MATING TYPE AND SPORULATION IN YEAST
I. MUTATIONS WHICH ALTER MATING-TYPE CONTROL OVER SPORULATION

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ABSTRACT

In *Saccharomyces cerevisiae*, meiosis and spore formation as well as mating are controlled by mating-type genes. Diploids heterozygous for mating type (**a**α) can sporulate but cannot mate; homozygous **aa** and $\alpha\alpha$ diploids can mate, but cannot sporulate. From an $\alpha\alpha$ diploid parental strain, we have isolated mutants which have gained the ability to sporulate. Those mutants which continue to mate as $\alpha\alpha$ cells have been designated CSP (control of sporulation). Upon sporulation, *CSP* mutants yield asci containing 4α spores. The mutant gene which allows $\alpha\alpha$ cells to sporulate is unlinked to the mating-type locus and also acts to permit sporulation in **aa** diploid cells. Segregation data from crosses between mutant $\alpha\alpha$ and wild-type **aa** diploids and *vice versa* indicate (for all but one mutant) that the mutation which allows constitutive sporulation (*CSP*) is dominant over the wild-type allele. Some of the *CSP* mutants are temperature-sensitive, sporulating at 32°, but not at 23°. In addition to *CSP* mutants, our mutagenesis and screening procedure led to the isolation of mutants which sporulate by virtue of a change in the mating-type locus itself, resulting in loss of ability to mate.

For heterothallic strains of *Saccharomyces cerevisiae*, the ability of haploid cells to mate is determined by their genetic constitution at the mating-type locus (Lindegren and Lindegren 1943). Cells of mating-type **a** can mate efficiently with α cells, whereas mating occurs at low frequency ($<10^{-6}$) between two α cells and apparently never between two **a** cells (Roman and Sands 1953; Roman, personal communication). The complementarity of mating behavior between **a** and α yeast cells thus ensures that diploid cells will normally be heterozygous (**a**α) for mating type. For meiosis and sporulation to occur, the presence of both mating-type genes is essential, because **aa** and $\alpha\alpha$ diploid cells cannot sporulate, whereas **a**α diploids can.

Upon transfer to sporulation medium, many of the initial metabolic responses of **aa** and $\alpha\alpha$ cells resemble those of **a**α diploids (Kane, Roth and Erwin 1972; Hopper *et al.* 1974). However, the **aa** and $\alpha\alpha$ cells fail to replicate their nuclear DNA (Roth and Lusnak 1970) and to undergo the subsequent meiotic events such as genetic recombination (Friis and Roman 1968; Roth and Fogel 1971), nuclear division, and spore formation (Roman and Sands 1953). Because the conditions which induce sporulation by **a**α cells (nitrogen starvation in the

presence of a nonrepressing carbon source) leave **aa** and $\alpha\alpha$ cells as resting diploid cells, it is evident that both mating-type alleles act to control the developmental response to this environmental stimulus (starvation for N). In order to analyze the nature of this control, we have isolated mutants in which the control system has altered properties. Diploids homozygous for mating type were mutagenized and then screened for the ability to undergo meiosis. Mutant clones were identified by their ability to undergo meiotic segregation of recessive alleles for cycloheximide and canavanine resistance that were present in the parental **aa** and $\alpha\alpha$ diploid strains. Screening for the ability of diploid cells to produce *can r*, *cyh r* colonies has allowed us to isolate mutants which are capable of ascus and spore formation as well as meiosis.

Some of the mutants retained the mating type of the parental diploid (**aa** or $\alpha\alpha$), whereas others lost the ability to mate either with **a** or α cells. The former allow sporulation to bypass the normal (**a**α) mating-type control while, in the latter group, mutations have affected the mating-type locus itself, either directly by chemical mutagenesis or indirectly through the action of a homothallism gene which arose by mutation.

Part of this research has been reported in a preliminary form (HALL and HOPPER 1973; HOPPER and HALL 1973).

MATERIALS AND METHODS

Yeast Strains: Table 1 lists the yeast strains used, their origin and properties. Mutant isolation experiments were done with diploids derived from strain AP-1-**a**α, obtained by crossing A364A × α_1 131-20. A364A is an **a** haploid (HARTWELL 1967); α_1 131-20 an α haploid segregant of diploid **a**α strain 131-20 (SIMCHEN, SALTS and PIÑON 1973). Diploids homozygous for mating type (AP-1-**aa** and AP-1-$\alpha\alpha$) were derived from AP-1-**a**α by a 1-min UV irradiation (18.4 erg/mm^2/sec) of 1ml cells at 1 × 10^8 cell/ml with stirring in H_2O to induce mitotic recombination (~90% survival).

Mitotic recombinants in which either **a** or α mating phenotype was expressed were scored by their ability to yield prototrophic diploids when crossed with **a** or α haploid strains containing complementary auxotrophic markers. In order to identify them positively as **aa** or $\alpha\alpha$ diploids, cells from those UV-irradiated colonies which could mate were mated with known $\alpha\alpha$ or **aa** diploids to form tetraploid strains which were sporulated and dissected. An ascus containing all 4 (Type I) non-mating diploid spores capable of sporulation (i.e., **a**α cells) could result only if the unknown parent in the cross were homozygous diploid for mating type (Figure 1A). If either parent was monosomic for mating type, no more than 2 of the 4 diploid spores would be **a**α mating type [i.e., monosomy would result in no Type I asci (Figure 1B)] When the putative **aa** strain was crossed to a known $\alpha\alpha$, 13 asci gave 5 cases of Type I asci : 3 of Type II : 5 of Type III. For the putative $\alpha\alpha$ × known **aa**, the ratio was 3:2:3 for asci. These segregations confirm that the cells tested are, respectively, **aa** diploid (AP-1-**aa**) and $\alpha\alpha$ diploid (AP-1-$\alpha\alpha$).

The procedure used to screen for mutants affected in control of sporulation is based upon meiotic segregation of recessive drug-resistance markers (SHERMAN and ROMAN 1963) present in **aa** and $\alpha\alpha$ diploids *r/s* heterozygotes. In order to confirm heterozygosity of the *can* and *cyh* loci, AP-1-**aa** and AP-1$\alpha\alpha$ (phenotypically sensitive for both drugs) were mated to diploids homozygous for mating type and for canavanine and cycloheximide sensitivity (HK-15 and HK-2). Upon sporulation each tetraploid colony was able by recombination to give rise to some homozygous *r/r* colonies capable of growth on both canavanine and cycloheximide and thereby confirming heterozygosity for both loci in AP-1-**aa** and AP-1-$\alpha\alpha$.

