ENHANCED GENE CONVERSION AND POSTMEIOTIC SEGREGATION IN PACHYTENE-ARRESTED *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Previous study has demonstrated that incubation of yeast cells of strain AP-1 in sporulation medium at 36° permits them to begin meiosis but that they become arrested at pachytene and undergo enhanced intragenic recombination between *ade2* heteroalleles. Tetrad analysis was undertaken to characterize the altered program of meiotic recombination more widely. In one set of experiments, pachytene-arrested cells were permitted to resume sporulation upon transfer to the permissive temperature. In the resulting asci, both postmeiotic segregation and gene conversion were increased several-fold at a number of loci relative to unarrested controls, whereas reciprocal recombination increased two- to threefold. Another set of experiments analyzed the genetic consequences of inducing the pachytene-arrested cells to revert directly to mitotic growth without completion of meiosis. The appearance of homozygous sectors from heterozygous markers revealed that these cells had become committed to appreciable recombination but that reciprocal exchange was less frequent than in normal asci. Taken together, the data indicate that pachytene arrest rendered the cells committed to enhanced recombination upon resumption of sporulation but that most of the crossing over did not occur until release from the arrest. ——The genetic basis of pachytene arrest by AP-1 was investigated by mating each of its parents with progeny of strain Y55, which is able to sporulate at 36°. Both of these diploids sporulated at 36°, and asci from the one studied further exhibited 2:2 segregation of the sporulation defect, indicating that pachytene arrest is dependent on a recessive, temperature-sensitive allele at a chromosomal locus.

ALTHOUGH many of the mechanisms essential to meiotic recombination remain obscure, we have gained considerable understanding by integrating findings from a variety of organisms. Data from the Ascomycetes have proven particularly useful because all of the meiotic products are recovered together. Tetrad analysis has demonstrated that crossing over is often accompanied by nonreciprocal recombination, or gene conversion (scored as 1:3 and 3:1, or 2:6 and 6:2 asci), and postmeiotic segregation (usually scored as 3:5 and 5:3 asci). These findings stimulated the concept that recombination entails the formation of a Holliday intermediate, or crossed strand-exchange form, in which single strands of DNA become base paired with homologous partners on either side of a common junction (HOLLIDAY 1964). Electron microscopy

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has revealed the existence of such forms in recombining bacteriophage DNA (VALENZUELA and INMAN 1975) and in some eukaryotic plasmids, including the 2-μm DNA plasmid in meiotic yeast (BELL and BYERS 1979). Further insight has been gained from extensive tetrad analysis in *Saccharomyces cerevisiae*. Here, gene conversion and postmeiotic segregation events are accompanied in approximately half of the tetrads by the reciprocal recombination of flanking markers (FOGEL *et al.* 1979), confirming the association of hybrid DNA with crossing over. These tetrad data also indicate that hybrid molecules are not formed in reciprocal pairs, such as would result from branch migration after formation of the crossed strand-exchange form. It may, therefore, be the case that most hybrid DNA is formed by invasion of one duplex by a single strand displaced from the other (MESELSON and RADDING 1975). Nevertheless, alternative models have not been excluded and the molecular mechanisms of crossing over remain obscure (STAHL 1979; SZOSTAK *et al.* 1983).

We have gained a better understanding of meiotic recombination from the analysis of meiotic mutants, which have been isolated in a variety of organisms (BAKER *et al.* 1976). Studies of female Drosophila have, for example, revealed that mutants defective in crossing over can be identified by the nondisjunction of nonexchange bivalents in the first meiotic division (LINDSLEY and SANDLER 1977). Many of these mutants are also defective in the distribution of recombination events, as might result from failure at an early stage of crossing over. In contrast, *mei9* maintains a normal distribution despite a marked decrease in reciprocal recombination. CARPENTER (1982) has recently found that this mutation also causes a substantial increase in intragenic recombination, presumably because an abnormally large amount of hybrid DNA is formed. It is an intriguing possibility that the enhancement of intragenic recombination results from excess activity by the same mechanism that results in gene conversion at sites of crossing over in the fungi.

Mutational analysis of meiosis has also been undertaken in yeast and has resulted in the characterization of several *spo* mutations, which fail at various phases of sporulation (ESPOSITO and ESPOSITO 1978), as well as *rec* (RODARTE-RAMON 1972), *mei* (ROTH 1973) and *con* (FOGEL and ROTH 1974) mutants, which may be defective more specifically in recombination processes. Certain radiation-sensitive (*rad*) mutations also have specific defects in meiotic recombination (GAME *et al.* 1980). In addition, many of the *cdc* mutants are unable to complete sporulation for a variety of reasons (SIMCHEN 1974). Analysis of the recombinational effects of these and other mutants has employed the capability of yeast to undergo reversion to mitosis after having become committed to meiotic recombination (SHERMAN and ROMAN 1963). Using this assay, ZAMB and ROTH (1977) investigated the effect of blocking DNA synthesis by exposing a *cdc8* homozygote to 36° and found that meiotic gene conversion requires chromosomal DNA replication. SCHILD and BYERS (1978) demonstrated a similar failure of recombination in another replication-defective mutant, *cdc21*, which is defective in thymidylate synthetase; on the other hand, *cdc7*, which is defective in the initiation of DNA replication in mitosis, prevented gene conversion without inhibiting premeiotic DNA replication.

In the course of these studies, we found that cells arrested by various *cdc* mutations, although capable of reverting to mitosis, are unable to resume meiosis and form complete asci of viable spores when transferred to the permissive temperature. On the other hand, a "wild-type" strain (AP-1) became arrested in pachytene upon transfer to 36° but remained capable (when returned to 23°) either of reverting to mitosis or resuming sporulation (BYERS and GOETSCH 1982). Assays for the production of adenine prototrophs by intragenic recombination between *ade2* heteroalleles revealed enhanced gene conversion among cells recovering from the arrest by either route. Because of the possibility that an understanding of this phenomenon might shed light on the recombinational mechanism, we have sought to examine the genetic consequences of the arrest in more detail. The tetrad data reported here demonstrate that pachytene arrest is accompanied by widespread alterations of both reciprocal and nonreciprocal recombination.

