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Gene Conversion in Synchronous Yeast Cell Pedigrees

by

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INTRODUCTION

The availability of the four strands from meiotic tetrads has enabled a detailed characterization of meiotic intragenic recombination (reviewed by Whitehouse and Hastings, 1965; Fogel and Hurst, 1967). This process possesses the following primary characteristics. (1) Recombination between alleles in trans array is largely non-reciprocal. Thus the double mutant strand expected from a classical exchange between the mutant sites rarely occurs concomitantly with a prototrophic strand. Instead, prototrophic revertants mainly show $3^+ : 1^-$ segregations for one of the alleles (gene conversion). (2) Conversion does not necessarily occur with equal frequency at the two allelic sites. Rather, conversion displays a directional polarity. (3) Conversional events are correlated with enhanced recombination of flanking markers. The revertant strand is generally involved in these exchanges at a frequency greater than that expected by chance alone.

Similarities are apparent in the comparable mitotic data from Saccharomyces cerevisiae. Thus a double mutant segregant accompanies prototroph formation in only a small fraction of the revertants from heteroallelic diploids (Roman, 1956a). Also, coincident associations of conversion with recombination of flanking markers have been described (Fogel and Hurst, 1963).

However, mitotic intragenic events are generally studied with selective procedures, typically in revertant diploid clones (reviewed by Pritchard, 1963). While this is necessitated by the rarity of spontaneous mitotic recombination, the amount of information obtained is limited not only by the operational failure to detect events which do not terminate in prototrophy, but by the recovery of only one of the participating strands in one-half of the prototrophic diploids. The pattern of mitotic events cannot therefore be fully specified. Moreover the question remains whether fundamentally similar recombinational mechanisms are operative during meiosis and mitosis. While the latter question can be resolved with techniques facilitating the complete recovery of all cellular products associated with prototroph formation, such systems have generally been inadequately marked (Roman, 1963). Consequently, the relationship between intra- and intergenic recombination remains unclarified.

Mortimer (1959) has shown that X-rays stimulate prototroph formation in heteroallelic diploids. The number of prototrophs rises linearly over a defined dose range (Manney and Mortimer, 1964; Hurst, Fogel and Weinstock, 1967). In addition Esposito (1967) has observed that the number of X-ray induced prototrophs fluctuates in a characteristic manner during the

cell cycle; the frequency is maximum just prior to the initiation of DNA synthesis, declines during replication and is minimal at the completion of replication. In this study X-ray induced histidine-1 prototrophic cells, rather than clones, were selected from synchronous prereplicative yeast cultures and the first four division products subsequently separated. Such pedigrees were isolated from both a linear and non-linear portion of the X-ray prototroph induction curve and from different stages during the cell cycle. The results support the view that similar recombinational mechanisms occur in meiotic and X-ray treated mitotic cells. Evidence is presented that mitotic prototroph formation can occur during the two-strand stage and that selection for mitotic histidine prototrophs results in a high frequency of irregular segregations at a closely linked cistron. Further, no significant differences were observed between X-ray derived mitotic prototrophs isolated at different stages during the cell cycle.

MATERIALS AND METHODS

Strains

The diploid clones utilized in these experiments were synthesized by Dr. D. Hurst and Dr. S. Fogel from the following parental ascospores:

Z2367- Z842-5B x A806-7B

α + + his1-315 + arg6 thr2 leu1 + + ade1
 a ura3 thr3-1 + his1-1 ade6 gal2 adel

Z3735- A3681-2D x A3671-1A

ura3 thr3-1 + his1-315 + arg6 try2 + ade6 gal2
 + + thr3-2 + his1-1 + + leu1 + +

uvsl adel
 + adel

For details of the origin of the two histidine-1 alleles and Z2367 see Fogel and Hurst (1967). They located the his1-315 mutant at the proximal end of the cistron with respect to the centromere by the flanking marker arrangement in prototrophic asci containing the double histidine-1 mutant.

Diploid Z3735 was synthesized for the present study. The thr3-2 allele was isolated in this laboratory following treatment with the mutagen ethylmethanesulfonate. The recovery of a tetrad from Z3735 with a reciprocal exchange between the mutant sites tentatively places the thr3-2 mutant distal to the thr3-1 mutant with respect to the centromere (Hurst and Fogel, unpublished data).

The ura3 (uracil), thr3 (threonine and methionine), his1 (histidine), arg6 (arginine), and try2 (tryptophan) markers are located on linkage group V in this order, as determined by Mortimer and Hawthorne (1966). The map distances are: ura3-5.0-centromere-34.0-thr3-2.4-his1-10.0-arg6-20.8-try2 (Hawthorne and Mortimer, 1960; Fogel and Hurst, 1967). Aside from the loci leu1 (leucine) and ade6 (adenine) which are both located in linkage group VII, the remaining markers segregate independently of each other (Mortimer and Hawthorne, 1966). The uvs mutant (ultraviolet light-sensitive) was isolated by Dr. D. Hurst and is not centromere linked.

Media

For details of the solid media used in these experiments see Roman (1956b), Fogel and Hurst (1963), and Hurst and Fogel (1964). Liquid starvation medium is described by Williamson and Scopes (1960) and contains, per liter: 0.34 g calcium chloride.2H₂O, 0.75 g potassium chloride, 0.5 g magnesium chloride.

Mutagenesis

The thr3-2 allele was isolated according to the following procedure (Lindgren, Hwang, Oshima and Lindgren, 1965). An 18 hr haploid culture was washed in distilled water and resuspended in 10 ml of 0.05M phosphate buffer, pH 9.0 at a

concentration of about 2×10^8 cells per ml. Ethylmethane-sulfonate (0.3 ml) was added, and the culture was incubated at room temperature with shaking for 60 min. The suspension was washed twice in 10 ml of a 6% solution of sodium thio-sulfate and post-incubated for 60 min in yeast extract-peptone-dextrose (YEPD). The culture was then diluted in sterile distilled water so that platings of 0.1 ml would yield approximately 60 clones per plate on complete medium. After 72 hr the resultant clones were replica-plated to complete medium lacking threonine. About 3000 clones were examined. Auxotrophic mutant clones, identified by their failure to grow on the selective medium, were crossed with strains carrying known thr3 alleles. Allelism was indicated by the absence of complementation in the resultant diploids.

Isolation of a Synchronous Fraction of Cells

A method for obtaining synchronously dividing cultures of Saccharomyces cerevisiae has been described by Williamson and Scopes (1962). Their procedure involves the removal of small cells from a stationary phase population consisting primarily of large unbudded cells by repeated centrifugation in a 15% mannitol solution. The remaining large cell fraction is then subjected to several cycles of feeding and starvation. In the present work it was more expedient to use the small cells.

These can be rapidly removed from the population as a homogeneous fraction, completely uniform in size. Such cells possess the advantage of dividing synchronously upon inoculation into fresh medium without prior cycles of feeding and starvation. The synchronous cultures used in the experiments reported here were obtained according to the following procedure. One clone from a synthetic complete streak plate was inoculated into 10 ml of double strength YEPD contained in a 50 ml Erlenmeyer flask at a concentration of about 100 cells per ml. The culture was incubated for 48 hr at 30° with shaking. After three washings, the cells were resuspended in 5 ml of sterile distilled water at a concentration of about $6-8 \times 10^8$ cells per ml. Such stationary populations consist of both budded and unbudded cells, the latter being broadly divisible into four classes on the basis of cell size. The suspension was then slowly centrifuged for approximately 2 min leaving only the smallest cells in the supernatant. A swinging-bucket centrifuge (International Model CL) was used. Since the exact speed and time of centrifugation vary with the concentration of cells and the proportion of small cells in the population, these parameters must be determined for each culture individually. The supernatant was decanted and inspected microscopically. Any sample containing discernible heterogeneity in size, as

well as any sample containing more than 0.5% budded cells, was discarded. This step is critical since cells visibly larger than the isolated fraction will contribute to the asynchrony of the culture. The original pellet was resuspended in 5 ml distilled water and the centrifugation repeated until the requisite cell number was isolated. About 5×10^7 cells were isolated at each centrifugation. However the efficiency of the procedure can be improved by centrifuging several such suspensions simultaneously and combining the respective yields.

The synchronous division of the isolated fraction was established as follows. The cells were inoculated into single strength YEPD at a concentration of 4×10^7 cells per ml and incubated at room temperature. At 15 min intervals, samples were removed and diluted with sterile distilled water containing 2 drops per ml of a 37.5% solution of formalin (Esposito, 1967). These were then scored for the percentage of budding cells as well as for cell number. Since separation of the bud from the mother cell does not usually occur until one or both cells begin to bud again (Burns, 1954; Williamson and Scopes, 1960; Williamson, 1964), the latter measurement is indicative of the duration of synchrony. The increase in the number of budding cells with time was observed with an oil immersion objective. Only cells with visible buds were

scored. A Coulter Counter (Model B) was used to measure the increase in the number of cell units. To obtain uniform separation of the mother and daughter cell at the completion of the second budding cycle, all measurements on samples observed to contain double units were preceded by a 10 sec exposure to ultrasonication (Williamson, 1964; Esposito, 1967). A Branson Ultrasonicator set at a power setting of one was used.

