The Physiology of Mutants Derepressed for Sporulation in *Saccharomyces cerevisiae*

By FRANCOISE VEZINHET,* JANE H. KINNAIRD
AND IAN W. DAWES

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

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The *spd 1* mutants of *Saccharomyces cerevisiae* are affected in the initiation of sporulation since they sporulate under conditions in which the wild-type does not. They are unable to grow on a range of non-fermentable carbon substrates, but can grow on ethanol. On others, including acetate, glycerol, lactate and pyruvate, the mutants sporulate abundantly, even in rich media. The mutation does not affect the uptake of glycerol or the synthesis of the enzymes concerned with its entry into general metabolic pathways. It probably affects a central metabolic function concerned with the metabolism of some, but not all, non-fermentable carbon sources. The *spd 1* mutation is also expressed in haploids. When haploid or diploid cells are transferred to media containing only non-fermentable carbon substrates they become arrested in the G1 phase of the cell cycle before, or at, the execution point for the *cdc 28* mutation. In diploids homozygous for the *spd 1* gene these conditions also lead to a lowering of the repression by the nitrogen source. The *spd 1* mutants are therefore probably not affected directly in events unique to the initiation of sporulation, but in areas of metabolism closely connected with both the arrest of cells in the G1 phase of the cell cycle and the control of induction of sporulation.

INTRODUCTION

Mutants of *Saccharomyces cerevisiae* which are derepressed for sporulation have been described previously (Dawes, 1975); these sporulate under certain conditions in which the wild-type does not. Those affected in a gene designated *spd 1* sporulate after reaching stationary phase on media containing glucose as carbon source, but unlike the wild-type, sporulation is initiated immediately when they are resuspended in a complete medium containing glycerol as the carbon source. The sporulation of these mutants is not altered by the mutation insofar as the duration of the process, the sequence of events, the number of spores per ascus and the viability of spores is concerned, and they appear to be modified in a metabolic or control function involved only in the initiation of sporulation.

Initiation is a complex of metabolic and genetic events. It requires at least the presence of *a* and α mating-type alleles, the arrest of cell growth in the G1 stage of the cell division cycle and the specific derepression of sporulation. While growth and sporulation are exclusive processes, the arrest of vegetative growth is not necessarily followed by the induction of sporulation, since this is specifically repressed by controls dependent on some form of glucose repression (Miller, 1963; Vezinhét, 1970) and of nitrogen repression (Miller, 1963; Piñon, 1977). Moreover, cells can only switch to alternative development processes if they are in the G1 stage of the cell cycle (between cytokinesis and the initiation of DNA synthesis). Reid & Hartwell (1977) showed with haploid strains that the transition between the mitotic cell cycle and the two other processes of mating, and of entry into

* Present address: Laboratoire de Recherches de la chaire de Génétique, INRA-ENSA, 34060 Montpellier, Cedex, France.

stationary phase, occurs only during G1 before the step mediated by the product of the *cdc 28* gene. Furthermore, once diploid cells have performed the function determined by the *cdc 28* gene product they are committed to mitosis and cannot undergo sporulation until they have completed the cell cycle (Shilo *et al.*, 1978). In this paper the physiology of a diploid strain homozygous for the *spd 1* mutation and the wild-type have been compared to determine which of the various controls over the initiation of sporulation are affected by the *spd 1* mutation.

METHODS

Yeast strains. The wild-type parental strain S41 of *Saccharomyces cerevisiae* ($\frac{a}{\alpha} \frac{HO_a}{HO_a} \frac{HM}{HM} \frac{arg\ 4-1}{arg\ 4-1} \frac{cyh\ 1}{cyh\ 1}$) was obtained from Professor H. O. Halvorson, Brandeis University. Strain ID16D ($\frac{a}{\alpha} \frac{HO_a}{HO_a} \frac{HM}{HM} \frac{arg\ 4-1}{arg\ 4-1} \frac{cyh\ 1}{cyh\ 1} \frac{spd\ 1-1}{spd\ 1-1}$) was derived from S41 by ultraviolet mutagenesis and selected by an ether selection technique (Dawes & Hardie, 1974). Homothallic strains were used since they permit the isolation of recessive mutations which would not otherwise have been recovered (Esposito & Esposito, 1969). Strain FV2D1 (*a, arg 4-1, tyr 1, his 7, ura 1, ade 1,2, lys 2, spd 1-1, cdc 28-1*) was a segregant from the mating of ID16D with ts 185-3-4 (*a ade 1,2, ura 1, tyr 1, his 7, lys 2, gal 2, cdc 28-1*) which was supplied by The Berkeley Yeast Genetic Stock Culture Collection. Gene symbols are those proposed at the IVth International Yeast Genetics Conference, Osaka, Japan (von Borstel, 1969); additionally, *cdc* refers to genes affecting the cell division cycle, *HO_a* and *HM* to genes involved in homothallism, and *spd* to genes concerned with depression of sporulation.

