MITOTIC RECOMBINATION AND HETEROALLELIC REPAIR IN SACCHAROMYCES CEREVISIAE[1]

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Received March 30, 1964

A diploid yeast cell, heteroallelic and auxotrophic for some requirement and conventionally heterozygous for linked or unlinked markers, regularly proliferates two major segregant classes during clonal development: prototrophs for the heteroallelic marker and mitotic recombinants for the heterozygous markers.

The first category, variously designated heteroallelic repair, allelic recombination, intragenic recombination, gene conversion, reversion, or simply mutation, may be detected by selective or nonselective techniques yielding partial or total recovery of all cellular products associated with prototroph production. Regardless of the analytical technique employed, however, the reciprocal double mutant expected of classical recombination is observed only rarely. Since prototrophs arise from heteroallelic combinations at rates several orders of magnitude higher than from the corresponding homoallelic combinations (ROMAN 1958; ROMAN and JACOB 1958; KAKAR 1963), back mutational origin lacked plausibility and "nonreciprocal recombination," implying that recombination between homologues may be imprecise, has gained acceptance as still another "neutral" term to describe prototroph formation. Evidence for the nonreciprocal nature of the recombination accompanying prototrophy has now accumulated for several fungi (see review by ROMAN [1963]); in yeast (ROMAN 1958); in Aspergillus (PRITCHARD 1960); in Neurospora (MITCHELL 1955; CASE and GILES 1958; STADLER 1959; MURRAY 1963; STADLER and TOWE 1963); in Ascobolus (RIZET, LISSOUBA and MOUSSEAU 1960; LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962); and in Sordaria (OLIVE 1959). Verification of this nonreciprocal phenomenon has led to a conceptual vacancy, vigorously filled by hypotheses such as the switch hypothesis (FREESE 1957), fixed or effective pairing regions (PRITCHARD 1960; STAHL 1961), the polaron (RIZET, LISSOUBA and MOUSSEAU 1960), and the modified polaron (STADLER and TOWE 1963). An alternative set of hypotheses, suggested by the observation of second-site alterations within the cistron in bacteriophages (CRICK, BARNETT, BRENNER and WATTS-TOBIN 1961), and of intrachromosomal exchange (PETERSON and LAUGHNAN 1963; NANCE 1963), has seldom received evaluation comparable to that lavished upon copy-choice replication as the mechanism underlying nonreciprocal recombination. The idea that prototroph formation might result from both equal and unequal crossing over between homologues, and from intrachromosomal exchange mechanisms that do

[1] This study was aided by Public Health Service Grant RG-06979.

not involve homologue interactions, has also been suggested by LAUGHNAN (1961) and PETERSON and LAUGHNAN (1963).

The second segregant class observed during clonal development is a consequence of mitotic recombination. Typically, under conditions of total recovery, the products of mitotic recombination are strictly reciprocal. However, mitotic recombination is generally detected by recessive homozygosis for heterozygous input markers under conditions of partial recovery (review by PRITCHARD 1963). Mitotic recombination, first described in Drosophila by STERN (1936), and originally reported in yeast by JAMES and LEE-WHITING (1955), has been extensively studied in yeast by ROMAN (1956), ROMAN and JACOB (1958), HURST and FOGEL (1962), FOGEL and HURST (1963), and WILKIE and LEWIS (1963). In addition, similar parasexual recombination has been reported in Aspergillus (PONTECORVO, ROPER, HEMMONS, MACDONALD, and BUFTON 1953; PONTECORVO and KÄFER 1958), and in Ustilago (HOLLIDAY 1961). Mitotic recombination in yeast is enhanced in frequency by ultraviolet light (UV) (HURST and FOGEL 1962; WILKIE and LEWIS 1963), but positive correlation between recessive homozygosis frequency and meiotic gene-centromere map distance, observed by the former authors and by PONTECORVO and KÄFER (1958) in Aspergillus, was not reported by the latter authors. Mitotic recombination has also been induced in Aspergillus by alkylating agents (MORPURGO 1963).

WILKIE and LEWIS consider four mechanisms for somatic homozygosis and the consequences of each. They are: (1) Mitotic crossing over, in which a pair of homologues synapse, crossing over occurs at the four-strand stage, and centromere disjunction is mitotic; (2) Nondisjunction of centromeres at mitosis; (3) Meiotic crossing over, meiotic centromere disjunction, and restitution of diploidy; and (4) Crossing over at the two-strand stage, meiotic centromere disjunction, and restitution of diploidy. From their data, WILKIE and LEWIS postulate that the third mechanism, meiotic crossing over and meiotic centromere behavior, followed by restitution of diploidy, is most satisfactory.

Earlier observations reporting coincidence between mitotic recombination and heteroallelic prototroph formation (FOGEL and HURST 1963) led to the hypothesis that both processes shared a common cellular precondition, perhaps synapsis or the onset of meiosis. The experiments described here were designed to explore this issue more critically and additionally to compare UV induced diploid prototrophs with spontaneous prototrophs, and diploid prototrophs analyzed by tetrad analysis with haploid prototrophs derived from sporulated material. The bearing of the results on the induction of mitotic recombination and the mechanisms of mitotic recombination and heteroallelic prototroph formation is discussed.

MATERIALS AND METHODS

The diploid clones utilized in these experiments were isolated as single prototrophic clones on synthetic media deficient for appropriate nutrilites. For media details see ROMAN (1956) and FOGEL and HURST (1963). The crosses, made on agar plates containing yeast extract-peptone-dextrose (YEPD), were replica-plated to appropriate media for zygote isolation. The hi_{1-1} mutant originated from DR. D. HAWTHORNE at the University of Washington (Seattle). The

hi_{1-7} mutant was obtained from DR. R. MORTIMER at the University of California (Berkeley) and is derived from his mutant strain JB151. After several crosses involving the mutant strains, two diploids were synthesized by combining haploid parents as follows:

Z1218: A4289A *a UR THR* hi_{1-7} *AR* is_1 tr_2 ad_2 *ME AD AD*
A3382C *α* ur_3 thr_3 hi_{1-1} ar_6 *IS* tr_2 ad_2 me_1 ad_3 ad_6
Z1707: A5881A *a* ur_3 thr_3 hi_{1-1} *AR TR* le_1 *AD GAL* ad_1
A5930B *α UR THR* hi_{1-7} ar_6 tr_2 *LE* ad_6 gal_2 ad_1

The markers uracil (*ur*), threonine (*thr*), histidine (*hi*), arginine (*ar*), isoleucine (*is*), and tryptophan (*tr*) are linked on chromosome V in the order given by HAWTHORNE and MORTIMER (1960), the Carbondale Yeast Genetics Conference (1963), KAKAR (1963) and MAGNI (1963). The map distances published by these authors are: ur_3-5-centromere-34-thr_3-1-hi_1-9-ar_6-12-is_1-9-tr_2. However, MAGNI (1963) reports the *thr-hi* interval as 7.8 map units. The loci leucine-1 (*le*) and adenine-6 (*ad*) are located in linkage group VII; the le_1 marker is 1 centimorgan from the centromere, while the ad_6 locus is located 26 centimorgans from the centromere in the opposing arm. The ad_3 locus is reported as mitotically linked to adenine-6 by ROMAN (1956). The galactose-2 (*gal*) locus is located on linkage group XII, unlinked to the centromere, while the methionine-1 (*me*) locus segregates independently of all other markers used in these diploids.

The resultant red diploid of Z1218 required histidine, tryptophan and adenine and was further purified by single clone reisolation from a synthetic complete (SC) streak plate. Cells of the single clone were washed, counted and 0.1 ml appropriately diluted in sterile distilled water so that 0.1 ml platings would yield approximately 250 clones per plate on SC as viable count controls. The undiluted remainder was: (a) plated immediately on histidineless media at a concentration of 2×10^5 cells per plate; and (b) treated in water on a shaker device, utilizing a Hanovia Letheray (No. 24500) as an ultraviolet (UV) source. The incident energy measured by a Latarjet UV dosimeter was 13 ergs/mm^2/sec. After irradiations of 15 and 45 seconds respectively, aliquots were removed and plated on SC and histidineless media. Treated cells were plated in dimmed light to avoid photoreactivation. After incubation in the dark at 30°C for four to five days, colonies were counted. The histidine prototrophs were transferred to YEPD master plates (2 percent agar), incubated 24 hours and replica-plated, using the velvet-transfer replica technique, to an appropriate series of SC plates deficient for single nutrilites. Included in each series was a sporulation induction plate. It contained, on a per liter basis, potassium acetate (10 g), yeast extract (2.5 g) and glucose (0.5 g) (MCCLARY, NULTY and MILLER 1959). The total procedure assays recessive homozygosis for all heterozygous markers in parental and prototrophic clones and provides asci for meiotic analysis (FOGEL and HURST 1963) of any particular clone. The same reisolation procedure was followed for Z1707, which was red and required histidine and adenine. However, this diploid was plated directly on histidineless media, and was never irradiated.