DNA Measurements

DNA was extracted from approximately $1-2 \times 10^8$ cells according to the method described by Ogur, Minckler, Lindegren and Lindegren (1952) except that the ether extraction was omitted (Schneider, 1945). The cells were washed twice in 0.5 ml of cold 70% ethyl alcohol and in 0.5 ml of cold 2% perchloric acid. DNA was extracted three times in 0.3 ml of 5% perchloric acid at 70° for 20 min. The amount of DNA in each sample was assayed colorimetrically with the diphenylamine reagent (Burton, 1956). Equal volumes of diphenylamine reagent and sample were used (0.9 ml). Calbiochem Grade A highly polymerized DNA served as the standard. This was hydrolyzed at 70° for 30 min in a 5% solution of perchloric acid. The diphenylamine reaction was linear with DNA concentrations between 2.5 and 30.6 μg .

Isolation of Diploid Prototrophs

A technique for the detection and isolation of asci containing prototrophic spores has been published by Fogel and Hurst (1967). This procedure was modified to allow the recovery of prototrophic cells originating during mitosis, as well as all cell products arising from the same event. A suspension of synchronous cells was streaked onto agar slabs of synthetic complete medium lacking histidine. Alternatively, the culture was plated at a concentration of about 2.5×10^5 cells per plate on histidineless medium. Slabs were then made from each plate. The slabs were incubated in moisture chambers at 30° for 6 hr and examined at 1 hr intervals thereafter for an additional 18 hr period. Prototrophic cells, detected by the appearance of a bud, were transferred to a fresh slab of histidineless agar and the mother cell, first bud, and the next division products of these, separated. The cells were positioned on the agar in a convenient order suggested by Burns (1954). Less than 1.0% of the initial auxotrophic population has a sufficient histidine pool to form a bud on histidineless medium. In contrast, auxotrophic products associated with prototroph formation typically undergo a single division on histidineless medium to yield two auxotrophic cells. Presumably this reflects a cytoplasmic continuity between the mother cell

and its bud. The slabs were transferred to YEPD plates and incubated 48 hr. The subsequent clones were replica-plated to an appropriate series of synthetic complete plates lacking single nutrients and scored for all parentally heterozygous markers. In addition, each series contained a potassium acetate sporulation plate (McClary, Nulty and Miller, 1959). All phenotypically different clones derived from a single cell, or the subsequent clones arising from the mother cell and first bud, were selected for sporulation, microdissection and tetrad analysis. The snail enzyme technique of Johnston and Mortimer (1958) was used to digest the asci for dissection. Asci with four viable ascospores were chosen from each clone and scored for all parentally heterozygous markers. The operations yielded approximately 3,500 unselected tetrads from the two diploids.

The parental allele present in the prototrophic cell as well as the heteroalleles present in any auxotrophic cells were identified by allele testing (Hurst and Fogel, 1964). This recombinational test is dependent on the difference in prototroph frequency between homoallelic and heteroallelic diploids (Roman, 1958). Allele testing involves crossing each ascospore clone to a and α tester strains of each allele initially present in the heteroallelic cistron. The

resulting zygotes, isolated on media selective for the diploid, are transferred to YEPD plates and after 24 hr of incubation, replica-plated to the appropriate omission medium for prototroph detection. Plates are scored after 72 hr. Spores homoallelic to the tester strain, or double mutant spores, operationally homoallelic to both tester strains, will produce few or no prototrophic papillations in the resultant diploid cells. The results of the his allele tests were confirmed by crossing each ascospore clone to a third tester strain, his1-270, which complements his1-315 but yields a typical prototrophic papillation pattern when combined with his1-1.

X-Ray Irradiation

The isolation of spontaneously arising mitotic prototrophs is complicated by the difficulty in ascertaining the number of independent events devoid of clonal organization. Manney and Mortimer (1964) have shown that X-ray treated heteroallelic diploids display a dose dependent linear increase in the number of mitotic prototrophs. To minimize the proportion of clonally related prototrophs as well as to ensure the recovery of each viable cell associated with prototroph formation, all cultures were treated with non-lethal X-ray doses. Virtually all cells retain the ability to form colonies at the doses administered. Cultures were

irradiated on the surface of agar plates with soft X-rays delivered from a Machlett OEG-60 unfiltered beryllium window X-ray tube operated at 50 kV and 40 mA. The dose rate is 22 r per sec as measured with an air extrapolation chamber (Mortimer, 1953).

Spontaneous Prototroph Titer

Prototrophic pedigrees were isolated from synchronous cultures treated with either a) 3.3 Kr or b) 6.6 Kr X-rays, or 3.3 Kr X-rays following incubation in c) YEPD or d) starvation medium supplemented with 1 μ g histidine per ml (SMH). The number of spontaneously arising HIS prototrophs in each untreated culture was determined by plating suspensions on histidineless medium at a concentration of about 5×10^5 cells per plate. The respective values, expressed as prototrophs per million cells, are: 9, 5, 10 and 9. Since cell division does not occur during the preincubation periods, any variation in the spontaneous prototroph frequency will not contribute to clonal organization.

X-Ray Induced Prototrophy As A Function Of Preincubation Time In Medium

A suspension of synchronous cells was diluted with an equal volume of starvation medium supplemented with histidine or double strength YEPD. At 30 min intervals samples were removed, washed and resuspended in sterile distilled water

at a concentration of about 5×10^6 cells per ml. The cells were then spread on the surface of histidineless plates and irradiated with a fixed dose of 3.3Kr. Cell counts, as well as a determination of the percent budded cells, were made on each sample.

The same conditions were used where X-ray induced prototrophic pedigrees were derived from preincubated cultures. Such pedigrees were isolated from cultures grown in YEPD for 60 min or SMH for 270 min. The 60 min preincubation time was selected because auxotrophic cells of Z2367 have sufficient residual histidine to bud on histidineless medium following growth periods in YEPD of 90 min or longer. It was initially believed that the 270 min SMH culture might represent an unbudded post-replicative culture.

Statistical Tests

The chi square test of significance was used to evaluate the homogeneity among two or more populations. Chi square values were computed from contingency tables. Values corresponding to the 0.05 level were considered significant. Yates' correction was uniformly used in all tests with one degree of freedom.

Ninety percent confidence intervals were used to estimate the population mean from the sample data. Such limits were computed according to

$$c = \bar{X} \pm t_{\alpha} s_{\bar{X}}$$

where c is the confidence limit, $\bar{X}$ is the arithmetic sample mean, t_{α} is the tabular Student's t -distribution value, determined by the number of degrees of freedom associated with t and some previously selected level of significance, and $s_{\bar{X}}$ is the estimated standard error of the arithmetic mean.

