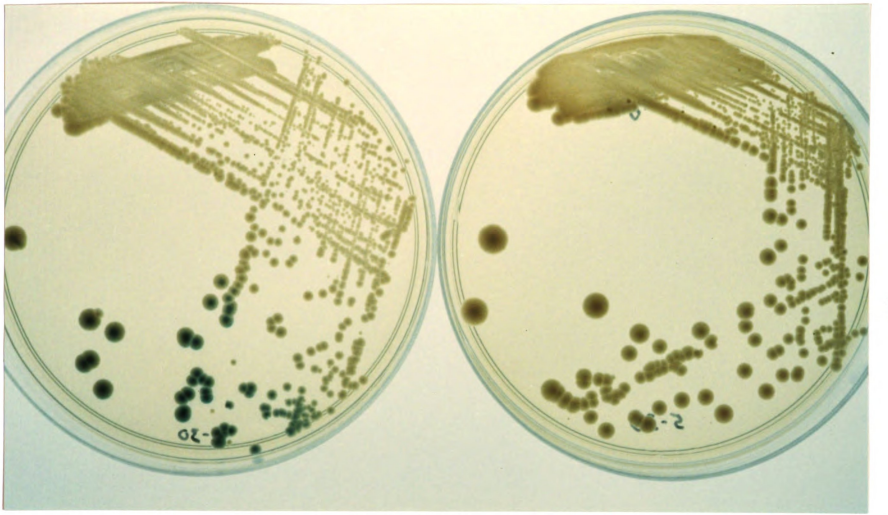


Figure 2

Figure 2. Growth of various experimental strains after exposure to sporulation conditions at 34°C. This plate was prepared exactly as the one pictured in Figure 1, except the incubation temperature during sporulation was 34°C.

1. W66-8A
2. SCMS7-1
3. R9
4. R92W
5. SL484
6. SL641
7. 6
8. 98
9. 48
10. 440
11. 57
12. 731
13. 1940
14. 1912
15. 98FOA-1
16. 98FOA-2
17. 98FOA-3
18. 1912-T1
19. 1940-T2
20. 204
21. 208
22. 2636
23. 2930
24. 469
25-29. R92W/pEMBB



a b

Figure 3

Figure 3. *RME1* expression in a *spo17* haploid and a *spo17* homozygous diploid. YEPD plates were inoculated with 100 ul of 2% X-gal and streaked with a) 2210-3 and b) 221a3.

REFERENCES

REFERENCES

Beggs, J.D., J. van den Berg, A. van Ooyen and C. Weissman. 1980. Abnormal expression of chromosomal rabbit Beta-globin gene in *Saccharyomyces cerevisiae*. Nature *283*:835-840.

Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zollar, S. Powers and M. Wigler. 1987. The *S. cerevisiae CDC25* gene product regulates the *RAS*/adenylate cyclase pathway. Cell *48*:789-799.

Clancy, M.J., B. Buten-Magee, D.J. Straight, A.L. Kennedy, R.M. Partridge, and P.T. Magee. 1983. Isolation of genes expressed preferentially during sporulation in the yeast *Saccharyomyces cerevisiae*. Proc Nat Acad Sci USA *80*:3000-3004.

Colonna, W.J., and P.T. Magee. 1978. Glycogenolytic enzymes in sporulating yeast. J Bacteriol *134*:844-853.

Dawes, I.W., and I.D. Hardie. 1974. Selective killing of vegetative cells in sporulated yeast cultures by exposure to diethyl ether. Mol Gen Genet *131*:281-289.

DeFeo-Jones, D., E.M. Scolnick, R. Koller, and R. Dhar. 1983. *ras*-related gene sequences identified and isolated from *Saccharyomyces cerevisiae*. Nature (London) *306*:707-709.

Esposito, R.E. 1987. Seminar given at the University of Michigan, Ann Arbor, on March 2, 1987.