The homozygous mating type triploid strain AU-155 (**aaa**) was constructed in the following manner: U-155 (**aa**) was mated on YEP with α haploid S141-S22. Three zygotes were selected

TABLE 1

Strains utilized

Strain	Mating type	Ploidy	Genotype*	Used for	Source‡
56a	**a**	haploid	*iso1*, *try1*, *arg4*	mating analysis	L. HARTWELL
S2072α	α	haploid	*leu1*, *thr4*, *try1*, *arg4*	mating analysis	L. HARTWELL
S400D	**a**	haploid	*met1*, *try1*, *iso1*	mating analysis	L. HARTWELL
S288C-2F	α	haploid	*iso1*	mating analysis	L. HARTWELL
M25-3D	**a**	haploid	*ade6*, *lys5*, *ura1*	mating analysis	P. WHITNEY
M25-18B	α	haploid	*lys1*, *his6*, *ura1*	mating analysis	P. WHITNEY
AP-1-aα	aα	diploid	(*ade1*, *ade2*, *ura1*, *his7*, *lys2*,	parent strain	A364A† Xα_1 131-20†
AP-1-aα	**aa**	diploid	*tyr1*, *gal1*) × (*ade2*, *cyh2*,	mutant parent strain	Derived from AP-1-α**a**
AP-1-$\alpha\alpha$	$\alpha\alpha$	diploid	*can1*, *leu1*)	mutant parent strain	by mitotic recombination
HK-2	**aa**	diploid	*lys1/lys1*	tetraploid analyses involving diploid mutants	H. KLEIN
HK-15	$\alpha\alpha$	diploid	*lys1/lys1*		H. KLEIN
U-155	**aa**	diploid	*leu1/leu1*, *tyr5/tyr5*, *met 13/met 13*,	tetraploid analysis involving diploid mutants	M. UNGER
			lys2/+, *lys5/+*, *ade5, 7/+*, *ade2/+*, *tyr1/+*, *cyh2/s*, *cyl 15/+*, *tyr 3/+*	tertaploid analysis involving haploid mutant segregants	Derived from U-155 (**aa**) × α parent by mitotic recombination
AU-155	**aaa**	triploid	*ul55* × *leu1*, *tyr5*, *cyh2*, *met 13*, *lys5*, *tyr3*, *ade5,7*		
A-2-1C	$\alpha\alpha$	triploid	as AP-1-aα; *CSP/csp*$^+$		Mutant in group A
A-169-$\alpha\alpha$	$\alpha\alpha$	triploid	as AP-1-aα; *CSP/csp*$^+$		Mutant in group A
A-169-**aa**	**aa**	triploid	as AP-1-aα; *CSP/csp*$^+$		Derived by outcross of A-169-$\alpha\alpha$ × AP-1-**aa** and sporulation

TABLE 1 (Continued)

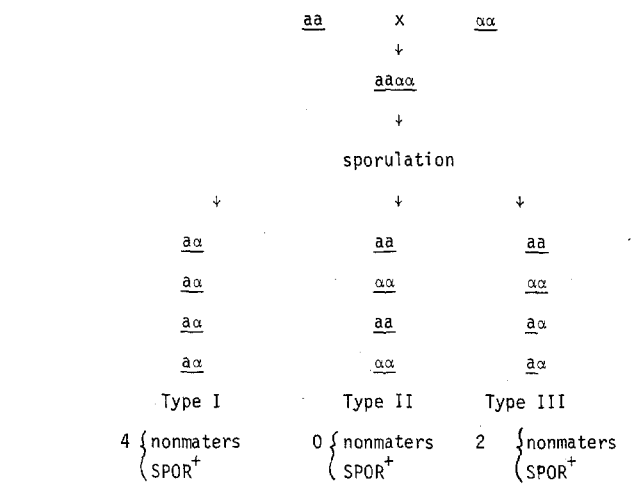
Strain	Mating type	Ploidy	Genotype*	Used for	Source‡
A-169-$\alpha\alpha$-3a	α	haploid	*gal1*, *leu1*, *cyh2 CSP*	haploid DNA analysis	Segregants of A-169-$\alpha\alpha$ ascus
A-169-$\alpha\alpha$-3b	α	haploid	*lys2*, *tyr1*, *CSP*	haploid × **aaa** triploid	No. 3 dissection
A-169-$\alpha\alpha$-3c	α	haploid	*gal1*, *lys2*, *tyr1*, *his7*, *leu1*, *csp*$^+$	tetraploid analyses	
A-169-$\alpha\alpha$-3d	α	haploid	*his7*, *cyh2*, *csp*$^+$		
A-169-$\alpha\alpha$-4a	α	haploid	*gal1*, *lys2*, *tyr1*, *csp*$^+$	haploid DNA analysis	Segregants of A-169-$\alpha\alpha$ ascus
A-169-$\alpha\alpha$-4b	α	haploid	*gal1*, *lys2*, *tyr1*, *his7*, *leu1*, *cyh2*, *csp*$^+$	haploid DNA analysis	No. 4 dissection
A-169-$\alpha\alpha$-4c	α	haploid	*leu1*, *CSP*	haploid DNA analysis	
A-169-$\alpha\alpha$-4d	α	haploid	*his7*, *cyh2*, *CSP*	haploid DNA analysis	
B-40-2a	$\alpha\alpha$	haploid	as AP-1-$\alpha\alpha$		Mutant in group B
B-174g	$\alpha\alpha$	haploid	as AP-1-$\alpha\alpha$		Mutant in group B
C-33	$\alpha\alpha$	haploid	as AP-1-$\alpha\alpha$		Mutant in group C
C-1018	**aa**	haploid	as AP-1-$\alpha\alpha$		Mutant in group C
D-135a	$\alpha\alpha$	haploid	as AP-1-$\alpha\alpha$		Mutant in group D

* Genetic nomenclature is as described by MORTIMER and HAWTHORNE (1973).

† The *ade2* markers in A364A and $\alpha_1$31-20 are heteroalleles.

‡ L. HARTWELL, P. WHITNEY, H. KLEIN, and M. UNGER are all at the Department of Genetics, University of Washington, Seattle, Washington.