Heteroallele identification tests: A representative series of each prototrophic class was sporulated, dissected and scored for all parentally heterozygous markers. Only asci with four viable ascospores were chosen for analysis beyond dissection. Spore survival was 90 to 95 percent, and therefore 80 to 85 percent of all asci dissected had four surviving spores. The linkage relationships of markers flanking the hi_1 locus, thr_3 and ar_6, are thus specified for each clone. In addition, the resulting ascosporal clones were tested to identify the surviving hi_1 allele by a modification of ROMAN'S (1958) technique. The allele identification test depends upon the differences in prototroph frequency on histidineless media between homoallelic diploids, hi_{1-1}/hi_{1-1} or hi_{1-7}/hi_{1-7}, and the heteroallelic diploids, hi_{1-1}/hi_{1-7}. Homoallelic diploids produce few or no prototrophic papillations within 48 hours after replica-plating about 1×10^8 cells, while heteroallelic diploids typically yield 20 to 100/10^6 cells. For Z1218, depending upon mating type, each ascospore derivative was crossed to *a* or *α* hi_{1-1} ad_1 and *a* or *α* hi_{1-7} ad_1 on solid media, and after incubation overnight was replica-plated to adenineless media. The white, adenine-independent zygotes were removed to a YEPD master and subsequently replica-plated to a histidineless plate and scored for homo- or heteroallelism. For experiments with Z1707, the allele testers carried ad_2 with hi_{1-1} and hi_{1-7}, to facilitate zygote selection on adenineless media. This technique allowed for rountine detection of either input allele or the double mutant without ambiguity.

Operationally, the double mutant, $hi_{1\text{-}1,7}$, yields few or no prototrophic papillations with either the $hi_{1\text{-}1}$ or the $hi_{1\text{-}7}$ tester. All double mutants were verified by retest.

A large series of prototrophic ascosporal derivatives were crossed to wild type and analyzed by ascus dissection as a test for suppressor mutations resulting in histidine independence. No unlinked suppressors were detected. Closely linked nonspecific or allele-specific suppressors, detectable as aberrant tetrad segregations for *HI*, were not found among the prototrophs tested. Intracistronic suppressor mutations, second-site mutations in the sense of CRICK, BARNETT, BRENNER and WATTS-TOBIN (1961), which could have been detected by these methods, were not observed among the prototrophs derived from heteroallelic combinations.

RESULTS

Meiotic mapping: The pooled meiotic tetrad data accumulated in this laboratory for the linked markers of chromosome V are presented in Table 1. These data derive from 419 diploid clones involving 3178 asci, all characterized by four-spored survival and normal 2:2 segregations for all markers. The sources of these asci are: 1559 asci from 176 clonal derivatives of Z1707; 576 asci from 113 clones of Z1218; and 1043 asci from 130 different diploids. The latter are mostly tests for suppressor mutations among prototrophs. Not all markers were heterozygous in every diploid. Not included in these data are any asci displaying aberrant segregation for any marker. About 1 to 2 percent of all segregations were aberrant. The frequency of aberrant segregations observed in this study is similar to that reported by HAWTHORNE and MORTIMER (1960). The map distances are taken to be equal to $(NPD+\frac{1}{2}T)/N$ (PERKINS 1953). No corrections for chromosome or chromatid interference have been made. The following gene sequence and map distances are indicated: $thr_3 - 2 - hi_1 - 10 - ar_6 - 8 - is_1 - 7 - tr_2$. HAWTHORNE and MORTIMER (1960) report ur_3 to be 5 map units to the left of the centromere, and thr_3 34 units to the right. Since in the long centromere-*thr* interval a crossover is expected in almost every tetrad, the *ur* marker does not constitute a useful indicator of events within the *thr-hi-ar* cluster and is therefore not shown in this table or in some of those following. Nevertheless, ur_3 was routinely scored, since it serves to assay nondisjunctions.

TABLE 1

Tetrad linkage data for the markers on chromosome V

Gene pair	Parental ditype (PD)	Nonparental ditype (NPD)	Tetratype (T)	$(NPD+\frac{1}{2}T)/N$
thr_3–hi_1	1670	0	77	.0220
thr_3–ar_6	1681	2	516	.1182
thr_3–is_1	82	0	56	.2029
thr_3–tr_2	746	39	995	.3014
hi_1–ar_6	1379	2	357	.1039
hi_1–tr_2	669	26	787	.2831
ar_6–is_1	117	0	23	.0821
ar_6–tr_2	1112	18	735	.2067
is_1–tr_2	213	1	32	.0691

Effect of UV upon HI *prototrophy and recessive homozygosis:* The effects of UV treatment of diploid Z1218 on survival and histidine independence are shown in Table 2. Although survival, compared to the unirradiated control, dropped to 73 percent after 45 seconds of UV exposure, the prototrophic clone titer increased sixfold. The increment due to irradiation may be evaluated by subtracting the prototrophs present in the unirradiated population (60 per 2×10^6) from the numbers of prototrophs in the irradiated populations. From these data it is apparent that irradiation increases the *HI* prototroph titer.

Recessive homozygosis frequencies in the population of Z1218 plated on SC are collected in Table 3. In this unselected population the total recessive clone titer ranges from 13/2000 in control clones to 39/2000 with 45 seconds of UV. The increment due to UV is 26/2000, and the greatest increase is seen for the independently segregating, non-centromere-linked marker, me_1, from 3/2000 to 17/2000. Thus, UV increases the titer of both *HI* prototrophs (Table 2) and the titer of mitotic recombinants (Table 3). These results are consistent with those reported by FOGEL and HURST (1963).

The distribution among *HI* prototrophs of recessive homozygosis for flanking markers and for the unlinked me_1 is shown in Table 4. This population, selected for primary events at the *HI* locus leading to the production of *HI* prototrophy, displays a high frequency of recessive homozygosis. Of the 506 *HI* prototrophs 126 or 20 percent show such recombination. This frequency contrasts to that observed in Table 3 (a sum of 52 in 4000 unselected clones) and demonstrates that the proportion of recessive homozygosis is significantly greater in the selected subpopulation than in the unselected parental population. However, a distinct increment in recessive homozygosis is not observed between the unirradiated controls and the population which received 45 seconds of UV (by subtraction of the controls from the irradiated). The proportion of *HI* prototrophs also recessive for one or more of the input heterozygous markers actually decreases from

TABLE 2

The effect of ultraviolet irradiation on survival and heteroallelic repair in Z1218

Ultraviolet treatment	Percent survival on SC media	Number of cells plated on HI⁻ media	Number of *HI* prototrophic clones
None	100	2×10^6	60
15 sec	96	2×10^6	114
45 sec	73	2×10^6	352

TABLE 3

Recessive homozygosis in an unselected population of Z1218 after ultraviolet irradiation

Ultraviolet treatment	Number of clones tested	Recessive clones homozygous for					Total recessive clones
		ur-3	*thr*-3	*ar*-6	*is*-1	*me*-1	
None	2000	2	4	4	0	3	13
45 sec	2000	3	9	7	3	17	39

30/60, or 50 percent in the control population to 67/334, or 20 percent in the population which received 45 seconds of UV. Thus, selection for *HI* prototrophs yields a high coincidence of recessive homozygosis for heterozygous input markers. UV appears to reduce the proportion of recessive clones among prototrophs. Conceivably, irradiation reduces homozygosity by repairing potential crossovers already present in the unirradiated population, or alternatively the additional induced exchanges are compensatory and restore the original marker sequence. The relatively high coincidence of recessive homozygosity for the unlinked me_1 gene with homozygosity for one or more markers on chromosome V, 40/49, might be construed as evidence for mitotic linkage of me_1 with chromosome V.

Tetrad analysis of HI *prototrophs from Z1218:* The genetic constitutions of the *HI* prototrophs from Z1218, derived by tetrad dissection and subsequent allele identification tests, are recorded in Table 5. Since not every prototrophic clone was resolved by tetrad analysis, the numbers do not reflect proportional frequencies of each type in the prototroph population. Rather, analysis of at least one representative of each phenotypic class, in control and experimental groups alike, was attempted. The data from the various irradiated populations and the controls are pooled in Table 5, since the sample sizes were too small for detection of significant trends. Not every prototroph sporulated, particularly those drawn from the 45 second UV population. The results signify that *HI* prototrophs derived from a single diploid clone constitute a highly heterogeneous genotypic array. Homozygosis may be present for the proximal marker (*thr* hi_{1-1} *ar/thr HI AR*), the distal marker (*thr* hi_{1-7} *ar/THR HI ar*), or the prototrophic derivative of the input heteroallelic locus itself (*thr HI AR/THR HI ar*). The reciprocal double mutant, $hi_{1-1,7}$, was not observed among the 104 *HI* prototrophic clones analyzed. Of particular interest are those diploids in which the surviving allele emerges in coupling with the input flanking markers with which it was in repulsion in the original diploid: input was *thr* hi_{1-1} *ar/THR* hi_{1-7} *AR*, output *THR* hi_{1-1} *AR* or *thr* hi_{1-7} *ar*. On a conventional basis, these presumably arise by multiple crossovers in the short *thr-ar* interval.

