Initiation of Sporulation in *Saccharomyces cerevisiae*. Mutations Preventing Initiation

By GEOFFREY R. CALVERT AND IAN W. DAWES*

Department of Microbiology, University of Edinburgh, Edinburgh EH9 3JG, UK

(Received 24 November 1983)

Mutants of *Saccharomyces cerevisiae* that are unable to initiate sporulation, but can continue vegetative growth under conditions in which the wild-type strain sporulates, have been isolated and characterized. The mutations arose spontaneously as suppressors of the *spd1* mutations, restoring the ability of *spd1* mutants to grow on glycerol, and also spontaneously in cultures of a wild-type diploid strain undergoing sporulation in continuous culture. The mutations all conferred asporogeny, and were recessive in this respect to the wild-type, but dominant in acting as suppressors of the *spd1* mutation. They fell into three complementation groups which corresponded to three unlinked loci, designated *spo50*, *spo51* and *spo53*. None of these mutations was closely linked to the other initiation mutations defined by the *spd1*, *spd3*, *spd4*, *cdc25*, *cdc28* loci, nor to the cell size control mutations *whi1* and *whi2*. Loose linkage was detected between *spd1* and *spo53*, and *spo50*, *spd3* and *spo53* were linked to their respective centromeres. The *spo50*, *spo51* and *spo53* mutations are not nonsense suppressors. Mutations in all three genes conferred similar highly pleiotropic phenotypes including: asporogeny; dominant suppression of both *spd1* and *spd3* mutations; aberrant cell morphology and viability loss on starvation; constitutive ability to reduce tetrazolium (which is subject to carbon source repression in the wild-type); and complete repression of the synthesis of several polypeptides that are subject to carbon source repression in the wild-type strain and derepressed in *spd1* mutants derepressed for sporulation. A diploid strain homozygous for the *spo50* mutation did not undergo either premeiotic DNA replication or meiotic recombination when transferred to sporulation media.

INTRODUCTION

To study the mechanisms controlling initiation of yeast sporulation, it is important to obtain as many different classes of mutation as possible which affect the process. Mutations leading to derepression of sporulation have been described previously (Dawes, 1975; Shilo *et al.*, 1978; Vezhinet *et al.*, 1979; Dawes & Calvert, 1984). Initiation mutants that fail to sporulate under conditions in which the wild-type does are intrinsically more difficult to recognize. A large number of asporogenous mutants have been isolated (Esposito & Esposito, 1969) and partially characterized, but from the way these mutants were selected it is not possible to determine whether any was the result of a mutation affecting initiation of sporulation. Mutations to asporogeny (*spo*) may affect sporulation at any of a number of stages, and they can be ordered in a temporal sequence according to the morphological stage at which they lead to arrest (Esposito *et al.*, 1970; Esposito & Esposito, 1974; Esposito & Klapholtz, 1981). However, none can be definitely assigned to initiation since there are no early events known which can be used as markers for initiation.

Asporogenous mutants defective in initiation can, however, be selected. It is possible to obtain populations of cells growing under conditions in which a proportion of the cells are sporulating. If the cells are dividing with a specific fission rate of ν , and are being initiated to

Abbreviations: TTC, triphenyltetrazolium chloride; KAc, potassium acetate.

sporulate at a specific rate κ , then the population of wild-type cells will have a reduced effective specific number growth rate (approximately $\nu - \kappa$). If a mutation occurs preventing the cells from initiating sporulation, the mutant will have an effective division rate of ν , and will outgrow the wild-type cell, and also any later-blocked asporogenous mutants, since these will also have reduced growth rate. This can only succeed if there are some common elements regulating the decision by cells to initiate cell division on the one hand and sporulation on the other. Studies with derepressed mutants have indicated that there may indeed be common elements and that this approach is feasible, since these mutants are unable to grow on many non-fermentable substrates, but sporulate extensively instead (Vezinhet *et al.*, 1979). These mutants cultured on such substrates frequently give rise to double mutants in which a second mutation to asporogeny suppresses the derepression on mutation and restores the ability of the cell to grow on non-fermentable substrates (Dawes & Calvert, 1984). A second approach to isolating initiation-defective mutants relies on the behaviour of sporulating micro-organisms in continuous culture, and is analogous to the methods used to isolate the very early-blocked *spoO* mutants in *Bacillus subtilis* (Piggot & Coote, 1976). *Bacillus subtilis* can be maintained in a chemostat such that some cells are sporulating while the rest maintain vegetative growth (Dawes & Mandelstam, 1970), and under these conditions the population growth rate is less than the division rate of the vegetative cells, since cells initiated to sporulation cease to contribute to population growth (Dawes & Thornley, 1970). In such populations, only asporogenous *initiation* mutants have a selective advantage, since all of their progeny can divide; any other (later-blocked) *spo* mutations would not confer this advantage. *Saccharomyces cerevisiae* can also undergo sporulation in continuous culture (Calvert & Dawes, unpublished) and hence it should be possible to obtain *spo* initiation mutants from wild-type strains in this way.

