MATING TYPE AND SPORULATION IN YEAST. II. MEIOSIS, RECOMBINATION, AND RADIATION SENSITIVITY IN AN $\alpha\alpha$ DIPLOID WITH ALTERED SPORULATION CONTROL

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ABSTRACT

In wild-type *S. cerevisiae*, diploid cells must be heterozygous at the mating-type locus in order to sporulate. In the preceding paper, we described a number of mutants (CSP mutants), isolated from nonsporulating **aa** and $\alpha\alpha$ parent strains, in which sporulation appeared to be uncoupled from control by mating type. The characterization of one of these mutants (*CSP1*) is now extended to other processes controlled by mating type. This mutant is indistinguishable from $\alpha\alpha$ cells and unlike **a**α cells for mating factor production and response, zygote formation, intragenic mitotic recombination, and for X-ray sensitivity. The mutant apparently undergoes a full round of DNA synthesis in sporulation medium, but with delayed kinetics. Only 20% of the cells complete sporulation. Among spores in completed asci, the frequency of both intra- and intergenic recombination is the same as it is for spores produced by **a**α cells. However, experiments in which cells were shifted from sporulation medium back to minimal growth medium gave a frequency of meiotic recombination between *ade2* or *leu2* heteroalleles only 25% to 29% as high for *CSP1* $\alpha\alpha$ diploid or *CSP1* **aa** disomic cells as for **a**α diploid or disomic cells. Because the latter result, indicating recombination defectiveness, measured recombinant production in the entire cell population, whereas the result indicating normal recombination sampled only completed spores, we infer that all meiotic recombination events occurring in the population of *CSP1* $\alpha\alpha$ cells are concentrated in those few cells which complete sporulation. This high degree of correlation between meiotic recombination and the completion of meiosis and sporulation suggests that recombination may be required for proper meiotic chromosome segregation in yeast just as it appears to be in maize and in Drosophila

In heterothallic yeast strains, sex is determined by the alleles of mating type present in a given cell (LINDEGREN and LINDEGREN 1943). Cells of mating-type **a** mate with cells of mating-type α and *vice versa*. Cells containing both mating-type alleles, **a**α diploids, do not mate but are able to undergo meiosis and sporulation. Diploid yeast cells homozygous for the mating-type alleles can mate, but cannot sporulate (ROMAN and SANDS 1953).

We have isolated yeast mutants with aberrant control of the sexual cycle. As a consequence of mutation in a gene unlinked to mating type, diploid **aa** and $\alpha\alpha$ strains became able to sporulate (HOPPER and HALL 1975). In this paper, we report an extensive characterization of one such *CSP* (control of sporulation)

mutant which was isolated from a wild-type (nonsporulating) $\alpha\alpha$ diploid parent, AP-1-$\alpha\alpha$.

The focus of this characterization is upon those processes, all related to genetic exchange and transmission, which distinguish **a**α from homozygous mating-type diploid strains. The mating reaction of homozygous mating-type cells appears to have as its basis the production of specific mating factors by both **a** and α mating types and a complementary response to these substances by cells of the opposite mating type (DUNTZE *et al.* 1973; MACKAY and MANNEY 1974a, b; WILKINSON and PRINGLE 1974). **a**α diploids can undergo sporulation and also the related meiotic processes of DNA replication (ROTH and LUSNAK 1970), genetic recombination (ROTH and FOGEL 1971; HOPPER *et al.* 1974) and chromosome segregation while $\alpha\alpha$ cells cannot undergo any of these processes. For induced intragenic recombination and X-ray radioresistance in mitotic cells, there is a quantitative difference; **a**α cells perform these processes more efficiently than **aa** or $\alpha\alpha$ cells (LASKOWSKI 1960; FRIIS and ROMAN 1968).

For each of the above processes we have compared the behavior of wild-type **a**α and $\alpha\alpha$ strains to that of A-169-$\alpha\alpha$, a *CSP1* mutant which appears to undergo a full round of meiotic DNA replication, but sporulates with only 20% efficiency. Particular attention has been given to the extent and nature of the recombination defectiveness (as compared to **a**α cells) of the *CSP1* mutant. Mitotic DNA repair and recombination are defective (or $\alpha\alpha$-like) and the frequency of intragenic meiotic recombination measured for the whole population of sporulating *CSP1* mutant cells is correspondingly low. However, a normal (**a**α-like) extent of recombination occurs in those cells (20%) which produce ascospores. These results suggest that meiotic recombination and chromosome segregation, which necessarily depend upon the prior occurrence of meiotic DNA synthesis, also are coordinately dependent upon some process which **a**α cells do efficiently and *CSP1* mutant cells do inefficiently.

MATERIALS AND METHODS

Yeast strains: Parental strains AP-1-**a**α and AP-1-$\alpha\alpha$ and the *CSP1* mutant A-169-$\alpha\alpha$ (all heteroallelic for *ade2*) were described in the previous communication (HOPPER and HALL 1975). All of the strains have a common genetic background.

Construction of disomic strains: The disomic strains used to study meiotic recombination were made by crosses between Z4521-96C, and α *CSP1* or α *csp*$^+$ haploid segregants of A-169-$\alpha\alpha$. Z4521-96C, obtained from R. ROTH (ROTH 1973), is disomic for chromosome III, homozygous for **a** mating type, and heteroallelic for *leu2*:

$$\frac{+ \quad leu2\text{-}27}{leu2\text{-}1 \quad +}\ \begin{matrix}\cup\\ \cap\end{matrix}\ \frac{\mathbf{a}}{\mathbf{a}} \qquad \underline{\qquad ade2\text{-}1. \qquad}$$

CSP1 mutant haploid segregants from A-169-$\alpha\alpha$ were identified by their ability to synthesize DNA in sporulation medium (HOPPER and HALL 1975). A-169-$\alpha\alpha$-4a (α, *gal1*, *lys2*, *tyr1*, *csp*$^+$) A-169-$\alpha\alpha$-4d (α, *his7*, *cyh2*, *CSP1*) each were mated with the disomic strain. Following sporulation of the $2n+1$ **aa**α hybrids, asci containing two disomic (**aa** or **a**α) leucine-requiring segregants were identified by mating-type analysis of the segregants. **aa** segregants were determined to be either *CSP1* or *csp*$^+$ by scoring for the ability to synthesize DNA during "sporulation". All disomic strains were further confirmed to be heteroallelic at *leu2* by the ability to give rise