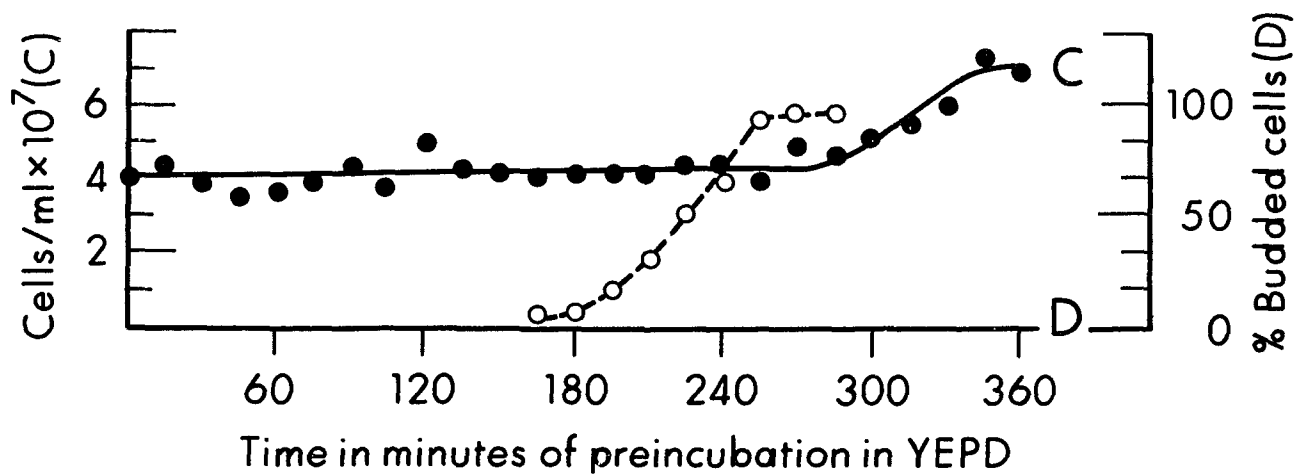
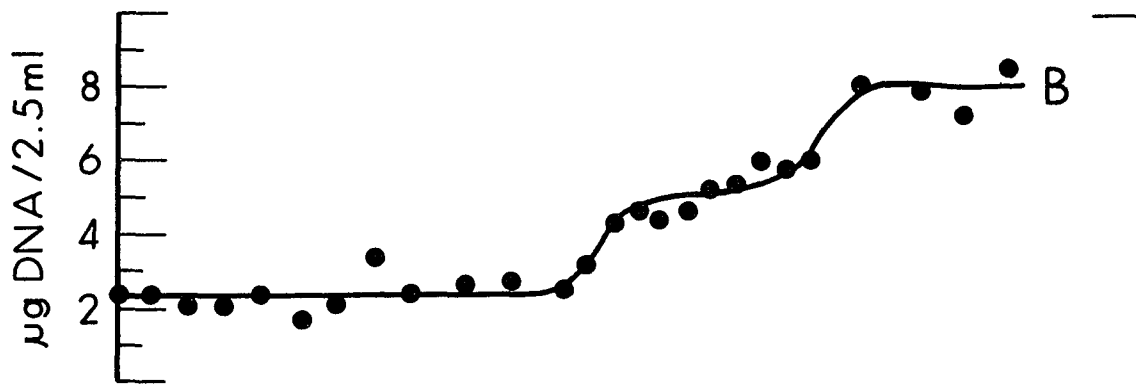
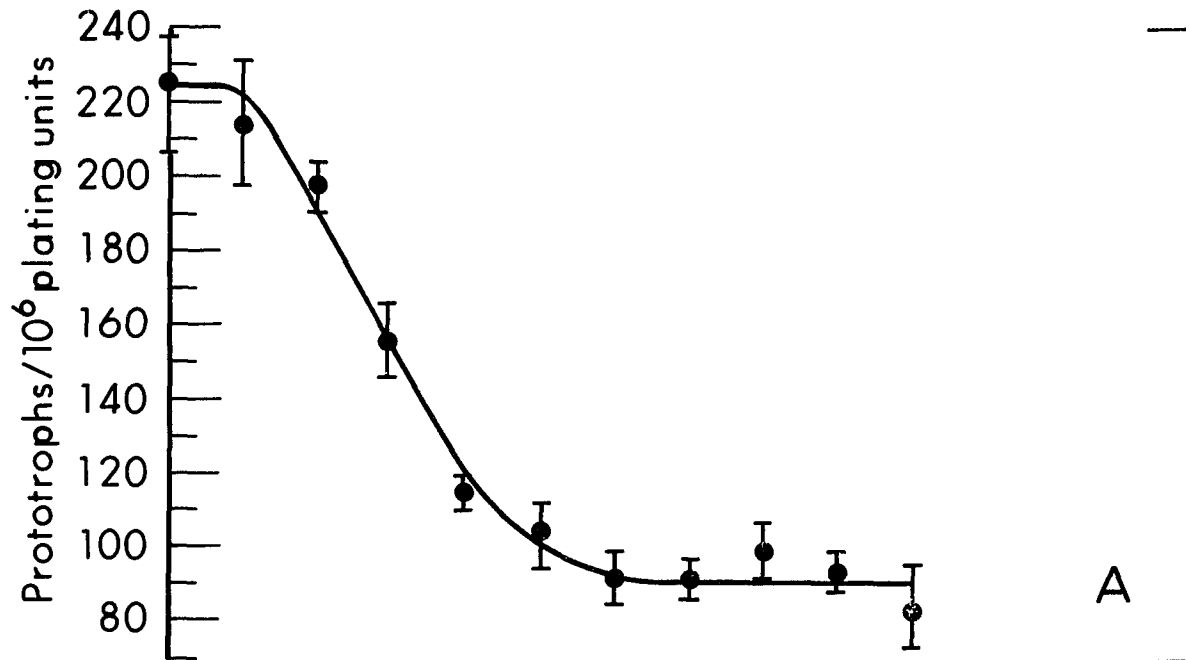
RESULTS

The Cell Cycle

Synchronously dividing cultures of Z2367 in YEPD were sampled at 15 min intervals for a period of 6 hr. The increase in the percentage of budded cells in the culture is shown in Figure 1D. Budded cells first appear after 195 min of growth and within a subsequent 60 min period they represent 90 to 95% of the culture. The second budding cycle, measured as an increase in the number of cell units, begins at approximately 285 min and is completed within 45 to 60 min. A more precise estimate of the initiation and length of this cycle is complicated by the separation of attached cells in the formalin solution just prior to the appearance of a visible bud.

The increase in the amount of DNA per sample with time is also shown in Figure 1B. Each point on the curve represents the average DNA value in duplicate samples from one culture. DNA synthesis is initiated at the time budded cells are first apparent and it is completed within 30 min. Thus the initial culture is probably prereplicative, with a lag period of 195 min, and divides synchronously, with the amount of DNA doubling within 30 min.

Figure 1. Relation of HIS prototroph number, DNA synthesis and cell division in diploid Z2367 to incubation time in YEPD. Prototrophy (upper curve A) was scored in 3.3Kv X-ray treated samples. DNA synthesis (middle curve B) and cell number (bottom curves C, D) were measured in untreated samples of the same culture. Error bars are 90% confidence limits.



The mean value of the DNA content per diploid cell is calculated to be $2.45 \pm 0.42 \times 10^{-14}$ g and $2.71 \pm 0.39 \times 10^{-14}$ g in two independently grown cultures of Z2367. The mean estimate values are based on the average determination at each of 12 and 15 points, respectively, prior to the initiation of DNA synthesis. While these values are lower than those available from heterogeneous diploid populations, ($4.9 \pm 0.60 \times 10^{-14}$ g; Ogur, Minckler, Lindegren and Lindegren, 1952), the discrepancy is undoubtedly contributed to by the difficulty in estimating the number of replicated cells in an asynchronous culture. Moreover, the amount of cytoplasmic DNA may be variable.

The 3.3Kr X-ray induction of his1 prototrophs at different times in the cell cycle of Z2367 is shown in Figure 1A. These values were not corrected for control revertants. The number of prototrophs per 10^6 cells falls sharply as a function of preincubation time in YEPD and attains a minimal level at 180 min. A comparison with the period of DNA synthesis in this same culture (Figure 1B) indicates that the minimum coincides with the initiation of DNA replication.

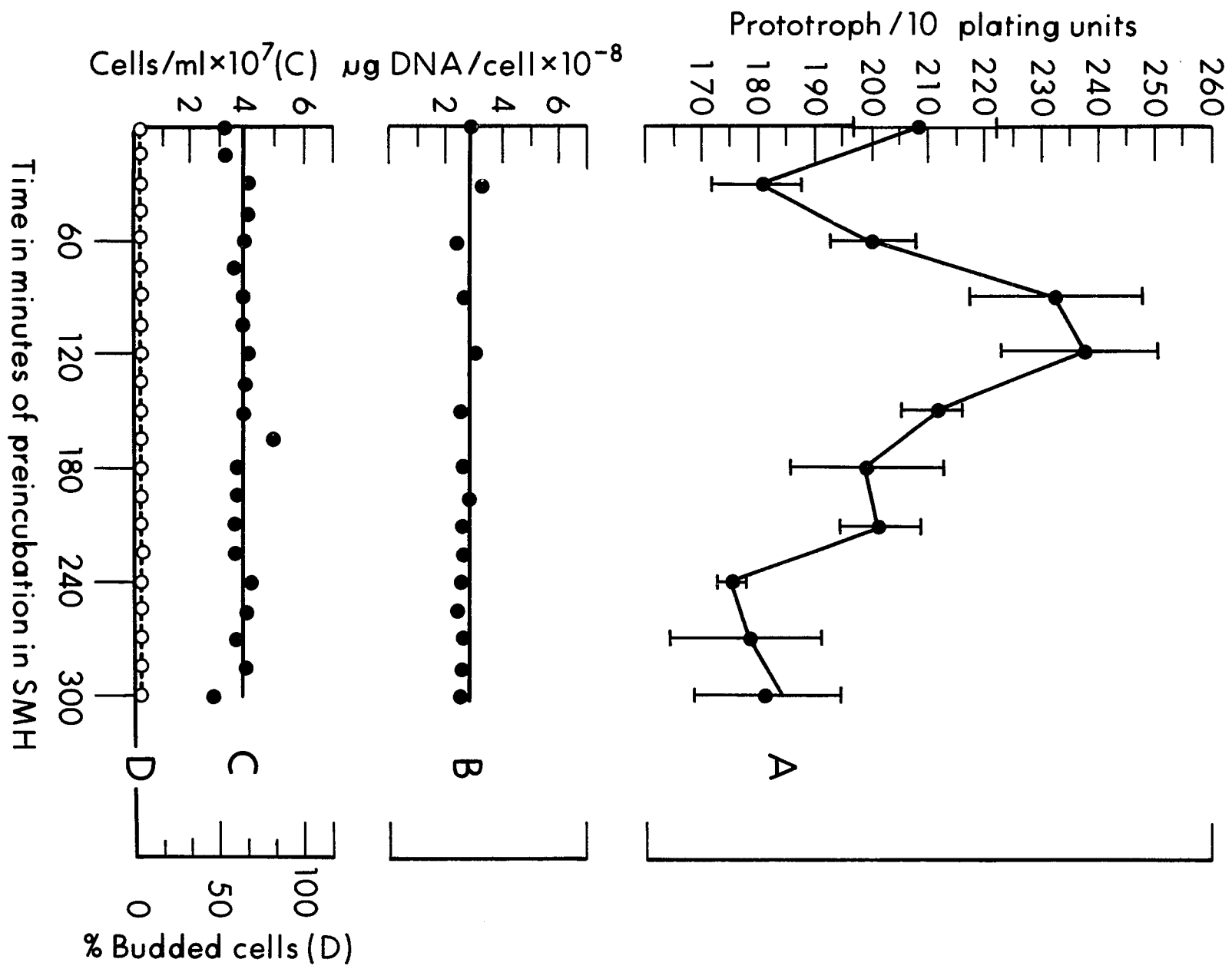
Similar curves were observed by Esposito (1967) for the tyrosine-1 cistron after treating a heteroallelic diploid with non-lethal doses of ultraviolet light. However, the

number of prototrophs recovered during the cell cycle could be differentially increased with the addition of a small amount of tyrosine (2 μ g per ml) to the tyrosineless plating medium. The resultant curve showed a maximum number of prototrophs just prior to DNA synthesis. This level fell during replication and was minimal at the completion of replication.