This paper describes the isolation of putative *spo* initiation mutations using the methods outlined above, and the subsequent biochemical and genetic characterization of the mutations. These mutations confer a number of interesting properties that set them apart from other *spo* mutations.

METHODS

Organisms. The main strains used in addition to those given in Dawes & Calvert (1984) included ST34 (*MATa cdc28-15 aro1 lys2 cyh1* from S. Reed, Santa Barbara University of California, USA), BR207-7a (*MATa cdc25-3 adel/ade2 ura1 trp1 his1 arg4 ilv1*) and BR220-169a (*MATd cdc35-4 adel/ade2 ura1 trp1 arg4*) from J. Pringle (University of Michigan, Ann Arbor, USA). Strains carrying the *whi1* and *whi2* size control mutations (Carter & Sudbery, 1980; Sudbery *et al.*, 1980) were obtained from P. Sudbery (University of Sheffield, UK).

Media. Complex media contained, (l⁻¹): 10 g yeast extract, 20 g bacto-peptone and 20 g carbon source (YEPD contained glucose; YEPA, potassium acetate; YEPGal, galactose) except YEPG (30 ml glycerol) and YEPE (20 ml ethanol). Minimal medium contained, (l⁻¹): 1.5 g Difco yeast nitrogen base (without amino acids or ammonium sulphate), 5 g ammonium sulphate and 20 g glucose, and was supplemented with appropriate mixtures of amino acids to assign auxotrophic markers. Where needed, media were solidified with agar (20 g l⁻¹). Potassium acetate sporulation medium (KAc) was that of Fast (1970).

Continuous cultivation. Strains were grown in a New Brunswick C-30 bench-top fermenter (305 ml working volume). Temperature was maintained at 30 °C using a cooling probe and a thermistor-controlled heating element. Agitation was maintained at 800 r.p.m. and the culture was aerated with sterile water-saturated air at 78 culture volumes h⁻¹. Dilution rate was controlled by a peristaltic pump (Pharmacia) and samples were taken directly from the culture vessel using a hooded sampler, discarding the first sample, which contained material from the sampling line dead space. Cultures were grown under galactose limitation using a medium containing, (l⁻¹): 1 g galactose (autoclaved separately); 1.5 g Difco yeast nitrogen base (without amino acids or ammonium sulphate); 2 g ammonium sulphate, 0.05 g L-arginine; 3 g KH₂PO₄; and 5 g K₂HPO₄.

Tetrazolium reduction. By adapting the method of Ogur *et al.* (1957), strains were tested for their ability to reduce tetrazolium dye. Cultures grown on agar plates were overlaid with 15 ml molten agar (50 °C) containing (l⁻¹): 0.1 g triphenyltetrazolium chloride (TTC) and 20 g agar buffered at pH 7.2 with 0.1 M-potassium phosphate. Clones of tetrazolium-reducing cells became red within 2 to 3 h.

Genetic analysis. Standard techniques of genetic analysis were used (Mortimer & Hawthorne, 1975). Crosses involving homothallic diploid strains were made as described by Dawes & Calvert (1984).

RESULTS

*Isolation of 'revertants' from a homozygous *spd1* diploid*

Once mutations affecting a process such as initiation of sporulation are available, further mutations affecting the process can be obtained by selecting for 'reversion' of the original mutant phenotype and screening for the occurrence of second-site mutations suppressing the initial mutation. For *spd* mutations leading to derepressed sporulation this approach is facilitated by their instability: homozygous *spd1* diploids plated on media on which they cannot grow, but on which they sporulate extensively, spontaneously give rise to colonies at a frequency of around 4×10^{-4} (Dawes & Calvert, 1984).