MATERIALS AND METHODS

Strains: Strain AP-1 is the product of a cross between A364a (*MATa ade1 ade2-1 ura1 tyr1 his7 lys2 gal1*) and α131-20 (*MATα ade2-R8 cyh2 can1 leu1 ura3 MALx*) (HOPPER *et al.* 1974). Strain Y55 (GARVIK and HABER 1978) was provided by J. HABER.

Media: YEPD medium contained 20 g/liter of glucose, 20 g/liter of bactopeptone and 10 g/liter of yeast extract. YPA presporulation growth medium contained 10 g/liter of potassium acetate, 20 g/liter of bactopeptone and 10 g/liter of yeast extract. SPM sporulation medium contained 3 g/liter of potassium acetate and 0.2 g/liter of raffinose. Diagnostic media, sporulation medium for plates and the standard methods for testing genetic markers have been described by MORTIMER and HAWTHORNE (1975).

Sporulation: Cultures were grown in YPA by rotary agitation in flasks at 23° to a density of approximately 8 × 10⁶ cells/ml (monitored by optical density). They were harvested by centrifugation, washed in water, resuspended in SPM at the original cell density and again incubated on the rotary shaker.

Reversible pachytene arrest at 36°: To arrest AP-1 cells in pachytene, cultures were placed at 36° upon transfer to SPM. (Although the restrictive temperature will be designated as 36° in this report, a temperature of 36.5° was often employed.) Progress of the cells through meiosis was assayed by electron microscopic observation of cell lysates spread on an aqueous surface (GOETSCH and BYERS 1982). Pachytene was defined in these spreads as the presence of synaptonemal complex from telomere to telomere in all chromosomes. The efficiency of sporulation was determined by phase-contrast microscopy, examining 200 cells at 500× for each determination. A finding of "no sporulation" signifies that no asci were seen in approximately 1000 cells.

Shifting the arrested cells from 36° to 23° permitted reversal of the arrest and resumption of sporulation. We have previously shown (DAVIDOW, GOETSCH and BYERS 1980) that prolonged arrest of meiosis at 36° before reversal resulted in a decrease in the average number of spores per ascus, whereas supplementation of the arrested cells with fresh SPM increased the proportion of four-spored asci without significantly changing the frequency of recombinant adenine prototrophs or postmeiotic segregants. Therefore, we added fresh SPM at the time of the shift for the largest ascus dissection experiment presented in RESULTS. Transfer of the arrested cells to YEPD permitted resumption of mitotic growth. Treatment with ultrasound at 100 watts for 10 sec did not significantly affect viability but enhanced the separation of cells for micromanipulation and platings.

Spore treatments: For random spore preparations, the SPM cultures were first washed in water and pretreated for 10 min in 0.1 M 2-mercaptoethanol, 0.02 M ethylenediaminetetraacetic acid and 0.2 M tris(hydroxymethyl)aminomethane hydrochloride, pH 9. They were then incubated in glusulase (Endo Laboratories) at a 1/10 dilution in water for 1 hr to remove ascal walls and lyse vegetative cells. Reconstruction experiments demonstrated that fewer than one in 10⁶ vegetative

cells survived this treatment. The spores were sonicated on ice 3 × 15 sec at 100 watts before dilution to disperse clumps and were plated on YEPD or adenine-deficient growth media to measure the prototroph frequency.

The method of DAWES and HARDIE (1974) for ether-killing vegetative cells was used to detect segregation of the ability to sporulate at 36° in some crosses. Sporulation plates were replica plated to YEPD agar in glass Petri dishes, which were then exposed to ether vapor in a closed vessel for 3–5 hr. Only sporulated colonies survived the treatment and grew into dense patches.

The individual ascus method of dissection was described previously (DAVIDOW, GOETSCH and BYERS 1980). Sporulated cultures were pretreated (as described before) and streaked on YEPD dissection plates. Individual, intact, four-spored asci were micromanipulated onto a segment of the plate coated with 1/5 × glusulase to digest the ascal walls. Each ascus was then dissected on the same plate. Dissection plates were used directly as master plates in replica plating to score marker segregation (FOGEL et al. 1979). Scoring for ura1, ura3, ade1, gal1 and MAT required prior cross-stamping with appropriate testers (two pairs of strains contained the needed combination of markers), whereas the other markers were scored directly on the first replica. The detection of post-meiotic segregations (PMS) as sectored colonies may have been less efficient for the markers requiring cross-stamping.

RESULTS

The 36° pachytene arrest of AP-1 cells in sporulation medium could be reversed by either of two pathways. Resumption of meiotic sporulation was induced by shifting the culture to a lower (permissive) temperature while leaving the cells in SPM. Alternatively, transfer of the cells to YEPD growth medium at either temperature permitted return to vegetative growth without completion of sporulation. The recombinational effects of reversible pachytene arrest were assayed after both pathways of recovery.

Effect of the pachytene arrest on recombination in asci

Four aspects of genetic recombination were examined in the asci from reversibly arrested cells: (1) heteroallelic recombination between two mutant alleles to generate a wild-type gene (at ade2), (2) gene conversion (non-2:2 ratios) at 12 heterozygous loci assayed in dissected tetrads, (3) reciprocal recombination between linked genes and in gene-centromere intervals, and (4) PMS—the generation of half-sectored colonies from single ascospores.