A. Homozygous Mating-Type Cross and Tetraploid Segregation



B. Monosomic Mating-Type Cross and Segregation

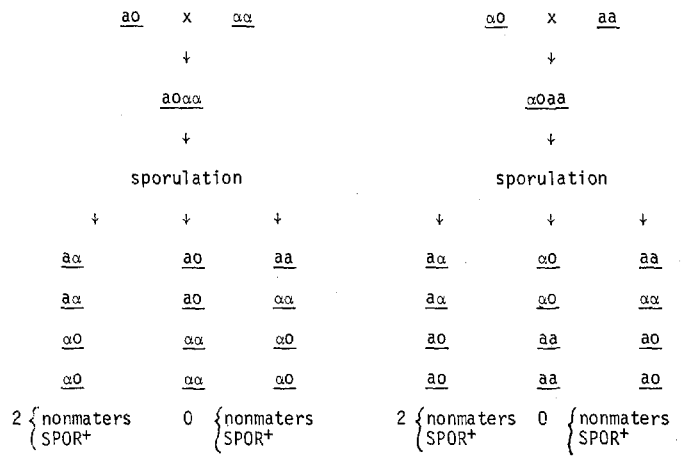


FIGURE 1.—Predictions for tetraploid mating type segregation if (A) both diploid parents are homozygous for opposite mating types. Frequencies for Type I: Type II: Type III asci are reported to be (ROMAN, PHILLIPS and SANDS 1955) 48.2; 9.5; 42.3 (B) segregation of mating type if one parent is monosomic for mating type. Type I asci only result from case A.

by micromanipulation. The zygote colonies were tested for **aa**α mating type by lack of mating with M25-18B. One confirmed triploid **aa**α was grown to stationary phase and UV-irradiated for 1 min as described above for AP-1-**aa** and AP-1-$\alpha\alpha$. Mitotic recombinants presumed to have the genotype **aaa** were scored by their ability to mate with M25-18B and thereby yield prototrophic zygotes. Mating type triploidy was confirmed by the pattern of segregation of mating types following mating with α haploids. Out of 60 asci produced by sporulating the progeny of AU-155 (**aaa**) $\times$ α haploid crosses, fifty-seven asci segregated 2 **a**α: 2**aa** and 2 asci segregated 3 **aa**: 1 $\alpha\alpha$. Only one ascus gave aberrant mating type segregation.

Media: The following media were used in growth, sporulation, mating, and mutant selection. Agar plates contained 1.5% agar in addition to the other components.

YEP-glucose growth medium was composed of 2% glucose, 2% bactopeptone (Difco), 1% yeast extract (Difco), 40 μg/ml adenine, and 40 μg/ml uracil.

Min-minimal glucose growth plates were composed of 2% glucose, 0.6% yeast extract. Min + Ura contained 20 μg/ml uracil.

PSP2-acetate growth medium, as described by ROTH and HALVORSON (1969), contained 0.67% yeast nitrogen base; 1% yeast extract, 1% potassium phthalate, 1% potassium acetate, and was supplemented with 40 μg/ml adenine and 40 μg/ml uracil.

Canavanine- and cycloheximide-containing selection medium was composed of 2% glucose, 0.67% yeast nitrogen base, 40 μg/ml adenine, 40 μg/ml uracil, 10 μg/ml histidine, 60 μg/ml isoleucine, 60 μg/ml leucine, 40 μg/ml lysine, 10 μg/ml methionine, 10 μg/ml tryptophan, 50 μg/ml threonine, 50 μg/ml tyrosine, 60 μg/ml phenylalanine, 40 μg/ml canavanine, and 20 μg/ml cycloheximide.

SPOR — 5 types of sporulation medium were utilized. SPOR—sporulation medium contained 0.2% potassium acetate, and 0.02% raffinose (SIMCHEN, SALTS and PIÑON 1973).

SPOR + auxotrophic requirements (A) was the same as above supplemented with 20 μg/ml each of uracil, histidine, lysine, tyrosine, and leucine.

SPOR + auxutrophic requirements (B) was SPOR medium supplemented with 20 μg/ml each of leucine, tryptophan, methionine, lysine, and tyrosine.

SPOR + yeast extract (A) contained 1% potassium acetate, 0.25% yeast extract, 0.1% glucose, 100 μg/ml adenine, 100 μg/ml uracil, and was further supplemented by 20 μg/ml each histidine, lysine, tyrosine, and leucine.

SPOR + yeast extract (B) was as (A), but supplemented by 20μg/ml leucine, tryptophan, methionine, lysine, and tyrosine.

Sporulation procedure: For sporulation of glucose-grown cells, the cells were grown in YEP until early stationary phase (1×10^8 cells/ml), collected and washed 2 times with H_2O by centrifugation, and resuspended into sporulation medium at 1–2 $\times$ 10^7 cells/ml. Conditions for acetate growth and subsequent sporulation of logarithmic cultures were those of ROTH and HALVORSON (1969).

Initially SPOR was used for sporulation of all yeast cultures. Both in liquid and on solid SPOR medium, at 30°, AP-1-aα asci first appear at 11 hr after the transfer to sporulation medium. Ascus formation reaches a plateau at approximately 17 hr, at which time about 70% of the cells have formed primarily 4-spore asci. At 23°, sporulation is completed within approximately 24 hr. Later, however, we found that some aα diploid strains arising from meiotic segregation of tetraploids were incapable of sporulation on SPOR. In many cases, sporulation could be restored by supplementing the sporulation medium with yeast extract + auxotrophic requirements (see *Media*); A or B was used, depending upon the auxotrophic markers segregating in given cross. Sporulation on these media is somewhat slower than on SPOR. Since cells divide on these media prior to sporulation, SPOR + yeast extract could not be used when measuring premeiotic DNA synthesis. Therefore, for those experiments SPOR + auxotrophic requirements medium was employed. AP-1-aα and *CSP* mutant A-169-$a\alpha$ sporulate to the same extent on supplemented medium as on SPOR.

DNA assay: The DNA content of yeast cells was analyzed by the diaminobenzoic acid (DABA) procedure (KISSANE and ROBINS 1958) adapted to yeast by C. MILNE (1972). For each time point, three 1-ml samples (1.5–2.0 $\times$ 10^7 cells/ml) were hydrolyzed in 1 N NaOH, for 24 hr at room temperature. The hydrolyzed samples were chilled, TCA precipitated and collected at 4° by centrifugation and subsequently washed with 1 ml 5% cold TCA, 0.1 M KAC in 95% ETOH, and 100% ETOH. The washed pellet was dried at 60° and then reacted with 62 mg decolorized (by Norite) DABA in 100 μl H_2O for 30 min at 60°. After 30 min, 2.0 ml 0.6 N $HClO_4$ was added to the DABA-DNA solution. The fluorescence (excitation at 408 nm and emission at 508 nm) was determined on an Aminco Bowman Spectrophotofluorometer.