Culture media. Complex media (YEP/carbon source) contained 1% (w/v) yeast extract, 2% (w/v) peptone and the carbon source specified. Defined minimal media (YNB/carbon source, YNB-N/carbon source and buffer/carbon source) contained, respectively, Difco yeast nitrogen base without amino acids (6.7 g l⁻¹), yeast nitrogen base without amino acid and without (NH₄)₂SO₄ (1.7 g l⁻¹) and potassium phosphate buffer (67 mM, pH 7.0). The carbon source was added as indicated in the Results. L-Aspartate was used as a nitrogen source at a concentration of 1 g l⁻¹. L-Amino acids and nucleotide bases were supplemented as needed at 10 µg ml⁻¹ (L-tyrosine, L-phenylalanine) or 20 µg ml⁻¹ (adenine, L-histidine, L-arginine, uracil) or 40 µg ml⁻¹ (L-lysine).

Assay of glycerol uptake. Strains were pre-grown on YEP/glucose, harvested in exponential phase, washed once and resuspended in a fivefold dilution of YEP/glycerol. After 5 h adaptation to this medium [¹⁴C]-glycerol was added to 0.4 µCi ml⁻¹. At intervals, 1 ml samples were filtered, and the filters were washed with water (3 × 10 ml), dried under an infrared lamp and counted in a toluene-based scintillation fluid [4.0 g 2,5-diphenyloxazole and 0.5 g 1,4-di-2-(5-phenyloxazolyl)benzene per litre toluene].

Preparation of crude enzyme extracts. For glycerol kinase and glycerol-3-phosphate dehydrogenase assays, crude extracts were prepared as follows. Cells were harvested by centrifuging, washed twice in 0.1 M-Tris/HCl pH 7.4 buffer and broken by agitation with glass beads. About 80% breakage was achieved by suspending the pellet in an equal volume of buffer and adding acid-washed glass beads (40 mesh) to about 5 mm below the liquid level in the glass centrifuge tube. This mixture was vibrated by inserting the probe of a Vibromix below the surface of the glass beads. The breakage tube was cooled in ice/water, and cycles (usually five) of vibrating for 1 min followed by 1 min rest were used. The broken cell extract was removed from the glass beads by centrifuging through a fine stainless-steel screen, and then centrifuged at 14000 g for 20 min. The supernatant was used for assay.

Assays of arginase, NAD⁺-dependent glutamate dehydrogenase and glutamine synthetase were carried out on extracts of freeze-dried cells.

Enzyme assays. Glycerol kinase [EC 2.7.1.30; ATP:glycerol 3-phosphotransferase] was estimated by determining [¹⁴C]glycerol 3-phosphate produced from [¹⁴C]glycerol according to the method of Newsholme *et al.* (1967) with the modification of Sprague & Cronan (1977). Glycerol-3-phosphate dehydrogenase [EC 1.1.99.5; glycerol-3-phosphate:(acceptor) oxidoreductase] was assayed by the method of Lin *et al.* (1962). Arginase (EC 3.5.3.1; L-arginine amidinohydrolase) was assayed according to the method of Middelhoven (1964), except that the urea produced was estimated by the diacetyl monoxime method of Moore & Kauffman (1970). NAD⁺-dependent glutamate dehydrogenase [EC 1.4.1.2; L-glutamate:NAD⁺ oxidoreductase (deaminating)] and glutamine synthetase [EC 6.3.1.2; L-glutamate: ammonia ligase (ADP-forming)] were assayed according to the methods of Ferguson & Sims (1971).

Protein estimation. The protein concentration in cell extracts was assayed by the Folin method of Lowry, using bovine serum albumin as standard.

Fig. 1. Growth and sporulation of the wild-type and homozygous *spd 1* mutant diploid strains on complex media. Cells growing on YEP/glucose were harvested in early-stationary phase, washed twice with acetate buffer and resuspended in the different media, containing YEP and the carbon source indicated (% w/v), to a turbidity at 600 nm of 0.1. Growth was measured by the turbidity increase after 24 h, and sporulation was estimated 48 h after inoculation: □, growth of wild-type S41; ▨, growth of *spd 1* mutant ID16D; ▩, sporulation of wild-type S41; ▧, sporulation of *spd 1* mutant ID16D.