TABLE 1

Construction of disomic strains

Disomic strain	Cross obtained from	Meiotic DNA synthesis	Mitotic recombination at *leu2*	
d-**aa**-6d	A-169-αα-4a × Z4521-96c	—	+	**aa** *leu2-1/leu2-27 csp*$^+$
d-**aα**-1b	A-169-αα-4a × Z4521-96c	+	+	**aα** *leu2-1/leu2-27 csp*$^+$
d-**aα**-1c	A-169-αα-4a × Z4521-96c	+	+	**aα** *leu2-1/leu2-27 csp*$^+$
d-**aα**-1a	A-169-αα-4d × Z4521-96c	+	+	**aα** *csp* genotype unknown
d-**aα**-2a	A-169-αα-4d × Z4521-96c	+	+	**aα** *csp* genotype unknown
d-**aa**-5b	A-169-αα-4d × Z4521-96c	+	+	**aa** *leu2-1/leu2-27* CSP
d-**aa**-4d	A-169-αα-4d × Z4521-96c	+	+	**aa** *leu2-1/leu2-27* CSP

to mitotic recombinant *leu* prototrophs upon exposure to UV. These strains are summarized in Table 1.

Growth media and sporulation: Growth media and sporulation conditions were also described in the previous communication. The following additional media were utilized for genetic analysis of the strains: Complete medium contains 1.5% agar, 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 threonine, 50 μg/ml tyrosine, 60 μg/ml phenylalanine, 10 μg/ml arginine. To score for particular markers, the appropriate nutrient is omitted from this medium. Cycloheximide medium is YEP plus 20 μg/ml cycloheximide. Galactose plates contain 1% galactose, 1% bactopeptone, 1% yeast extract, 1.5% agar, 40 μg/ml adenine, 40 μg/ml uracil, and enough bromthymol blue to give the solution a light green color. Colonies capable of growth on this medium cause the agar surrounding the colonies to turn yellow.

Mating analysis: The analysis of response to **a** factor was as described by WILKINSON and PRINGLE (1974). A concentrated preparation of supernatant from **a** cells (prepared by WILKINSON and PRINGLE) was diluted 1 to 10 into cultures of vegetative (10^7 cells/ml) yeast cells. At ½-hr intervals (for 2½ hr) aliquots were removed from the growing culture and sonicated for 15 sec. The percent of unbudded cells was analyzed by light microscopy. Mating-factor production was assayed similarly to factor response. Yeast cultures were grown to stationary phase in YEP (2×10^8 cells/ml). The cells were removed from the growth medium by centrifugation; the clear cell supernant was used as a source of mating factor and tested on cells of known mating type.

X-ray killing: Cells were grown in YEP to stationary phase, sonicated 4 times for 20 sec to release buds from mother cells, and diluted in H_2O. At appropriate times duplicate samples containing approximately 200 cells were spread onto YEP plates which were irradiated in a Picker X-ray apparatus delivering 140 r/sec at 50KV and 20ma. The irradiated cells were allowed to grow at 32° for 4 days, at which time the average number of surviving colonies per plate was determined.

DNA analysis: The DNA content of yeast cells was analyzed as described previously (HOPPER and HALL 1975).

Meiosis I and II: The cells were stained with giemsa by the method of ROBINOW and MARAK (1966) as modified by L. HARTWELL (1970).

Recombination: Mitotic intragenic recombination between *ade2* or *leu2* heteroalleles was measured as the frequency of induced prototroph production. Cells were grown in YEP to stationary phase. One-ml cells at 2×10^8 cells/ml were exposed to 0-, 1- or 2-min UV irradiation (18.4 erg/mm^2/sec) in order to induce mitotic recombination (ROMAN and JACOB 1958; FRIIS and ROMAN 1968). Duplicate samples of 10^2 cells were spread onto YEP plates and duplicate samples of 10^6 cells onto complete minus adenine or complete minus leucine plates. The colonies were allowed to grow at 32° for 3 days, at which time the average number of prototroph colonies on complete minus adenine or leucine plates per 10^6 surviving colonies on YEP was determined.