Mutagenesis: Ethylmethanesulphonate (EMS) mutagenesis was as described by FINK (1970). In order to inactivate the excess EMS the mutagenized culture was washed by centrifugation 2 times with 6% sodium thiosulfate. After mutagenesis, the culture was diluted approximately

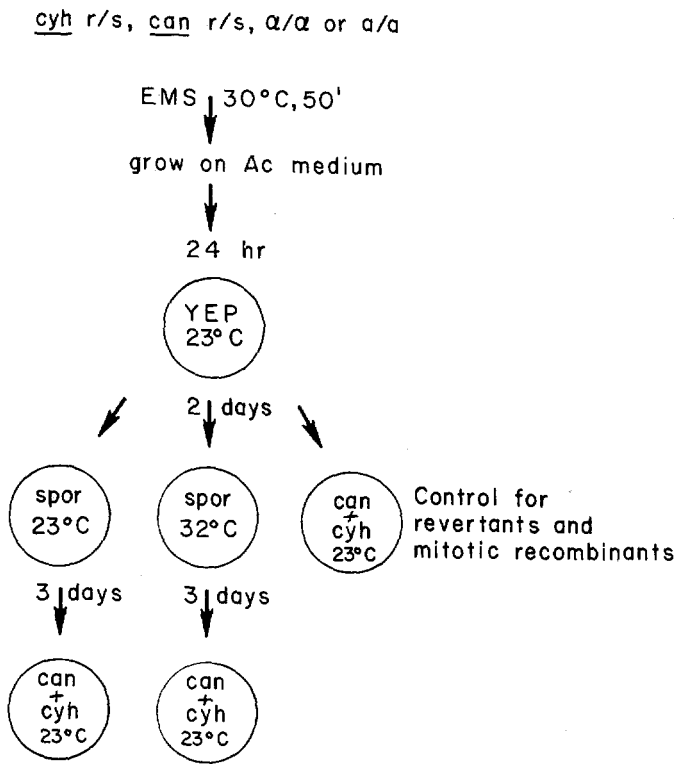


FIGURE 2.—Protocol for isolation of CSP mutants. Details are given in MATERIALS AND METHODS.

1 to 50 or 100 into PSP2 medium and allowed to grow about 15 hr at 30° to a cell density of 2–4 × 10^7.

Mutant isolation procedure: Mutants were isolated by the selection protocol shown in Figure 2. Mutagenized AP-1-**aa** or AP-1-$\alpha\alpha$ log phase cells capable of growth on PSP2 were spread onto 60-100 YEP agar plates to yield 200–400 colonies/plate and allowed to grow at room temperature, The YEP plates were replica plated onto 2 plates containing sporulation medium (SPOR) and then onto 1 containing canavanine-cycloheximide selection medium. Canavanine- and cycloheximide-resistant double revertants or mitotic recombinants were scored on these selection plates after a three-day incubation at 23°. Colonies on the 2 sporulation plates were allowed to "sporulate" at 23° and 32° respectively. After 3 days the sporulation plates were each replicated onto canavanine- and cycloheximide-containing medium. These selection plates were also incubated at room temperature for 3 days. Colonies capable of growth on selection plates *only* after exposure to sporulation medium were regarded as potential mutants. Colonies capable of growth on only one of the selection plates (i.e., only one of the 2 temperatures of sporulation led to the ability of cells to grow on selection medium) were considered to be putative *ts* mutants. The corresponding sporulation colonies on the YEP master plate were picked, cloned 2–3 times on YEP, and again tested for the ability to undergo meiotic segregation and ascus formation.

RESULTS

Isolation of mutants: In the first two mutant hunts, mutagenized AP-1-$\alpha\alpha$ (*can s/r cyh s/r*) cells were screened for the ability to sporulate by the procedure

detailed in MATERIALS AND METHODS (Figure 2). Of 20,000 mutagenized colonies tested in the first mutant hunt, 9 produced growth on the selection plates. Of these, 3 colonies were capable of meiosis and spore formation. Three apparently had become *can r/r* and *cyh r/r* by reversion and/or mitotic recombination, since the corresponding unsporulated diploid clones scored as *can r*, *cyh r*. Three additional colonies produced no asci after cloning; these were not further studied. In the second mutant hunt, of 100,000 mutagenized colonies, 40 produced growth on the selection plates. Of these, 26 were capable of meiosis and spore formation, 6 were mitotic recombinants or revertants and 8 were incapable of sporulation after being cloned.

An additional complication was encountered when the procedure was used to select mutants from mutagenized AP-1-**aa** cells. As previously reported (HOPPER *et al.* 1974), AP-1-**aa** produces 1–2% asci and AP-1-$\alpha\alpha$ <0.05% asci upon 3 days' incubation on sporulation agar. A low level of sporulation by **aa** and $\alpha\alpha$ cells has been previously reported (ZAKHAROV and KOŽINA 1967). With the selection procedure given in MATERIALS AND METHODS, unmutagenized AP-1-$\alpha\alpha$ cells gave no growth when replicated from sporulation agar to canavanine and cycloheximide. However, upon replication, all AP-1-**aa** colonies gave rise to diffuse and patchy growth on the canavanine and cycloheximide selection plates. Therefore, in the mutant hunt with mutagenized **aa** cells, the only colonies retained and analyzed were the two which gave rise to solid growth on the selection plates.

The mutants obtained from three mutant hunts have been categorized on the basis of sporulation and mating-type characteristics into 4 groups: A, B, C, and D. Table 2 summarizes these characteristics. Mutants known to be of independent origin are marked †.

Group A and B mutants—mating and sporulation characteristics: Group A and B mutants retain the mating-type specificity of the $\alpha\alpha$ or **aa** strain from which they were derived. Mating response was as efficient in the mutants as in the parental strains, as analyzed by the kinetics and extent of zygote formation and of mating factor production and response (HOPPER, KIRSCH and HALL 1975). If the diploid mutant strains are genotypically identical to their parents at the mating-type locus, then sporulation of the mutants would be expected to yield asci in which all four spores have the same mating type as the parental diploid strain. To determine whether mutant ascospores retain the parental mating type, two mutants from each group were sporulated and the resulting asci were dissected. Mutant A-169-$\alpha\alpha$, derived from AP-1-$\alpha\alpha$, had good spore viability (3 or 4 viable spores/ascus) and produced 4 spores of mating type α in each ascus dissected. An A-169-**aa** strain derived from an outcross (see below) produced 4 spores of mating type **a** in each ascus (5 asci dissected). Spores from strains A-2-1c, B-40-2a, and B-174g had poorer viability,* but in every case only α spores were recovered (see Table 2). The mating response of haploid segregants from Group A and B mutants was the same for matings performed at 23° as at 32°.

* It is likely that poor haploid viability is due to the segregation of recessive lethals, since diploid cells were mutagenized.