Esposito, R.E., N. Frink, P. Bernstein, and M.S. Esposito. 1972. The genetic control of sporulation in *Saccharomyces*. Mol and Gen Genet *114*:241-248.

Esposito, M.S. and R.E. Esposito. 1974. Genes controlling meiosis and spore formation in yeast. Genetics *78*:215-225.

Esposito, M.S. and R.E. Esposito. 1978. Aspects of the genetic control of meiosis and ascospore development inferred from the study of *spo* (sporulation-deficient) mutants of *Saccharyomyces cerevisiae*. Biologie Cellulaire *33*:93-101.

Esposito, R.E., and S. Klapholz. 1981. Meiosis and ascospore development. p. 211-287. *In* J.N. Strathern, E.W. Jones, and J.R. Broach (ed.), The molecular biology of the yeast *Saccharyomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Field, J., J.I. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I.A. Wilson, R. Lerner, and M. Wigler. 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharyomyces cerevisiae* by

use of an epitope addition method. Mol Cell Biol *8*:2159-2165.

Friis, J., and H. Roman. 1968. The effect of mating-type alleles on intragenic recombination in yeast. Genetics *59*:33-36.

Game, J. 1976. Yeast cell-cycle mutant *cdc21* is a temperature sensitive thymidilate auxotroph. Mol Gen Genet *146*:313-315.

Hartwell, L.H. 1974. *Saccharyomyces cerevisiae* cell cycle. Bact Rev *38*:164-198.

Herskowitz, I., and Y. Oshima. 1981. Control of cell type in *Saccharyomyces cerevisiae*: mating type and mating type interconversion. p. 181-209. In J.N. Strathern, E.W. Jonew, and J.R. Broach (ed.), The molecular biology of the yeast *Saccharyomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Heywood, P. and P.T. Magee. 1976. Meiosis in protists. Some structural and physiological aspects of meiosis in algae, fungi, and protozoa. Bacteriol Rev *40*:190-240.

Holaway, B., D. Lehman, D. Primerano, P.T. Magee and M.J. Clancy. 1985. Sporulation-regulated genes of *Saccharyomyces cerevisiae*. Curr Genet *10*:163-169.

Hopper, A.K., P.T. Magee, S.K. Welch, M. Friedman, and B.D. Hall. 1974. Macromolecular synthesis and breakdown in relation to sporulation and meiosis in yeast. J Bacteriol *119*:619-628.

Hopper, A.K., J. Kirsch and B.D. Hall. 1975. Mating-type and sporulation in yeast. II. Meiosis, recombination, and radiation sensitivity in an α/α diploid with altered sporulation control. Genetics *80*:61-76.

Iino, Y., and M. Yamamoto. 1985a. Negative control for the initiation of meiosis in *Schizosaccharomyces pombe*. Proc Nat Acad Sci USA *82*: 2447-2451.

Iino, Y., and M. Yamamoto. 1985b. Mutants of *Schizosaccharomyces pombe* which sporulate in the haploid state. Mol Gen Genetics *198*:416-421.

Jensen, R.. G.F. Sprague, Jr., and I. Herskowitz. 1983. Regulation of yeast mating-type conversion: feedback control of *HO* gene expression by the mating-type locus. Proc Nat Acad Sci *80*:33035-3039.

Kaback, D.B., and L.R. Feldberg. 1985. *Saccharyomyces cerevisiae* exhibits a sporulation-specific temporal pattern of transcript accumulation. Mol Cell Biol *5*:751-761.

Kassir, Y. and G. Simchen. 1976. Regulation of mating and meiosis in yeast by the mating type locus. Genetics *82*:187-206.

Kassir, Y., D. Granot, and G. Simchen. 1988. *IME1*, a positive regulator gene of meiosis in *S. cerevisiae*. Cell *52*:853-862.

Kaufer, N.F., V. Simanis and P. Nurse. 1985. Fission yeast *Schizosaccharomyces pombe* correctly excises a mammalian RNA transcript intervening sequence. Nature *318*:78-80.