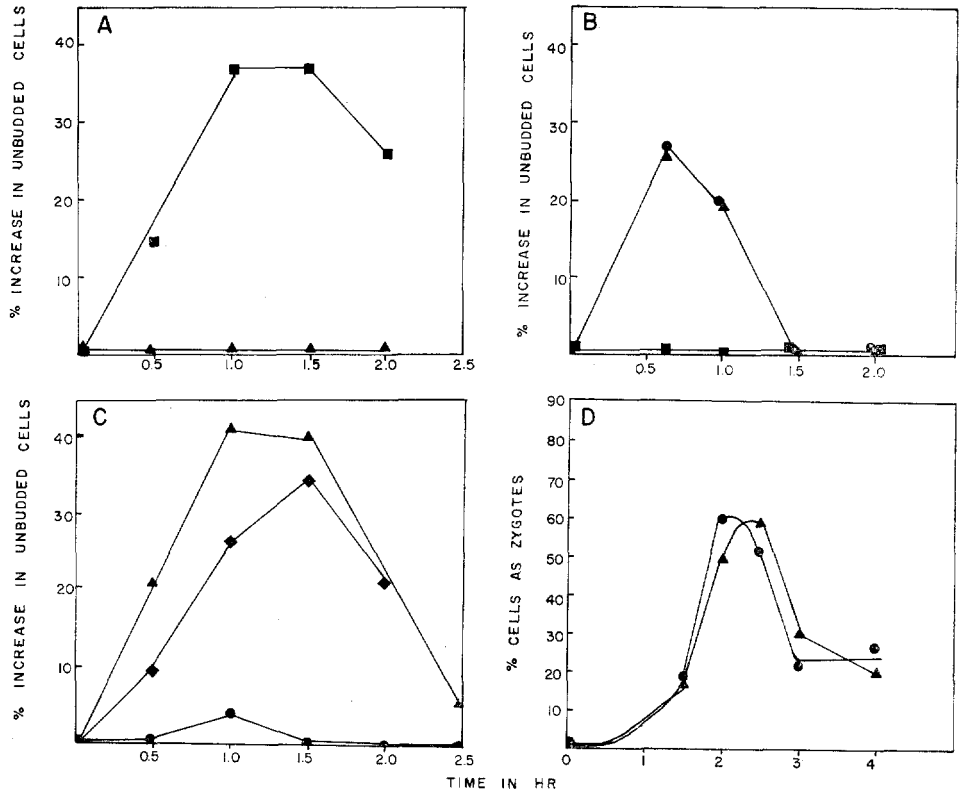

FIGURE 1.—Mating characteristics of A-169-$\alpha\alpha$. A. Production of **a** factor—AP-1-**aa** and mutant A-169-$\alpha\alpha$ cultures were grown in YEP to stationary phase. Cells were removed from the media by centrifugation. The clear supernatant was diluted 1–10 into vegetative cultures of S288C-27 (α haploid) cells (in YEP at 10^7 cells/ml). At ½-hr intervals aliquots of S288C-27 cells were analyzed for the percent unbudded cells (see MATERIALS AND METHODS). ■-■-■, test for **a** factor in AP-1-**aa** supernatant; ▲-▲-▲, test for **a** factor in mutant A-169-$\alpha\alpha$ supernatant. B. Production of α factor. AP-1-**aa** (■-■-■), AP-1-$\alpha\alpha$ (●-●-●), and A-169-$\alpha\alpha$ (▲-▲-▲) cultures were grown in YEP to stationary phase. Cells were removed from the media by centrifugation. The clear cell supernatant was diluted 1–10 into vegetatively (YEP ~ 10^7 cells/ml) growing cultures of AP-1-**aa**. At ½-hr intervals aliquots of the **aa** cells were analyzed for percent unbudded cells as above. C. Response to **a** factor—Concentrated **a** factor (WILKINSON and PRINGLE 1974) was diluted 1–10 into vegetatively (PSP2) growing cultures of A-169-$\alpha\alpha$ ▲-▲-▲; ◆-◆-◆, S288C-27 (α haploid; see HOPPER and HALL 1975); and ●-●-●, S400D, (**a** haploid). At ½-hr intervals aliquots were removed, fixed in saline-formaldehyde, and analyzed for percent unbudded cells. D. Zygote formation. Cells were grown in YEP. At ~ 10^7 cells/ml equal volumes of AP-1-**aa** were mixed with AP-1-$\alpha\alpha$ (●-●-●) or A-169-$\alpha\alpha$ (▲-▲-▲). The cells were allowed to continue growth on a rotary shaker at 30°. At 1-hr intervals, 0.5-ml samples were taken and mixed with an equal volume saline formaldehyde. The percentage of cells as zygotes at each point in each of the two cultures was analyzed by light microscopy.

Meiotic intragenic recombination was measured similarly to mitotic intragenic recombination (SHERMAN and ROMAN 1963), except that samples were taken at appropriate times from cells in sporulation medium at 10^7 cells/ml, diluted, and spread onto YEP (or complete plates) and complete minus adenine or complete minus leucine plates.

For assaying intragenic recombination in completed single spores, asci in distilled H_2O were

digested with glusulase (1 to 10 dilution of glusulase (Endo Laboratories) for 24 hr at room temperature), washed free of glusulase by centrifugation, resuspended in distilled H_2O and then sonicated 4 times for 20 sec each to release and disaggregate spores. This procedure lyses all unsporulated diploid cells as assayed by light microscopy. The frequency of prototrophic recombinants in the spore suspension was assayed as described above.

Meiotic intergenic recombination in completed spores was measured as the frequency of recombination between the recessive heterozygous genetic loci: *gal1-lys2*, *lys2-tyr1*, *tyr1-his7*, *leu-1-cyh2*. Colonies resulting from the dissection of 4 spored asci of AP-1-aα or A-169-$\alpha\alpha$ were streaked into complete minus lysine, tryptophan, histidine and leucine plates as well as galactose and cycloheximide plates. The frequency of recombination was taken as: (T + 6 NPD)/2(PD+NPD+T) (MORTIMER and HAWTHORNE 1969).

RESULTS

Mating behavior of CSP1: A comparison was made between the CSP mutant and wild-type cells with respect to α mating factor production, response to **a** mating factor, and zygote formation with an **a** tester strain. The ability of culture supernatant from the mutant cells to inhibit budding of the **aa** strain was nearly equal, both in extent and kinetics of inhibition, to that produced by culture supernatant from $\alpha\alpha$ cells (Figure 1B). A-169-$\alpha\alpha$ culture supernatant did not inhibit budding of α cells (Figure 1A). Figure 1C shows that the response of A-169-$\alpha\alpha$ to **a** factor is similar to that of a wild-type α strain.

The kinetics and extent of zygote formation were nearly identical for matings of $\alpha\alpha$ and the *CSP1* mutant with an **aa** tester strain (Figure 1D). A-169-$\alpha\alpha$ also shows normal $\alpha\alpha$ specificity of zygote formation; no zygotes have been detected either within A-169-$\alpha\alpha$ cultures or upon mixing A-169-$\alpha\alpha$ with aα cells.

The three tests performed show that the *CSP1* mutant exhibits normal $\alpha\alpha$-like behavior both in mating and in factor production and response.

Induced mitotic intragenic recombination and resistance to X-rays: Mitotic aα cells exceed **aa** and $\alpha\alpha$ cells both in their ability to undergo induced intragenic mitotic recombination (FRIIS and ROMAN 1968) and to withstand X-ray damage to DNA (LASKOWSKI 1960). The level of ultraviolet-induced mitotic intragenic recombination between *ade2* heteroalleles was measured for A-169-$\alpha\alpha$, $\alpha\alpha$, and aα strains (Figure 2). The yield of *ade*$^+$ recombinants for a given dose of ultraviolet radiation (slope of the lines in Figure 2) was 3- to 4-fold higher for *CSP* or *csp*$^+$ aα cells than for *CSP* or *csp*$^+$ $\alpha\alpha$ cells.

aα cells are more X-radioresistant than are **aa** and $\alpha\alpha$ cells (LASKOWSKI 1960). Mutant A-169-$\alpha\alpha$ has a killing curve identical to that of its $\alpha\alpha$ parent (Figure 3).