Heteroallelic recombination: The frequency of adenine prototrophic spores from reversibly arrested cells increased with time of exposure to the restrictive temperature (36°) up to approximately eight or nine times the level in control spores not arrested at pachytene (Table 1). A cytological time course (Table 2) revealed that significant numbers of pachytene figures were first present in these cultures at 9 hr after transfer to SPM at 36°; by 12 hr virtually all of the cells that were undergoing meiosis had reached pachytene. We conclude from these observations that cells subjected to reversible arrest at 36° undergo enhanced levels of intragenic recombination only if they are left at the elevated temperature until after they have entered pachytene.

Gene conversion and PMS in dissected tetrads: More than 100 asci with four viable spores were dissected and analyzed from each of three parallel subcultures exposed to the restrictive temperature for 0, 9 or 21 hr. To eliminate the possibility of false tetrads, preselected asci were individually micromanipulated to selected positions on the dissection plate before wall removal and

TABLE 1

Heteroallelic recombination in spores from reversibly arrested cells

Experiment I		Experiment II	
Time at 36° (hr)	Adenine prototrophs (per 10^6 viable spores)	Time at 36° (hr)	Adenine prototrophs (per 10^6 viable spores)
0	39	0	42
9	44	7	45
15	260	9	55
21	290	11	144
		13	145
		15	244
		17	303
		19	296
		21	397

Cultures of AP-1 were placed in SPM at 36° for the indicated time before shifting to 23° to complete sporulation. Cultures remained in SPM a total of 48–72 hr before harvest.

TABLE 2

Cytological time course at the restrictive temperature

Time at 36° (hr)	% nuclei in pachytene
0	0
3	0
6	0
9	12
12	76

Time points for electron microscopic cytology were taken from an AP-1 culture transferred to SPM at 36° at $T = 0$. The first 50 nuclei observed were scored from each time point.

ascospore dissection (see MATERIALS AND METHODS). The segregations of each of 12 markers in the tetrads (Table 3) showed a dramatic increase in non-2:2 segregation at all loci in the reversibly arrested preparation. In asci from cells left at 23° or downshifted after only 9 hr at 36°, all markers showed a low level of non-2:2 segregation, ranging from 0 for several loci to 2% for *leu1* and *can1*. All of the markers showed increased non-2:2 segregation in the culture from 21 hr at 36° with frequencies ranging from 4% for *gal1* and *MALx* to 21% for *ura1*. The increase in non-2:2 segregation averaged over all of the markers was approximately 15-fold.

PMS in spores from reversibly arrested pachytene cultures also showed a particularly striking increase over the controls (Table 4). The distribution of these postmeiotic segregations among the different heterozygous markers was nonuniform. More than half of the 126 PMS occurred at *lys2* or *his7*, whereas five of the markers, including mating type, had none. Overall, PMS increased about 20-fold in the 21-hr group; 41 PMS occurred in 404 spores from asci with all four spores viable, whereas the incomplete asci had a slightly, but not significantly, higher rate of 85 PMS in 726 viable spores.

TABLE 3

Non-2:2 segregations in asci from reversibly arrested cells

A. Cultures with less than 10 hr at 36° (234 asci)

Marker	9 hr at 36° (123 asci)		Always at 23° (111 asci)			Combined % non-2:2
	3:1	1:3	3:1	5:3	1:3	
ade1	0	1	0	1	1	1.3
gal1	0	0	0	0	0	<0.4
lys2	0	0	0	0	0	<0.4
tyr1	0	1	0	0	0	0.4
his7	0	0	0	0	0	<0.4
MAT[a]	0	0	0	0	0	<0.4
ura3	0	1	0	0	1	0.9
can1	1	2	1	1	0	2.1
leu1	1	1	1	0	2	2.1
cyh2	0	0	0	0	0	<0.4
ura1	0	2	0	0	2	1.7
MAL	0	0	0	0	0	<0.4
Totals	2	8	2	2	6	

B. 21 hours at 36° (101 asci)

Marker	4:0	7:1	ab6:2	3:1	5:3	ab4:4	3:5	1:3	0:4	% non-2:2 (excluding 4:0 and 0:4)
ade1	0	0	0	4	0	0	1	8	0	12.9
gal1	1[b]	0	0	3	0	0	0	1	0	4.0
lys2	1[b]	0	0	2	4	1	3	3	0	13.0
tyr1	1[b]	0	0	2	4	0	2	5	0	13.0
his7	1[b]	1	0	2	5	2	4	1	0	15.0
MAT[a]	0	0	0	8	0	0	0	1	0	8.9
ura3	0	0	0	4	0	0	0	10	1	14.0
can1	0	0	1	13	0	0	1	1	1	16.0
leu1	0	0	0	3	0	0	0	3	0	5.9
cyh2	0	0	0	3	2	0	3	1	0	8.9
ura1	0	0	0	10	3	0	0	8	0	20.8
MAL	0	0	0	4	0	0	0	0	0	4.0
Totals		1	1	58	18	3	14	42		

Entries are the number of asci in each class.

[a] α regarded as "wild type" for tabulation purposes, so 3:1 means 3α:1**a**.

[b] A single ascus was homozygous for all four chromosome *II* markers. This is assumed to represent a mitotic event in subsequent calculations, as are the 0:4 cases.

Viability of ascospores was found to decrease with time after 9 hr at 36°. In the previous experiment, the viability was 93, 91 and 60% in the 23° control, 9 hr at 36° and 21 hr at 36° cultures, respectively. In a smaller experiment in which points were taken at 0, 15 and 23 hr, the 15-hr point had both intermediate viability, 78%, and an intermediate frequency of PMS, five PMS/219 viable spores, compared with the 23-hr group with 54% viability and 12 PMS/121 viable spores.