The effect of histidine supplementation on his1 prototroph induction by 3.3Kr X-rays was studied with cultures of Z2367 preincubated in starvation medium supplemented with histidine (SMH). The results are shown in Figure 2. The number of prototrophs varies with the time of preincubation in this medium; a peak in the number of prototrophs is observed at 120 min followed by a decrease and a subsequent minimum at 240 min. This general pattern is unaltered when the 90% confidence limits are computed (Figure 2).

Figure 2 shows however, that neither cell division nor DNA synthesis occurs in starvation medium supplemented with histidine. Thus, it may be concluded that the variation in prototroph number is not directly dependent on the DNA cycle. Rather, a variation in the activity of some other physical or biochemical process is indicated.

Figure 2. Relation of HIS prototroph number to DNA content and cell number in diploid Z2367 in starvation medium with added histidine. Prototrophs (upper curve A) were induced with 3.3Kr X-rays. DNA content (middle curve B) and cell number (bottom curve C, D) were measured in untreated samples. Error bars are 90% confidence limits.



MEIOTIC ANALYSIS OF X-RAY INDUCED PROTOTROPHIC PEDIGREES

The distribution of genotypes among pedigrees containing at least one HIS prototrophic cell was studied at different times in the cell cycle and with different X-ray doses. Synchronous prereplicative cultures of Z2367 were either treated directly with 3.3Kr or 6.6Kr X-rays, or preincubated in YEPD for 60 min or starvation medium with added histidine for 270 min prior to irradiation with 3.3Kr. A total of 268 independently arising prototrophic pedigrees were isolated and subsequently analyzed in the four experiments.

The clones arising from the mother cell, first bud and any additional phenotypically distinct product were sporulated and dissected. The derived genotypes are shown in Tables 1-4. The cell order is arbitrarily chosen since the prototrophic unit was frequently detected after the bud had attained the size of the mother cell. A single polyploid pedigree is not included.

The 268 pedigrees could be distributed into 98 types, probably reflecting both the unrelatedness and the highly heterogeneous nature of the events. These were further grouped into three general categories possessing the following characteristics. Class I: pedigrees consisting entirely of progeny prototrophic at the his locus. Class II: pedigrees

TABLE I. Genotypes* of X-ray induced prototrophic pedigrees from diploid Z2367.**

		<u>+</u>	<u>his1-315</u>	<u>+</u>	<u>arg6</u>	<u>try2</u>		
		<u>thr3-1</u>	<u>+</u>	<u>his1-1</u>	<u>+</u>	<u>+</u>		
		<u>Parental</u>						
		<u>+</u>	<u>-</u>	<u>+</u>	<u>-</u>	<u>-</u>		
		<u>-</u>	<u>+</u>	<u>-</u>	<u>+</u>	<u>+</u>		
Type	Mother cell	<u>Number of pedigrees</u>					Total	Number with lethal sector†
		6.6Kr	3.3Kr	YEPD	SMH			
1	<u>+(+)+ - -</u> <u>- + - + +</u>	7	10	21	7	45		10
2	<u>+(+)+ - -</u> <u>(+)+ - + +</u>	0	1	0	0	1		0
3	<u>+(+)+ - -</u> <u>-(- +)+ +</u>	1	0	0	0	1		0
4	<u>+(+)+ - -</u> <u>(+ - +)+ +</u>	0	0	0	1	1		0
5	<u>+(+)+ - -</u> <u>(+ - + - -)</u>	0	0	0	1	1		1
6	<u>+(+)+ - -</u> <u>- +(+)+ +</u>	1	0	2	0	3		0
7	<u>+ - + - -</u> <u>- +(+)+ +</u>	9	8	9	7	33		9
8	<u>+ - + - (+)</u> <u>- +(+)+ +</u>	0	0	1	0	1		0
9	<u>(-) - + - -</u> <u>- +(+)+ +</u>	0	0	1	0	1		0
10	<u>+(+ -) - -</u> <u>- +(+)+ +</u>	1	0	0	1	2		1
11	<u>+(+ -) - (+)</u> <u>- +(+)+ +</u>	1	0	0	0	1		0
12	<u>(- +)+ - -</u> <u>(+)+ - + +</u>	1	3	2	0	6		2
13	<u>(- +)+ - -</u> <u>- + - + +</u>	1	1	4	1	7		3
14	<u>+ - +(+ +)</u> <u>- +(+ - -)</u>	2	2	1	1	6		2
15	<u>+ - + - -</u> <u>- +(+ - -)</u>	0	1	0	1	2		1
16	<u>+ - (- + +)</u> <u>- +(+ - -)</u>	0	2	1	1	4		2
17	<u>- + + - -</u> <u>+ + + + +</u>	0	0	1	0	1		0

TABLE 1. Continued

		<u>+</u>	<u>his1-315</u>	<u>+</u>	<u>arg6</u>	<u>try2</u>		
		<u>thr3-1</u>	<u>+</u>	<u>his1-1</u>	<u>+</u>	<u>+</u>		
		<u>Parental</u>						
		<u>+</u>	<u>-</u>	<u>+</u>	<u>-</u>	<u>-</u>		
		<u>-</u>	<u>+</u>	<u>-</u>	<u>+</u>	<u>+</u>		
		<u>Number of pedigrees</u>						
<u>Type</u>	<u>Mother cell</u>	<u>6.6Kr</u>	<u>3.3Kr</u>	<u>YEPD</u>	<u>SMH</u>	<u>Total</u>	<u>Number with lethal sector†</u>	
18	<u>(-)- + - -</u> <u>(+)(+)+ +</u>	1	0	2	1	4	1	
19	<u>+(+)(+ +)</u> <u>- + -(- -)</u>	1	1	0	0	2	1	
Total Class I-A		26	29	45	22	122	33	

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands, where the recovered strands are identifiable.

** The table describes the genotypes of pedigrees with diploid progeny cells of the same type.

† Includes lethal sectoring of any of the first four division products.

TABLE 2. Genotypes* of X-ray derived pedigrees with two unique HIS prototrophic progeny cells from diploid Z2367.

	<u>+</u>	<u>his1-315</u>	<u>+</u>	<u>arg6</u>	<u>try2</u>						
	thr3-1	+	his1-1	+	+						
	<u>Parental</u>					<u>Number of Pedigrees</u>				Number with lethal sector**	
	<u>+</u>	<u>-</u>	<u>+</u>	<u>-</u>	<u>-</u>	6.6Kr	3.3Kr	YEPD	SMH		
	-	+	-	+	+						
Type	Mother	cell	First	bud					Total	sector**	
20	<u>+(+) + - -</u>	<u>(- +) + - -</u>	1	0	0	0	1	0			
	<u>(+) + - + +</u>	<u>- + - + +</u>									
21	<u>+(+) + - -</u>	<u>(- +) + - -</u>	1	0	0	0	1	0			
	<u>- + - + +</u>	<u>- + - + +</u>									
22	<u>+(+) + - -</u>	<u>(- +) + - -</u>	1	0	0	0	1	0			
	<u>(+ -) - + +</u>	<u>- (-) - + +</u>									
23	<u>+(+) + - -</u>	<u>+ - + (+ +)</u>	0	0	1	0	1	1			
	<u>- (- +) + +</u>	<u>- + (+ - -)</u>									
24	<u>+(+) + - -</u>	<u>(+ - +) + +</u>	1	0	0	0	1	0			
	<u>+ - + - -</u>	<u>- + (+) + +</u>									
25	<u>+(+) + - -</u>	<u>+ - + - -</u>	1	0	0	0	1	0			
	<u>- (- +) + +</u>	<u>- + (+) + +</u>									
26	<u>+(+) + - -</u>	<u>+ (+ -) - -</u>	0	0	1	1	2	0			
	<u>- + - + +</u>	<u>- + (+) + +</u>									
27	<u>+(+) + - -</u>	<u>(- +) + - -</u>	0	1	0	0	1	0			
	<u>- + (+) + +</u>	<u>- + (+) + +</u>									
28	<u>+(+) + - -</u>	<u>+ (+) + (+ +)</u>	0	0	0	1	1	0			
	<u>(+) + - + +</u>	<u>(+) + - (- -)</u>									
29	<u>+ - + - -</u>	<u>+ - + - (+)</u>	0	1	0	0	1	0			
	<u>- + (+) + +</u>	<u>- + (+) + +</u>									
30	<u>(- +) + - -</u>	<u>(- +) + - -</u>	2	1	1	1	5	1			
	<u>(+) + - + +</u>	<u>- + - + +</u>									
31	<u>(- +) + - -</u>	<u>+ - (- + +)</u>	1	0	0	0	1	0			
	<u>- (-) - + +</u>	<u>- + (+ - -)</u>									
Total Class I-B						8	3	3	3	17	2
32	<u>+(+) + - -</u>	<u>+(+) + - -</u>	0	1	0	0	1	0			
	<u>- + - + +</u>	<u>- (- +) + +</u>									
33	<u>+(+) + - -</u>	<u>+(+) + - -</u>	0	1	1	0	2	1			
	<u>- + - + +</u>	<u>- + (+) + +</u>									
34	<u>+(+) + - -</u>	<u>+ - + - -</u>	0	1	0	0	1	0			
	<u>- + (+) + +</u>	<u>- + (+) + +</u>									
35	<u>+ - + - -</u>	<u>+ - (-) - -</u>	0	2	0	0	2	0			
	<u>- + (+) + +</u>	<u>- + (+) + +</u>									