With respect to the ability of mitotic cells to carry out intragenic recombination and for X-ray killing, as for mating, the *CSP1* mutant is clearly $\alpha\alpha$-like in its behavior.

Meiotic DNA synthesis: The quantity of DNA accumulated in *CSP1* cells (either A-169-**aa** or A-169-$\alpha\alpha$) following a shift to sporulation medium is the same as for aα cells; however, the kinetics of DNA increase differ in two respects. Meiotic DNA synthesis begins at 2 to 3 hours for aα cells, at 8 hours for A-169-$\alpha\alpha$; the duration of meiotic S phase is 6 hours for aα and 10 to 11 hours for *CSP1* cells (Figure 4). Isopycnic centrifugation experiments (data not shown) have shown

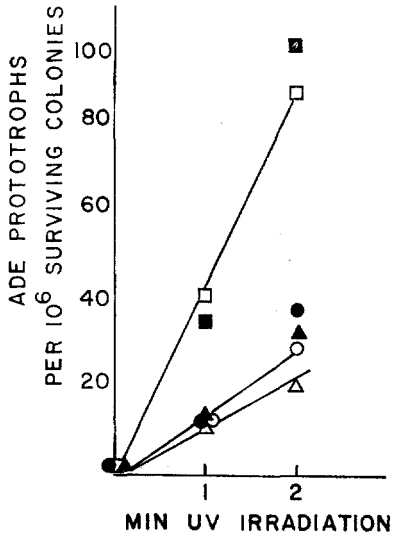

FIGURE 2.—UV-induced mitotic recombination. Cells were grown on YEP to stationary phase, washed with H_2O 2X by centrifugation, and resuspended at 10^8 cells/ml. One-ml aliquots were UV-irradiated (18.4 erg/mm²/sec) for 0, 1, or 2 min. Duplicate samples of the irradiated cells were plated onto YEP (~ 10^2 cells/plate). The colonies were allowed to grow at 23° 3 days, at which time the average number of recombinant, adenine prototrophs per 10^6 surviving colonies was determined. ■-■-■, □-□-□, aα recombination for two independent determinations ●-●-●, o-o-o- A-169-$\alpha\alpha$ recombination; ▲-▲-▲, △-△-△, AP-1-$\alpha\alpha$ recombination.

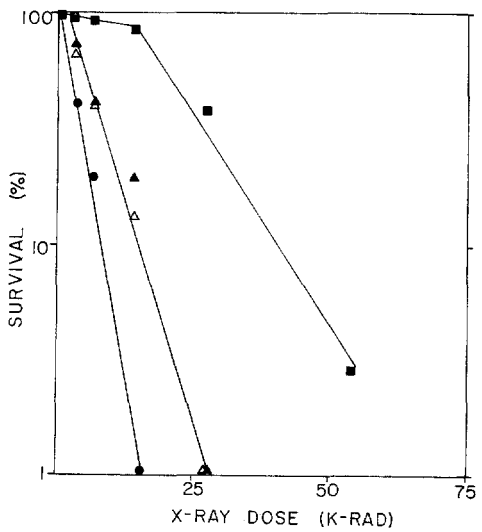

FIGURE 3.—X-ray resistance. Cells were grown in YEP to stationary phase, sonicated 3 × 15 sec to separate mother cells and buds, and spread onto YEP plates at approximately 2–4 × 10^2 cells/plate. The cells were immediately exposed to X-rays at the given dose. Irradiated cells were allowed to grow at 23° or 30° 2–3 days, at which time the average percent surviving colonies per X-ray dose was determined. ■-■-■, AP-1-aα; ▲-▲-▲, AP-1-$\alpha\alpha$; ●-●-●, A364A (a haploid cells); △-△-△, A-169-$\alpha\alpha$ mutant cells.

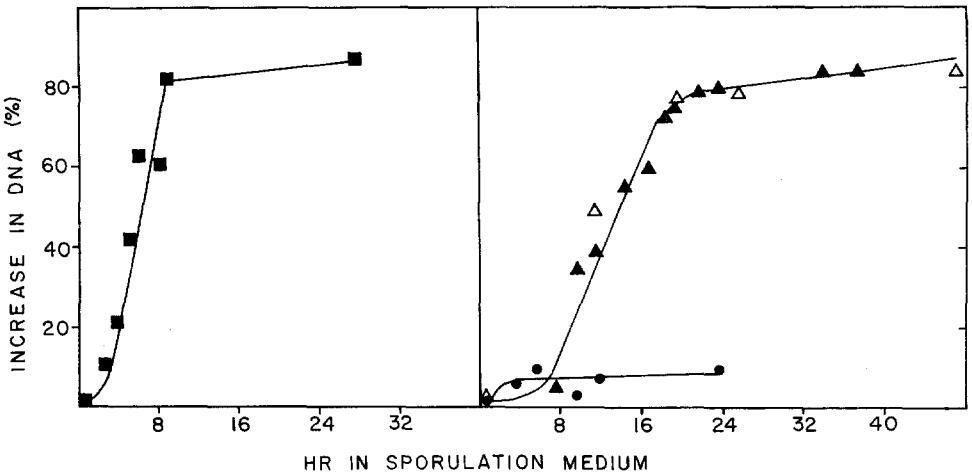

FIGURE 4—Increase in DNA content for sporulating AP-1-**a**α (■-■-■); mutant A-169-$\alpha\alpha$ (▲-▲-▲); A-169-**aa** (△-△-△); and AP-1-$\alpha\alpha$(●-●-●) cells. The yeast cells were maintained in logarithmically growing condition (PSP2) at 30° for 30 hr. The cells at a density of 1–2 × 10^7 cells/ml were collected onto a millipore filter, washed with 1 vol. sporulation medium, and resuspended at the same cell density in sporulation medium. Cells were sporulated at 30° on a rotary shaker. At indicated times, 3 1-ml aliquots of sporulating cells were taken and analyzed for the average percent increase in DNA content by the DABA procedure previously described (HOPPER and HALL 1975).

that the major part of the DNA made both by **a**α and A-169-$\alpha\alpha$ cells in sporulation medium has the buoyant density of nuclear DNA.

Except for the difference in timing, *CSP1* is **a**α-like with respect to meiotic DNA synthesis. Both the quantity and nature of the DNA synthesized would seem to indicate that most of the mutant *CSP1* cells in a sporulating culture possess the $4N$ complement of DNA needed to undergo meiotic chromosome segregation.