Patently, UV increased the titer of both prototrophs and mitotic recombinants,

TABLE 4

Diploid phenotypes for linked and unlinked markers among prototrophic HI *clones, Z1218*

$$\frac{UR_3 \text{ c } THR_3\ hi_{1-7}\ AR_6\ is_1}{ur_3 \text{ c } thr_3\ hi_{1-1}\ ar_6\ IS_1} \quad \frac{ME_1}{me_1}$$

UV treatment	*HI* prototrophs tested	Phenotypes												*me*, with linked marker	*me*, without linked marker	Total coincident with Hi+
		UR THR AR IS	ur THR AR IS	UR THR AR is	ur THR AR is	UR THR ar IS	ur THR ar IS	UR thr AR IS	ur thr AR IS	UR thr AR is	ur thr AR is	UR thr ar IS	ur thr ar IS			
None	60	30	1	6	2	6	1	0	1	4	5	0	1	12	3	30
15 sec	112	83	0	10	2	6	1	4	0	2	2	0	1	9	1	29
45 sec	334	267	0	13	8	12	1	10	3	6	6	2	1	19	5	67
Total	506	380	1	29	12	24	3	14	4	12	13	2	3	40	9	126

TABLE 5

Meiotic analysis of 104 HI *prototrophic diploids Z1218*

$$\frac{THR_3\ hi_{1-7}\ AR_6}{thr_3\ hi_{1-1}\ ar_6}$$

Genotype	Number	Genotype	Number
$\frac{thr\ hi_{1-1}\ ar}{THR\ HI\ AR}$	28	$\frac{THR\ hi_{1-7}\ AR}{THR\ HI\ AR}$	1
$\frac{thr\ HI\ ar}{THR\ hi_{1-7}\ AR}$	26	$\frac{THR\ hi_{1-7}\ ar}{THR\ HI\ ar}$	1
$\frac{thr\ HI\ ar}{THR\ HI\ AR}$	2	$\frac{thr\ HI\ ar}{THR\ hi_{1-7}\ ar}$	3
$\frac{thr\ hi_{1-1}\ ar}{thr\ HI\ AR}$	11	$\frac{thr\ HI\ AR}{THR\ HI\ ar}$	1
$\frac{thr\ HI\ ar}{thr\ hi_{1-7}\ AR}$	4	$\frac{thr\ HI\ ar}{THR\ HI\ ar}$	1
$\frac{thr\ HI\ AR}{thr\ hi_{1-7}\ AR}$	2	$\frac{THR\ hi_{1-7}\ AR}{thr\ HI\ AR}$	1
$\frac{thr\ HI\ ar}{thr\ HI\ ar}$	1	$\frac{thr\ hi_{1-7}\ ar}{THR\ HI\ ar}$	3
$\frac{thr\ HI\ AR}{thr\ HI\ AR}$	1	$\frac{thr\ HI\ AR}{THR\ hi_{1-7}\ ar}$	3
$\frac{thr\ HI\ ar}{thr\ hi_{1-1}\ AR}$	1	$\frac{thr\ hi_{1-7}\ AR}{THR\ HI\ ar}$	1
$\frac{THR\ HI\ ar}{THR\ hi_{1-7}\ AR}$	2	$\frac{THR\ hi_{1-1}\ ar}{thr\ HI\ AR}$	9
$\frac{THR\ hi_{1-1}\ ar}{THR\ HI\ AR}$	1	$\frac{thr\ HI\ ar}{THR\ hi_{1-1}\ AR}$	1

but three related issues required elucidation: (1) Are individual *HI* prototrophs derived by plating a single clone related to each other by common cell lineage and therefore do they reflect a few clonally organized events, or are they, for the most part, products of independent events? (2) Is there a specific "marker effect" due to the outside markers *thr* and *ar* being present in *cis* rather than *trans* configuration? (3) Is the proportion of recessive homozygotes among prototrophs constant? Resolution of these issues was initiated by synthesizing a second diploid strain with the flanking markers in repulsion, instead of coupling. The resultant diploid, Z1707, also contained le_1, a centromere-linked gene on chromosome VII, and gal_2, an unlinked marker on chromosome XII. This diploid was not irradiated in any of the following experiments and the study with this material therefore represents an analysis of spontaneous events. Diploid clones of Z1707 derived from a streak plate on SC (each clone presumably arising from a single cell), were transferred to a YEPD master and replica-plated to histidine-

less media. A total of 72 *HI* prototrophic papillations were randomly taken from 28 different clonal derivatives of Z1707, but not more than three papillations were taken from any one clone. A complete analysis for recessive homozygosis in these prototrophs is recorded in Table 6. The frequency of recessive homozygosis is seen to be much lower, 10 percent (7/72), than the comparable clone of Z1218 (Table 4, no irradiation, 30/60, 50 percent). All 72 prototrophic clones were analyzed by ascus dissection for genetic constitution (Table 7). Since genotypic heterogeneity in this sample of largely independent events is essentially similar to that seen in Z1218 (Table 5), it is tentatively concluded that each prototroph is, in effect, unrelated to any other and therefore probably originates as an independent event. Moreover, no marker effect due to flanking markers present in repulsion in this experiment, compared to coupling in Table 5, is evident.

The mutants, hi_{1-1} and hi_{1-7}, are readily ordered by considering the double mutant classes recorded in Table 7. The diploid, *thr HI ar tr/THR* $hi_{1-1,7}$ *AR TR*, may be interpreted as resulting from a single reciprocal recombination between the two mutants. Thus, the suggested mutant sequence along the chromosome is: thr_3 hi_{1-7} hi_{1-1} ar_6 tr_2. In addition, one prototroph, otherwise diploid, was trisomic for the ur_3-tr_2 chromosome and recessive for the tr_2 marker. Some of the ascosporal derivatives of this prototroph gave heteroallelic responses on histidineless media. Another prototroph carried an apparently spontaneous recessive lethal gene on an unlinked chromosome, since it regularly yielded two surviving spores in each tetrad.

A single diploid clone of Z1707 was also plated on histidineless media. A total of 1178 *HI* prototrophic clones derived from this plating were analyzed for proportions of recessive homozygosis (Table 8). Representative samples within each class were also studied by tetrad analysis (Table 9). The recessive homozygosis frequency among *HI* prototrophs was again low (65/1178, 5.5 percent), and heterogeneity among prototrophs similar to that observed in Z1218 (Table 5) and in the 72 papillations of Z1707 (Table 7) is evident. Possibly, clonal organization of prototrophs may be indicated by the relatively high frequency of diploids homozygous for the wild allele of *HI* yet heterozygous for the flanking markers (*thr HI ar tr/THR HI AR TR*). However, comparison of Tables 7 and 9 reveals that essentially the same distribution of genotypes among prototrophs is found regardless of whether the prototrophs are derived from independent clones or from a single clone. The numbers of prototrophs in each genotypic class, however, could indeed reflect clonal organization.

TABLE 6

Outside-marker homozygosis in 72 prototrophic papillations of Z1707

Homozygous for				
thr_3	ar_6 tr_2	tr_2	Normal phenotype	Total
1	5	1	65	72

TABLE 7

Meiotic analysis of 72 prototrophic papillations, Z1707

$$\frac{thr_3\ hi_{1-1}\ AR\ TR}{THR\ hi_{1-7}\ ar_6\ tr_2}$$

Genotype	Number	Genotype	Number
$\frac{THR\ HI\ AR\ TR}{THR\ hi_{1-7}\ ar\ tr}$	1	$\frac{THR\ HI\ ar\ tr}{THR\ hi_{1-1}\ AR\ TR}$	1
$\frac{thr\ hi_{1-1}\ AR\ TR}{THR\ HI\ AR\ TR}$	1	$\frac{thr\ hi_{1-7}\ AR\ TR}{THR\ HI\ ar\ tr}$	2
$\frac{thr\ hi_{1-1}\ ar\ tr}{THR\ HI\ AR\ TR}$	1	$\frac{THR\ hi_{1-1,7}\ AR\ TR}{THR\ HI\ ar\ tr}$	1
$\frac{THR\ HI\ AR\ tr}{THR\ hi_{1-7}\ AR\ TR}$	1	$\frac{THR\ hi_{1-1,7}\ AR\ tr}{THR\ HI\ ar\ TR}$	1
$\frac{thr\ hi_{1-7}\ AR\ TR}{thr\ HI\ ar\ tr}$	1	$\frac{thr\ HI\ AR\ TR}{THR\ hi_{1-7}\ ar\ tr}$	22
$\frac{thr\ HI\ ar\ tr}{THR\ hi_{1-7}\ ar\ tr}$	2	$\frac{THR\ hi_{1-1}\ ar\ tr}{thr\ HI\ AR\ TR}$	2
$\frac{thr\ HI\ ar\ tr}{THR\ hi_{1-7}\ AR\ TR}$	2	$\frac{thr\ HI\ ar\ tr}{THR\ HI\ ar\ tr}$	1
$\frac{thr\ HI\ ar\ tr}{THR\ hi_{1-1}\ AR\ TR}$	3	$\frac{THR\ HI\ ar\ tr}{THR\ HI\ ar\ tr}$	1
$\frac{thr\ HI\ ar\ tr}{THR\ hi_{1-1,7}\ AR\ TR}$	1	$\frac{THR\ HI\ AR\ TR}{THR\ HI\ AR\ TR}$	1
$\frac{thr\ hi_{1-1}\ AR\ TR}{THR\ HI\ ar\ tr}$	25	trisomic (*tr/tr/tr*)	1
$\frac{THR\ hi_{1-7}\ ar\ tr}{THR\ HI\ ar\ tr}$	1		