TABLE 2

Sporulation and mating characteristics of spore mutants

Group	Mutant number	Derived from	Mutant hunt*	% Sporulation 23°	% Sporulation 32°	Mating type 23°	Mating type 32°	Mating type of spores	Remarks
AP-1-$\alpha\alpha$	Control			70	70	nonmater	nonmater	2a and 2α	
AP-1-aα	Control			<0.05	<0.05	$\alpha\alpha$	$\alpha\alpha$	α	
AP-1-**aa**	Control			1-2%	1-2%	**aa**	**aa**	**a**	
A	A-2-1c†	$\alpha\alpha$	1	5	5	$\alpha\alpha$	$\alpha\alpha$	α	2 or 3 spores viable/4-spore ascus
	A-111	$\alpha\alpha$	2	5	5	$\alpha\alpha$	$\alpha\alpha$		
	A-113	$\alpha\alpha$	2	20	20	$\alpha\alpha$	$\alpha\alpha$		
	A-136a	$\alpha\alpha$	2	10	10	$\alpha\alpha$	$\alpha\alpha$		
	A-169†	$\alpha\alpha$	2	20	20	$\alpha\alpha$	$\alpha\alpha$	4α	3 or 4 spores viable/4-spore ascus
	A-1094†	***aa***	3	30	30	**aa**	**aa**		
B	B-40-2a†	$\alpha\alpha$	1	0	1–5	$\alpha\alpha$	$\alpha\alpha$	α	3 spores viable from 7 4-spore asci
	B-174g†	$\alpha\alpha$	2	0	1–5	$\alpha\alpha$	$\alpha\alpha$	4α	2, 3, or 4 spores viable 4-spore ascus
	B-155c	$\alpha\alpha$	2	0	5–10	$\alpha\alpha$	$\alpha\alpha$		
	19 others	$\alpha\alpha$	2	0	1–5	$\alpha\alpha$	$\alpha\alpha$		
C	C-33†	$\alpha\alpha$	1	>60	>60	nonmater	nonmater	α	4 viable spores/10 4-spore asci
	C-1018†	**aa**	3	40	40	nonmater	nonmater	α & α	1 or 2 viable spores/4-spore ascus
D	D-135a†	$\alpha\alpha$	2	>60	>60	nonmater	nonmater	*nonmaters*	mainly 3 and 4 viable spores/4-spore ascus

* In mutant hunt 1, 20,000 $\alpha\alpha$ colonies were screened for meiotic segregation and ascus formation; mutant hunt 2, 100,000 $\alpha\alpha$ colonies; and mutant hunt 3, 15,000 **aa** colonies.

† Known to be of independent origin.

Group A mutants form asci more slowly and to a lower extent (5–30% asci after 2–3 days) than does wild-type strain AP-1-**a**α. Group B mutants sporulate (at 32°) both more slowly and to a lower extent than mutants in Group A. In addition, mutants in Group B are temperature-sensitive for sporulation, forming 1–5% asci in 3 days at 32°, but no detectable asci at 23° (even after 6 days on sporulation plates). In contrast to AP-1-**a**α cells, which form almost exclusively 4-spore asci, both Group A and B mutants form only a small proportion of 4-spore asci; 1-, 2-, and 3-spore asci predominate.

Genetic analysis of mutants in Groups A and B: The mutations in these strains appear to have uncoupled sporulation from the requirement for **a**α mating type. We use the symbol *CSP* to denote these mutant genes. A straightforward analysis of the segregation of *CSP* genes in crosses is difficult because haploid *CSP* cells cannot sporulate and diploid **a**α cells can sporulate whether or not they have received the *CSP* marker. To obtain **aa** and $\alpha\alpha$ diploid segregants appropriate for scoring the *CSP* phenotype, we crossed $\alpha\alpha$ Group A and B mutants with a *csp*$^+$ **aa** strain (U-155). The resulting tetraploids, heterozygous for *CSP*, yield, upon sporulation, three types of asci (Table 3), two of which contain **aa** and $\alpha\alpha$ diploid spores. These were identified by mating tests and then tested for inheritance of the *CSP* marker by replication onto sporulation agar and subsequent examination for ascus formation.

For both of the Group A and one of the Group B mutations, an appreciable fraction of both **aa** and $\alpha\alpha$ segregant colonies were able to sporulate (Table 3). These results indicate that these *CSP* mutations are not limited to $\alpha\alpha$ strains for their phenotypic expression. Furthermore, the nearly equivalent frequencies of *CSP* diploids among **aa** and $\alpha\alpha$ segregants indicate that the *CSP* mutations in

TABLE 3

Tetraploid analysis of CSP *mutants*

Experiment:			
$\alpha\alpha$ *CSP* × **aa** +	→ tetraploid	→ sporulation	→
Type 1	Type II	Type III	
aα	**aa**	**aa**	
aα	$\alpha\alpha$	$\alpha\alpha$	
aα	**aa**	**a**α	
aα	$\alpha\alpha$	**a**α	
48.2	9.5	42.3*	

Results: *CSP* mutant	Fraction of spore colonies able to sporulate		
	aa	$\alpha\alpha$	**a**α
A-169-$\alpha\alpha$	7/17	4/15	50/53
A-2-1c	3/11	5/13	24/28
B-40-2a	3/8	4/7	18/20
B-174g	1/12	0/12	26/30

* Published frequencies of ROMAN, PHILLIPS and SANDS 1955.

A-169-$\alpha\alpha$, A-2-1c, and B-40-2**a** are not linked to the mating-type locus. For mutant B-174g, the *CSP* phenotype was not efficiently recovered among the progeny of a cross. However, one *CSP* **aa** segregant was recovered, indicating that the mutant gene or genes can function in diploids homozygous for either mating type.

Besides showing that the *CSP* mutations studied are neither functionally dependent upon mating type nor linked to it genetically, the data in Table 3 can be used to determine whether the mutants are heterozygous for a dominant mutation, homozygous for a dominant mutation, or homozygous for a recessive mutation. Sporulation of the heterozygous *CSP* **aa**$\alpha\alpha$ tetraploid would yield, for an initially homozygous or heterozygous *CSP* mutation, the distributions of ascus types shown in Table 4B. The proportions of *CSP*/*CSP*, *CSP*/+ and +/+ segregants that can be calculated from these distributions apply equally to total

TABLE 4

Tetraploid analysis of CSP *mutants*

A. Experiment:

Diploids resulting from the cross: $\alpha\alpha$ *CSP* × **aa** + → tetraploid → sporulation →

B. Predictions:

If homozygous:

CSP/*CSP*		*CSP*/+		*CSP*/+		*CSP*/*CSP*		*CSP*/*CSP*
CSP/*CSP*		*CSP*/+		*CSP*/+		*CSP*/*CSP*		*CSP*/+
+/+		*CSP*/+		*CSP*/+		+/+		*CSP*/+
+/+		*CSP*/+		*CSP*/+		+/+		+/+
1	:	2		4	:	1	:	4
centromere-linked				unlinked to centromere				

If heterozygous:

CSP/+	*CSP*/*CSP*
CSP/+	+/+
+/+	+/+
+/+	+/+
100%	0%
93%*	7%*

Type of mutant	Total % **aa** and $\alpha\alpha$ expected to sporulate
Recessive	17 (20*)
Homozygous dominant	83 (80*)
Heterozygous dominant	50 (45*)

C. Results:

Mutant:	Fraction **aa** & $\alpha\alpha$ sporulation†	% **aa** & $\alpha\alpha$ sporulation†	†Corrected % **aa** & $\alpha\alpha$ sporulation†
A-169-$\alpha\alpha$	11/32	35	37
A-2-1c	8/24	33	36
B-40-2a	7/15	47	52
B-174g	1/24	4	4

* Value for tetravalent pairing and markers unlinked to centromere

† Corrected for 100% **a**α sporulation

segregants or to **aa** and $\alpha\alpha$ segregants, because the *CSP* loci are unlinked to mating type. Finally, by specifying whether *CSP* is dominant or recessive, the expected frequency of sporulation-capable **aa** and $\alpha\alpha$ segregants can be calculated for each of the three cases (Table 4B).