Meiosis and sporulation: At present cytological measurement of chromosome segregation is not possible for meiotic yeast cells. A gross analysis of chromatin body movement which accompanies meiosis can, however, be carried out by Giemsa staining (ROBINOW and MARAK 1966). Upon microscopic examination of Giemsa-stained preparations, "binucleate cells" are seen following Meiosis I and "tetranucleate cells" following Meiosis II. In contrast to the transient presence of binucleate cells in **a**α cultures shortly after DNA synthesis begins (Figure 5A), these structures appear quite late, then accumulate slowly and continuously in A-169-$\alpha\alpha$ cultures (Figure 5B). The nuclear counts indicate that, at 52 hours, 50% of the *CSP1* mutant cells appear to have completed meiosis I and 20% appear to have completed meiosis II. Of the total cells, 18% have become 1- or 2-spore asci and 4% have become 3- or 4-spore asci. Thus the meiotic processes which follow DNA synthesis are not carried out efficiently by the *CSP1* mutant.

Meiotic recombination in ascospores: Intergenic and intragenic recombination during meiosis of *CSP1* mutant and **a**α cells were compared by measuring the frequency of recombinants in ascospores produced by each strain. For each of

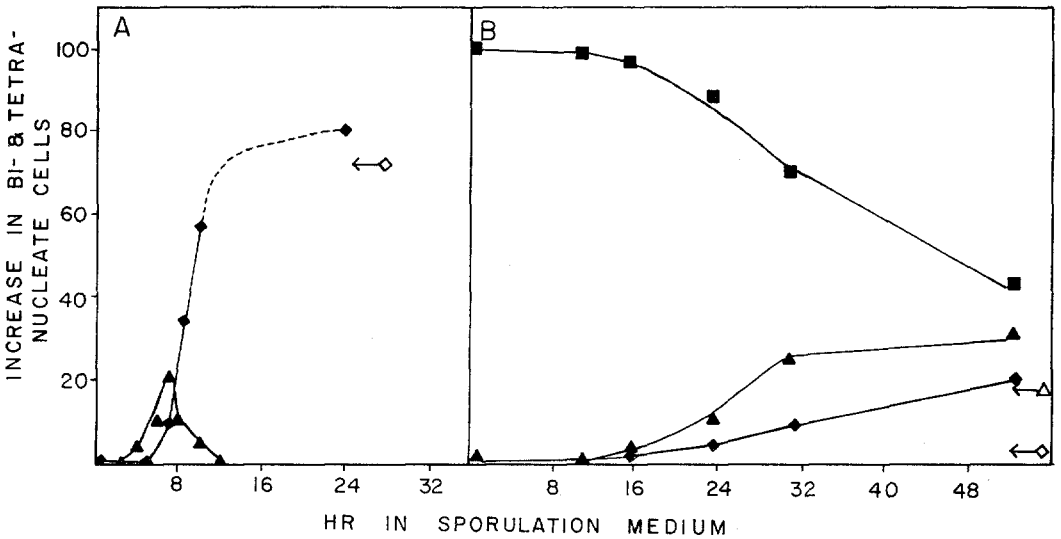

FIGURE 5.—Increase in bi- and tetranucleate cells during sporulation. AP-1-aα (A) and A-169-$\alpha\alpha$ (B) cultures were maintained logarithmically and sporulated as described for Figure 4. At indicated times, 0.1-ml aliquots of cells were removed from a sporulating culture and fixed with 0.9 ml formaldehyde-saline. The cells were fixed to slides, stained with Giemsa stain, and the percent cells appearing binucleate (▲-▲-▲); 3- or 4-nucleate (◆-◆-◆), or mononucleate (■-■-■) scored. No discrimination between 1- and 2-nucleate and 1- and 2-spored asci or 3- and 4-nucleate and 3- and 4-spored asci was made. Final percent asci are indicated by open symbols: ◇-3 or 4 spores/ascus; △—1 and 2 spores/ascus.

four pairs of weakly-linked markers, recombination map distances were calculated from the relative frequencies of PD (parental ditype), NPD (nonparental ditype) and T (tetratype) ascus segregation patterns (Table 2). For 3 of the 4 regions of the map, the distances were nearly equal in the two strains. For the *leu1–cyh2* interval, *CSP1* had more recombination than did aα. The number of *ade*$^+$ intragenic recombinants recovered by plating on complete minus adenine medium was 77 per 10^6 spore colonies for the *CSP1* mutant and 76 per 10^6 for aα random spore colonies.

By scoring for recombinant genotypes among haploid spore nuclei, which are the completed products of meiosis, we have found that both intergenic and intragenic recombination occur with equal frequency in *CSP1* and aα cultures. This result appears paradoxical in view of the quantitative defectiveness of *CSP1* mutant compared to aα cells, both as regards mitotic recombination and the ability to undergo meiosis and spore formation.

In order to determine whether the apparent non-defectiveness of meiotic recombination applies both to those *CSP1* mutant cells which do complete ascospore formation and to those which do not, we have attempted to extend our recombination measurements to include the entire culture of "sporulating" cells.

Meiotic recombination in sporulating populations: Intragenic recombination is stimulated by exposing aα diploid cells to sporulation medium and then, before

TABLE 2

Meiotic recombination in CSP1

A. *Intergenic recombination*

Markers Chromosome II	Published* recombination dist.	AP-1-aα PD:NPD:T	AP-1-aα Recombination distance	A-169-$\alpha\alpha$ PD:NPD:T	A-169-$\alpha\alpha$ Recombination distance
Centromere—*gal1*	6 cM				
gal1—lys2	>50 cM	9:1:21	44	13:2:26	46
lys2—tyr1	45 cM	8:0:23	38	15:0:29	33
tyr1—his7	45 cM	11:2:20	49	7:1:34	48
Chromosome VII					
Centromere—*leu1*	< 1 cM				
leu1—cyh2	>50 cM	9:0:19	34	12:3:30	>50

B. *Intragenic recombination*

	aα	A-169-$\alpha\alpha$
No. *ade* prototrophs per 10^6 completed spores	76	77
Max. no. *ade* prototrophs per 10^6 cells in sporulation medium	290†	66†
Percent viable colonies after 64 hr in sporulation medium‡	100	39
Max. no. *ade* prototrophs per 10^6 viable colony-forming units	290	170
Max. no. *ade* prototrophs per 10^6 viable colony-forming units corrected for avg. no. spores per ascus§	78	81

* MORTIMER and HAWTHORNE (1969)

† Essentially the same numbers of recombinants are obtained whether normalizing to the number of cells capable of growth on YEP, complete with glucose, or complete with acetate.