TABLE 2. Continued

	<u>+</u>	<u>his1-315</u>	<u>+</u>	<u>arg6</u>	<u>try2</u>						
	thr3-1	+	his1-1	+	+						
	<u>Parental</u>										Number with lethal sector**
	<u>+</u>	<u>-</u>	<u>+</u>	<u>-</u>	<u>-</u>						
	-	+	-	+	+						
Type	Mother cell	First bud	<u>Number of Pedigrees</u>					Total			
			6.6Kr	3.3Kr	YEPD	SMH					
36	<u>+</u> <u>-</u> <u>+</u> <u>(+ +)</u>	<u>(- +)</u> <u>+</u> <u>-</u> <u>-</u>	0	1	0	1	2	1			
	<u>-</u> <u>+</u> <u>(+ - -)</u>	<u>(+)</u> <u>+</u> <u>-</u> <u>+</u> <u>+</u>									
37	<u>+</u> <u>(+)</u> <u>+</u> <u>(+ +)</u>	<u>+</u> <u>-</u> <u>+</u> <u>(+ +)</u>	0	0	0	1	1	0			
	<u>-</u> <u>+</u> <u>(+ - -)</u>	<u>-</u> <u>+</u> <u>(+ - -)</u>									
38	<u>+</u> <u>-</u> <u>(- + +)</u>	<u>(- +)</u> <u>+</u> <u>-</u> <u>-</u>	0	2	1	0	3	2			
	<u>-</u> <u>+</u> <u>(+ - -)</u>	<u>(+)</u> <u>+</u> <u>-</u> <u>+</u> <u>+</u>									
39	<u>(- +)</u> <u>+</u> <u>-</u> <u>-</u>	<u>(-)</u> <u>-</u> <u>+</u> <u>-</u> <u>-</u>	0	1	1	0	2	0			
	<u>(+)</u> <u>+</u> <u>(+)</u> <u>+</u> <u>+</u>	<u>(+)</u> <u>+</u> <u>(+)</u> <u>+</u> <u>+</u>									
40	<u>+</u> <u>(+)</u> <u>+</u> <u>(+ +)</u>	<u>+</u> <u>(+)</u> <u>+</u> <u>(+ +)</u>	0	0	1	0	1	0			
	<u>-</u> <u>+</u> <u>(+ - -)</u>	<u>-</u> <u>+</u> <u>-</u> <u>(- -)</u>									
Total Class I-C			0	9	4	2	15	4			
41	<u>+</u> <u>(+)</u> <u>+</u> <u>-</u> <u>-</u>	<u>+</u> <u>-</u> <u>+</u> <u>-</u> <u>-</u>	1	0	0	0	1	0			
	<u>-</u> <u>+</u> <u>-</u> <u>+</u> <u>+</u>	<u>-</u> <u>+</u> <u>(+ - -)</u>									
42	<u>+</u> <u>(+)</u> <u>+</u> <u>-</u> <u>-</u>	<u>+</u> <u>-</u> <u>+</u> <u>-</u> <u>-</u>	0	0	1	0	1	0			
	<u>-</u> <u>+</u> <u>-</u> <u>+</u> <u>+</u>	<u>-</u> <u>+</u> <u>(+)</u> <u>+</u> <u>+</u>									
43	<u>-</u> <u>+</u> <u>(+ - -)</u>	<u>+</u> <u>-</u> <u>+</u> <u>(+ +)</u>	0	1	0	0	1	1			
	<u>-</u> <u>+</u> <u>(+ - -)</u>	<u>+</u> <u>(+)</u> <u>+</u> <u>(+ +)</u>									
Total Class I-D			1	1	1	0	3	1			

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands, where the recovered strands are identifiable.

** Includes lethal sectoring of any of the first four division products.

TABLE 3. Genotypes* of X-ray derived pedigrees with his auxotrophic and prototrophic cells from diploid Z2367.

		+ his1-315		+ arg6 try2						
		thr3-1	+	his1-1	+	+				
Type	<u>Parental</u>		First bud	<u>Number of Pedigrees</u>					Number with lethal sector**	
	Mother cell			6.6Kr	3.3Kr	YEPA	SMH	Total		
44	<u>+ (+) + - -</u>	<u>+ - + - -</u>	9	2	13	6	30	11		
	<u>- + - + +</u>	<u>- + - + +</u>								
45	<u>+ (+) + - (+)</u>	<u>+ - + - -</u>	1	0	0	0	1	0		
	<u>- + - + (-)</u>	<u>- + - + +</u>								
46	<u>+ (+) + - -</u>	<u>+ - + - -</u>	1	0	1	0	2	1		
	<u>(+) + - + +</u>	<u>- + - + +</u>								
47	<u>+ (+) + - -</u>	<u>+ - + - -</u>	0	1	0	0	1	0		
	<u>(+) + - + +</u>	<u>(+) + - + +</u>								
48	<u>+ (+) + - -</u>	<u>+ - (-) - -</u>	0	0	1	0	1	0		
	<u>- + - + +</u>	<u>- + - + +</u>								
49	<u>+ (+) + - -</u>	<u>+ (+ -) - -</u>	0	1	0	0	1	0		
	<u>- + - + +</u>	<u>- (- +) + +</u>								
50	<u>+ (+) + - -</u>	<u>(-) - + - -</u>	0	0	0	1	1	1		
	<u>- + - + +</u>	<u>- + - + +</u>								
51	<u>+ (+) + - -</u>	<u>+ - + - -</u>	1	0	0	0	1	0		
	<u>- + - + +</u>	<u>- (-) - + +</u>								
52	<u>+ (+) + - -</u>	<u>+ - + - -</u>	1	0	1	1	3	0		
	<u>- (- +) + +</u>	<u>- (- +) + +</u>								
53	<u>+ (+) + - -</u>	<u>(+) + - + +</u>	1	0	0	0	1	1		
	<u>(-) - + - -</u>	<u>(+) + - + +</u>								
54	<u>+ (+) + - -</u>	<u>+ - + - -</u>	0	0	0	1	1	0		
	<u>- (- +) + +</u>	<u>- + - + +</u>								
55	<u>+ (+) + - -</u>	<u>+ (+ -) - -</u>	0	1	0	0	1	0		
	<u>- (- +) + +</u>	<u>- (-) - + +</u>								
56	<u>+ (+) + - -</u>	<u>(+ - +) + +</u>	1	0	0	0	1	0		
	<u>+ (+) + - -</u>	<u>(+ - +) + +</u>								
57	<u>+ (+) + - -</u>	<u>- + - + +</u>	1	0	0	0	1	0		
	<u>+ (+) + - -</u>	<u>- + - + +</u>								
58	<u>+ (+) + - -</u>	<u>(+) + - + +</u>	1	0	0	0	1	0		
	<u>(- +) + - -</u>	<u>- + - + +</u>								
59	<u>+ (+) + - -</u>	<u>- + - + +</u>	1	0	0	1	2	1		
	<u>(- +) + - -</u>	<u>- + - + +</u>								
60	<u>+ (+) + - -</u>	<u>+ (+ -) - -</u>	1	0	0	1	2	1		
	<u>- + (+) + +</u>	<u>- + - + +</u>								
61	<u>+ - + - -</u>	<u>+ - + - -</u>	1	3	2	2	8	2		
	<u>- + (+) + +</u>	<u>- + - + +</u>								