Lawns of the homothallic diploid strain 54 (*spd1* homozygote) grown on YEPD plates after replica-plating onto YEPG plates gave numerous papillae after incubation for two weeks. From a genetic analysis of 34 independently isolated clones it was found that more than half of these retained the *spd1* mutation (Table 1) and the revertant phenotype was the result of the acquisition of a second, unlinked mutation. In 10 of the 20 such cases the second-site mutation was present in heterozygous state, and these strains grew on YEPG and sporulated on sporulation plates. On dissection of asci from these, diploids homozygous for the mutation suppressing the inability of *spd1* strains to grow on YEPG were obtained. These were found to be asporogenous under all conditions, and the mutation suppressing *spd1* was therefore acting as a recessive *spo* mutation.

Ten of the 'revertants' were asporogenous on isolation and were found to have resulted from the acquisition of an *spo* mutation in the homozygous state. These homozygous diploids were difficult to analyse by meiotic analysis, since back-crossing to wild-type strains could only be achieved in some of them, and this involved incubating them for an extended time on sporulation medium before attempting to mate them to a wild-type haploid. Under these conditions a very few spores formed in the *spo* strains and these could be induced to mate to give diploids heterozygous at the *HO* (homothallism), *spd1* and *spo* loci. These were sporulated and dissected to isolate the *spo* mutation in an *SPD1* background. For three independent *spo* mutations tested in this way, complementation tests were performed by isolating *MATa* and *MATα* haploids carrying the *spo* mutation. All three mutations from revertant strains R42, R65 and R77 failed to complement each other and were presumed to represent different alleles of the same locus. From the dissections used to characterize R42, strain 69.10C, a homothallic diploid homozygous for the *spo50* mutation, was obtained and used in subsequent analysis.

*Isolation of 'revertants' from a haploid *spd1* strain*

Isolation of the *spo* mutations in a homothallic diploid strain led to considerable difficulty in genetic analysis. This problem was largely avoided by isolating 'revertants' from a haploid strain, 168.2D (*MATa spd1-1 met1*) grown on YEPG medium. These 'revertant' strains were back-crossed to a wild-type strain, 168.2A (*MATa arg4-1*), and the resultant cross dissected. Clones with genotypes *MATa spo arg4-1* and *MATα spo met1* were obtained and mated, and each resultant cross was tested for its ability to sporulate. From 20 isolates four classes were obtained; these included: three mating-deficient strains which could not be analysed further; three wild-type revertants producing four normally-sporulating clones from each ascus; six unstable 'revertants' which after mating and dissection had recovered the *spd1-1* phenotype; and six stable 'revertants'. On backcrossing to the wild-type strain, the *spd1-1* mutation was recoverable from these latter isolates, indicating that the mutation restoring growth on YEPG was at a second site unlinked to the *spd1-1* locus. When these second-site suppressor mutations were present in the homozygous state, they conferred asporogeny.

*Isolation of an *spo* mutant from continuous culture*

Strain S41 was grown in a galactose-limited chemostat culture, as described in Methods. The culture reached a peak of sporulation of 35% asci, which was maintained for approximately 100 h, after which asporogenous mutants appeared in the culture and these very rapidly became the predominant organisms. These mutants were obtained in pure culture and proved to have very similar characteristics to the other asporogenous mutants described above.

Table 1. *Analysis of spontaneous 'revertants' of a homozygous spd1 diploid strain*

Strain 54 was plated on YEPG plates at high cell density. After incubation for two weeks papillae were picked, purified by streaking on YEPG plates and tested for their ability to sporulate on plates of sporulation medium. Asci from those isolates that sporulated to any extent were dissected and the progeny scored for derepressed sporulation, normal sporulation or absence of sporulation on appropriate media. Three classes of mutant were obtained.

No. of mutants isolated	Percentage sporulation	Nos of tetrads			Presumed genotype
		Wild-type	Derepressed	Asporogenous	
14	70-90	4	0	0	$\frac{SPD1}{SPD1}$
10	10-50	0	2	2*	$\frac{spd1-1}{spd1-1} \frac{spo}{SPO}$
10	0	—	—	—	$\frac{spd1-1}{spd1-1} \frac{spo}{spo}$

* These asporogenous strains had highly pleiotropic phenotypes similar to those observed for the totally asporogenous mutants.

Table 2. *Growth and sporulation phenotypes of diploids carrying different combinations of spd1 and spo50 mutations*

Strains with the genotypes listed below were constructed from the parent strains: S41 (wild type), 54 (*spd1*) or 69.10C (*spo50*), or from the progeny of appropriate crosses between these strains. At least 5 isolates with each genotype were obtained, and the extent of sporulation was assessed on YEPG and KAc media. The figures indicate the range of sporulation frequencies observed.