Reciprocal recombination between linked genes: Strain AP-1 is heterozygous for five pairs of linked markers (Table 5), the closest pair being *ura3* and *can1*,

TABLE 4

PMS in spores from reversibly arrested cells

Marker	9 hr at 36° (628 spores)	Always at 23° (537 spores)	21 hr at 36° (1130 spores)
ade1	0	1	5
gal1	0	0	0
lys2	0	0	47
tyr1	0	0	12
his7	0	1	34
mat	0	0	0
ura3	1	0	0
can1	0	1	6
leu1	0	0	0
cyh2	0	0	12
ura1	0	0	10
MAL	0	0	0
Total	1	126	3

Results from all viable ascospores from each time point are included, not just the asci with four viable spores included in Table 3. Entries are the number of sectored spore colonies scored for each genetic marker.

TABLE 5

Reciprocal exchange between markers in asci from reversibly arrested cells

Chromosome	Interval	9 hr at 36°	Always at 23°	21 hr at 36°
II	gal1–lys2	33:84:6	27:76:8	16:61:13
II	lys2–tyr1	39:80:3	40:68:3	16:52:19
II	tyr1–his7	34:80:8	24:84:3	19:58:10
V	ura3–can1	72:47:0	60:48:1	19:48:5
VII	leu1–cyh2	25:87:9	17:84:7	20:52:19

The results are expressed as numbers of parental ditype to tetratype to nonparental ditype asci for each pair of markers. Asci with gene conversions for one of the given markers were not included.

which are approximately 20 cM apart on chromosome *V*. The results on reciprocal recombination from the group left 9 hr at 36° and the 23° control are similar, but the 21-hr group showed increased crossing over in all five intervals. Three of the pairs (*gal1-lys2*, *lys2-tyr1* and *leu1-cyh2*) no longer displayed linkage, in that the parental ditype asci did not significantly exceed nonparental ditypes. The distances for the other two pairs (*tyr1-his7* and *ura3-can1*) increased by 1.5-fold and 2.2-fold, respectively, as calculated using the mapping formula of PERKINS (1949). Therefore, although recombination clearly increased in all five intervals, the upper limit on the relative increase could not be established.

Crossing over in gene-centromere intervals: Four of the genetic markers in AP-1 are tightly centromere linked; in addition, mating type is loosely centromere linked (Table 6). Enhanced recombination also occurs in some of these intervals at the pachytene arrest. Three of the four tightly linked markers experi-

TABLE 6

Crossing over in gene-centromere intervals in asci from reversibly arrested cells

Chromo-some	Gene	9 hr at 36°		Always at 23°		21 hr at 36°	
		FDS:SDS	% SDS	FDS:SDS	% SDS	FDS:SDS	% SDS
I	ade1	112:10	8.2	100:10	9.1	83:6	6.7
II	gal1	112:11	8.9	103:8	7.2	80:16	16.7
III	MAT	67:56	45.5	62:49	44.1	39:53	57.6
V	ura3	105:17	13.9	95:15	13.6	61:25	29.1
VII	leu1	116:5	4.1	103:5	4.6	83:12	12.6

Determination of the pairs of spores that were sisters was based solely on the segregation patterns at the four loci that were most tightly centromere linked. When not all four segregations were concordant, the interpretation giving the fewest second division segregations (SDS) was chosen. FDS:SDS denotes ratio of first to second division segregation.

enced two- to threefold increases in second division segregation, whereas the segregation of the fourth, *ade1*, was not significantly changed. Mating type showed a slight (30%) increase in second division segregation.

Disjunctional errors and spore death: Occasional disomic spore colonies were recovered, always in asci with at least one dead spore. One type of disomic product was recognized because it was unable to mate, did not sporulate significantly and was found in an ascus yielding one *MATa* spore colony, one *MATα* colony and a dead spore. These nonmaters were interpreted as disomes for chromosome *III* heterozygous for *MAT*. The first nonmating colony found was "force-mated" to a haploid tester by selection for prototrophic complementation and the resulting colony sporulated. The results indicated trisomic segregation for mating type and normal diploid segregation for seven other heterozygous markers. Seven nonmaters were recovered among 1720 spore colonies from pachytene-arrested cultures, compared with one nonmater among 1739 colonies from cultures that were at 36° for less than 10 hr. In the seven asci with nonmating colonies that had three viable spores, the two haploids of opposite mating type were sisters in two cases and nonsisters in five, as judged by centromere markers. Therefore, the chromosome segregation defect was not a simple failure of chromosome *III* to disjoin at meiosis II, which would always have left the two maters as sisters.

Colonies that papillated markedly for canavanine or cycloheximide resistance were presumed heterozygous and, therefore, probably disomic for chromosomes *V* and *VII*, respectively. Five colonies that retested as giving marked papillation when replica plated from large steaks were found in pachytene-arrested material compared with only one in control material.

Among the first 20 asci from the 21-hr group that had only two viable spores, five pairs of survivors were sisters and 15 were nonsisters. We can, therefore, rule out simple nondisjunction at meiosis I, which would leave only sisters, as the main cause of this class of asci. In the first 233 asci dissected in the 21-hr group there were 14, 35, 73, 58 and 53 asci containing 0, 1, 2, 3 and 4 viable spores, respectively. This distribution contains significantly more

TABLE 7

Distributions of events per ascus in the 21-hr group

	Events per ascus							
Events included	0	1	2	3	4	5	6	7
PMS								
Observed	69	24	7	1	0	0	0	0
Expected	68	27	5.5	0.7	0.07			
Gene conversions								
Observed	34	42	19	4	1	1	0	0
Expected	37	37	19	6.2	1.5	0.3		
Both combined								
Observed	24	36	27	9	2	2	0	1
Expected	25	35	24	11	4	1.1	0.3	0.05

Entries are the number of asci that contained the indicated number of events. Aberrant 4:4 segregations were counted as two PMS events. The 7:1 segregation counted as one PMS plus one conversion. If the aberrant 4:4 cases were counted as one event, the data would fit a Poisson distribution slightly better; however, none of the tabulations above differ significantly from a Poisson distribution.

asci in both extreme classes (0 and 4) than would be expected from the binomial distribution if each spore death was completely random, but the cause of spore death remains unknown.