TABLE 3. Continued

		+	his1-315	+	arg6	try2			
		thr3-1	+	his1-1	+	+			
		<u>Parental</u>							
		+	-	+	-	-			
		-	+	-	+	+			
Type	Mother cell	First bud	<u>Number of Pedigrees</u>				Total	Number with lethal sector**	
			6.6Kr	3.3Kr	YEPD	SMH			
62	<u>+ - + - -</u>	<u>+ - + - -</u>	1	0	1	1	3	2	
	<u>- + (+) + +</u>	<u>- (- +) + +</u>							
63	<u>- + (+) + +</u>	<u>+ - + - -</u>	1	0	0	0	1	1	
	<u>(+ - +) + +</u>	<u>+ - + - -</u>							
64	<u>+ - + - -</u>	<u>+ - + - -</u>	0	0	1	0	1	0	
	<u>- + (+) + +</u>	<u>(+ - + - -)</u>							
65	<u>+ - + - -</u>	<u>+ (+ -) - -</u>	0	0	1	2	3	0	
	<u>- + (+) + +</u>	<u>- + - + +</u>							
66	<u>+ - + - -</u>	<u>+ - + - -</u>	0	0	1	0	1	1	
	<u>- + (+) + +</u>	<u>(+ - +) + +</u>							
67	<u>+ - (-) - -</u>	<u>+ - + - -</u>	0	0	1	0	1	0	
	<u>- + (+) + +</u>	<u>- + - + +</u>							
68	<u>(- +) + (+ +)</u>	<u>(-) - + (+ +)</u>	0	1	0	0	1	0	
	<u>- + - (- -)</u>	<u>- + - (- -)</u>							
69	<u>+ (+) + (+ +)</u>	<u>(-) - + - -</u>	0	1	0	0	1	0	
	<u>- + (+) + +</u>	<u>- + - (- -)</u>							
70	<u>(- +) + - -</u>	<u>(-) - + - -</u>	2	0	2	1	5	0	
	<u>(+) + - + +</u>	<u>(+) + - + +</u>							
71	<u>(- +) + - -</u>	<u>+ - + - -</u>	0	1	2	1	4	1	
	<u>(+) + - + +</u>	<u>- + - + +</u>							
72	<u>(- +) + - -</u>	<u>(-) - + - -</u>	1	1	0	0	2	1	
	<u>- + - + +</u>	<u>- + - + +</u>							
73	<u>(- +) + - -</u>	<u>(- + -) - -</u>	0	0	1	1	2	1	
	<u>- + - + +</u>	<u>- + - + +</u>							
74	<u>(- +) + - -</u>	<u>- + - + +</u>	0	1	0	0	1	0	
	<u>+ (+ - + +)</u>	<u>- + - + +</u>							
75	<u>(- +) + - -</u>	<u>(+) + - + +</u>	1	0	0	0	1	0	
	<u>+ - + - -</u>	<u>(+) + - + +</u>							
76	<u>+ - + (+ +)</u>	<u>+ - + (+ +)</u>	0	0	0	1	1	1	
	<u>- + (+ - -)</u>	<u>- + - (- -)</u>							
77	<u>+ - + (+ +)</u>	<u>+ - + (+ +)</u>	0	1	0	0	1	0	
	<u>- + (+ - -)</u>	<u>- (- + - -)</u>							
78	<u>(- +) + - -</u>	<u>(+) + - + +</u>	0	1	0	0	1	0	
	<u>(- +) + - -</u>	<u>(+) + - + +</u>							
79	<u>+ - (- + +)</u>	<u>+ - + (+ +)</u>	0	1	0	0	1	0	
	<u>- + (+ - -)</u>	<u>- + - (- -)</u>							

TABLE 3. Continued

		+	his1-315	+	arg6	try2			
		thr3-1	+	his1-1	+	+			
		<u>Parental</u>							
		+	-	+	-	-			
		-	+	-	+	+			
Type	Mother cell	First bud	<u>Number of pedigrees</u>				Total	Number with lethal sector**	
			6.6Kr	3.3Kr	YEPD	SMH			
80	<u>+(+)(+ +)</u> - + -(- -)	<u>+(+ - + +)</u> - + -(- -)	1	0	0	0	1	0	
81	<u>+(+)(+ +)</u> - + - + +	<u>+(+ -) - -</u> - + -(- -)	1	0	0	0	1	0	
82	<u>+(+)(+ +)</u> - + -(- -)	<u>+ - + - -</u> - + - + +	0	1	0	0	1	0	
83	<u>+(+)(+ +)</u> - + -(- -)	<u>+ - +(+ +)</u> - + -(- -)	0	1	0	1	2	1	
84	<u>(- + -) - -</u> (+)(+)+ +	<u>(- + -) - -</u> - + - + +	0	0	1	0	1	0	
85	<u>+(+ -) - -</u> (+)(+)+ +	<u>+(+ -) - -</u> (+)+ - + +	0	0	1	0	1	0	
86	<u>+(+ -) - -</u> (+)(+)+ +	<u>+ - + - -</u> - + - + +	0	0	0	1	1	1	
87	<u>+ - + - -</u> (+)(+)+ +	<u>+ - + - -</u> - + - + +	0	0	0	1	1	1	
88	<u>+ - + - -</u> (+)(+)+ +	<u>(-) - + - -</u> - + - + +	0	1	0	0	1	0	
Total Class II			28	19	30	23	100	29	

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands.

** Includes lethal sectoring of any of the first four division products.

TABLE 4. Genotypes* of X-ray induced HIS prototroph-containing pedigrees with three unique progeny cells from diploid Z2367.

+ thr3-1 his1-315 + + his1-1 arg6 + try2 +										
Parental + - + - - - + - + +										
Number of Pedigrees 6.6Kr 3.3Kr YEPD SMH Total Number with lethal sector**										
Type	Mother cell	First bud	Other		6.6Kr	3.3Kr	YEPD	SMH	Total	Number with lethal sector**
89	+(+)+ - - (- +)+ - -	(+)+ - + + - + - + +	- + - + + - + - + +	†	1	1	0	0	2	1
90	(- +)+ - - - + - + +	+ - +(+ +) - + - (- -)	+(+)+ - - - + - + +	†	1	0	0	0	1	0
91	+(+)+ - - +(+)+ - -	(+)+ - + + - + - + +	+(+)+ - - (+)+ - +(-)	‡	1	0	0	0	1	0
92	+ - (- + +) - + (+ - -)	- + - (- -) + - +(+ +)	(- +)+ - - (+)+ - + +		1	0	0	0	1	1
93	+(+)+ - - - + - + +	(-)- + - - - + - + +	+ - + - - - + - + +		0	0	0	1	1	0
94	+(+)+(+ +) - + (+)+ +	+(+ -)- - +(+ -)- -	+(+ -)- - - + - (- -)	†	0	0	1	0	1	0
95	+(+)+ - - - + - + +	+(+ - + +) - + - (- -)	+ - + - - - + - + +	‡	0	0	1	0	1	0
96	- + + - - + - + - -	- + + - - + + + - -	- + + + - + + + - - - + + + + + + + + +		0	0	1	0	1	1
97	+(+)+ - - - + - + +	+ - + - - - + - + +	+(+ -)- - - + - + +		0	0	1	0	1	0
98	(- + +(+ +)) - + - (- -)	(-)- + - - - + - + +	+ - + - - - + - + +		0	0	1	0	1	0
Total Class III					4	1	5	1	11	3

TABLE 4. Continued

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands, where the recovered strands are identifiable.

** Includes lethal sectoring of any of the first four division products.

† Isolated from first bud.

‡ Isolated from mother cell.

containing his auxotrophic as well as prototrophic cells.

Class III: pedigrees containing three or more cells genetically distinct at the his locus or for the markers flanking the his locus.

Among the pedigrees comprising the first class, the predominant types are those in which the mother and daughter cells possess the same parental his allele and the same outside markers. These represent subgroup A of Class I.

The remainder were distributable into three additional subgroups. Class I-B includes pedigrees where the same his allele accompanies the prototrophic strand but different flanking marker arrangements characterize the mother and daughter cells. Class I-C consists of pedigrees which have identical flanking marker arrays, but different his alleles are present in the mother and daughter cells. Class I-D pedigrees contain progeny cells that differ with respect to both the accompanying his allele and the flanking markers.