RESULTS

Growth and sporulation of the spd 1 mutant on complex media

Preliminary characterization of the derepressed sporulation (*spd 1*) mutants in *Saccharomyces cerevisiae* indicated that they were affected in their ability to grow and sporulate on complex media containing glycerol as the energy source (Dawes, 1975). These results have now been extended to a range of other carbon sources, to determine whether the *spd 1* mutation modifies the general pattern of carbon source effects on sporulation. The carbon sources tested were: glucose, which is known to repress sporulation strongly; galactose, glycerol, pyruvate, lactate and ethanol, which support sporulation to a limited extent; and acetate, which is the best carbon source for inducing sporulation (Miller, 1957; Vezinhet, 1970). The utilization of many of the above carbon sources is subject to some form of carbon catabolite repression and the possibility that the *spd 1* phenotype is due to an alteration in this repression has been examined. Results for growth and sporulation on complex media, containing the different carbon sources, of the homozygous *spd 1* mutant ID16D and its wild-type parent S41 are given in Fig. 1. The phenotype of the *spd 1* mutation is characterized by poor growth and a rapid and abundant sporulation, and this was observed on glycerol, pyruvate, acetate and lactate. On galactose or ethanol, the growth of the mutant was more or less reduced but sporulation did not occur within 48 h. Sporulation of the mutant was sometimes observed at very low frequency on galactose. On glucose, the mutant grew as well as the wild-type and did not sporulate within 48 h. On two other substrates, dihydroxyacetone and succinate, the growth of both the wild-type and the mutant was very poor (results not shown in Fig. 1). Sporulation of the mutant was only observed on those substrates which did not support its growth. On these substrates the wild-type grew well and did not sporulate, except on acetate which is known to induce sporulation with great efficiency.

Growth and sporulation of the spd 1 mutant on defined minimal media

Yeast extract and peptone contain compounds which yeast can use as sources of carbon for growth, and we therefore tested both the wild-type and mutant strains for their ability to grow and sporulate on defined minimal media. These tests included media lacking a nitrogen source required for growth (Table 1).

Table 1. *Growth and sporulation of the wild-type and spd 1 diploid mutant strains on defined minimal media*

Conditions were similar to those described in Fig. 1, except that growth was estimated 48 h after inoculation.

Medium composition		Wild-type S41		<i>spd 1</i> mutant ID16D	
Base of medium	Carbon source (% w/v)	Growth (turbidity at 600 nm)	Sporulation (% asci)	Growth (turbidity at 600 nm)	Sporulation (% asci)
YNB	Glucose (2)	4.05	0	5.10	0
	Galactose (2)	4.55	0	3.55	1
	Glycerol (3)	0.57	18	0.39	41
	Pyruvate (1)	1.25	7	0.64	38
	Acetate (1)	1.20	0	0.72	20
	Lactate (1)	0.58	13	0.32	35
	Ethanol (1)	3.45	0	1.97	4
YNB-N	Glucose (2)	2.05	1	2.02	1
	Galactose (2)	1.50	3	1.32	12
	Glycerol (3)	0.60	16	0.46	46
	Pyruvate (1)	0.75	28	0.37	63
	Acetate (1)	0.89	50	0.34	53
	Lactate (1)	0.44	13	0.29	49
	Ethanol (1)	1.52	5	0.95	14
Phosphate buffer (67 mM, pH 7)	Glucose (2)	1.01	1	1.02	2
	Galactose (2)	0.95	11	0.96	11
	Glycerol (3)	0.42	19	0.50	17
	Pyruvate (1)	0.66	19	0.55	45
	Acetate (1)	0.77	46	0.90	48
	Lactate (1)	0.39	9	0.55	17
	Ethanol (1)	1.02	13	1.00	36

On YNB minimal media, the *spd 1* phenotype is expressed on the same substrates as found using complex media. On glycerol, pyruvate, acetate and lactate, the growth of the wild-type was very poor, but growth of the mutant was always less. The poor growth of the wild-type was associated with some sporulation on these substrates (except on acetate, on which cell lysis occurred) but sporulation of the mutant occurred at much higher frequency. Growth and the absence of sporulation was observed for both the wild-type and the mutant on glucose, galactose and ethanol under these conditions. On YNB-N minimal media, the absence of a nitrogen source prevented growth of the wild-type and the mutant. On all substrates, except glucose, sporulation was induced. On acetate, the wild-type and the mutant sporulated to the same extent, but on the other substrates the mutant sporulated to a greater degree than the wild-type. In buffer, the results were very similar. However, the sporulation of the mutant was reduced on glycerol and lactate which are not good substrates for sporulation and which might not permit formation of spores and asci even if meiosis was induced. Moreover, we observed that in buffer, sporulation was very dependent on presporulation conditions and on the physiological state of cells at the moment of transfer to the buffer.