Distribution of events per ascus: If the striking enhancement of gene conversion and PMS in the 21-hr group arose as a consequence of the pachytene arrest experienced by the entire population of cells, then the distribution of these events should have been equally distributed. Conversely, if the effect were restricted to a subset of aberrant cells, then the events might have been coincident, being clustered into a few asci. Analysis of the distributions of conversion events, PMS events and the sum of both events (Table 7) showed that the population of asci was homogeneous. None differed significantly from a Poisson distribution expected if each event occurred randomly within the full set of asci analyzed.

Multiple PMS at individual loci: Although the overall distribution of multiple PMS was homogeneous, there was a significant coincidence of multiple PMS at individual loci. Among the asci with four viable spores, there were three asci with two PMS for a single marker (two aberrant 4:4 tetrads and one aberrant 6:2 tetrad) and four asci with two PMS for each of two different markers. The one completely viable ascus with three PMS had an aberrant 4:4 at *his7* and a single PMS at *cyh2*. Among the incompletely viable asci, there were 12 showing multiple PMS: five had two PMS at the same locus, two had two pairs each (a total of four sectored colonies from each ascus) and the final five had two different markers sectoring. (In two of these latter five, the same spore colony contained both events. So, for seven of ten partial asci with PMS in a pair of spores, the same marker was involved in each member of the pair.) Statistical analysis demonstrated that the hypothesis that the multiple PMS at individual loci occurred by chance could be rejected ($P \leq 0.005$).

Effect of the pachytene arrest on cells returned to mitotic growth

Cells at the 36° pachytene arrest in sporulation medium rapidly resumed budding growth upon transfer to YEPD medium. The large meiotic cells, which constituted approximately 75% of the culture from 21 hr at 36°, began to bud after about 4 hr at 23°, and by 7 hr most of the cells (40 of 44) on the YEPD observation plate had divided at least once. [The remaining micromanipulated cells (4 of 44) were inviable.] By contrast, cells left in sporulation medium and shifted to room temperature began to form asci at 10 hr after the downshift. The smaller cells, comprising 25% of the population, presumably were the last daughters of larger cells following the shift into nitrogen-deficient sporulation medium. These small cells showed lower viability and none of the recombinational effects, which will be described, when shifted to YEPD, so they were not used in subsequent micromanipulation experiments.

The ability of the larger cells to revert to budding growth upon transfer to vegetative medium was not immediately lost upon shifting the SPM culture to 23°. Cells left in SPM for 16 hr at 36° followed by 3 hr at 23° were still able to resume budding upon transfer to YEPD. Cytological examination revealed that the cells subjected to this regimen remained in pachytene throughout the entire period of SPM.

Effects on recombination upon reversion to mitotic growth were measured in two ways. Reversibly arrested cells were either plated on adenine-deficient vegetative growth medium to measure heteroallelic reversion or plated on YEPD medium to form colonies, which were subsequently replica plated to reveal sectoring for the various heterozygous markers in AP-1.

Adenine prototrophs: The frequency of prototrophs detected upon shift to growth medium (Table 8) increased as the cells began to reach pachytene (9 hr) and rose further with continued arrest until at least 17 hr. The frequency of prototrophs upon return to mitotic growth also increased slightly upon an intermediate shift to 23° in SPM, going from 380 to 470 prototrophs/10^6 in an SPM culture originally held at 36° for 22 hr and then transferred to 23° for 3 hr. At times when all meiotic cells had reached pachytene (12 hr or more) the ratio of prototrophs in cells reverting to mitosis (Table 8) to those in spores from cells permitted to complete meiosis (Table 1) remained about 3:1 to 4:1. If the mitotically reverting cells had become committed to the same level of recombination as in the completed spores from the same time point, the expected ratio would have been about 6:1. This approximation is derived from the facts that (1) each cell at pachytene has four times as many chromatids as a spore, (2) the mitotically reverting cells will consist predominantly of *ade1/ ADE1* heterozygotes, whereas one-half of the *ADE2* recombinant spores will be *ade1* and, therefore, unable to grow and (3) only approximately ¾ of the plating units had reached pachytene. We note however that our random spore preparations contained some pairs of spores, which would artifactually raise the apparent level of prototrophy among spores.

Sectored colonies: Pachytene-arrested cells plated onto YEPD medium resumed mitotic growth, and the resulting diploid colonies were replica plated to detect colonies sectored for markers heterozygous in the parental strain. Such colonies

TABLE 8

Adenine prototrophs in cells returned to mitotic growth

Time at 36° in SPM	Prototrophs/10^6 viable cells
0	1.1
7	14
9	150
11	330
13	580
15	780
17	1200
19	1210
21	1170

Cells in SPM at 36° for the indicated amount of time were plated on YEPD to determine the viable cell titer and on synthetic medium lacking adenine to determine the titer of prototrophs.

TABLE 9

Source of sectored colonies determined by dissection

Marker sectored	Conversion	Crossover
lys2	3	6
tyr1	3	8
his7	2	19
can1	1	5
cyh2	2	10
leu1	2	4
ura1	1	6
ura3	0	2
Totals	14	60

Forty-three sectored colonies that had sectored for a total of 74 markers (after 21 hr incubation in SPM at 36° followed by release on YEPD) were sporulated and dissected. If the half showing the dominant trait was homozygous or heterozygous, it was scored as resulting from a crossover or gene conversion, respectively. Three colonies that sectored for mating type (and, therefore, would not sporulate) in addition to one of the above markers were not included.

might have arisen either from reciprocal recombination between a marker and the centromere followed by appropriate disjunction or from gene conversion to the recessive allele at the four-strand stage. To distinguish between these possibilities, unbudded, pachytene-arrested cells were micromanipulated to a grid pattern on YEPD plates, and the resulting colonies were replica plated for sectors. This procedure ensured that each sectored colony arose from a single cell. Both halves of the sectors were subcloned, sporulated and dissected. The side showing the recessive trait was always homozygous and was not analyzed further in most cases. The side showing the dominant trait could be either homozygous or heterozygous, representing occurrence of either a cross-over or a conversion, respectively. Of 74 sectors dissected, 60 were caused by a crossover and 14 by a conversion event (Table 9). The ratios of crossovers to conversion events in immediate shifts to YEPD from 36° SPM did not differ significantly from those in shifts to 23° for an additional 3 hr in SPM.