The same general distribution is observed irrespective of the X-ray dose or the particular cell cycle stage from which the prototrophs were derived. In all instances the number of Class I pedigrees (total 157) is greater than or equal to the combined number in Class II and Class III (total 111). However, the absence of an his auxotrophic cell

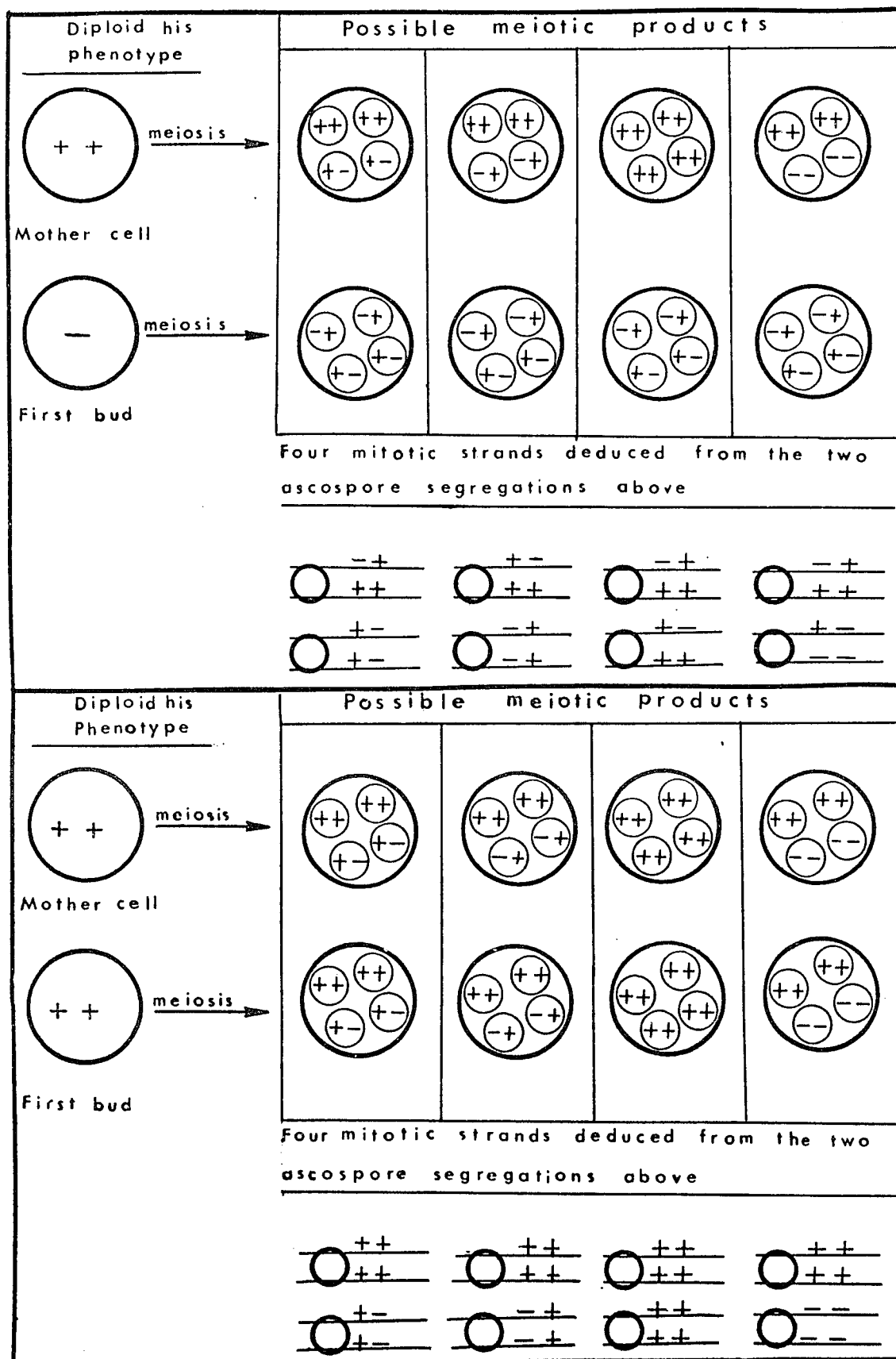
in the Class I data cannot be attributed to a high pre-existing prototrophic titer. In the present experiments the spontaneous prototroph titers never exceeded 10 per 10^6 cells. The total number of cells observed was approximately 1.8×10^6 . Thus, approximately 18 of the 268 pedigrees can be accounted for in this way. Similarly, Class I is not totally explainable by first division lethal cell sectoring. While single cell deaths among the first four cell products were detected in 72 of the 268 pedigrees, these are random with respect to the three classes. These data are included in Tables 1-4.

From their analyses of mitotic recombinants, Wilkie and Lewis (1963) and Hurst and Fogel (1964) concluded that mitotic recombinational events originate through a chromosomal pairing mechanism similar to the one occurring during meiosis. The 268 mitotic HIS pedigrees will therefore be examined for the primary genetic patterns observed among meiotically arising HIS prototrophs (Fogel and Hurst, 1967).

Origin of HIS Prototrophs

The genotypes of both clones obtained from the mother cell and first bud were resolved by tetrad analysis and allele identification tests. Figure 3 illustrates possible meiotic his segregations when the pedigree contains either HIS prototrophic and auxotrophic progeny or only HIS

Figure 3. Typical meiotic his segregations in tetrads from clones arising from the mother cell and first bud. Ascospore genotypes are illustrated for pedigrees with prototrophic and auxotrophic progeny (above) or only prototrophic decedents (bottom). The genotype of each diploid clone was determined from the meiotic segregations. The four strands at the first mitotic metaphase were deduced from the genotypes of the mother cell and first bud. It is assumed that additional exchanges with the centromere did not occur. ++ is prototroph, - is auxotroph, -+ is his1-315 +, +- is + his1-1.



prototrophic derivatives. The four strands that were probably present at the first mitotic metaphase, inferred from tetrads derived from the two diploid clones, are also shown in Figure 3. In the following analysis reference is made to the his allele distribution among these four mitotic strands.

Table 5 shows the number of pedigrees containing the double mutant strand expected from an exchange between the two alleles, as well as the number displaying proximal or distal allele conversion. These values are separately summed for each class in each experiment. The analysis classifies only those pedigrees which are uniquely specified when either the his allele distribution or the flanking marker arrays is considered (total 236). The remaining 32 are scored as uncategorized in Table 5 and they will be described separately. Of the 236 pedigrees, 72 (type 36, Class I-C, Table 2; types 41, 42, Class I-D, Table 2; types 44-47, 49, 50, 53, 61, 69-72, 75, 76, 79, 82, 83, 86-88, Class II, Table 3; types 90, 93, Class III, Table 4) were most simply characterized on the basis of all of the recovered input his alleles as follows: Reciprocal Recombinants: Pedigree genotypes containing one prototrophic strand, one double mutant strand and one each of the parental his alleles. The flanking markers are reciprocally

TABLE 5. Classification of the 268 X-ray induced HIS prototroph-containing pedigrees of Tables 1-4.

Class	6.6Kr					3.3Kr					YEPD					SMH					Total				
	Cp	Cd	Cpd	R	U	Cp	Cd	Cpd	R	U	Cp	Cd	Cpd	R	U	Cp	Cd	Cpd	R	U	Cp	Cd	Cpd	R	U
I-A	10	12	1	0	3	16	10	0	2	1	27	14	3	1	0	9	9	0	1	3	62	45	4	4	7
I-B	4	0	0	2	2	1	1	1	0	0	1	0	0	0	2	2	0	0	0	1	8	1	1	2	5
I-C	0	0	0	0	0	0	0	4	2	3	0	0	3	1	0	0	0	2	0	0	0	0	9	3	3
I-D	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	0	0
II	21	3	0	0	4	9	5	1	1	4	22	5	0	0	3	13	5	0	0	5	65	18	1	1	15
III	3	0	0	1	0	1	0	0	0	0	2	0	1	0	2	1	0	0	0	0	7	0	1	1	2
Total	38	15	2	3	9	27	16	7	5	7	52	19	8	2	7	25	14	2	1	9	142	64	19	11	32

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* Convention on notation:

Cp = Proximal allele conversion
Cd = Distal allele conversion
Cpd = Proximal-distal allele conversion
R = Reciprocal recombinant
U = Uncategorized

recombined in the prototrophic and double mutant strands, and both alleles exhibit $2^+ : 2^-$ segregation. Reciprocal recombinant pedigrees will contain the following genotypes: ++, 315,1, 315 +, + 1. Proximal Allele Convertants: Pedigrees with one prototrophic strand, one proximal allele and two distal alleles. These imply segregations of $3^+ : 1^-$ for the proximal allele accompanied by $2^+ : 2^-$ segregation for the distal allele. The genotype of such pedigrees is: ++, 315 +, + 1, + 1. Distal Allele Convertants: Pedigrees containing one prototrophic strand, one distal allele and two proximal alleles. These show segregations of $3^+ : 1^-$ for the distal allele accompanied by $2^+ : 2^-$ segregation of the proximal allele. Distal convertant pedigrees are represented as: ++, + 1, 315 +, 315 +. Proximal-Distal Allele Convertants: Pedigrees containing two prototrophic strands, one distal allele and one proximal allele. These show $3^+ : 1^-$ segregations for both the proximal and distal alleles and contain the following genotype: ++, ++, + 1, 315 +. Where a parentally marked prototrophic chromosome (total 47/72) can be used to specify the conversional event, the assignment is identical to the one based on the above scheme.