Comparison of the results obtained on the different defined media with those found using complex media showed that under conditions which lead to an absence of growth the mutant responds in a markedly different way to the wild-type, the latter never sporulating to the same extent as the mutant.

Table 2. Growth of the mutant and wild-type strains on the different ingredients of YEP/glycerol

Conditions were similar to those described in Fig. 1, except that growth was estimated 48 h after inoculation.

Medium composition			Growth (turbidity at 600 nm)	
Yeast extract (%, w/v)	Peptone (%, w/v)	Glycerol (%, v/v)	Wild-type S41	<i>spd 1</i> mutant ID16D
1	—	—	0.99	1.15
—	2	—	0.49	0.35
1	2	—	1.22	1.23
1	—	3	2.82	1.55
—	2	3	2.14	0.55
1	2	3	5.55	1.82

Fig. 2. Uptake of [^{14}C]glycerol by wild-type (○) and homozygous *spd 1* mutant (●) diploid strains. Cells growing on YEP/glucose were harvested in exponential phase, washed once and resuspended in a fivefold dilution of YEP/glycerol. After 5 h incubation in this medium [^{14}C]glycerol was added ($0.4 \mu\text{Ci ml}^{-1}$) and uptake was assayed immediately (time 0) and at intervals thereafter, as described in Methods. The data have been corrected for non-specific adsorption of glycerol to the filters.

Metabolism of glycerol

One possible explanation for the nature of the *spd 1* phenotype is that the mutation affects the assimilation of certain carbon sources. Such a mutation would have to affect either a metabolic pathway common to all of the substrates concerned, or an element regulating the synthesis or activity of the enzymes and transport systems needed to assimilate the substrates. To investigate these possibilities further, glycerol metabolism in the *spd 1* strain was compared with that in the wild-type, since glycerol is the substrate on which the *spd 1* mutation is very clearly expressed.

In complex media, glycerol is used for growth by the wild-type but not by the mutant. By comparing growth of these on different combinations of the components of YEP/glycerol, it was found that the small amount of growth of the mutant on YEP/glycerol occurred mainly at the expense of yeast extract and little of the glycerol was consumed (Table 2). It seems unlikely that the poor growth of the mutant on glycerol was due to a defect in the uptake of glycerol since the mutation does not solely affect glycerol metabolism and also the mutant uses glycerol as a carbon source to support sporulation (it does not sporulate in YEP alone). It is possible that the *spd 1* mutation pleiotropically affects an element regulating the synthesis or activity of enzymes and/or transport systems needed in the assimilation of the various carbon sources used. Mutations of this type affecting cata-

Table 3. *Activities of enzymes involved in glycerol catabolism in the wild-type and mutant strains*

Cells growing exponentially in YEP/glucose were harvested and resuspended in YEP/glycerol. Enzymes were assayed immediately (0 h) and 5 h after resuspension.

Strain	Enzyme activity [nmol min ⁻¹ (mg protein) ⁻¹]			
	Glycerol kinase		Glycerol-3-phosphate dehydrogenase	
	0 h	5 h	0 h	5 h
Wild-type S41	0.092	1.43	2.08	6.34
<i>spd 1</i> mutant ID16D	0.067	2.60	0.53	7.68

Table 4. *Effect of various components on growth of the wild-type and mutant strains on defined medium*

Cells growing on YEP/glucose were harvested in early-stationary phase and resuspended in the medium indicated. Growth was estimated 48 h after resuspension.

Component added (%, w/v)	Growth (turbidity at 600 nm)			
	Wild-type S41		<i>spd 1</i> mutant ID16D	
	YNB	YNB/glycerol	YNB	YNB/glycerol
Glucose (15 × 10 ⁻⁶)	0.14	0.47	0.34	0.22
Yeast extract (0.1)	—	1.82	—	0.34
Yeast extract (0.2)	—	2.70	—	0.53
Yeast extract (0.5)	0.53	5.42	0.55	1.51
Peptone (0.2)	—	0.82	—	0.46
Peptone (2.0)	0.32	2.56	0.30	0.69
Pyruvate (0.5)	0.78	3.16	0.32	0.51
Acetate (0.5)	0.42	0.63	0.50	0.47
Citrate (0.5)	0.08	0.19	0.08	0.10
Succinate (0.5)	0.07	0.31	0.08	0.10
Malate (0.5)	0.09	1.61	0.08	0.18
Glyoxylate (0.5)	0.08	0.13	0.08	0.10
Glutamate (0.5)	0.09	0.52	0.08	0.13
Aspartate (0.5)	0.12	3.71	0.08	0.19
None	0.09	0.10	0.08	0.10