TABLE 10

Time course of sectoring

A. Number of sectors with time at 36° in SPM.

Hr at 36°	Marker							Total colonies examined
	lys2	tyr1	his7	can1	cyh2	leu1	ura	
0	0	0	0	0	0	0	0	310
7	0	0	0	0	0	0	0	494
9	1	0	4	4	9	0	0	419
11	1	0		11	28	0	0	497
13	3	1	11	25	22	0	4	436
15	5	2	26		35	1	1	423
17	6	4	25	22	42	0	6	376
19	9	7	26	16	41	2	7	440
21	7	7	52	53	60	1	5	448

B. Sectoring with additional 3 hr in SPM at 23°

Hr at 36°	Hr at 23°	Marker								Colonies examined
		lys2	tyr1	his7	can1	cyh2	leu1	gal1	ura	
16	0	4	4	11	7	6	1	0	2	104
16	3	12	8	17	16	22	3	0	15	98
20	0	9	9	18	10	27	3	0	14	143

Cultures of AP-1 in SPM were kept at 36° for the indicated time. Then they were either plated directly on YEPD or incubated in SPM at 23° an additional 3 hr before plating. The YEPD plates were then replica plated to the appropriate synthetic media to detect sectored colonies. Entries under each locus represent the total number of colonies sectored for that phenotype.

Easily scored markers were selected for further analysis of the frequency of sectoring among cells held in SPM at 36°. Sectoring was not observed at 7 hr or earlier but arose at 9 hr and increased with increasing time of arrest (Table 10A). Sectoring frequencies also increased when cultures were shifted from 36° in SPM to 23° SPM for 3 hr before the final transfer to YEPD plates (Table 10B). However, sectoring also increased approximately twofold in cells left at the pachytene arrest for 4 more hours, so the release to 23° did not permit a substantially greater extent of crossing over in cells recoverable by reversion to mitosis. The markers that are tightly centromere linked, *leu1* and *gal1* (Table 6), sectored very rarely (Table 10B), indicating that the first division following plating of the pachytene-arrested cells was mitotic rather than meiotic. Most of the sectored colonies on uracil-deficient medium were, therefore, assumed to be *ura1* sectors. This was proven to be the case by dissection in seven of nine uracil sectors.

Included among the count of sectored colonies were colonies showing only the recessive, auxotrophic phenotype. These colonies, which accounted for between 1/10 and 1/3 of the totals in several experiments, may have resulted from the inviability of one of the products of the first division on the YEPD plate. If this were true, then an equal proportion of reciprocal exchanges should have resulted in colonies homozygous for the dominant marker, when

the alternative sister cell died. Correcting the data in Table 10 for the small number of such events would not have increased our estimate of reciprocal exchange sufficiently to negate the conclusions in the following section.

Level of reciprocal recombination: Heterozygous markers unlinked to centromeres should segregate randomly, causing sectoring for the marker at a frequency of ⅓. For experiments in which the bulk SPM culture, containing only 75% meiotic cells, was plated directly onto YEPD, a sectoring frequency of 25% would be expected. In our experiments, only *cyh2* (Table 10B) attained this level of independence from the centromere. This suggested either that only a subset of the cells were undergoing the full meiotic level of recombination or that the entire population was recombining at a restricted level. To distinguish these possibilities, we asked whether cells that had sectored for a given marker had also undergone sectoring for other, unlinked markers. Cells that had been plated directly onto YEPD from 36° in SPM and sectored for one of *his7*, *cyh2*, *can1* or *ura* were tabulated for the sectoring frequency of the other three traits combined and in no case was the full level found. The 25 canavanine-resistant sectored colonies detected in one experiment (Table 10A) came the closest to this level, with 16 total additional sectors, whereas 25 would be expected. Therefore, individual cells must have undergone sectoring without achieving the full meiotic frequency of recombination in their entire genome.

The genetic basis of the pachytene arrest

Since AP-1 grows well vegetatively at 36° in either glucose or acetate growth medium, we could attribute the pachytene arrest to a specific meiotic defect and wished to determine whether a single gene is responsible for it. A brief test of various laboratory strains revealed that many others fail to sporulate at 36° but that Y55 is competent at this temperature. Since Y55 is prototrophic and homothallic, spore to cell matings were followed microscopically to obtain the hybrids with the two parents of AP-1: α131-20 and A364a. Both hybrids sporulated at 36°.

The hybrid between Y55 and A364a showed poor spore viability and was not analyzed further. Among 20 homothallic progeny of the hybrid Y55 × α131-20, ten could sporulate at both 30° and 36°, eight could sporulate only at 30° and two failed to sporulate appreciably at either temperature. In general, those able to sporulate at 36° sporulated to a higher extent at 30° than the others, although the ranges did overlap (30–80% sporulation *vs.* 5–70%, respectively). One temperature-sensitive and one temperature-resistant homothallic progeny strain were crossed in spore to spore matings. Fourteen asci of the resulting hybrid, homozygous for *HO* (the homothallism gene), were dissected. All eight asci with four viable spores segregated 2:2 for temperature sensitivity for sporulation, and the incomplete asci were consistent with this result. All strains grew well vegetatively at 36°. Since the temperature-sensitive parent arrested in pachytene at 36°, we conclude that an allele at a single genetic locus is responsible for the difference between a 36° pachytene arrest and completion of sporulation at 36°. We denote the dominant allele conferring temperature resistance as *PAC1* and the recessive allele as *pac1*.