Klapholz, S. and R.E. Esposito. 1980a. Isolation of *spo12-1* and *spo13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics *96*:567-588.

Klapholz, S. and R.E. Esposito. 1980b. Recombination and chromosome segregation during the single division meioisis in *spo12-1* and *spo13-1* diploids. Genetics *96*:589-611.

Klar, A.J.S., J.N. Strathern, J.R. Broach, and J.B. Hicks. 1981. Regulation of transcription in expressed and unexpressed mating type cassettes of yeast. Nature *289*:239-244.

Langford, C., W. Nellen, J. Niessing, D. Gallwitz. 1983. Yeast is unable to excise foreign intervening sequences from hybrid gene transcripts. Proc Nat Acad Sci USA *80*:1496-1500.

Law, D.T.S., and J. Segall. 1988. The *SPS100* gene of *Saccharomyces cerevisiae* is activated late in the sporulation process and contributes to spore wall maturation. Mol Cell Biol *8*:912-922.

MacKay, V. and T.R. Manning. 1974. Mutations affecting sexual conjugation and related processes in *Saccharyomyces cerevisiae*. II. Genetic analysis of nonmating mutants. Genetics *76*:273-288.

Mandelstam, J. 1971. Recurring patterns during development in primitive organisms. Symp Soc Exp Biol *25*:1-26.

Matsumoto, K., I. Uno, and T. Ishikawa. 1983. Initiation of meiosis in yeast mutants defective in adenylate cyclase and cyclic-AMP dependent protein kinase. Cell *32*:417-423.

Mills, D. 1980. Quantitative and qualitative analysis of polyadenylated RNA sequences during meiosis of *Saccharyomyces cerevisiae*. In Tenth International Conference on yeast genetics and molecular biology, Louvain-la-Neuve, Belgium. p. 55- ....

Mitchell, A. and I. Herskowitz. 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature *319*:738-742.

Miyake, S., N. Sands and S. Sato. 1971. Biochemical changes in yeast sporulation. II. Acetate metabolism. Dev Growth Differ *12*:285-296.

Moens, P.B., R.E. Esposito and M.S. Esposito. 1974. Aberrant nuclear behavior at meiosis and anucleate spore formation by sporulation-deficient (*spo*) mutants of *Saccharyomyces cerevisiae*. Exp Cell Res *83*:166-174.

Nasmyth, K.A., K. Tatchell, B.D. Hall, C. Astell, and M. Smith. 1981. A physical analysis of mating type loci. Cold Spring Harbor Symp Quant Biol *45*:961.

Nasmyth, K.A. 1982. Molecular genetics of yeast mating type. Ann Rev Genet *16*:439-500.

Nurse, P. 1985. Mutants of the fission yeast *Schizosaccharomyces pombe* which alter the shift between cell proliferation and sporulation. Mol Gen Genet *198*:497-*502*.

Percival-Smith, A., and J. Segall. 1984. Isolation of DNA sequences preferentially expressed during sporulation in *Saccharyomyces cerevisiae*. Mol Cell Biol 4:142-150.

Percival-Smith, A., and J. Segall. 1986. Characterization and mutational analysis of a cluster of three genes expressed preferentially during sporulation of *Saccharyomyces cerevisiae*. Mol Cell Biol *6*:2443-2451.

Percival-Smith, A., and J. Segall. 1987. Increased copy number of the 5'end of the *SPS2* gene inhibits sporulation of *Saccharyomyces cerevisiae*. Mol Cell Biol *7*:2484-2490.

Powers, S., T. Kataoka, O. Fasano, M. Goldfarb, J. Strathern, J. Broach, and M. Wigler. 1984. Genes in *S. cerevisiae* encoding proteins with domains homologous to the mammalian *ras* proteins. Cell *36*:607-612.

Primerano, D.A., G. Muthukumar, S.H. Suhng, and P.T. Magee. 1988a. Manuscript in preparation.