bolite repression or nitrogen repression have been described in both prokaryotes (Perlman & Pastan, 1969) and the eukaryote *Aspergillus nidulans* (Arst & Cove, 1973). We therefore studied the uptake of [¹⁴C]glycerol over a short period (10 min) into cells which had previously been incubated in a fivefold dilution of YEP/glycerol to ensure adaptation to this substrate (Fig. 2). The rate of incorporation of isotope was similar in both the wild-type and the mutant.

Catabolism of glycerol proceeds via glycerol 3-phosphate to dihydroxyacetone phosphate through the action of glycerol kinase and glycerol-3-phosphate dehydrogenase, respectively (Sprague & Cronan, 1977). The activities of these enzymes were determined in both the wild-type and the mutant 5 h after transfer of exponentially growing cells from YEP/glucose to YEP/glycerol, by which time the wild-type was adapted to growth on glycerol (Table 3). We observed an extensive derepression of glycerol catabolic enzymes after 5 h on YEP/glycerol but there was no significant differences between the *spd 1* mutant and the wild-type.

From these results it can be concluded that the *spd 1* mutation does not affect either the uptake of glycerol into the cell, or the induction and synthesis of the enzymes specifically concerned with its assimilation. The possibility remains, however, that the activity of one

Table 5. Effect of NH_4^+ concentration on sporulation of the mutant and wild-type strains in the presence of glycerol, pyruvate or lactate

Cells growing on YEP/glucose were harvested in early-stationary phase, washed with NaCl (9%, w/v) and resuspended in 67 mM-phosphate buffer (pH 7) containing glycerol (3%, v/v), sodium pyruvate (1%, w/v) or sodium lactate (1%, w/v), and $(\text{NH}_4)_2\text{SO}_4$ at the concentrations indicated. Sporulation and viability were estimated at 48 h. [The highest concentration of $(\text{NH}_4)_2\text{SO}_4$ (35 mM) was that usually used in growth media.]

Sporulation medium	$(\text{NH}_4)_2\text{SO}_4$ concn (mM)	Wild-type S41		<i>spd 1</i> mutant ID16D	
		Sporulation (% asci)	Viability (%)	Sporulation (% asci)	Viability (%)
Phosphate buffer + glycerol	0	22	99	22	99
	0.5	25	99	23	99
	1	23	99	27	99
	2	9	99	26	99
	5	10	98	64	97
	35	11	98	17	96
Phosphate buffer + pyruvate	0	18	99	33	99
	0.5	17	86	30	99
	1	14	75	38	99
	2	14	85	29	99
	5	23	75	28	92
	35	18	77	33	99
Phosphate buffer + lactate	0	2	98	15	100
	0.5	3	99	11	99
	1	1	98	9	99
	2	2	99	9	99
	5	2	99	11	92
	35	1	99	5	97

of these enzymes is affected in the mutant, but if so the activity of other enzymes specifically involved in the utilization of acetate and lactate must also be modulated in the same way. It is more likely that the lesion in *spd 1* strains affects a central metabolic reaction which is essential for the utilization of all of the substrates on which the *spd 1* phenotype is expressed.

Defined media for the growth of wild-type strains on glycerol

One of the problems found in studying the strains reported here was the inability of the wild-type to grow on glycerol as the sole carbon source (i.e. in YNB/glycerol). In an attempt to identify a compound present in yeast extract or peptone which stimulated growth on glycerol, particular components were added to the defined medium (Table 4). Glucose added at the concentration found in yeast extract was ineffective, but the response of growth to the addition of yeast extract was proportional to the amount added, indicating that the wild-type needs an additional compound present in yeast extract. The action of different intermediates of the tricarboxylic acid cycle, or compounds directly related to this cycle, was tested; pyruvate, malate and aspartate were found to improve growth of the wild-type on glycerol minimal medium. L-Malate had only a small effect, while pyruvate could itself act as a carbon source, and the most noticeable effect followed the addition of L-aspartate. Patel & Miller (1972) observed that ammonium sulphate is not used as nitrogen source when glycerol is the carbon source and that glutamic acid supports growth. We observed that L-aspartate is more effective than L-glutamate and confirmed that ammonium sulphate is not used when glycerol is the main carbon source since, regardless of the concentration of aspartate in a medium, addition of ammonium sulphate did not greatly increase the yield of wild-type cells.