TABLE 8

Recessive homozygosis in 1178 HI *prototrophs of Z1707*

Recessive homozygosis for									
gal	*thr*	*ar*	*tr*	*thr ar tr*	*ar tr*	*ur*	*le*	Normal phenotype	Total
1	16	6	3	14	24	1	0	1113	1178

ANALYSIS

Any hypothesis formulated to embrace these data must take into account the following observations: (1) high frequencies of new combinations for the surviving and wild-type *HI* allele with the flanking markers; (2) homozygosity for the proximal *thr* marker along with heterozygosity for the distal *ar* gene, but persistent heterozygosity for the centromere-linked ur_3 marker; (3) homozygosity for the distal ar_6 and tr_2

TABLE 9

Meiotic analysis of HI *prototrophs of Z1707 (from Table 8)*

$$\frac{thr_3\ hi_{1-1}\ AR\ TR}{THR\ hi_{1-7}\ ar_6\ tr_2}$$

Genotype	Number	Genotype	Number
THR hi_{1-7} *ar tr* / *THR HI AR tr*	1	*THR* hi_{1-7} *ar tr* / *THR HI ar tr*	1
THR HI AR TR / *thr* hi_{1-1} *ar* tr	4	*THR HI ar tr* / *thr* hi_{1-1} *AR tr*	1
thr HI ar tr / *THR* hi_{1-7} *AR TR*	4	*THR HI ar TR* / *THR* hi_{1-1} *ar TR*	1
thr HI ar tr / *thr* hi_{1-1} *AR TR*	9	*thr HI AR TR* / *THR* hi_{1-7} *ar tr*	12
thr HI ar tr / *thr* hi_{1-1} *ar tr*	11	*thr* hi_{1-7} *ar tr* / *thr HI AR TR*	1
thr HI ar tr / *THR* hi_{1-7} *ar tr*	14	*thr HI AR TR* / *thr* hi_{1-7} *AR TR*	1
thr HI ar tr / *THR* hi_{1-7} *ar TR*	5	*thr HI ar tr* / *THR HI AR TR*	15
thr HI ar tr / *THR* hi_{1-1} *ar tr*	1	*thr HI AR TR* / *thr HI ar tr*	1
thr HI ar tr / *thr* $hi_{1-1,7}$ *AR TR*	2	*thr HI ar tr* / *thr HI ar tr*	1
thr hi_{1-1} *AR TR* / *THR HI ar tr*	3	*thr HI ar tr* / *THR HI ar tr*	2
thr hi_{1-1} *ar tr* / *THR HI ar tr*	1		

markers; (4) Homozygosity for the distal *ar* marker and heterozygosity for the more distal *tr* marker; (5) homozygosis for the wild-type *HI* alleles, but heterozygosity for the flanking markers; (6) the predominant absence of the double mutant, $hi_{1\text{-}1,7}$, among prototrophic diploids; and (7) differences in the frequency of recessive homozygosis among prototrophs derived from different diploids.

Meiosis and the classical theory of crossing over provide a conceptual basis for the observed recombinations between homologous chromosomes. Meiosis in diploids, however, generally results in haploid cell products. But the prototrophic clones described in these experiments were sporulatable and demonstrably diploid on tetrad analysis. Hence, to assume an essentially meiotic origin for these clones must entail either preservation or restitution of the diploid condition. Restitution of diploidy by random fusions of haploid products or from ordinary first division products involving reductive centromeric disjunctions must be rejected, since the le_1 and ur_3 markers, closely linked to their respective centromeres, are not rendered homozygous with frequencies predicted for such events: 0.25 for *ur/ur* or *le/le* with random haploid fusions, or 0.5 for

the same markers following first division disjunctions. Thus, preservation of diploidy is assumed to be reasonable. We therefore propose that the origin of mitotic recombinants involves a conventional replication-pairing-exchange sequence or onset of meiosis, followed by a mitotic centromere disjunction at the ensuing anaphase. This whole process we term *parameiosis. Parameiosis is mitotic centromere disjunction preceded by the chromosomal events associated with the first meiotic prophase.* This hypothesis accounts for the observed reciprocality of crossing over, allows for the maintenance of diploidy and explains homozygosis for heterozygous input markers. The evidence for generalized chromosome synapsis is the high coincidence of mitotic recombination for unlinked markers observed in this study (me_1, Table 4), and in FOGEL and HURST (1963). Though this hypothesis does not bear directly on the mechanism of heteroallelic repair, it is reasonable to assume that at least some *HI* prototrophs could arise from conventional crossing over between heteroalleles during parameiosis. This notion is supported by the recovery of the double mutant (Tables 7 and 9).

The preceding hypothesis implies that the diagnostic differences between meiosis and parameiosis involve centromere behavior and absence of haploid cell progeny. During mitotic clonal development, it is assumed, some cells enter into meiosis, and since up to a point commitment to meiosis is reversible, diploidy is maintained by mitotic centromere disjunction. In heterothallic yeasts, a consequence of parameiotic crossing over between the mating-type locus and its centromere (20 map units) would be the production of diploid clones which could not sporulate. Furthermore, in older clones, perhaps, the onset of meiosis might be followed by the commitment to meiosis and production of haploid derivatives (ascospores). These expectations were tested by plating three 21-day old clones of Z1707 from SC onto histidineless media and SC, followed by mating-type tests of all derived clones. The prototroph titers of the three clones were approximately $120/10^6$ (123, 120 and 116 per 10^6 cells plated). The *HI* prototroph phenotypes derived from these clones are shown in Table 10. Three major observations are drawn from Table 10. (1) Prototrophs derived from aged clones are predominantly haploid by mating-type test, with haploidy frequencies of 72 percent (215/300), 71 percent (249/350) and 76 percent (914/1196) respectively. (2) High recessive homozygosis frequencies are observed among the diploid prototrophs. The proportion of diploid prototrophs recessive for one or more markers ranged from 32 percent (32/101) for the second clone to 41 percent (115/282) for the third; this contrasts with 10 percent and 4.8 percent for comparable younger clones (Tables 6 and 8) of the same diploid. (3) The distribution of diploid recessive homozygosis among the three tested clones are widely divergent. Clone 1 had only six classes of diploid recessive prototrophs. Clone 2 had 14 such classes (only eight are shown, the remainder are grouped in the "other" category). Clone 3 distributed recessive diploid homozygotes into 50 phenotypic classes (40 in the "other" category), including multiple recessiveness for 3, 4, 5, 6 and 7 markers. Since the plated clones must have been partly sporulated, it is apparent that the selective plating of asci from a heteroallelic diploid yields predominantly haploid prototrophs and is not complicated by intra-ascal fusions.

The phenotypes of the clonal progeny derived from the same three aged clones plated simultaneously on SC media are given in Table 11. The low haploidy frequencies (7/828, 3/480, 6/936) suggest that spore fusions, restoring diploidy, must occur following germination of ascospores on synthetic complete media. A similar striking reduction in diploid recessive homozygotes to about 1 percent (nine such clones in

TABLE 10

Distribution of ploidy and phenotypes among HI *prototrophs derived from aged clones of Z1707*

Clone No.	Normal diploid phenotype	Recessive diploid phenotype												Total diploid	Haploid	Total prototrophs
		thr	*ar*	*tr*	*ur*	*le*	ad_6	*tr* ad_6	*ar tr*	*ar tr le*	*thr ar tr*	*thr ur le*	Other*			
1	56	22	0	0	0	0	0	1	3	1	1	1	0	85	215	300
2	69	5	3	2	1	2	3	0	6	2	0	0	8	101	249	350
3	167	9	4	1	3	3	4	1	9	5	3	0	73	282	914	1196

* For explanation see text.

821 diploids from Clone 1), is also evident. The 16 haploid clones in Table 11 were distributed into 15 genotypic classes, mostly multiply recessive. Taken together, the data in Tables 10 and 11 indicate that an aged clone may contain as many as three kinds of cells: parameiotic cells (detectable as diploid and recessive for heterozygous input markers), cells committed to meiosis (detectable as haploid cell progeny), and ordinary vegetative or mitotic cells of normal phenotype. Tentatively, all the clones scored in Table 10 are considered as either parameiotic or meiotic. The clones of Table 11, however, may include vegetative cells which never entered into meiosis, as well as parameiotic and meiotic cells. In addition, diploid clones formed by fusions of ascospores may also be present.