‡ The exact extent of A-169-$\alpha\alpha$ viability is difficult to accurately assess since many of the cells are budded; in such cases both a cell and a bud must die in order to score as a lethal event.

§ 3.7 spores/avg. aα ascus; 2.1 spores/avg. *CSP1* ascus

meiosis has occurred, shifting the cells back to vegetative growth medium (SHERMAN and ROMAN 1963). This procedure makes possible the scoring of "meiotic" recombination events in diploid cells even if the cells are genetically or physiologically incapable of completing ascus development (ESPOSITO and ESPOSITO 1974). By experiments of this type, we have made a comparison of the abilities of the total population of *CSP1* and aα diploid cells to undergo meiotic recombination. Using as a test system intragenic recombination between two *ade2* heteroalleles, we found a fourfold higher recombination frequency both for *CSP* and *csp*$^+$ aα cells than for $\alpha\alpha$ *CSP1* mutant cells.

Taken at face value, these results (Figure 6A; data not shown for *CSP1/CSP1* aα cells) suggest that there is a meiotic recombination deficiency in total populations of *CSP1* mutant cells even though normal recombination frequencies are observed among spores in completed *CSP1* mutant asci. It appears from quantitative comparisons (Table 2B) that *all* of the *ade*$^+$ recombinant colonies observed

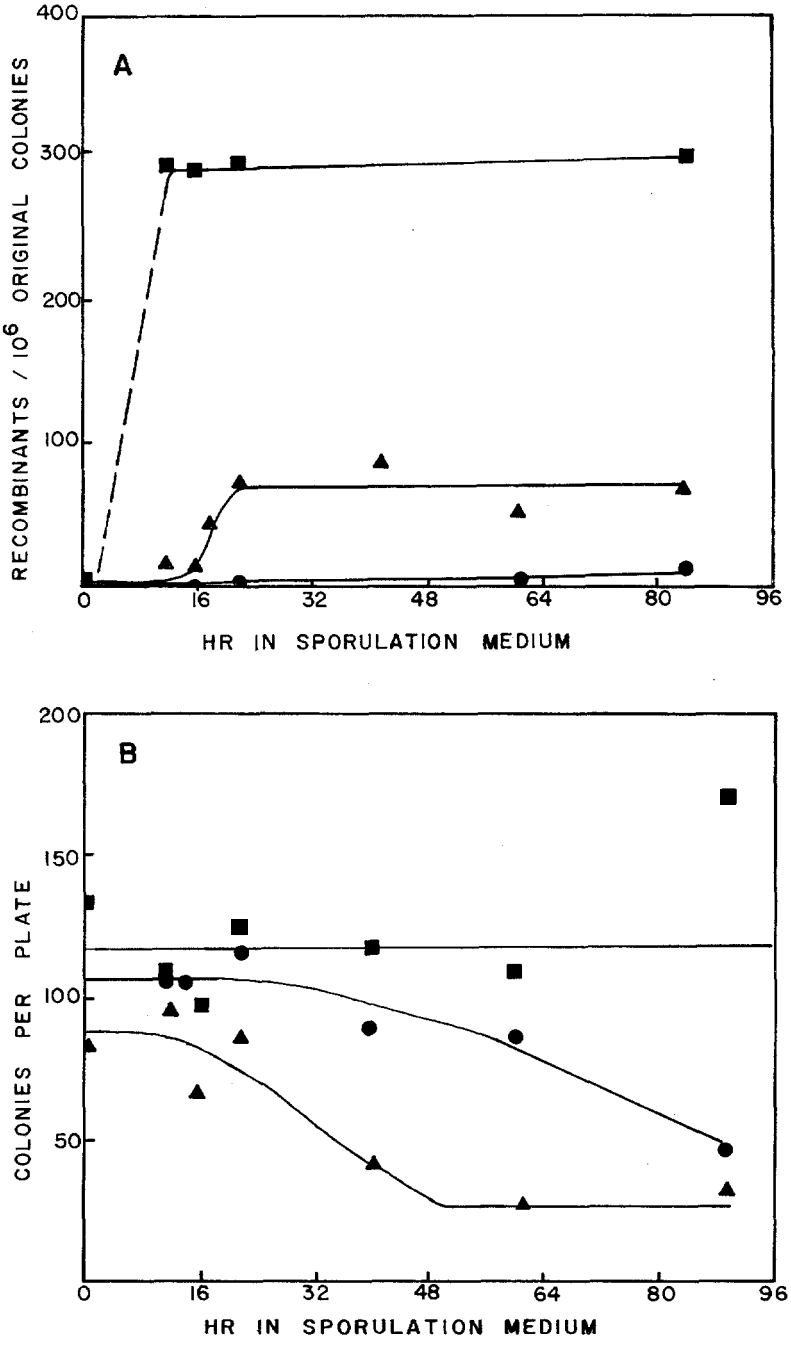

FIGURE 6—Intragenic recombination and viability of sporulating populations. Cultures were grown to stationary phase (2×10^8 cells/ml), washed, and resuspended in sporulation medium at 2×10^7 cells/ml. At indicated times duplicate aliquots of cells were removed from the sporulating culture, diluted in H_2O, and spread onto YEP and complete minus adenine media.

by shifting *CSP1* mutant cultures back to vegetative growth medium can be accounted for by complete asci (which can form colonies if *ade*$^+$) or by those cells which would eventually form asci. Thus it appears that the 80% fraction of cells which fail to sporulate do not produce any recombinants at all. However, the procedure for detecting recombinants is heavily biased against detecting recombinant cells which cannot complete sporulation. With this procedure, both *ade*$^+$ meiotic cells and asci containing *ade*$^+$ spores produce colonies and are scored as recombinant organisms. However, the *CSP1* cells which have not completed sporulation lose colony-forming ability with increasing time in sporulation medium, whereas completed asci remain completely viable. In order to eliminate this bias, further comparisons between $\alpha\alpha$ *CSP1* and $\mathbf{a}\alpha$ capacity for meiotic recombination were carried out in disomic strains.