To determine whether *PAC1* also functioned in heterothallic strains the progeny of Y55 × α131-20 were cross-stamped with two haploid mating testers that did not sporulate at 36° when crossed to each other. The mating plate was replica plated to a sporulation plate and tested for the presence of spores by the ether-killing procedure of DAWES and HARDIE (1974). Among the 14 viable heterothallic spore colonies, all four allele combinations at *MAT* and *PAC1* were inferred in approximately equal numbers. All three complete asci segregated 2:2 for both *PAC1* and *HO* independently. Therefore, it is likely that this single genetic factor functions in heterothallic diploids and is responsible for the different sporulation abilities of Y55 and AP-1. Efforts to map *pac1-1* have been frustrated by decreased sporulation ability under permissive conditions upon repeated backcrosses with A364a (E. SHUSTER, personal communication), so the genetic basis of the arrest requires further study in other genetic backgrounds.

DISCUSSION

Effects of reversible pachytene arrest on marker segregation in ascospores: Previous work has demonstrated a striking enhancement of intragenic recombination between *ade2* heteroalleles among cells recovering from pachytene arrest either by reversion to mitosis or resumption of sporulation (BYERS and GOETSCH 1982). In the present study, tetrad analysis of the sporulation products has shown that transient pachytene arrest sufficient to cause an eightfold enhancement of gene conversion in *ade2* also results in marked enhancement of gene conversion and PMS at several loci (Table 3). The precise magnitude of enhancement is obscured by the low level of these events in control asci. But we find by averaging over all loci that gene conversions (1:3 and 3:1 asci) were increased 11-fold and that the range of their occurrence (4–21%) was non-overlapping with the range in the control (0–2%). The relatively high frequency of non-2:2 segregation in the arrested population raises the possibility that false asci may have contributed to the total, but use of the individual ascus dissection technique (see MATERIALS AND METHODS) ensures that the apparent gene conversions are *bona fide*. PMS (3:5 and 5:3 asci) was strongly enhanced, rising about 20-fold from 0.046% per locus per spore in control meiosis to 0.92% in the arrested population. Changes in the frequency of reciprocal recombination were of smaller magnitude but clearly significant. Crossing over increased in all five intervals between genes (Table 5) and in four of five gene-centromere intervals (Table 6, with the *ADE1*-centromere distance statistically unchanged), but the measured increases were less than threefold.

The foregoing alterations of marker segregation at several loci suggest that the overall mechanism of meiotic recombination was profoundly altered by transient pachytene arrest. Determining the implications may be facilitated by considering whether the observed alterations represent novel processes or simply the exaggeration of events occurring normally during meiosis. In the latter case, we would not expect to find substantial departures from the thoroughly characterized patterns of marker segregation in yeast tetrads described by

FOGEL *et al.* (1979). In fact, increased PMS did not lead to an abundance of aberrant 4:4 tetrads, which would have indicated the novel formation of symmetric heteroduplexes. Neither did there appear to be any departure from parity in the direction of gene conversion. Although too few conversion events were available to demonstrate that the direction of conversion was random at individual loci (Table 3B), the occurrence of gene conversion to either allele at 11 of 12 loci indicates approximate parity, as in normal yeast meiosis. We note also that some loci experienced increased gene conversion but no PMS. Mating type, for example, is never found to undergo PMS in normal tetrads (FOGEL *et al.* 1979); nor did it occur in the present experiments. Analysis of cloned sequences from the *MAT* locus (NASMYTH *et al.* 1981) has established that the alternative alleles, *MAT*a and *MAT*α, show substantial nonhomology. We do not know whether other loci failing to display PMS possess similar extents of nonhomology, but it is clear that the enhanced population is not aberrant in this regard. The data are consistent, in fact, with the premise that both gene conversion and PMS are derived from a common precursor, a heteroduplex (HOLLIDAY 1964; FOGEL *et al.* 1979), which is unusually prevalent in these cells. If the probability of resolution to one product or the other were unbiased, we would expect equivalent increases in the two frequencies. As noted earlier, the control data are insufficient to confirm the apparent tendency toward a greater increase in PMS than gene conversion. If a larger sample were to demonstrate an inequality of enhancement, one might suspect that the mechanism for excision repair had become saturated by the increased amount of heteroduplex upon which it is postulated to act.

Although PMS and gene conversion may not have been enhanced differentially, reciprocal recombination clearly increased less than either of these. The relative paucity of crossing over might indicate that the enhanced values for PMS and gene conversion arose by a distinct mechanism, but this seems unlikely since reciprocal recombination was not unaltered. Moreover, abundant data from normal tetrads have established an essential association between nonreciprocal events and crossing over (FOGEL *et al.* 1979). The Aviemore model (MESELSON and RADDING 1975) suggests an explanation for these and other data. It is postulated that crossing over is initiated by invasion of one duplex by single-stranded DNA donated by the other. In the context of this model, two explanations for the present data present themselves. One is that pachytene arrest leads to longer segments of strand invasion than in the normal case, so that the overall extent of heteroduplex associated with each crossover is increased. Alternatively, initiation events may be more numerous at the arrest but less frequently productive, often being aborted rather than proceeding to formation of a crossed strand exchange form, which may be an essential intermediate in crossing over. The occurrence of unproductive asymmetric events is not unprecedented, for it is well established that positive interference is displayed by crossovers but not by gene conversions (STADLER 1959; MORTIMER and FOGEL 1974). Although positive interference is readily detectable in yeast tetrad data, its significance in limiting the frequency of crossing over has not been established (SNOW 1979). Nevertheless, it may well be the case

that chiasma interference would prevent many incipient recombinational events from proceeding to crossing over from an asymmetric stage productive of gene conversions and postmeiotic segregants if crossing over were increased substantially. On the other hand, progress from the asymmetric phase to crossing over may be specifically limited by the defect causing pachytene arrest. The isomerization step proposed in the model by MESELSON and RADDING (1975) could, for example, be defective.