The observation of haploid *HI* prototrophs among the cells of aged clones generates the prediction that plating of deliberately sporulated material (asci) for prototrophs would yield high haploid prototroph frequencies and afford meiotic random strands for comparison with the parameiotic data described earlier. This expectation was tested by plating sporulated Z1707 cells from solid sporulation media onto histidine-less media, followed by analysis of the *HI* prototroph yield for genotype by replica-plating and for haploidy by mating-type test. A direct count indicated that four-, three-, two- and one-spored asci (or unsporulated cells), were in proportions .24, .25, .21 and .30 among the plated cells. The *HI* prototroph titer was 1 per 200 plating units. The expectation of haploidy was fully borne out: only 16 diploid *HI* prototrophs were observed among the 2500 analyzed. The genetic constitutions of the 2484 haploid *HI* prototrophs are shown in Table 12, along with their proposed modes of origin. The haploid prototrophs are distributed into eight classes according to the associated linked markers. Two genetic intervals are estimated by these data: thr_3–ar_6, or region I–III, which flanks the *HI* locus and has a standard length of 12 map units, and the more

TABLE 11

Distribution of ploidy and phenotypes in aged clones of Z1707 plated on synthetic complete media

Clone No.	Diploid, normal phenotype	Diploid, recessive for							Haploid recessive	Total clones
		gal	*gal HI*	*thr*	*ar*	*tr*	*ad*	*ar tr*		
1	812	3	1	3	1	1	0	0	7	828
2	475	2	0	0	0	0	0	0	3	480
3	922	1	0	1	0	0	5	1	6	936

distal ar_6–tr_2, or region IV, 21 units in length on our standard map (Table 1). The recombination frequency among *HI* prototrophs in the thr_3–ar_6 interval was 58.3 percent (1447/2484), indicating that selection for prototrophy at the *HI* locus is positively correlated with selection for adjacent outside marker recombination. The total recombination in the ar_6-tr_2 interval was 15.5 percent (384/2484). However, when the region IV crossovers are assorted according to *thr–ar* genotype, it is found that the highest frequencies of crossing over in region IV are observed in those classes which are parental for *thr–ar* genotype: *THR–ar*, 20.4 percent (Type 3a); *thr–AR*, 14.5 percent (Type 4a). On the other hand, the lowest frequencies of crossing over in region IV are observed among prototrophs that are recombinant in region I–III: *THR–AR*, 10.8 percent (Type 1a); *thr–ar*, 12.8 percent (Type 2a). These observations suggest that positive chiasma interference is operative: a crossover in region I–III interferes with crossing over in the adjacent region IV.

All other markers in Z1707 segregated normally among the haploid prototrophs. It is apparent that in a given ascus only one ascospore could have been an *HI* prototroph, otherwise, many more diploids resulting from spore fusions would have been observed. If an *HI* prototrophic cell entered into meiosis, two *HI* spores with opposite mating types could be produced. Upon spore germination fusion would occur. The 16 diploid *HI* prototrophic clones observed in this experiment (10 with normal phenotype, 2 ar^-, 1 gal^-, 1 le^-, 1 ar^- tr^-, 1 thr^- tr^-) could have resulted from such ascospore fusions. The implications of these data on the proposed modes of origin of *HI* prototrophy will be discussed in a subsequent section.

The critical implication of the proferred hypothesis for mitotic recombination is that mitotic and meiotic recombination are fundamentally identical up to the point of first-division centromere disjunction, upon which the parameiotic process yields diploid progeny, while meiosis ultimately results in haploid cell products. The apparent differences in parameiotic and meiotic recombination frequencies reported in this

TABLE 12

Genotypes and proposed origin of HI *prototrophs derived from plating of Z1707 asci on* HI$^-$ *media*

I	II	III	IV	
thr_3	HI_{1-7}	hi_{1-1}	AR_6	TR_2
THR_3	hi_{1-7}	HI_{1-1}	ar_6	tr_2

Type	Genotype		Region I-III		Region IV		Proposed origin
			Parental	Recomb.	Parental	Recomb.	
1	THR_3 *HI* AR_6 TR_2		0	347	347	0	Rev. 1–7, III
1a	THR_3 *HI* AR_6 tr_2		0	42	0	42(.108)	Rev. 1–7, III, IV
2	thr_3 *HI* ar_6 tr_2		0	923	923	0	II
2a	thr_3 *HI* ar_6 TR_2		0	135	0	135(.128)	II, IV
3	THR_3 *HI* ar_6 tr_2		706	0	706	0	Rev. 1–7
3a	THR_3 *HI* ar_6 TR_2		186	0	0	186(.204)	Rev. 1–7, IV
4	thr_3 *HI* AR_6 TR_2		124	0	124	0	I, rev. 1–7, III/ II, III
4a	thr_3 *HI* AR_6 tr_2		21	0	0	21(.145)	I, rev. 1–7, III, IV/II, III, IV
	Total	2484	1037	1447	2100	384	
	Percent		.417	.583	.845	.155	

study may well result from assaying for parameiosis in a clonal background of vegetative cells. From Table 3, among 2000 untreated clones 13 recessive homozygotes were observed. Since an equal number of mitotic segregants could be expected to remain heterozygous after centromere disjunction (Figure 1), a total of 26 parameiosed clones were present in the 2000, or about 1 percent. Thus, in this comparatively young clone about 1 percent of the cells had entered into meiosis. Assuming this figure then, the prototroph titer in this population is recalculated as $30/10^4$ (Table 2), or 1/333, and compares favorably with the 1/200 titer estimated by ascus plating. During clonal aging, the proportion of parameiotic cells would attain some maximal value and then decline as the frequency of cells fully committed to complete meiosis rises linearly from a low initial value. Under these circumstances, comparison of the recessive homozygosis frequency among diploids for any marker, such as ar_6, with meiotic map distance from the centromere is properly made only between meiotic distance of the marker from the centromere and the corrected homozygosis frequency (doubled) among parameiosed cells. Such a comparison for ar_6, 46 map units from its centromere, in Table 3 yields $(4 \times 2)/20$ or 40 map units. In view of the sample sizes involved in Table 3, this calculation must be considered as only suggestive of an experimental rationale for verification of parameiosis as a quasi-meiotic process.

A slightly more sensitive test of the fundamental similarity between parameiotic and meiotic processes compares the distributions of outside marker recombination among *HI* prototrophic chromosomes derived from diploids (Tables 7 and 9) with haploid *HI* prototrophic chromosomes derived by plating asci (Table 12). The comparison is made by enumerating all *HI* prototrophic chromosomes in these tables, including *HI* homozygotes, and arraying the sums against the data of Table 12. A summary of the results is given in Table 13. Unfortunately, the data of Tables 7 and 9 were collected for different purposes and only Table 7 (72 papillations from 28 independent clones) approximates a valid random sample. The excess of *thr HI ar tr* types in Table 9, for example, came about through deliberate selection of particular recessive homozygotes ($thr^- ar^-$ and $thr^- ar^- tr^-$) for tetrad analysis. In addition, some clonal organization of prototrophs may be incorporated in these data. However, comparison of the data from Table 7 with that from Table 12 lends support to the suggestion that the underlying mechanisms are identical in parameiosis and meiosis.

TABLE 13

Comparison of HI *prototrophic chromosomes among diploids in Tables 7 and 9 with haploid* HI *chromosomes in Table 12*

HI chromosome type	Genotype	Z1707 random prototrophs (Table 7)	Z1707 clone plating (Table 9)	Z1707 ascus plating (haploids) (Table 12)
1	$THR_3\ HI\ AR_6\ TR_2$	5	15	347
1a	$THR_3\ HI\ AR_6\ tr_2$	1	1	42
2	$thr_3\ HI\ ar_6\ tr_2$	10	70	923
2a	$thr_3\ HI\ ar_6\ TR_2$	0	0	135
3	$THR_3\ HI\ ar_6\ tr_2$	33	8	706
3a	$THR_3\ HI\ ar_6\ TR_2$	1	1	186
4	$thr_3\ HI\ AR_6\ TR_2$	24	15	124
4a	$thr_3\ HI\ AR_6\ tr_2$	0	0	21
Totals		74	110	2484