Intragenic recombination in disomic strains: Both the mating-type and *leu2* loci are located on linkage group III. Consequently, $\mathbf{a}\alpha$ *leu2-1/leu2-27* heteroallelic disomic strains can yield leucine prototrophs by meiotic recombination even though monosomy for chromosomes I, II and IV–XVII prevents normal meiosis. Similarly **aa** or $\alpha\alpha$ disomes should exhibit recombination between *leu2* heteroalleles if the *CSP* mutation is present. Because the occurrence of meiosis in such strains causes lethal aneuploidy, only those recombinant cells which have not undergone chromosome segregation will yield colonies when aliquots of a sporulating disome culture are plated on growth plates lacking leucine. Thus, by using disomes rather than diploids (Figure 6) to compare meiotic intragenic recombination, the results should not be affected by the differential abilities of *CSP1* and $\mathbf{a}\alpha$ cells to complete sporulation.*

Disomic $\mathbf{a}\alpha$ and **aa** strains, heteroallelic for *leu2* (*leu2-1/leu2-27*) with and without the *CSP1* mutation, were constructed as described in MATERIALS AND METHODS. The strains were grown in YEP medium and transferred at stationary phase to sporulation medium. At appropriate times culture aliquots were returned to vegetative growth by plating on YEP and complete minus leucine agar. At these times samples were also taken for measurement of the increase in DNA content.

The kinetics of recombinant production differed for the 4$\mathbf{a}\alpha$ disomic strains tested. The maximum number of leucine prototrophs obtained, however, was very similar for all 4 strains (Table 3). The experimental values for recombination agree quantitatively with those reported previously by ROTH and FOGEL (1971). The kinetics for increase in *leu* prototrophs also differed in the 2 **aa** *CSP1* strains tested (Figure 7). For both **aa** *CSP1* cases the maximum number of meiotic recombinants is significantly lower than for $\mathbf{a}\alpha$ strains. Yet meiotic DNA synthesis in these strains was nearly equivalent to that of $\mathbf{a}\alpha$ disomic

* The authors thank DR. P. T. MAGEE for suggesting the disome experiments.

The colonies were allowed to grow at 32° 3 days, at which time the number of adenine prototroph colonies on minus adenine media (A) and the average number of viable colonies on YEP (B) was determined. ■-■-■, AP-1-$a\alpha$ sporulating cells; ▲-▲-▲, *CSP* mutant cells; ●-●-●, AP-1-$\alpha\alpha$ cells.

TABLE 3

Meiotic recombination of disomic strains

Strain	Max. no. *leu* prototrophs per 10^5 colonies	% Increase meiotic DNA synthesis
d-aα-1b	375	80
d-aα-1c	370	70
d-aα-1a	420	—
d-aα-2a	387	—
Avg.	391	
d-**aa**-6d	0	0
d-**aa**-5b	75	56
d-**aa**-4d	150*	69
Avg.	113*	
Avg. recombination **a**α / recombination **aa** *CSP1*	= 3.5	

*This number is probably an overestimation since d-**aa**-6d is a clumpy strain (not all the cells were separated by sonication) and consequently the no. colonies per YEP plate is underestimated.

strains (Table 3). An **aa** *csp*$^+$ control yielded no *leu* prototrophs per 10^5 colonies. These results strongly indicate that **aa** *CSP1* populations are deficient in intragenic meiotic recombination as compared to **a**α cells.

DISCUSSION

Our observations on mitotic and meiotic cells of an $\alpha\alpha$ *CSP1* diploid show that

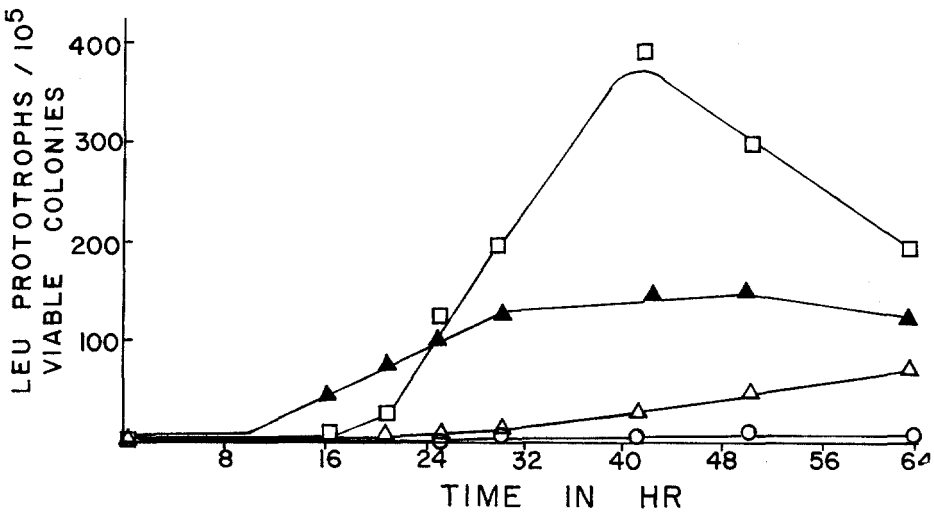

FIGURE 7.—Meiotic intragenic recombination in disomic strains. Disomic strains were grown and sporulated as for Figure 6. At indicated times duplicate aliquotes of cells were removed, diluted and spread onto YEP and complete minus leucine media. The colonies were allowed to grow at 32° 3 days, at which time the number of leucine prototroph colonies per 10^5 viable (YEP) colonies was determined. □-□-□, d-aα-1c (aα disome); ▲-▲-▲, d-**aa**-4d (**aa** disome, *CSP*); △-△-△, d-**aa**-5b (**aa** disome, *CSP*); o-o-o, d-**aa**-6d (**aa** disome, *csp*$^+$).

this strain is $\alpha\alpha$-like for the processes of mating, X-ray repair, and mitotic recombination; $a\alpha$-like for meiotic DNA synthesis and partially $a\alpha$-like for meiotic recombination and sporulation. If we consider **aa** and $\alpha\alpha$ diploid strains to differ from $a\alpha$ diploids by a pleiotropic regulatory deficiency, then an **aa** or $\alpha\alpha$ *CSP1* mutant diploid may be regarded as a partial "revertant" for mating-type-controlled gene expression. In this sense, a *CSP1* $\alpha\alpha$ diploid lacks $a\alpha$ function for induced mitotic recombination, X-ray radioresistance of DNA, meiotic recombination and spore formation and, as a consequence, cannot carry out these processes efficiently.