Strain genotype	Growth		Percentage sporulation (48h)	
	YEPD	YEPG	YEPG	KAc
$\frac{SPD1}{SPD1} \frac{SPO50}{SPO50}$	+	+	0-5	60-80
$\frac{spd1}{SPD1} \frac{SPO50}{SPO50}$	+	+	5-10	60-80
$\frac{spd1}{spd1} \frac{SPO50}{SPO50}$	+	—	70-95	70-95
$\frac{SPD1}{SPD1} \frac{spo50}{spo50}$	+	+	0-5	50-80
$\frac{SPD1}{SPD1} \frac{spo50}{spo50}$	+	+	0	0-5
$\frac{spd1}{spd1} \frac{spo50}{spo50}$	+	+	0	0-10

Dominance and complementation analysis

Diploids heterozygous for the above asporogenous mutations were constructed and tested for their ability to sporulate on sporulation medium. The mutations included those in the six haploid asporogenous isolates, the chemostat-isolated strain CH15, and the diploid 69.10C. All of these were recessive to the wild-type with respect to their effect on sporulation, and therefore complementation tests were done. It should be noted, however, that these mutations were dominant with regard to suppression of the *spd1* characteristic of poor growth on non-fermentable carbon sources. The growth and sporulation characteristics of diploids carrying the various combinations of wild-type and mutant alleles of *spd1* and *spo50* are summarized in Table 2.

Three complementation groups for these potential initiation *spo* mutations were established (Table 3). Crosses between strains carrying mutations from different groups all yielded diploids sporulating at a frequency over 50%, while those between strains carrying mutations in the same complementation group were asporogenous.

Table 3. Complementation analysis of mutations to asporogeny that suppress the *spd1* mutation

The numbers refer to strains in which independent mutations were first characterized. For haploid strains, opposite mating types were constructed from dissection of a cross to a wild-type haploid. For diploids 69.10C and CH15 the appropriate heterozygous diploids were constructed by crossing the very few spore progeny of each strain to the haploids. Heterozygous diploids were tested for sporulation on KAc sporulation plates; a plus sign indicates that complementation had occurred and sporulation (> 50%) was obtained.

Parent strain	Genotype designated											
69.10C	<i>spo50-1</i>	—										
r42	<i>spo50-2</i>	—	—									
r77	<i>spo50-3</i>	—	—	—								
2	<i>spo51-1</i>	+	*	*	—							
8	<i>spo51-2</i>	+	*	*	—	—						
4	<i>spo53-1</i>	+	*	*	+	+	—					
6	<i>spo53-2</i>	+	*	*	+	+	—	—				
10	<i>spo53-3</i>	+	*	*	+	+	—	—	—			
14	<i>spo53-4</i>	+	*	*	+	+	—	—	—	—		
CH15	<i>spo53-5</i>	+	*	*	*	+	*	*	—	*	—	
		69.10C	r42	r77	2	8	4	6	10	14	CH15	

* Not tested.

Linkage analysis for sporulation initiation mutations

From the heterozygous diploids constructed for the above complementation tests, representative strains were dissected to determine the linkage relationships between *spo50*, *spo51* and *spo53*. Additionally, the other mutations affecting sporulation initiation, including: *spd1*, *spd3*, *spd4* (derepressed sporulation mutations; Dawes & Calvert, 1984), *cdc25*, *cdc28*, *cdc35* (temperature-sensitive cell division cycle mutations; Hartwell, 1974; Reed, 1980; Shilo *et al.*, 1978), *whi1* and *whi2* (mutations affecting cell size; Sudbery *et al.*, 1980) were tested for linkage by meiotic analysis using tetrad dissection where this was feasible, or random spore analysis when lack of spore viability made tetrad analysis unfeasible. No close linkage of any of the above mutations was detected, although some linkage of *spd1* and *spo53* was inferred from the low frequency of non-parental ditypes in dissection (Table 4). From the data obtained, it can be calculated that *spo53* is about 35 cM from *spd1*. The *spd1* mutation is centromere-linked (Dawes & Calvert, 1984) and from this fact and the data in Table 4, *spo50*, *spd3* and *spo53* are also linked to their respective centromeres. The probable location of *spo53* is the right arm of chromosome IV, about 20 cM from the centromere, although poor spore viability has prevented a more definite assignment by tetrad analysis.