Origin of HI *prototrophs:* The *HI* prototrophic chromosomes may be partitioned into two groups: (1) recombinant for the flanking thr_3 and ar_6 markers (Types 1 and 2 in Table 12), and (2) nonrecombinant or parental for the flanking markers (Types 3 and 4, Table 12). A plausible initial hypothesis emerges from this observation; namely, prototrophs originate from two major sources, recombination between the heteroalleles and mutation ("reversion") of either input allele. Recombination between the heteroalleles should result in coupling for the recessive alleles of the flanking markers (Types 2 and 2a, Table 12). The relative revertibility of the input alleles is most directly estimated in the corresponding homoallelic diploids. However, when the $hi_{1\text{-}1}/hi_{1\text{-}1}$ homoallelic diploid was constructed and assayed for *HI* prototroph titer it yielded no prototrophs in 10^{11} cells, and is therefore tentatively considered a deletion. The $hi_{1\text{-}7}/hi_{1\text{-}7}$ homoallelic diploid yielded an *HI* prototroph titer of 1.6×10^{-9}. These observations, eliminating $hi_{1\text{-}1}$ as a significant source of prototrophs provided the basis for the proposed modes of origin of each *HI* prototrophic chromosome type indicated in Table 12. A dualistic origin of wild-type loci by recombination between heteroalleles or by reversion of $hi_{1\text{-}7}$ is also supported by the following observations in Table 12: (1) Where the proposed origin is reversion of $hi_{1\text{-}7}$ and simultaneous exchange in region III (Types 1 and 1a), high positive interference is manifested by reduction of additional crossovers in region IV to half the normal value, from 21 percent to 10.8. (2) Where crossing over between heteroalleles in region II is postulated (Types 2 and 2a), the interference with coincident crossover in region IV (12.8 percent) is almost as great. (3) Where reversion of $hi_{1\text{-}7}$ is postulated (Types 3 and 3a), the normal meiotic crossover rate of 20.4 percent in region IV is observed. Thus, among prototrophs demonstrably nonrecombinant for outside markers an origin requiring single or multiple exchanges between homologues in the heteroallelic vicinity is excluded. It is imaginable that the reverted $hi_{1\text{-}7}$ allele arises either prior to or coincident with, but not involving, conventional or unequal segmental interchange. (4) The Type 4 and 4a chromosomes might each have been formed in two ways, either as reversions of $hi_{1\text{-}7}$ concurrent with exchanges in regions I and III (and region IV for Type 4a), or by recombination between the heteroalleles coincident with crossing over in region III (and region IV for Type 4a). Some estimate of the recombination frequency in each interval may be achieved by partition of the Types 4 and 4a prototrophs according to the relative frequencies of recombination in region II and reversion of $hi_{1\text{-}7}$ within Types 1 through 3. The region II frequency (Types 2, 2a) is 1058/2339, or 0.45; $hi_{1\text{-}7}$ reversion is equal to 1281/2339 or 0.55. The total region I crossing over is then 145×0.55, or 80/2484, or 3.2 percent crossing over in region I (meiotic distance 2.2 percent, Table 1). The total region III crossing over is then 534/2484, or 21.5 percent (meiotic distance, 10.4 percent). These differences could be indicative of an enhanced probability of crossing over after reversion, or an excess of multiple exchanges in regions I and III. It is obvious that recombinant *HI* prototrophy requires chromosome synapsis (onset of meiosis) postulated as the initial phase of either parameiosis or meiosis and common to both. In view of the parameiotic recombinants observed among the postulated $hi_{1\text{-}7}$ revertant diploids in Table 13, onset of meiosis may occur in revertant cells. However, whether synapsis of homologues is necessary to or enhances the probability of reversion is not yet clear.

The above arguments regarding mitotic recombination and *HI* prototrophy are summarized as Figures 1 and 2. In Figure 1, the consequences of a primary recombinational event between the heteroalleles are shown for parameiosis (Tables 7 and 9), and for meiosis (prototrophs only in Table 12). The results of additional crossovers

in any genetic interval are not depicted, but are relevant to Tables 7 and 9. In Figure 1, the parameiotic prototrophs are shown as either $HI/hi_{1\text{-}7}$ or $HI/hi_{1\text{-}1,7}$. However, any proximal crossover involving the strand carrying centromere 1 with the strands carrying centromeres 3 or 4 would generate a 3-strand double exchange which would yield a cell with an $HI/hi_{1\text{-}1}$ constitution following mitotic centromere disjunction. Since the interval between the centromere and the *HI* locus is 36 meiotic map units, such crossovers would not be rare. In Figure 2, the consequences of reversion of the $hi_{1\text{-}7}$ allele are shown for parameiosis and meiosis but without additional crossovers in any interval. The parameiotic products in this figure are both $HI/hi_{1\text{-}1}$. However, here too an additional crossover between the centromere and the *hi* locus would allow for the production of cell products with an $HI/hi_{1\text{-}7}$ constitution. An estimate of the expected ratio of surviving alleles among parameiotic *HI* prototrophic diploids may be based on the following assumptions: (1) The mean exchange frequency in the 36 unit interval *hi*-centromere is 0.72; (2) Nonexchanges and exchanges of all types are Poisson distributed; (3) No interference is operative. From these assumptions the ratio of surviving alleles among reversional prototrophs is computed to be 4 $hi_{1\text{-}1}$: 1 $hi_{1\text{-}7}$, and among recombinational prototrophs 1 $hi_{1\text{-}1}$: 2 $hi_{1\text{-}7}$: 2 $hi_{1\text{-}1,7}$. These expectations are tested in Table 14 for the diploids of Tables 7 and 9. Among the *HI* prototroph types (1, 1a, 3, 3a) presumably all reversions of $hi_{1\text{-}7}$, the $hi_{1\text{-}1}$: $hi_{1\text{-}7}$ ratio, 34:7, is not significantly different from the expected 33:8. However, this computation does not apportion the surviving alleles associated with Type 4 prototrophs. Among the *HI* prototrophs which have been proposed as recombinants (Types 2 and 2a), the $hi_{1\text{-}1}$: $hi_{1\text{-}7}$: $hi_{1\text{-}1,7}$ ratio is 28:28:3 (or 28:28:5 if the two associated with Type 3 chromosomes are included). These ratios are significantly different from expected 1:2:2. Interference has not been considered in these calculations; the effects of interference would be expected to be greater among recombinational prototrophs than among reversional prototrophs. Of the five double mutants observed in this study, four show additional crossovers in the short *thr-hi* interval where they would be least expected. The significance of these observations is not yet clear. The last prototrophic diploid type observed in Tables 7 and 9, homozygous *HI/HI*, presumably represents parameiosis of already prototrophic cells, and might arise by "recycling". Thus, if a cell becomes an *HI* prototroph early in clonal development, either by recombination or reversion, and if the mating-type locus remains heterozygous, it could pass through a parameiotic cycle again. An additional possibility is crossing over at the chromosome level in parameiotic cells.

Origin of revertants: The precise nature of the $hi_{1\text{-}7}$ reversion remains to be elucidated and would depend upon whether the mutation itself was a duplication, deletion, transition or transversion. However, two observations suggest that some diploids containing reversional type prototrophic chromosomes may have undergone parameiosis: (1) Among the *HI* prototrophic chromosomes derived from diploids, the Types 1 and 3 (reversional) chromosomes are recombined for the tr_2 marker in four of 64 enumerated in Table 13. (2) Among the 41 chromosomes associated with Type 1 and Type 3 prototrophic chromosomes in Table 14, 33 are not recombined, one is a single crossover in region I, three in III, and one in IV; two are I, III doubles; and one is a I, III, IV triple crossover. Thus, at least eight of the 41 diploids in this group would have undergone parameiosis. These data imply that allele reversion may occur in parameiotic cells, but do not demonstrate that parameiosis is a requirement for reversion.

A comparison of the reversion titers of $hi_{1\text{-}7}$ in the heteroallelic diploid with the reversion titer of the $hi_{1\text{-}7}$ homoallelic diploid indicates that revertants occur in the

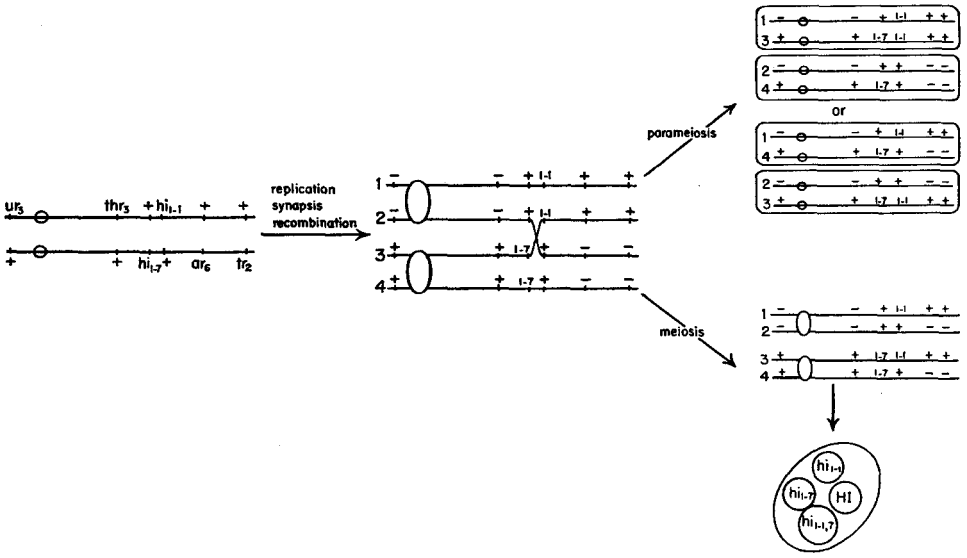

FIGURE 1.—*HI* prototroph formation by reciprocal recombination between heteroalleles in parameiosis and meiosis.