For three of the mutants, the experimentally observed percentages of *CSP* segregants range between 33% and 47%, whereas for the fourth, only 1/24 (4%) of the **aa** and $\alpha\alpha$ segregants sporulated. The three higher values lie between those expected for homozygous recessive *CSP* mutations (17% to 20%) and heterozygous dominant *CSP* mutations (45.5% to 50%). However, it is likely that the observed frequencies are depressed by effects of the genetic background. Crossing of the *CSP* mutant strains to **aa** strain U-155 apparently introduced genes which are deleterious for sporulation. As a result there was great variability even in the sporulation ability of **a**α segregants from the *CSP* $\times$ U-155 tetraploids. Assuming that the genetic background will affect sporulation of **aa**, $\alpha\alpha$, and **a**α offspring of a given tetraploid to the same extent, we have corrected the observed percentages of **aa** and $\alpha\alpha$ segregants which are phenotypically *CSP* upward to that which would have been observed if all the **a**α segregants of a particular cross had been able to sporulate. The corrected percentages (Table 4C) for three of the mutants tested most closely approximate those expected for dominant mutations present in one copy. For mutant B-174g, the lower percentage of sporulating colonies suggests that the mutation may be recessive.

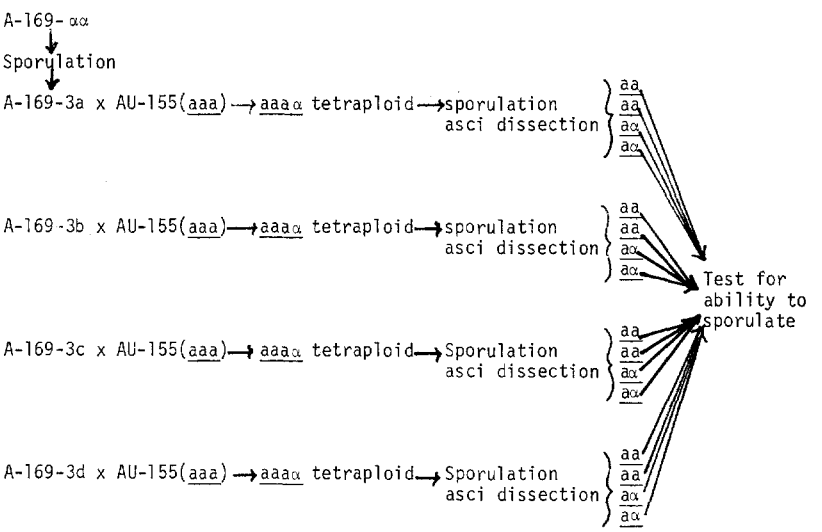
For one of the crosses, A-169-$\alpha\alpha$ $\times$ U-155 (**aa**) tetrad data is available. Out of 29 asci resulting from the sporulation of this tetraploid, 15 were of Type I (see Table 3) with respect to mating-type segregation, four of Type II, and ten of Type III.* For three of the four Type II asci, two of the four clones were capable of sporulation. Such segregation is consistent with the above conclusions that A-169-$\alpha\alpha$ is heterozygous for a dominant mutation. For one ascus, only one of the four mating spore clones was capable of sporulating. This could be due to the presence of deleterious genes for sporulation in one spore clone which was genetically *CSP* or, possibly, to homozygosity for *CSP* in the one spore clone capable of sporulation.

Genetic analysis of haploid segregants from CSP *mutant A-169-*$\alpha\alpha$: In the preceding section, crosses were performed between *CSP* mutant diploids and wild-type diploids of opposite mating type. Segregation of the *CSP* marker in these crosses was observed by testing **aa** and $\alpha\alpha$ segregants from the tetraploid for their ability to sporulate. The results indicated that mutant A-169-$\alpha\alpha$ was heterozygous for a dominant *CSP* mutation. Upon sporulation of A-169-$\alpha\alpha$, two *CSP* and two *csp*$^+$ α haploid spores should be present in each ascus. We have found two ways of testing haploid segregants to distinguish *CSP* from wild type. One, a phenotypic test, is a measurement of the "meiotic" DNA synthesis induced by placing the presumptive *CSP* haploid cells in sporulation medium. The other, a test of genotype, requires that each haploid segregant be crossed with a wild-type **aaa** strain. Diploid **aa** segregants of the hybrid **aaa**α tetraploid are then tested for

* These figures include 6-3 spore asci in which the mating type of the 4th spore was inferred from the mating type of the 3 viable spores. No unusual segregations of mating type were found in any of the asci for which there were 4 viable spores.

TABLE 5

A. Experiment:



B. Predictions:

a. If heterozygous dominant 2 of 4 A-169-αα spores give rise to tetraploids which segregate (45.5)* -50% aa SPOR$^+$ (CSP)

b. If recessive
   1. centromere linked: 4 of 4 spores give rise to 0% aa Spor$^+$ (CSP)
   2. unlinked to centromere: 4 of 4 spores give rise to 0%-(7%)* aa SPOR$^+$ (CSP)

C. Results:

A-169-3a x AU-155(aaa)→sporulation →

3/32 = 10% (19%)† aa Spor — A-169-3a is CSP

A-169-3b x AU-155(aaa)→sporulation →

4/19 = 21% (42%)† aa Spor — A-169-3b is CSP

A-169-3c x AU-155(aaa)→sporulation →

0/36 aa Spor$^+$ (aα sporulation = 66%) — A-169-3c is csp$^+$

A-169-3d x AU-155(aaa)→sporulation →

0/32 aa Spor$^+$ (aα sporulation = 87%) — A-169-3d is csp$^+$

*range of predictions includes extremes of 0% tetravalent to 100% tetravelent pairing for markers unlinked to centromeres.

†numbers in parentheses are normalized to 100% aα sporulation

their ability to sporulate. Those haploid clones which contain the *CSP* marker are identified by their ability to transmit this marker through the tetraploid to **aa** segregant progeny.