In the course of linkage tests, it was also shown that strains carrying any of the three *spo* mutations and *spd3* failed to express the *spd* phenotype. Thus all three suppressors (*spo50*, *spo51* and *spo53*) of *spd1* were also able to suppress *spd3*, implying that all of these mutations affect a common function in the cell.

The *spo50*, *spo51* and *spo53* mutations are not nonsense suppressors

The high frequency with which *spo50*, *spo51* and *spo53* arose in strains carrying *spd1*, and their ability to suppress *spd3* mutations, may have been the result of the *spd* mutations being nonsense suppressible alleles, and the *spo* mutations being amber, ochre or opal suppressors. This was ruled out in two ways. Diploids were constructed which were homozygous for *spd1* and various combinations of known amber, ochre and opal mutations, including: *arg4-17*, *his5-2*, *lys1-2* (ochre); *trp1-1*, *aro7-1* (amber); *ade5,7-u*; *his5'-u*; *leu2'-u*; *lys1-u* (opal; Hawthorne, 1976). On isolating at least 20 spontaneously arising asporogenous clones that grew on YEPG, none was found to have lost any of the auxotrophic requirements, showing that the mutations to asporogeny failed to suppress the nonsense-suppressible markers. In the same way nonsense suppressors selected by simultaneous suppression of several auxotrophic markers were found

Table 4. *Meiotic analysis of mutations affecting initiation of sporulation*

Diploids heterozygous for the various combinations of the mutations indicated were sporulated and dissected. Where feasible, tetrads were scored for the frequencies of parental ditype (PD), nonparental ditype (NPD) and tetratype (T) segregation. For the *cdc* mutations poor spore viability was a problem and random spore analysis was used instead.

	Tetrad analysis			Random spore analysis	
	PD	NPD	T	No. of spores	Spores of parental type (%)
<i>spd1</i> × <i>spo50</i>	4	5	10	93	(54)*
<i>spd1</i> × <i>spo51</i>	1	1	7	75	(64)*
<i>spd1</i> × <i>spo53</i>	14	1	15	68	(70)*
<i>spd3</i> × <i>spo50</i>	10	10	15	117	(62)*
<i>spd3</i> × <i>spo51</i>	7	5	6	89	(54)*
<i>spd3</i> × <i>spo53</i>	1	0	5	56	(50)*
<i>spo50</i> × <i>spo51</i>	9	3	11	134	(46)*
<i>spo50</i> × <i>spo53</i>	4	6	6	89	(62)*
<i>spo51</i> × <i>spo53</i>	4	5	20	129	(54)*
<i>spd1</i> × <i>whi1</i>	3	1	6	70	58
<i>spd1</i> × <i>whi2</i>	6	2	17	100	58
<i>spo50</i> × <i>whi1</i>	2	1	5	32	53
<i>spo50</i> × <i>whi2</i>	1	4	5	40	43
<i>spo51</i> × <i>whi2</i>	2	1	4	44	64
<i>spo53</i> × <i>whi1</i>	3	6	7	88	47
<i>spo53</i> × <i>whi2</i>	2	4	5	36	23
<i>spd1</i> × <i>cdc25</i>				39	24
<i>spd1</i> × <i>cdc35</i>				35	34
<i>spo50</i> × <i>cdc28</i>				17	47
<i>spo51</i> × <i>cdc25</i>				16	68
<i>spo51</i> × <i>cdc28</i>				29	48
<i>spo51</i> × <i>cdc35</i>				6	33
<i>spo53</i> × <i>cdc25</i>				11	45
<i>spo53</i> × <i>cdc28</i>				16	44
<i>spo53</i> × <i>cdc35</i>				13	61

* In these crosses only one class of parental spore type could be easily distinguished (*spd*), and the percentage given is double that for this class.

neither to suppress the YEPG growth phenotype of the *spd1* mutation nor to produce asporogeny. Some strains isolated sporulated poorly, but this can be accounted for by the known characteristics of some nonsense suppressor mutations (Rothstein *et al.*, 1976). In addition, the ochre-specific nonsense suppression mutation *SUP3*, and opal suppressors *SUP76* and *SUP85* (Hawthorne, 1976) were found not to suppress the *spd1* mutation. These results show that the second-site mutations to asporogeny are not nonsense suppressors, nor were any of the four alleles of the *spd1* mutation tested nonsense suppressible.