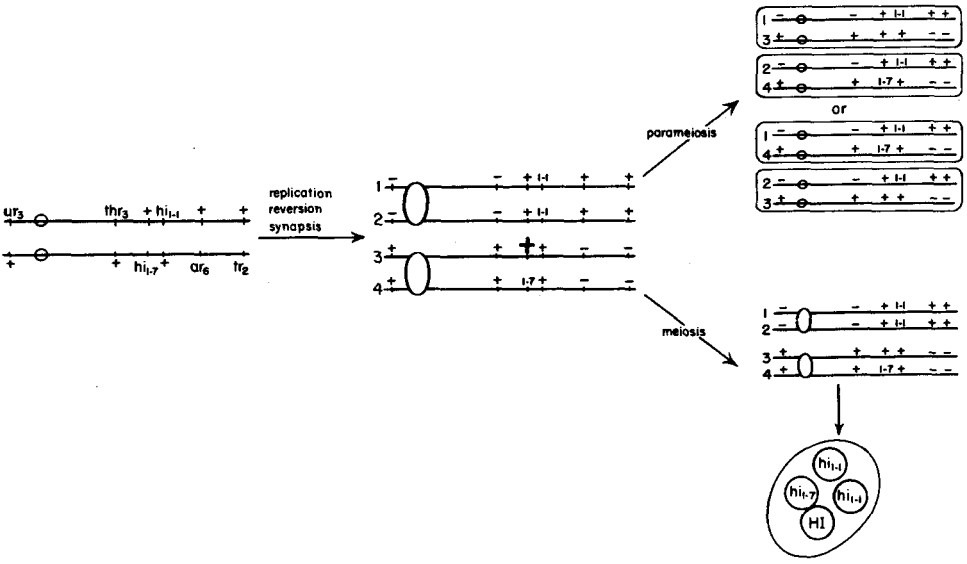

FIGURE 2.—*HI* prototroph formation by reversion of hi_{1-7} input allele in parameiosis and meiosis.

heteroallelic combination much more frequently than in homoallelic combination. The former titer is calculated as 33×10^{-6} ($0.55 \times 60 \times 10^{-6}$), while the latter is 1.6×10^{-9}. This difference leads to the suggestion that enhanced reversion in the heteroallelic diploid is perhaps a resultant of synaptic stresses due to unstable pairing relationships.

TABLE 14

Surviving hi_1 alleles among HI *prototrophic diploids (Data from Tables 7 and 9)*

Type	Prototrophic HI genotype	Surviving alleles among recombinants			Surviving alleles among revertants	
		hi_{1-1}	hi_{1-7}	$hi_{1-1,7}$	hi_{1-1}	hi_{1-7}
1	THR_3 *HI* AR_6 TR_2	0	0	0	2	1
1a	THR_3 *HI* AR_6 tr_2	0	0	0	0	2
3	THR_3 *HI* ar_6 tr_2	0	0	1	31	4
3a	THR_3 *HI* ar_6 TR_2	0	0	1	1	0
2	thr_3 *HI* ar_6 tr_2	28	28	3	0	0
2a	thr_3 *HI* ar_6 TR_2	0	0	0	0	0
	Totals	28	28	5	34	7
4	thr_3 *HI* AR_6 TR_2	(not apportioned)			2	36
4a	thr_3 *HI* AR_6 tr_2 *HI/HI*:22				0	0

DISCUSSION

Mitotic recombination: WILKIE and LEWIS (1963), in a study of UV and mitotic recombination in yeast involving several strains heterozygous for four to ten genes, present a theoretical consideration of the mechanisms of somatic homozygosis and their consequences. These mechanisms include mitotic crossing over, nondisjunction of centromeres at mitosis, meiotic crossing over and restitution, and crossing over at the chromosome level followed by meiotic centromere action and restitution. They conclude that "meiotic crossing over and segregation followed by nondisjunction or restitution at the second division" best explains their results, emphasizing that mitotic recombination occurs in cells in an incipient meiotic condition. We concur in the latter statement, yet our Figures 1 and 2, diagramming parameiosis, are identical to WILKIE and LEWIS' figure showing "mitotic crossing over". The differences between "mitotic crossing over" and "parameiosis" are not essentially semantic. The new term implies generalized homologue synapsis where the earlier phrase did not. At the same time, "parameiosis" is evocative of the fundamental similarity to meiosis; the difference lies wholly in centromere behavior. The evidence for the similarity of parameiosis and meiosis is as follows: (1) coincident homozygosis of markers on different chromosomes, suggesting independent assortment and therefore also generalized synapsis of homologues (FOGEL and HURST 1963; HURST and FOGEL 1962; WILKIE and LEWIS 1963); (2) correlation between homozygosis frequency and meiotic map distance, and between frequency of prototrophy in mitotic (diploid) and meiotic cells, provided corrections are made for the numbers of vegetative or unparameiosed cells in the clonal background; (3) positive correlation between frequency of homozygosis and marker to centromere distance in yeast (HURST and FOGEL 1962) and in Aspergillus (PONTECORVO and KAFER 1958), although not in the yeast data of WILKIE and LEWIS; and (4) the conspicuous absence of homozygosis for centromere-linked genes predicted by all the other mechanisms considered by WILKIE and LEWIS for somatic homozygosis. The absence of positive correlation between recessive homozygosis frequency and meiotic map distance in their data thus becomes critical. This difference cannot be ascribed to different ascertainment methods (in this study by selection of *HI* prototrophs, in WILKIE and LEWIS by UV), since HURST and FOGEL (1962) used UV, and PONTECORVO

and KAFER, no irradiation at all. Rather, the major difference lies in the prior growth history of clones before treatment. WILKIE and LEWIS used liquid yeast extract-peptone-dextrose, while in this laboratory initial clones are taken from solid SC. However, if parameiosed cells can "recycle", or if parameiotic cells are clonally organized by subsequent divisions after recombination, then the additional cell divisions in liquid (to ca. 2×10^8 cells/ml compared to 10^7 cells on solid) could act to obscure the correlations. Furthermore, it is not unlikely that liquid-grown cultures contain some sporulated cells.

Induction of parameiosis: In this study and in WILKIE and LEWIS (1963), the titer of recessive homozygotes was increased by UV. MORPURGO (1963) reports the same result in Aspergillus by treatment with alkylating agents. Similar effects are obtained by starvation (Tables 10 and 11) and apparently with mitomycin in Ustilago (R. HOLLIDAY and R. EASTON, personal communication). WILLIAMSON and SCOPES (1960) show that starvation perpetuates yeast cells in stationary phase, and that repeated alternate starvation and feeding will synchronize the division cycle. In view of the generality of mitotic recombination among fungi, and its extension to Drosophila (STERN 1936; ABBADESSA and BURDICK 1963), a rather speculative hypothesis is suggested. Regardless of phyletic level, cells in which stationary phase is either induced by treatment or perpetuated by starvation, or perhaps by embryonic cessation of growth, respond by entering into meiosis. If the cells or tissue are competent to produce haploid cell-progeny, such may be found, as in yeast; if not, then homozygous parameiotic segregants may be generated. Thus the nonspecific or physiological effects of UV, alkylating agents, mitomycin and starvation interfere with division cycle timing, perpetuate or induce stationary phase and thereby enhance the likelihood of initiating onset of meiosis. While it is highly speculative, this hypothesis is directly testable. Tissue cultures of metazoan cells, appropriately treated and heterozygous for suitable markers, perhaps biochemical or serological, should yield clonally organized mitotic segregants. The data of ABBADESSA and BURDICK (1963), when viewed in the light of the proposed hypothesis, are relevant. They found that X rays increased the frequency of abdominal mosaicism and that this effect was influenced by ontogenetic time. Such a result would be expected if tissue irradiated in stationary phase later began rapid division. But in rapidly dividing tissues a pronounced effect would not be expected. Of course, the hypothesis does not exclude specific effects of treatments like UV and alkylating agents upon enhancement of allelic reversion or recombination.

Heteroallelic repair: In this study, *HI* prototrophs were selected as both diploids and haploids. When the *HI* prototrophic chromosomes from diploids and haploids were compared, their overall similarities suggested that the distributions of types might be identical. Thus, all *HI* prototrophic chromosomes, regardless of source, may be classified as (1) recombinant for markers flanking the heteroallelic locus, or (2) nonrecombinant for the flanking markers.