Four haploid segregants dissected from a single A-169-$\alpha\alpha$ ascus (A-169-$\alpha\alpha$-3a,-3b,-3c,-3d) were each crossed with the triploid tester strain, AU-155(**aaa**). Following sporulation of the four tetraploids and dissection of several asci from each, the resulting diploid **aa** segregants were tested for their ability to sporulate. The results (Table 5) show that the ability to sporulate segregates 2:2. Hence A-169-$\alpha\alpha$ must be heterozygous for a dominant *CSP* mutation.

Meiotic DNA synthesis by Group A and B mutants: The extent of increase in DNA when cells are placed in sporulation medium distinguishes **a**α diploid strains from *csp*$^+$ diploids homozygous for mating type and from *csp*$^+$ haploids. The **a**α strains double their DNA, while the others show no appreciable increase. One mutant from each of groups A and B was analyzed for the ability to carry out meiotic DNA synthesis. After 52 hours in sporulation medium at 32°, mutant B-174g increased about 16% in DNA content. At 23° there was no increase in DNA content. Mutant A-169-$\alpha\alpha$ nearly doubled in DNA content when exposed to sporulation conditions. However, the rate of increase of DNA for A-169-$\alpha\alpha$ was slower than that for AP-1-**a**α. The rate and extent of meiotic DNA synthesis in strain A-169-**aa** (a *CSP* segregant from the tetraploid: A-169-$\alpha\alpha$ × AP-1-**aa**) was similar to that observed for A-169-$\alpha\alpha$ (Figure 3). The close similarity between these two strains, both as regards DNA synthesis and the efficiency of sporula-

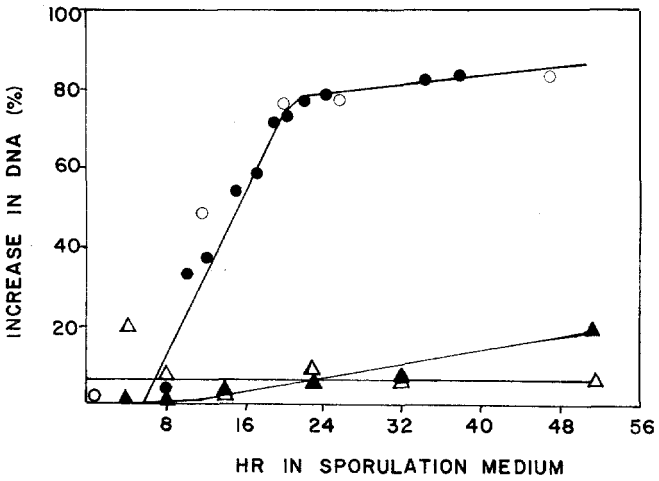


FIGURE 3.—Increase in DNA content during "sporulation" for group A and B mutants. The yeast strains were maintained in logarithmically growing condition (PSP2) at 30° for 30 hr. The cells at a density of 1–2 × 10^7 cells/ml were collected onto a millipore filter, washed with 1 vol SPOR, and resuspended at the same cell density in SPOR. Cells were sporulated at 23° (B-174g), 30° (A-169-$\alpha\alpha$, A-169-**aa**) or 32° (B-174g). At the times indicated 3–1 ml aliquots of sporulating cells were taken and analyzed for average percent increase in DNA content as detailed in MATERIALS AND METHODS. ▲-▲-▲, B-174g at 32°; △-△-△, B-174g at 23°; ●-●-●, A-169-$\alpha\alpha$; and O-O-O, A-169-**aa**.

tion, argues that both of these characteristics are specified by the A-169 mutation independently of the mating-type alleles (**aa** or $\alpha\alpha$) present.

Because the *CSP* mutation in A-169 allows premeiotic DNA synthesis to occur in $\alpha\alpha$ and **aa** diploids (Figure 3), the expression of a similar mutant phenotype might be expected in haploid strains as well. To test for this, four haploid segregant cultures (A-169-$\alpha\alpha$-3a, 3b, 3c, and 3d) were transferred to sporulation medium and samples were removed at appropriate times for DNA analysis. The results (Figure 4) show that two of the four cultures underwent increases of DNA content of 31% (A-169-$\alpha\alpha$-3a) and 50% (A-169-$\alpha\alpha$-3b), whereas the other two showed no increase. No asci were present in any of the four cultures. This analysis was repeated on the four segregants from another A-169-$\alpha\alpha$ ascus and on those from an A-169-**aa** ascus. Again, two of the four haploid cultures showed an increase in DNA. A-169-$\alpha\alpha$-4c increased in DNA by 50% and A-169-$\alpha\alpha$-4d by 65%, while the other two haploid cultures (A-169-$\alpha\alpha$-4a and α-4b) showed no significant increase in DNA content. A-169-**aa**-2a increased 44% in DNA content and A-169-**aa**-4d increased 81%; A-169-**aa**-2b and A-169-**aa**-2c exhibited no increase in DNA.

In the previous sections we concluded, by analyzing the ability of the diploid A-169-$\alpha\alpha$ mutant and its haploid segregants to transmit the *CSP* character through tetraploids to **aa** and $\alpha\alpha$ diploid segregants, that the *CSP* mutant gene segregated 2:2 during sporulation of the diploid. This conclusion is strongly

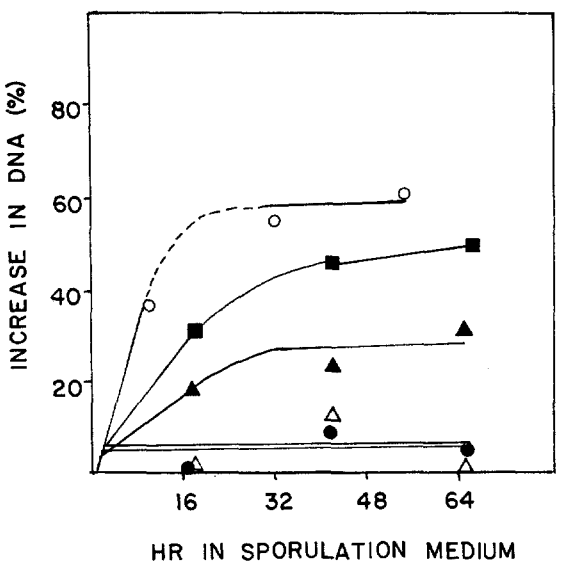


FIGURE 4.—Increase in DNA content for A-169-$\alpha\alpha$ haploid segregants. Cultures were grown overnight in YEP at 20° to stationary phase (1–2 × 10^8 cells/ml). The cells were collected by filtration, washed with H_2O 2 times, and resuspended in SPOR + auxotrophic requirements A at ~ 1 × 10^7 cells/ml. "Sporulation" proceeded at 30°. At the times indicated triplicate 1-ml samples were taken and analyzed for average DNA content as detailed in MATERIALS AND METHODS. O - O - O, AP-1-$a\alpha$; ▲-▲-▲ haploid segregant C-169-3a; ■-■-■, segregant A-169-$\alpha\alpha$-3b; ● - ● - ●, segregant A-169-$\alpha\alpha$-3c; ^ _ ^ _ △, segregant A-169-$\alpha\alpha$-3d.

supported by the observation that the *same* two spores of a given ascus (A-169-$a\alpha$-3a and A-169-$a\alpha$-3b) that are capable of transmitting the *CSP* genetic trait in a cross (Table 4) are the ones which phenotypically express *CSP* by carrying out meiotic DNA synthesis. Thus, these experiments provide three independent determinations that diploid strain A-169-$a\alpha$ is heterozygous for a *CSP* mutation which exerts a dominant phenotypic effect.