The defined medium YNB/glycerol/aspartate supported growth of the wild-type but remained ineffective in supporting growth of the *spd 1* mutant.

Table 6. *Effect of NH_4^+ concentration on sporulation of the mutant and wild-type strains in the presence of potassium acetate*

Conditions were similar to those described in Table 5, except that potassium acetate (1 %, w/v) was present in the sporulation medium.

Sporulation medium	$(\text{NH}_4)_2\text{SO}_4$ concn (mm)	Wild-type S41		<i>spd 1</i> mutant ID16D	
		Sporulation (% asci)	Viability (%)	Sporulation (% asci)	Viability (%)
Acetate	0	65	96	70	99
	0.5	44	87	75	99
	1	21	63	68	95
	2	1	49	27	76
	5	0	27	0	55
	35	0	10	0	18
Phosphate buffer + acetate	0	38	95	59	99
	0.5	22	81	51	98
	1	1	75	54	92
	2	2	69	54	94
	5	0	66	56	93
	35	0	59	37	92
YNB-N + acetate	0	58	99	69	99
	0.5	45	98	70	98
	1	53	98	73	98
	2	27	90	80	93
	5	0	50	61	88
	35	0	30	25	55

Table 7. *Nitrogen repression of inducible enzyme synthesis in the wild-type and homozygous *spd 1* mutant diploid strains*

Enzymes were assayed as described in Methods using mid-exponential phase cultures. The growth medium was YNB-N containing glucose (2%, w/v) and the nitrogen source indicated. The substrate for arginase was arginine, and for the other two enzymes was glutamate.

Nitrogen source	Enzyme activity [$\text{nmol min}^{-1} (\text{mg protein})^{-1}$]					
	Arginase		NAD ⁺ -dependent glutamate dehydrogenase		Glutamine synthetase	
	S41	ID16D	S41	ID16D	S41	ID16D
$(\text{NH}_4)_2\text{SO}_4$ (7.5 mm)	180	150	3.86	6.72	190	140
Substrate (20 mm)	960	890	28.94	32.79	880	790
Substrate (20 mm) + $(\text{NH}_4)_2\text{SO}_4$ (7.5 mm)	650	360	5.76	8.29	200	090

Nitrogen repression of sporulation

Sporulation in both the wild-type and the *spd 1* mutant is not only a response to the absence of growth on some substrates but is also the consequence of specific derepression conditions. As the mutant is still repressed by glucose, we studied nitrogen repression in the mutant in greater detail. Previous experiments (Dawes, 1975) indicated that nitrogen repression seems to be modified in the mutant; in these, the action of ammonium sulphate was tested in a medium supporting growth and the concentration of NH_4^+ at the time of initiation of sporulation was not known. In the present work we tested the effect of NH_4^+ on sporulation without growth in the presence of glycerol, pyruvate, lactate and acetate.

For the first three substrates the rate of induction of sporulation was low, particularly for lactate (Table 5). Neither sporulation nor the viability of cells seemed to be influenced by the NH_4^+ concentration in the medium. The *spd 1* mutant gave similar results to the wild-type, except that sporulation was higher than for the wild-type in most cases.

The response of sporulation to NH_4^+ , however, was very different when acetate was present as carbon source. This was tested under three different conditions: on potassium acetate alone, on potassium acetate buffered to pH 7 by a phosphate buffer, and on YNB-N/potassium acetate. The wild-type strain was very sensitive to the presence of NH_4^+ under these conditions; sporulation was markedly inhibited at concentrations from about 1 mM (depending on the medium) and the viability of the culture population was also considerably reduced (Table 6). These results are in accord with those of Piñon (1977).

The response of the *spd 1* mutant was, however, very different from that of the wild-type. On acetate alone this difference was less marked since NH_4^+ concentrations in excess of 2 mM inhibited sporulation and also reduced the viability of the cells. Lower concentrations of NH_4^+ did not affect sporulation to any appreciable extent. On buffered potassium acetate, and on potassium acetate containing vitamins and essential ions (YNB-N) the difference between the wild-type and mutant was very marked, since sporulation of the mutant was almost completely insensitive to NH_4^+ in the medium, and there was less of an effect of this ion on cell viability.