Primerano, D.A., A.L. Kennedy and P.T. Magee. 1988b. Manuscript in preparation.

Pringle, J.R. and L.H. Hartwell. 1981. The *Saccharyomyces cerevisiae* cell cycle. p.97-142. In J.N. Strathern, E.W. Jones J.R. Broach (ed.), The molecular biology of the yeast *Saccharyomyces cerevisiae*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Rine, J., G.F. Sprague, and I. Herskowitz. 1981. *rmel* mutation of *Saccharomyces cerevisiae*: map postion and bypass of mating type locus control of sporulation. Mol Cell Biol *1*:958-960.

Robinson, L.C., J.B. Gibbs, M.S. Marshall, I.S. Sigall, and K. Tatchell. 1987. *CDC25*: a component of the *RAS* adenylate cyclase pathway in *Saccharymyces cerevisiae*. Science *235*:1218-1221.

Roman, H. and S.M. Sands. 1953. Heterogeneity of clones of *Saccharyomyces* derived from haploid ascospores. Proc Nat Acad Sci USA *39*:171-179.

Roth, R. 1973. Chromosome replication during meiosis: Identification of gene function required for premeiotic DNA synthesis. Proc Nat Acad Sci USA *70*:3087-3091.

Roth, R. and K. Lusnak. 1970. DNA synthesis during yeast sporulation: Genetic control of an early developmental event. Science *168*:493-494.

Roth, R. and S. Fogel. 1971. A selective system for yeast mutants deficient in meiotic recombination. Mol Gen Genetics *112*:295-305.

Rothstein, R. 1983. One-step gene disruption in yeast. Meth Enzymol *101*:670-676.

Shortle, D., J.E. Haber, and D. Botstein. 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. Science *217*:371-371.

Simchen, G., R. Pinon, and Y. Salts. 1972. Sporulation in *Saccharomyces cerevisiae*: premeiotic DNA synthesis, readiness and commitment. Exp Cell Res *75*:207.

Simchen G., D. Idar, and Y. Kassir. 1976. Recombination and hydroxyurea inhibition of DNA synthesis in yeast meiosis. Mol Gen Genet *144*:21-27.

Simchen, G. 1974. Are mitotic functions required in meiosis? Genetics *76*:745-753.

Smith, L., L. Robbins, A. Kennedy and P.T. Magee. 1988. Identification and characterization of mutations affecting sporulation in *Saccharomyces cerevisiae*. Genet *120*:899-907.

Struhl, K. 1983. The new yeast genetics. Nature *305*:391-397.

St. John, T.P. and R.W. Davis. 1979. Isolation of galactose-inducible DNA sequences from *Saccharomyces cerevisiae* by differential plaque filter hybridization. Cell *16*:443-452.

Thorner, J. 1981. Pheromonal regulation of development in *Saccharomyces cerevisiae*. *In* J.N. Strathern, E.W. Jones, and J.R. Broach (eds.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Toda, T., I. Uno, T. Ishikawa, S. Powers, T. Kataoka, D. Broek, S. Cameron, J. Broach, K. Matsumoto, and M. Wigler. 1985. In yeast, RAS proteins are controlling elements of adenylate cyclase. Cell *40*:27-36.

Wang, H.T., S. Frackman, J. Kowalisyn, R.E. Esposito and R. Elder. 1987. Developmental regulation of *SPO13*, A gene required for separation of homologous chromosomes at meiosis I. Mol and Cell Biol *7*:1425-1435.

Wegstaff, J.E., S. Klapholz and R.E. Esposito. 1982. Meiosis in

haploid yeast. Proc Nat Acad Sci USA *79*:2986-2990.

Yamashita,I., and S. Fukui. 1985. Transcriptional control of the sporulation-specific glucoanylase gene in the yeast *Saccharomyces cerevisiae*. Mol and Cell Biol *5*:3069-3073.