Pleiotropic phenotypes of spo50, spo51 and spo53 mutations

Diploids homozygous for any of these three asporogenous initiation mutations exhibited similar highly pleiotropic phenotypes that set these mutations apart from others causing asporogeny.

Starvation response. One characteristic noticed in the routine handling of *spo50* and *spo51* mutants was their extensive loss of viability on prolonged incubation on plates and in storage on agar slopes kept at 4 °C. Fig. 1 illustrates the marked decrease in survival of these strains compared to the wild-type in cultures grown to maximum turbidity in YEPA medium. For the homozygous *spo50* diploid there were fewer than 0.1% survivors after 5 d further incubation compared with about 60% for the wild-type. Under these conditions the asporogenous mutants failed to adopt a normal stationary-phase morphology characterized by single unbudded cells (appearing phase-bright); instead they became aberrant, producing long processes without completing cytokinesis (Fig. 2). This aberrant morphology occurring under starvation

Fig. 1. Viability of mutant diploid strains on prolonged incubation. Strains were grown to stationary phase at 30 °C with shaking in YEPA medium, then monitored for change in viable cell numbers on continued incubation. ●, Homothallic diploid wild-type strain S41; ○, homozygous *spo50* diploid strain 69.10C; △, homozygous *spo51* diploid strain 246.1B; □, homozygous *spo53* diploid strain 247.2D.

Fig. 2. Photomicrograph of homozygous *spo50* diploid strain 69.10C grown to stationary phase. The pseudomycelial appearance of this strain does not begin to develop until stationary phase has been attained (the above was obtained from a culture incubated for 48 h in YEPA medium at 30 °C). Magnification approx. 1700 ×.

conditions was characteristic of *spo50*, *spo51* or *spo53* strains and may indicate that the cells were continuing cell growth, but that the cytokinesis and cell separation events of the cell division cycle were prevented.

The colonies of homozygous *spo50*, *spo51* or *spo53* diploids could easily be distinguished from

those of wild-type strains since they became a distinct brown colour after prolonged incubation on YEPD agar. The surface of these colonies often became crinkled and cells in the colonies were often tightly bound so that they were difficult to replicate. This was probably due to the pseudomycelial morphology of the strains seen after starvation.

Reduction of TTC. The *spd1* mutation may affect some aspect of mitochondrial activity (Vezinhet *et al.*, 1979) and in view of the ability of the *spo50*, *51* and *53* mutations to suppress the *spd1* mutation they may also affect mitochondrial activity. Several strains were therefore tested for their ability to reduce TTC via the respiratory chain (Slater *et al.*, 1963), and distinct differences were observed between *spd1*, *spo50*, *spo51*, *spo53* and wild-type strains. The wild-type strain did not reduce TTC when fermenting glucose, but reduced it strongly when growing by respiration of glycerol. The *spd1* strain 54, however, failed to reduce TTC on any medium tested and was apparently unable to respire. By contrast, strains carrying any of the above *spo* mutations were able to reduce TTC, even when grown on glucose. The characteristic was sufficiently reliable for its use as a diagnostic test in screening for *spo* initiation mutations, or scoring for their presence, and indicated that in such strains respiratory functions are constitutively derepressed.

Differences in protein composition. Several differences between the protein compositions of wild-type and *spd1* strains have been detected by polyacrylamide gel electrophoresis. One polypeptide of molecular weight about 21 000 whose appearance is partially derepressed in *spd1* strains was not produced by *spo50* mutants under any conditions of growth including those (on YEFG or YEPE) in which the wild-type strains do produce this polypeptide (Dawes & Calvert, 1984).

DISCUSSION

A further three genes, *SPO50*, *SPO51* and *SPO53*, have been implicated in the initiation of meiosis and sporulation, in addition to those previously reported, including *CDC25*, *CDC35*, possibly *CDC28* (Shilo *et al.*, 1978), *SPD1*, *SPD3* and *SPD4* (Dawes & Calvert, 1984). Mutation in any of these three *SPO* genes invariably resulted in cells trying to divide under conditions that normally favour sporulation. They act as by 'bypass' mutations suppressing derepressed sporulation in an *spd1* or *spd3* background and confer a growth advantage under conditions in which a proportion of a wild-type population would sporulate. Diploids homozygous for these *spo* mutations showed none of the morphological changes associated with sporulating cells and, moreover, a strain homozygous for the *spo50* mutation did not undergo premeiotic DNA replication or meiotic recombination under normal sporulation conditions, although a few very early sporulation-specific polypeptide changes have been detected in this strain (Ajam, 1981; Wright *et al.*, 1981). These changes occurred in wild-type cells well before they became committed to meiosis.