It can thus be concluded that the *spd 1* mutation reduces the NH_4^+ -mediated repression of sporulation under certain conditions. It is not known if there are any control elements common to both the nitrogen source effect on sporulation and to the repression by nitrogen compounds of the synthesis of certain inducible enzymes. We tested this possibility by studying the NH_4^+ repression of the synthesis of three enzymes—arginase, NAD^+ -dependent glutamate dehydrogenase and glutamine synthetase. These are all subject to repression by NH_4^+ (Middelhoven, 1970; Ferguson & Sims, 1971; Prival *et al.*, 1973) but perhaps in different ways (Dubois *et al.*, 1973). These enzymes were assayed under three growth conditions (Table 7) to determine the basal, induced and repressed levels. In the wild-type, arginase was induced five- to sixfold by growth on arginine as the sole nitrogen source, and 7.5 mM-ammonium sulphate repressed its synthesis to approximately half the fully-induced level. Growth on glutamate as sole nitrogen source led to a sevenfold induction of NAD^+ -dependent glutamate dehydrogenase and a fourfold increase in glutamine synthetase. Both enzymes were, however, more subject to repression by NH_4^+ than was arginase.

In the mutant strain, repression by NH_4^+ appeared to be similar to that for the wild-type. There was a difference between the basal level of the NAD^+ -dependent glutamate dehydrogenase of the wild-type and the mutant, but induction and repression of this enzyme was the same in both strains. Thus, there appears to be no effect of the *spd 1* lesion on the sensitivity of the mutant to repression of enzyme synthesis by NH_4^+ under the conditions tested.

Arrest of spd 1 mutants during the cell cycle

It is possible that the *spd 1* mutation affects the mitotic cell cycle in such a way that the cells are arrested at a particular point favourable to sporulation of diploids homozygous for the mutation. To test this it was necessary to use a defined minimal medium (rather than YEP/glycerol) on which the phenotype is clearly expressed. Cells were therefore shifted from YEP/glucose to the YNB-N/glycerol medium described above. Since *spd 1* haploids also express part of the phenotype in terms of their inability to grow, the effect of shifting cultures of wild-type and *spd 1* homozygous diploids and of wild-type and *spd 1* haploids was studied using cultures in the exponential phase of growth.

Exponentially growing cells of the wild-type shifted into YNB-N/glycerol/L-aspartate started to grow slowly after a lag phase of about 10 h and reached maximum numbers 48 h after inoculation. Cells of the diploid *spd 1* homozygote and the haploid *spd 1* strain were observed to complete the cell division in progress at the time of the shift and some were able to initiate and complete one more cell cycle. However, all cells finally accumulated in the culture at the end of the cell cycle as either unbudded cells, or cells with large buds. With the haploid *spd 1* strain (FV 2D1) the new bud formed often did not reach the size of the

mother cell and did not separate from it, and a lower percentage of unbudded cells was observed. Nevertheless both haploid and diploid accumulated in the G1 phase of the cell cycle on glycerol/aspartate medium.

Similar differences between the wild-type and the diploid and haploid mutants were seen when stationary phase cultures grown on YEP/glucose were shifted to the glycerol/aspartate defined medium, except that in this case the diploid did not grow and the cells began to sporulate as soon after the shift as they had reached the appropriate stage in the cell cycle, so that 60 to 80% asci were observed after 48 h incubation.

These experiments led us to conclude that in the haploid the *spd 1* mutation after a shift to the 'restrictive' condition (i.e. glycerol as carbon source), leads to an arrest of cells in the G1 phase of the cell cycle. This type of arrest is seen under conditions of nutrient starvation (Unger & Hartwell, 1976). It is possible to determine more precisely the stage in G1 at which the *spd 1* haploids are arrested, using constructed double mutants carrying both the *spd 1* and the *cdc 28-1* mutations. The latter is a temperature-sensitive mutation which at the restrictive temperature arrests cells early in the cell cycle prior to the initiation of DNA synthesis and before duplication of the spindle pole body (Hartwell, 1973; Byers & Goetsch, 1974). In such a double mutant there are two types of conditionality, one sensitive to temperature changes, the other to medium composition. If cells arrested in the restrictive growth medium for the *spd 1* mutation (i.e. on glycerol/aspartate mineral medium) at a permissive temperature for the *cdc 28-1* mutation (30 °C) are transferred to a permissive growth medium (YEP/glucose) at the restrictive temperature (37 °C), then either the cells will bud and continue through another cell cycle until they reach the G1 phase or they will not bud and will arrest before the step mediated by the *cdc 28-1* gene. In the former case, if budding of the *cdc 28-1 spd 1* double mutant does not occur, then the *spd 1* gene function is presumed to arrest the cells *after* the *cdc 28-1* gene function is required. Results obtained, however, indicate that the *spd 1* mutation arrested cells at least at, and probably before, the execution point of the *cdc 28-1* gene. The *spd 1 cdc 28-1* double mutant was arrested at the *spd 1*-mediated step in the cell cycle by growing it at 30 °C on the minimal glycerol/aspartate medium. At this state the culture was divided into two, one part was transferred to YEP/glucose and incubated at 30 °C, the other to YEP/glucose at 37 °C and bud formation was studied by direct microscopic examination. After 2 h at the permissive temperature for the *cdc 28-1* gene (30 °C) bud emergence had started and within 3 h 40 to 60% of the cells had small buds, depending on the experiment. In the same time at 37 °C only about 5% of the cells had buds and after 5 h at the restrictive temperature most of the cells displayed the characteristic phenotype of cells arrested at the execution point of the *cdc 28-1* gene.