What events occur during pachytene arrest? It has been evident for several decades that genetic recombination must precede the first meiotic division, but direct evidence for the time of its occurrence has remained elusive. Several experiments have exploited the effects of temperature shifts on recombination frequency (HENDERSON 1970). Because the frequency remains sensitive to alteration well into pachytene, one may conclude that recombination could not have been completed before this phase. The timing of meiotic events in yeast cells is accessible to analysis in cells caused to revert from the meiotic regimen to vegetative growth before completion of sporulation. SHERMAN and ROMAN (1963) demonstrated that such reversion after an appropriate interval in sporulation conditions results in nearly meiotic levels of recombination within cells that failed to complete meiosis or the formation of spores. Although recombination *per se* is not known to have occurred at the time of transfer, one may specify that the cells had become committed to recombination without having undergone commitment to the reductional division (ESPOSITO and ESPOSITO 1974). Our previous work has demonstrated that cells reverting from pachytene arrest had undergone substantial commitment to intragenic recombination in *ade2* (BYERS and GOETSCH 1982). In the present study, the production of sectored colonies from such cells has revealed commitment to recombination throughout the genome (Table 10). By subsequent sporulation and genetic analysis of the sectors we have found that many sectors had arisen by reciprocal recombination, so some commitment to reciprocal recombination had occurred at the arrest. But the segregation of other markers in these sectors indicated the occurrence of fewer reciprocal recombination events than would have occurred in normal meiosis. By contrast, resumption of the same meiosis as that from which sectored colonies were obtained yielded a substantial increase in crossing over relative to normal sporulation. The possibility that this difference in recombination resulted from selection of different subpopulations was examined by micromanipulation of arrested cells before analysis for sectoring. We found that sectors arose principally from the larger cells in the arrested culture. Because this is the same subset of cells that produced asci most frequently, we are assured that both sectoring data and tetrad data represent the same set of cells under the two modes of release. Clearly, commitment to crossing over was limited at pachytene arrest in comparison with the enhanced frequency that would ensue upon resumption of meiosis. Even when arrested cells were shifted to the permissive temperature (still in sporulation medium) for 3 hr before assay for sectoring (Table 10B), the commitment to reciprocal exchange remained low. Therefore, little, if any, crossing over had already occurred during pachytene arrest, despite the accumulating potential for en-

hanced recombination when sporulation was permitted to resume. These findings alone do not obviate the occurrence of crossing over before pachytene in normal meiosis, but they clearly demonstrate that the bulk of crossovers can arise at a stage later than the arrested state in cells subjected to pachytene arrest and release.

The striking enhancement of gene conversion and PMS among asci produced upon meiotic release may indicate the production during pachytene arrest of heteroduplex—the suggested precursor to nonreciprocal recombination (FOGEL *et al.* 1979). But, as in the case of reciprocal recombination, we are unable to distinguish the actual occurrence of these events from an increased potential for their manifestation when meiosis resumes. One possible means of establishing whether heteroduplex is already present at arrest would be to subject the chromosomal DNA to physical analysis. When the chromosomal DNA of similar meiotic cells was subjected to chemical crosslinking to stabilize against branch migration and cleaved with restriction endonucleases, branched forms joined in regions of homology were isolated by two-dimensional electrophoresis (BELL and BYERS 1983). The role of these branched forms remains obscure, but their presence during pachytene arrest demonstrates that homologous interactions—and possibly segments of heteroduplex DNA—are present during the stage of accumulating commitment to gene conversion.

What is the role of the PAC1 *product?* The ability of strain Y55 to complete sporulation at 36° while strain AP-1 undergoes pachytene arrest has permitted us to explore the genetic basis for the arrest. The 2:2 segregation of temperature sensitivity for sporulation implies that AP-1 must be homozygous for an allele (*pac*1-1), which is responsible for the arrest, whereas the dominant allele (*PAC1*) renders Y55 temperature resistant. A possible explanation for the phenotype of *pac*1 at elevated temperature is that is plays a direct role in the recombination process and is required for completion of crossing over. Regulatory mechanisms would then retard departure from pachytene until the deficiency in recombination had been corrected. However, the fact that completion of sporulation occurs efficiently in the absence of recombination in *rad50* homozygotes (GAME *et al.* 1980; KLAPHOLZ and ESPOSITO 1982) renders it unlikely that simply the lack of recombination would cause arrest. Another possibility is that the *PAC1* function is not directly related to recombination but is instead required for departure from pachytene, so that a failure of the function would simply prolong the period during which recombination could occur. Arrest at pachytene has been reported for several *spo* mutations (ESPOSITO and ESPOSITO 1978) as well as for two *cdc* mutations: *cdc4* (SIMCHEN and HIRSCHBERG 1977) and *cdc5* (SIMCHEN *et al.* 1981), the latter being reported to cause enhanced intragenic recombination. At least three *cdc* mutations defective in the "start" function (E. SHUSTER, personal communication) also cause arrest in pachytene. It is, therefore, clear that several genetic defects in addition to *pac1* lead to pachytene arrest. Present information does not permit us to specify any particular role for *pac1* among functions of this sort. We note, however, that the *pac1* defect is particularly amenable to reversal.

In addition to facilitating genetic analysis, this may indicate that the *pac1* arrest is similar to the prolonged pachytene state observed in normal meiosis (MOENS and RAPPORT 1971; GOETSCH and BYERS 1982).

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