While the *spo50*, *spo51* and *spo53* mutations promoted cell division at the expense of sporulation, diploids homozygous for them were apparently unable to adopt a normal stationary-phase configuration under starvation conditions. Wild-type cells in stationary phase fail to initiate sporulation (Hirschberg & Simchen, 1977) and assume distinctive shapes, chromosome structure (Piñon, 1978) and staining reactions (Schwartzentruber, 1977). Piñon suggested that stationary phase is not simply arrest in G1 of the cell cycle, but is a separate life cycle phase (designated G0) with specific changes adapting the cell to starvation. Bedard *et al.* (1981) doubted the existence of a separate G0 phase as they were unable to isolate mutants that were specific to it; our isolates may be such mutants.

The *spo50*, *spo51* and *spo53* genes therefore have a role in determining whether the cell initiates sporulation, continues division or adapts to starvation. They act in a positive way to promote the initiation of sporulation and adaptation to starvation conditions, as mutations in them leading to asporogeny and abnormal starvation morphology are recessive. However, the same mutations are at least partially dominant in suppressing the inability of *spd1* strains to grow on non-fermentable carbon sources.

Since mutation at any of the *spo50*, *spo51* or *spo53* loci suppresses any of the *spd1* or *spd3*

mutations, and since they restore to *spd* strains the ability to grow on glycerol, it is likely that all five mutations affect a common function in the cell. The *spd1* mutations appear to influence directly the mechanism by which cells monitor their nutritional status when respiring, and this may involve some mitochondrial function (Dawes & Calvert, 1984). The loss of glucose repression of TTC reduction in the *spo50*, *spo51* and *spo53* mutants supports this hypothesis and the mutants that have lost glucose control over mitochondrial activity recently described by Böker-Schmitt *et al.* (1982) should be considered in this context since they have a similar phenotype.

In summary, it seems likely that the decision between continued cell division or initiation of meiosis and spore formation depends on the level of some mitochondrial activity encoded by the nuclear *SPD* genes. A reduction in this activity by mutation could be compensated for by derepression of mitochondria under the control of the *SPO* genes described here. Sporulation is known to be dependent on respiration (Roth & Halvorson, 1969) or at least on some mitochondrial functions (Pratje *et al.*, 1979; Hartig & Breitenbach, 1980), yet mutations that lead to one form of respiratory deficiency (*spd1*, *spd3* and *spd4*) promote sporulation, while those probably resulting in derepressed mitochondrial activity (*spo50*, *spo51* and *spo53*) inhibit sporulation. There is therefore a more fundamental and complex regulatory connection between respiration and sporulation than the simple dependence of sporulation on respiration. Definition of this connection requires a detailed analysis of mitochondrial metabolism as cells are shifted from growth to sporulation conditions, using wild-type strains and the mutants described here.

This work was supported by grants from the SERC and an SERC studentship award to G. R. C.