DISCUSSION

The *spd 1* mutation has a highly pleiotropic phenotype. Vegetative growth of mutant strains was arrested on a range of different non-fermentable carbon substrates (except on ethanol) and homozygous diploids sporulated abundantly even in rich medium. Mutants able to sporulate in rich media have been reported recently by Shilo *et al.* (1978) and these appear to have some features in common with the *spd 1* mutants. With such pleiotropy it is difficult to distinguish between two possibilities: (i) that in *spd 1* mutants, sporulation is induced because growth is prevented by the mutation, or (ii) that the lesion leads directly to an induction of sporulation and precludes the cell from participating further in mitotic growth and division. For the *spd 1* mutation the former hypothesis is much more likely since haploid *spd 1* mutants are also unable to grow on a range of carbon substrates, and yet normal haploid strains are not known to undergo any events specific to sporulation. It is possible, however, that early events associated with sporulation remain to be detected in haploid strains under sporulation conditions. Since growth of the mutants was affected on a number of different substrates, and for at least one of these (glycerol) neither the uptake nor

synthesis of the primary enzymes needed for its catabolism was altered, it is likely that the mutation affects metabolic function rather than a repression system controlling carbon (or energy) source utilization or a metabolic pathway concerned solely with a particular carbon source. As all of the substrates on which the phenotype is most clearly expressed can only be utilized via respiratory pathways it is possible that the mutation is in a nuclear gene affecting some aspects of mitochondrial function. If this is the case then the function which is affected cannot be needed for sporulation (for which some mitochondrial activities are essential) or for utilization of ethanol on which the mutants grow well.

Nitrogen repression of sporulation is altered by the *spd 1* mutation, but this is not a primary effect for two reasons. First, the mutation is expressed on substrates on which NH_4^+ does not repress sporulation (e.g. glycerol, lactate and pyruvate). Since growth on glycerol requires a nitrogen source other than NH_4^+ it is possible that the above effect is due to a lowered rate of NH_4^+ uptake into the cell. Similarly on acetate medium the altered sensitivity of the mutant to NH_4^+ present in the medium may be a secondary effect arising from the modification to acetate metabolism. Secondly, Piñon (1977) and Durieu-Trautmann & Delavier-Klutcho (1977) observed that NH_4^+ does not inhibit the initiation of premeiotic DNA synthesis, but acts at two stages, the first during DNA synthesis, the other later in sporulation.

The *spd 1* mutation leads to conditions of starvation which cause the accumulation of large budded or unbudded cells in the medium. These cells are arrested in the cell cycle before, or at, the execution point of the *cdc 28-1* gene in such a way that meiosis proceeds directly following this arrest. Such an arrest in the G1 phase is not, however, a sufficient condition for sporulation, since on some media not supporting growth of the wild-type (e.g. YNB supplemented with either glycerol or lactate, or with acetate in the absence of NH_4^+) sporulation of the wild-type was much lower than that of the mutant. The condition of 'starvation' reached by cells of the mutant is therefore presumably different from that attained by the wild-type under the same medium conditions.

In summary, the *spd 1* mutation of *Saccharomyces cerevisiae* probably affects a central metabolic function concerned with the utilization of some, but not all, non-fermentable carbon sources. The lesion induces a particular form of starvation in which the cells are arrested in the G1 phase of the cell cycle before, or at, the execution point of the *cdc 28-1* mutation, under intracellular conditions which do not repress sporulation in the mutant. The *spd 1* mutants, while probably not affected directly in events unique to the initiation of sporulation, do, however, provide useful strains in which to study those areas of metabolism which are closely connected with both the initiation (repression) of sporulation, and also with the arrest of cells in the G1 phase of the cell cycle.

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