Two quite distinct hypotheses about $a\alpha$-dependent gene regulation seem potentially capable of explaining the meiotic defectiveness of $\alpha\alpha$ *CSP1* diploids:

1) The pleiotropic defect of $\alpha\alpha$ strains for all meiotic functions is ameliorated by the *CSP1* mutation. Consequently, the *CSP1* $\alpha\alpha$ diploid strain has a slightly reduced (as compared to $a\alpha$ diploid) level of expression of every $a\alpha$-dependent meiotic gene. For a complex morphogenetic process such as meiosis, a partial deficiency of many meiotic proteins will have a multiplicative effect; consequently, defectiveness should be least for early steps and greatest for late meiotic processes.

2) The meiotic defectiveness of *CSP1* $\alpha\alpha$ as compared to $a\alpha$ diploids may result from blockage of a single step in meiosis. The gene functions necessary to perform this step are, however, expressed at a low, leak-through level in $\alpha\alpha$ *CSP1* diploids; consequently, a few cells circumvent the block and go on to sporulate. Because recombination is the earliest step in meiosis for which $\alpha\alpha$ *CSP1* is noticeably defective and because subsequent meiotic processes are equally defective, but not more so, (see below) we hypothesize that meiotic recombination is the blocked step in $\alpha\alpha$ *CSP1* diploids and interpret our data to mean that recombination is necessary for subsequent steps in meiosis to occur. A dependence of meiotic chromosome segregation upon recombination has been observed both for Drosophila and maize. For several meiotic mutants of Drosophila, non-exchange tetrads were observed to undergo frequent chromosome nondisjunction (CARPENTER and BAKER 1974; SANDLER and LINDSLEY 1974). In asynaptic maize strains BEADLE (1933) observed results very similar to those we report here. Although chromosome pairing occurs to an abnormally low extent in such strains, the few progeny which result show a normal level of genetic recombination. An indication that recombination and meiosis in yeast may also be subject to an obligatory coupling is afforded by the *con1* mutant (FOGEL and ROTH 1974), which lacks meiotic intragenic recombination and is sporulation-defective. The two hypotheses outlined above differ both in regard to the number of steps in meiosis presumed to be under direct mating-type control and in the pattern of meiotic defectiveness to be expected for $\alpha\alpha$ *CSP1* diploids. The first supposes that genes acting at all stages of meiosis are uniformly subject to mating-type control, whereas the second implies that direct $a\alpha$ control acts only upon meiotic DNA synthesis and recombination. The first hypothesis also predicts that the fraction of $\alpha\alpha$ *CSP1* cells able to carry out each successive meiotic event will decrease in a steady progression; the second predicts that a large fraction of the

CSP1 mutant cells will complete meiotic events prior to the block, while a constant small fraction of cells will complete those meiotic events subsequent to the block. The three sets of observations which pertain most directly to these predictions are: the frequency of meiotic intragenic recombination (25% to 29% as high for *CSP1* $\alpha\alpha$ or **aa** as for **a**α cultures); the proportion of cells which become tetranucleate (25% as high for *CSP1* $\alpha\alpha$ as for **a**α) and the extent of ascus formation (25% as high for *CSP1* $\alpha\alpha$ as for **a**α). The greater degree of defectiveness for all three of these processes than for premeiotic DNA synthesis is consistent with both of the hypotheses. However, the fact that intragenic recombination, tetranucleate cell formation and ascus formation all occur to the *same* extent ($\alpha\alpha$ *CSP1* being 25% of **a**α) is clearly compatible with the second hypothesis and incompatible with the first. Thus it appears that $\alpha\alpha$ *CSP1* cells are blocked in meiosis after DNA replication and prior to recombination. This conclusion is strengthened by the data on UV-induced intragenic recombination in mitotic cells. For this process, $\alpha\alpha$ *CSP1* cells are also defective, as compared to **a**α cells, producing only 25% as many prototrophic recombinants for a given dose of ultraviolet light.

The meiotic phenotype of *CSP1* $\alpha\alpha$ diploids is most succinctly described as meiotic-recombination defective (with about 25% leakage). The mutant defect is pleiotropic for mitotic recombination and X-ray repair, suggesting that the **a**α-dependent gene products needed for meiotic and mitotic recombination may be the same. Similar pleiotropy has been observed for certain X-ray-sensitive yeast mutants, most notably for *rad52*. In **a**α diploid strains which are *rad52/rad52*, the high level of induced mitotic recombination typical of wild-type **a**α strains is not observed and the X-ray survival curve lacks the resistant shoulder typical of **a**α strains. For the homozygous *rad52* diploids, quite similar survival curves are obtained for **a**α and $\alpha\alpha$ cells (Ho and MORTIMER 1973); thus it appears that *rad52* is deficient in **a**α-dependent recombination and X-ray-radioresistant gene function. Of particular interest for this discussion is the fact that **a**α *rad52/rad52* diploids are partially sporulation-defective (GAME *and* MORTIMER 1974). The phenotypic similarity between $\alpha\alpha$ *CSP1* and **a**α *rad52/rad52* strains suggests that the same gene products are lacking or underproduced in both. It follows from this analogy that the lack of **a**α-dependent recombination function, which results in a low percentage of sporulation in $\alpha\alpha$ *CSP1*, is also what causes its X-ray survival curve to be $\alpha\alpha$-like rather than **a**α-like (Figure 1). The introduction into $\alpha\alpha$ *CSP1* of a constitutive (**a**α-independent) mutation for this recombination and repair function would be expected to suppress the sporulation defect of $\alpha\alpha$ *CSP1*. A mutation fitting this general description has already been described (ZAKHAROV and KOŽINA 1967; KOŽINA 1968). These authors report the isolation of diploid **aa** and $\alpha\alpha$ strains which sporulate slightly (<1% asci) and which are **a**α-like with respect to X-ray sensitivity and intragenic mitotic recombination.

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