Sporulation and mating characteristics of Group C and D mutants: The final level of ascus formation and kinetics of sporulation for Group C and D mutants are similar to those for the wild-type **a**α strain (AP-1-**a**α). After 16 hours on sporulation agar more than 60% (40% for C-1018) of the cells formed asci. Most of the asci contained four spores.

When mixed with cultures of any of the six tester strains (see MATERIALS AND METHODS) and incubated on YEP plates, Group C and D mutants formed no visible zygotes and gave rise to no prototrophs when the mating mixtures were spread on the appropriate minimal plates. By both of these criteria, Group C and D mutants lack the ability to mate.

Because the mutants in Groups C and D differ from their parent strains both in mating and sporulation phenotypes, a genotypic change from homozygous mating type to **a**α seemed a likely possibility. In that event, sporulation of these mutants would be expected to yield asci containing two **a** and two α spores. Of spores dissected from asci of Group C mutants, only 8/36 were viable for C-1018 and 4/40 for C-33. Both **a** and α mating types were found among the C-1018 spores; all four C-33 spores were of mating type α. Although these data are incomplete, they are consistent with the occurrence of a mating-type mutation from **aa** and $a\alpha$ to **a**α. Such mutations at the mating-type locus ($\alpha \leftrightarrows$ **a**) have been previously described (AHMAD 1952; ROMAN and SANDS 1953).

The single mutant in Group D, D-135a, unlike the Group C mutants, gave only non-mating meiotic segregant clones. The apparently complete inability of D-135a to give normal **a** and α progeny suggests that this mutant must have arisen by a more complex event than a single mutation at the mating-type locus ($a\alpha \rightarrow$ **a**α). Further evidence for the unusual nature of the Group D mutant is the observation that presumed "haploid" segregant cultures produced by sporulation of D-135a are themselves capable of sporulation. Upon further study it was determined that this strain resulted from the mutation of the heterothallic AP-1-$a\alpha$ strain to a homothallic diploid strain. The results of this study are presented in an accompanying paper (HOPPER and HALL 1975).

DISCUSSION

In order to undergo efficient meiotic DNA synthesis, as well as recombination, nuclear division and spore formation, wild-type diploid yeast cells must have an **a**α genetic constitution at the mating-type locus. However, it has been shown (KANE, ROTH and ERWIN 1972; HOPPER *et al.* 1974) that a number of the biochemical changes observed in sporulating **a**α cells aso occur in **aa** and $a\alpha$ diploids placed in sporulation medium. This similarity even extends to the majority of newly-synthesized proteins which we found to be indistinguishable, by SDS-

polyacrylamide gel electrophoretic analysis, for **a**α and non-sporulating diploid yeast cells. The similar pattern of gene expression may mean that only a few crucial sporulation genes require both **a** and α for their expression. Consistent with a small number of target genes for mating-type control over sporulation is the fact that mutation at a single locus can allow as much as 20% sporulation to occur in strains which lack **a**α mating type. We have given the name *CSP* (control of sporulation) to such mutant genes. GERLACH (1974) studied a similar mutant locus, which he named *sca* (sporulation capable). The *sca* mutant locus is recessive, while all *CSP* mutants, except possibly for B-174g, are dominant.

In attempting to infer the regulatory interconnections among mating-type genes, *CSP* loci, and structural genes for meiosis-specific and spore proteins, we have considered models for sporulation control in which mating type exerts either a positive or negative control over sporulation genes. We assume that the *CSP* mutations exert their effect through an intermediate gene product (SI) in the normal pathway between **a**α mating-type genes and sporulation proteins. SI would function in wild-type (*csp*$^+$)**a**α cells, CSP **aa** cells, and in *CSP* $\alpha\alpha$ cells. but not in **aa** or $\alpha\alpha$ wild-type cells. For both positive and negative models, the sequence of gene action on the control circuit is: **a**$\alpha \rightarrow$ SI $\rightarrow$ sporulation genes.

To construct a positive control model (AH model) consistent with the observation that *CSP* mutants are dominant, we assume that the mutant *CSP* locus makes a gene product, SI, which actively turns on sporulation genes. The wild-type SI must work in concert with gene products from **a** and α in order to activate sporulation genes. In *CSP* mutants, SI is structurally altered so that it can act as a positive regulator without **a** and α both being present.

In a negative control model (BH model) *CSP* and *csp*$^+$ are alleles of an operator site which regulates expression of a contiguous SI gene. *CSP* corresponds to o^0 and *csp*$^+$ to o^+. The *csp*$^+$ allele of this operator is repressed by a protein or proteins which are made in haploids, in **aa** and $\alpha\alpha$ diploids, but not in **a**α diploids. An essential difference between these two models is that SI *action* is positively regulated by mating type in the AH model, while SI *synthesis* is negatively controlled by mating type in the BH model. *CSP* mutations cause constitutivity of SI action in the former case and SI synthesis in the latter.

To obtain a more precise description of the *CSP* mutations and to determine their relationship to sporulation control, it will be necessary to determine how many different *CSP* loci there are. An essential next step in our analysis will be to perform the necessary tests of allelism between *CSP* mutants. The availability of a test for the *CSP* phenotype in haploid segregants (Table 5, Figure 4) makes it possible to determine whether or not single-gene segregation occurs in a cross between two different *CSP* mutant haploids.

To evaluate various models for sporulation control we intend to test for epistasis between *CSP* mutants and mutants blocked in sporulation (ESPOSITO and ESPOSITO 1969; ROTH and FOGEL 1971). Crosses between *spo*$^-$ and *CSP* mutants should also allow us to determine whether any essential sporulation genes are allelic or closely linked to the *CSP* locus. The identification of such a gene, corresponding to SI in the models we have presented, should make possible an analysis of its regulatory behavior.

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