The first group is taken to represent reciprocal recombination between the heteroalleles. The considerations upon which this classification was based are: (1) high frequency of recombination between *thr* and *ar* among haploid *HI* prototrophs (58 percent, Table 12); (2) detection of the double mutant type and the seriation of the heteroalleles in the sequence thr_3–hi_{1-7}–hi_{1-1}–ar_6; (3) positive interference in the *ar-tr* interval observed among presumptive recombinant prototrophs and not among presumptive revertant types (Table 12); (4) the proximity of the thr_3 locus to the histidine locus (2 map units), which suggests that no more than 2 percent of the *thr HI ar* chromosome types could be hi_{1-7} revertants simultaneously exchanged in the *thr-hi*

interval. A significant donation from hi_{1-1} revertants which had also crossed over in the *hi-ar* interval is not seriously considered, since this mutant has a very low reversion frequency. Furthermore, the additional crossover required in the distal interval (10 map units) precludes significant contributions of prototrophic chromosomes from this source.

The second type, nonrecombinant for flanking markers, are identical to those observed by MURRAY (1963) among methionine prototrophic chromosomes, by STADLER and TOWE (1963) in both asci and among random-spore cysteine prototrophs in Neurospora, and probably by RIZET, LISSOUBA and MOUSSEAU (1960) in Ascobolus (on different grounds), and by others (review by ROMAN 1963). This group, treated earlier as hi_{1-7} reversions since hi_{1-1} is considered a deletion, merits further discussion.

Nonrecombinant prototrophs of the general type under consideration, whether recovered from parameiotic diploids, random spores, or from asci lacking the reciprocal double mutant, have been termed conversions (LINDEGREN 1953; MURRAY 1963; STADLER 1959), mutations (MAGNI and VON BORSTEL 1962; MAGNI 1963), recombinants (RIZET, LISSOUBA and MOUSSEAU 1960; LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962; STADLER and TOWE 1963), and even reversions. Perhaps, as LAUGHNAN (1961) has suggested, the profusion of descriptive terminology stems from the absence of a convincing conceptual basis for the observations. However, the conceptual lacuna has not lacked for candidates. STADLER and TOWE enumerate the switch hypothesis (FREESE 1957), fixed pairing regions (STAHL 1961), the polaron (RIZET, LISSOUBA and MOUSSEAU 1960), and then add one, the modified polaron. Since this study also presents evidence for polarization of recombination, defined as marked asymmetry in the parentally marked classes of prototrophs, in Table 12, the temptation to add still another hypothesis is difficult to resist. However, all the hypotheses listed above share a common feature. *Uniformly they derive prototrophs from interactions between the homologous chromosomes.* But the critical evidence for such interaction is largely indirect. Included here are the findings of MAGNI and VON BORSTEL (1962) that mutation rates are 6 to 20 times higher in meiosis than in mitosis, and also the finding in this study that apparent reversions in heteroallelic combinations are more frequent than reversions in homoallelic diploids. Worse still, at least one reversion class from homoallelic diploids, namely, second-site mutations yielding complementing alleles (unpublished data), have not been observed among heteroallelic prototrophs. If our suggestion that mitotic recombinational events represent meiotic phenomena occurring in mitotic backgrounds is correct, then it would seem that the argument for synapsis and homologous chromosome exchange is strengthened. However, MAGNI's (1963) finding that a hemizygous locus reverts with a higher frequency meiotically than mitotically appears to weaken the case for meiosis as necessarily implying segmental exchange, since the interaction between homologues at or prior to synapsis is not necessarily limited to reciprocal breakage-reunion or unequal crossing over.

The alternative hypothesis, allele reversion, requires no exchange between homologues or discontinuity within the cistron. The critical datum bearing on this issue is the normal meiotic crossover rate observed in region IV among presumptive hi_{1-7} revertants (Table 12). If all prototrophs, recombinant and nonrecombinant alike, arise in a pairing-exchange sequence, then a 50 percent reduction in region IV map length would be predicted among nonrecombinant prototrophs. Since no interference effects are noted, the nonrecombinants are viewed as originating from processes other than conventional crossing over. Under these circumstances, the specific nature of the mutant allele, duplication, deletion, transition or transversion, should be known for

more accurate prediction. If the hi_{1-7} mutation represents a tandem duplication of the Bar type (PETERSON and LAUGHNAN 1963; NANCE 1963), then single intrachromosomal exchanges restoring wild type could occur. Synaptic stress resulting from pairing between homologues carrying a duplication and a deficiency might enhance the rate of intrachromosomal exchanges. Such events could not be expected to yield prototrophs recombined for outside markers. If two duplication mutants were close to each other on the chromosome and paired in heteroallelic combination, synaptic constraints might reduce the probability of crossing over between the mutants. Thus, the double mutant would be rare and only revertant prototrophs observed. On the other hand, two deletion mutants might revert with frequencies so low that only recombinant prototrophs are seen. Deletion mutants would be difficult to map against mutants reverting with appreciable frequencies, since gross prototroph titers would not be additive. In general, consistent mapping is attainable if reversion frequency is relatively constant and contributions from recombination are low. Unfortunately, two possible candidates for such comparison in Ascobolus, mutants 67 and 16 of Series 12 of RIZET, LISSOUBA and MOSSEAU (1960), are not shown on their map or in LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL (1962). Predictions concerning transitions and transversions are not clear at this time.

The preceding discussion on heteroallelic repair leads to the following observations. In a heteroallelic diploid, two mutants of independent origin and perhaps of different types, duplications, deletions, transitions or transversions are brought into *trans* configuration. In addition to the particular combination of types represented, any specific mutant pair would differ from any other in map distance within the cistron. If two mutants complement each other in the diploid, prototrophs from random spore populations furnish the basis for fine structure mapping. If they do not complement, prototrophs may be selected from plating the diploid or random spores. In any case, the resultant prototroph population may be heterogeneous depending upon mutant types and donations from recombination and reversion. The selected prototrophs could arise from exchange or nonexchange mechanisms. The exchange mechanisms are: (1) normal reciprocal crossing over, which yields a wild type and double mutant; (2) oblique crossing over (in a duplication) which yields a wild type and double mutant also; and (3) intrachromosomal exchange, which yields a wild type but does not produce a double mutant. The nonexchange mechanisms would include insertion or deletion of bases (replicational errors). These would not yield a double mutant either. Consistent mapping and double mutant recovery would depend upon the mutant types paired and the distances between mutants. The reversion hypothesis also seems to account for high negative interference, since every reversion is scored as a double crossover. The data of STEINBERG and EDGAR (1962) on bacteriophages could be viewed as an admixture of reversions and allelic recombinants.

The relationship of the time of reversion to the times of recombination and replication is not clear. The curves of SHERMAN and ROMAN (1963), showing two periods during meiosis at which allelic recombination might occur, now appear (M. ESPOSITO, personal communication) to be a composite of parameiotic diploid prototrophs along with haploid prototrophs in the early phase of the meiotic cycle with only haploid prototrophs accumulating at a constant rate in the later portion of the cycle. MAGNI (1963) shows association of reversion of homoallelic hi_{1-1} (not the same hi_{1-1} used in this study) with chromosomal exchange between thr_3–ar_6 among random spores, and some negative interference even in the distal ar_6–is_1 interval. At the moment, these data are not reconcilable with those reported here.

Conclusions: The following conclusions appear to follow from consideration of the data and the discussion. (1) *HI* prototrophs arise from two sources, classical recombination between the heteroalleles or reversions (intrachromosomal events) of the hi_{1-7} mutant. Enhancement of reversion by oblique pairing of homologous chromosome regions during meiosis, or polarization of reversion, has, however, not been excluded. (2) Mitotic recombinants arise by generalized chromosome synapsis and crossing over in a four-strand stage at normal meiotic rates accompanied by regular mitotic centromere disjunction. The process has been termed parameiosis.

SUMMARY

Two diploids, heteroallelic at the histidine-1 locus (hi_{1-1}/hi_{1-7}), were prepared in coupling and repulsion for closely linked flanking thr_3 and ar_6 markers, with an additional marker, tr_2, distal to ar_6, heterozygous in the second diploid. The first diploid was treated with ultraviolet irradiation (UV) which increased the *HI* prototroph frequency, but with increased UV dose the proportion of recessive homozygosis among prototrophs decreased. The second diploid was plated for prototrophs as a young clone, and as an aged clone, and was sporulated and plated for haploid prototrophs. Tetrad analysis of the diploid *HI* prototrophs revealed high genotypic heterogeneity. The double mutant expected of reciprocal recombination was also found in low frequencies. Prototrophs from the aged clone were about 70 percent haploid, and the remaining diploids showed high recessive homozygosis for heterozygous input markers, reflecting a high incidence of meiosis in the clone. The haploid *HI* prototrophs from asci were distributed into eight genotypic classes, unequal in size. The frequencies of crossing over in the distal *ar-tr* interval indicated that positive interference was observed in some genotypic classes (postulated as recombinant between the heteroalleles) and not in others (postulated to be reversions of the hi_{1-7} input allele). The hi_{1-1} mutant is considered as a deletion. These observations are discussed with respect to hypotheses involving exchanges between homologues or intrachromosomal events.—It is proposed that mitotic recombination reflects the occurrence of generalized chromosome synapsis (the onset of meiosis) followed by mitotic centromere disjunction. This process has been called parameiosis.

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