REFERENCES

- AJAM, N. (1981). *Regulation of sporulation in Saccharomyces cerevisiae*, pp. 151–174. PhD thesis, University of Edinburgh, UK.
- BEDARD, D. P., JOHNSTON, G. C. & SINGER, R. A. (1981). New mutations in the yeast *Saccharomyces cerevisiae* affecting completion of 'start'. *Current Genetics* **4**, 205–214.
- BÖKER-SCHMITT, E., FRANCISCI, J. & SCHWEYEN, R. S. (1982). Mutations releasing mitochondrial biogenesis from glucose repression in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **151**, 303–310.
- CARTER, B. L. A. & SUDBERY, P. (1980). Small-sized mutants of *Saccharomyces cerevisiae*. *Genetics* **96**, 561–566.
- DAWES, I. W. (1975). Study of cell development using derepressed mutations. *Nature, London* **255**, 707–708.
- DAWES, I. W. & CALVERT, G. R. (1984). Initiation of sporulation in *Saccharomyces cerevisiae*. Mutations causing derepressed sporulation and G1 arrest in the cell division cycle. *Journal of General Microbiology* **130**, 605–613.
- DAWES, I. W. & THORNLEY, J. H. M. (1970). Sporulation in *Bacillus subtilis*. Theoretical and experimental studies in continuous culture systems. *Journal of General Microbiology* **62**, 49–66.
- ESPOSITO, M. S. & ESPOSITO, R. E. (1969). The genetic control of sporulation in *Saccharomyces*. I. The isolation of temperature-sensitive sporulation-deficient mutants. *Genetics* **61**, 78–89.
- ESPOSITO, M. S. & ESPOSITO, R. E. (1974). Genes controlling meiosis and spore formation in yeast. *Genetics* **78**, 215–225.
- ESPOSITO, M. S., ESPOSITO, R. E., ARNAUD, M. & HALVORSON, H. O. (1970). Conditional mutants of meiosis in yeast. *Journal of Bacteriology* **104**, 202–210.
- ESPOSITO, R. E. & KLAPHOLZ, S. (1981). Meiosis and ascospore development. In *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, pp. 211–287. Edited by J. N. Strathern, E. W. Jones & J. R. Broach. New York: Cold Spring Harbor Laboratory.
- FAST, D. (1973). Sporulation synchrony in yeast. *Journal of Bacteriology* **116**, 925–930.
- HARTIG, A. & BREITENBACH, M. (1980). Sporulation in mitochondrial *oxi3* mutants of *Saccharomyces cerevisiae*. A correlation with the genetic map. *Current Genetics* **1**, 97–102.
- HARTWELL, L. H. (1974). *Saccharomyces cerevisiae* cell cycle. *Bacteriological Reviews* **38**, 164–198.
- HAWTHORNE, D. C. (1976). UGA mutations and UGA suppressors in yeast. *Biochimie* **58**, 179–182.
- HIRSCHBERG, J. & SIMCHEN, G. (1977). Commitment to the mitotic cell cycle in yeast in relation to meiosis. *Experimental Cell Research* **105**, 245–252.
- MORTIMER, R. & HAWTHORNE, D. C. (1975). Genetic mapping in yeast. *Methods in Cell Biology* **11**, 221–234.
- OGUR, M., ST. JOHN, R. & NAGAI, S. (1975). Tetrazolium overlay technique for population studies of respiration deficiency in yeast. *Science* **125**, 928–929.
- PIGGOT, P. J. & COOTE, J. G. (1976). Genetic aspects of bacterial endospore formation. *Bacteriological Reviews* **40**, 908–962.
- PIÑON, R. (1978). Folded chromosomes in non-cycling yeast cells. Evidence for a characteristic G0 form. *Chromosoma* **67**, 263–274.

- PRATJE, E., SCHULZ, R., SCHNIERER, S. & MICHAELIS, G. (1979). Sporulation of mitochondrial respiratory-deficient *mit⁻* mutants of *Saccharomyces cerevisiae*. *Molecular and General Genetics* **176**, 411–415.
- REED, S. I. (1980). The selection of *Saccharomyces cerevisiae* mutants defective in the start event of cell division. *Genetics* **95**, 561–577.
- ROTH, R. & HALVORSON, H. O. (1969). Sporulation of yeast harvested during logarithmic growth. *Journal of Bacteriology* **98**, 831–832.
- ROTHSTEIN, R. J., ESPOSITO, R. E. & ESPOSITO, M. S. (1976). The effect of ochre suppression on meiosis and ascospore formation in *Saccharomyces*. *Genetics* **85**, 35–54.
- SHILO, V., SIMCHEN, G. & SHILO, B. (1978). Initiation of meiosis in cell-cycle initiation mutants of *Saccharomyces cerevisiae*. *Experimental Cell Research* **112**, 241–248.
- SLATER, T. F., SAWYER, B. & STRÄULI, U. (1963). Studies on succinate-tetrazolium reductase system. III. Points of coupling of four different tetrazolium salts. *Biochimica et biophysica acta* **77**, 383–393.
- SUDBERY, P. E., GOODEY, A. R. & CARTER, B. L. A. (1981). Genes which control cell proliferation in the yeast *Saccharomyces cerevisiae*. *Nature, London* **288**, 401–404.
- SWARTZENDRUBER, D. E. (1977). A bromodeoxyuridine (BUdR)-mithramycin technique for detecting cycling and non-cycling cells by flow microfluorimetry. *Experimental Cell Research* **109**, 439–443.
- VEZINHET, F., KINNAIRD, J. H. & DAWES, I. W. (1979). The physiology of mutants derepressed for sporulation in *Saccharomyces cerevisiae*. *Journal of General Microbiology* **115**, 391–402.
- WRIGHT, J. F., AJAM, N. & DAWES, I. W. (1981). The nature and timing of sporulation-specific protein changes in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* **1**, 910–918.