The remaining 164 classified pedigrees require that additional assumptions be made. Pedigrees where the genotype

distributions ++, ++, + 1, + 1; ++, ++, 315 +, 315 +; ++, ++, ++, ++, or ++, ++, 315,1, 315,1 define the four strands recovered from the mother cell and first bud, comprise 135 of these (types 1, 2, 5-9, 12-14, 16-19, Class I-A, Table 1; types 20-22, 27-31, Class I-B, Table 2; Types 57-59, 78, Class II, Table 3; type 89, Class III Table 4). All of the progeny cells show the same his genotypes.

The proportion of lethal sectoring in this fraction (34/135 or 25.2%) is comparable to that in the remaining population (38/133 or 28.6%). It is likewise in agreement with the 25% frequency observed by Beam (personal communication) in unselected pedigrees derived from heterogeneous cultures of Z2367 treated with 7.2Kr X-rays. Thus induced mitotic prototroph formation does not appear to be associated with increased cell death. Moreover, the occurrence of a lethal sector does not necessarily imply the loss of a genetically distinct product. While it is unlikely that the observed his distributions are uniformly attributable to partial strand recovery, some fraction of the 135 pedigrees undoubtedly reflect both detected and undetected nuclear or cellular death.

Parental flanking marker combinations are associated with both the prototrophic chromosomes and the accompanying his auxotrophic strands in 80 of the 135 pedigrees (types

1, 5, 7, 57). Since a parental relationship is observed between the flanking markers and the recovered his allele as well, the prototrophic event is most simply interpreted from the accompanying allele. Thus with one exception (type 5), the 80 such pedigrees have been described in Table 5 as proximal convertants or distal convertants when the respective allele was his1-1 or his1-315. The results are entirely in agreement when the assignment is made from the prototrophic chromosome. The type 5 pedigree is exceptional in that the isolated diploid cells are homozygous for each of the three linked loci. This pattern presumably reflects the observed cell death in the single pedigree of this type. It is therefore scored in Table 5 according to the flanking marker arrays in the prototrophic strand.

Pedigrees of this general type containing only HIS prototrophic strands (type 6; total 3) could be scored as proximal-distal convertants, consistent with the recovery of both parental marker arrays.

The remainder of the 135 pedigrees (types 2, 7-9, 12-14, 16-22, 27-31, 58, 59, 78, 79; total 52) contain one or more strands with non-parental arrangements of outside markers. Where the reciprocal array is recovered (types 12, 14, 16-22, 58, 78, 79; total 31) the pedigree has been defined by the

parental his allele. The his allele has likewise been used to specify 21 non-parental pedigrees (types 2, 8, 9, 13, 27-31, 59) in which a reciprocally related product is not present. One or both of the recovered prototrophic strands display parental marker combinations in 14 of these. The arrays are consistent with the his allele assignment. Lethal sectoring was observed in only 5 such pedigrees (3 of 7 type 13; 1 of 5 type 31; 1 of 2 type 59).

Multiple events at the his cistron can be detected in the remainder of the 237 pedigrees. Those containing three prototrophic strands and one proximal or one distal allele (types 33, 34, 37, 39, 40, Class I-C, Table 2; type 43, Class I-D, Table 2; type 96, Class III, Table 4; total 19), or two prototrophic strands, one double mutant strand and one distal allele (type 38, Class I-C, Table 2; total 3) exhibit the following genotypes: ++, ++, ++, 315 +; ++, ++, ++, + 1, ++, ++, 315,1, + 1. Two prototrophic strands invariably show identical outside marker arrays, even where non-parental arrangements are observed. Pedigrees displaying the first two his genotypes have been categorized as proximal-distal allele convertants. While $3^+:1^-$ segregations for the proximal allele characterize pedigrees of the third genotype, one specifiable event results in the formation of reciprocally

related products at the his cistron. Since the resultant double mutant chromosome and recombinant marker arrays are compatible with the criteria used to designate reciprocal recombinants, this pedigree type has arbitrarily been scored in this category in Table 5. Pedigrees containing only one prototroph strand but in which $1^+ : 3^-$ segregations for the second allele are observed as well (types 48, 62-64, 66, 73, 77, 80, 81, 84, 85, Class II, Table 3; total 14) display the following genotypes: ++, 315,1, + 1, + 1, ++, 315 +, 315 +, 315 +; ++, + 1, + 1, + 1. With the exception of type 64, the centromere linked ura3 marker segregated normally. These are categorized in Table 5 according to the event associated with the formation of the prototrophic strand. Such classifications are most simply derived from both the flanking marker combinations and the his allele distribution. The remaining pedigrees each display three progeny cells with different his genotypes (types 91, 92, 95, 97, Class III, Table 3; total 4). The following pairs of histidine strands were recovered: Cell 1. ++ / ++, Cell 2. ++ / + 1, Cell 3. + 1 / + 1 (type 91); Cell 1. ++ / + 1, Cell 2. 315 + / + 1, Cell 3. + 1 / + 1 (types 95, 97); Cell 1. ++ / 315,1, Cell 2. ++ / + 1, Cell 3. 315 + / + 1 (type 92). Since mitotic chromosome assortment

is expected to be random, the four strands participating in prototroph formation cannot be uniquely identified. However, the first two will be classified as proximal convertants when either prototrophic-auxotrophic cell combination is analyzed. The third type is arbitrarily scored as a reciprocal recombinant.

Assuming random centromere disjunction, reciprocally related strands originating during or subsequent to DNA duplication will segregate to different daughter cells one-half the time. The actual number of reciprocal recombinant pedigrees may therefore be underestimated in Table 5 if the resultant doubly mutant cell is itself non-viable and fails to produce a viable bud. Nevertheless, it is apparent that double mutant strands accompany the prototrophic chromosome in only a small fraction of the categorized pedigrees. This proportion varies from 5.2% (3/58) at 6.6Kr to 9.3% (5/54) at 3.3Kr to 2.4% in 3.3Kr cultures preincubated in YEPD (2/82) or in starvation medium with histidine (1/42). Thus non-reciprocal events for one or both of the alleles (total 225) were more than 20 times as numerous as those generating a double mutant strand (total 11). The proportion of such pedigrees (11/236 or 4.7%) is not significantly different from the number of spontaneous prototrophic asci observed in this diploid to contain double mutant spores (22/290

or 7.6%, Fogel and Hurst, 1967). Apparently, X-rays do not enhance the relative frequency of reciprocal inter-allelic events.

The four populations are homogeneous with respect to the relative number of proximal and distal conversions and may be treated as a single sample. The summed data (last columns Table 5) contain 142 proximal convertants, 64 distal convertants, 19 proximal-distal convertants and 11 reciprocal recombinants. Thirty-two pedigrees are uncategorized. Two-thirds of the non-reciprocal prototrophs originate through conversions involving the proximal allele (161/225). The observed proximal polarization, mainly from Class II, is consistent with that reported for this cistron among meiotic prototrophs (Fogel and Hurst, 1967).

The respective conversion frequencies of the proximal and distal heteroalleles in Class I-A (Table 5 last columns) however, are 59.5% (66/111 or 62 proximal convertants and 4 proximal-distal convertants) and 44.2% (49/111 or 45 distal convertants and 4 proximal-distal convertants). These values are not significantly different. Thus conversion is equally frequent at both sites among pedigrees of Class I-A. The absence of polarity is suggested as well by the equal frequency with which each parental marker array is associated

with prototrophy (50 and 38 for the arrays associated with the proximal and distal sites, respectively, in the parent diploid). Although a slight excess of proximal allele convertants appears in the YEPD-preincubated culture (30:17), the chi square value is not significant at the 0.05 level. Class II however is characterized by an asymmetric distribution of the two parentally marked prototrophic chromosomes. Thus the marker combination initially associated with the proximal his allele is recovered nearly three times as often as the complementary input array (42:15, respectively). This inequality is likewise apparent in Table 5 where conversions of the proximal allele (total 66) are three times as frequent as of the distal allele (total 19). The number of proximal to distal conversions ranges from 10:6 and 13:5 at 3.3Kr and in starvation medium with added histidine, respectively, to 21:3 and 22:5 at 6.6 Kr and in YEPD, respectively. These ratios, which include proximal-distal as well as single site conversional events, do not differ significantly.

Although polarity may be detected among the entire Class I pedigrees when proximal-distal conversions are not considered (70 proximal conversions: 46 distal conversions), the fraction of proximal convertants is significantly less than in Class II. Thus polarity in the total data is contributed to mainly by the Class II pedigrees.

Twenty-one pedigrees in addition to the 19 proximal-distal convertants require a second event to have occurred at the his cistron. However this does not necessarily result in a second unique HIS prototrophic strand. Such pedigrees were arbitrarily scored in Table 5 according to the event associated with prototroph formation only. Of these, three (type 38, Class I-C, Table 2) contain two prototrophic strands, one distal allele and one double mutant strand. Respective segregations of $3^+:1^-$ and $2^+:2^-$ are observed for the proximal and distal alleles. Since both prototrophic chromosomes display identical recombinant marker arrays, the two strands may be related through replication. It is suggested that proximal allele conversion as well as reciprocal recombination occurred in these pedigrees. A similar pattern is observed in two of three cells in pedigree type 92 (Class III, Table 4). Fourteen pedigrees contain only one prototrophic strand but they display aberrant segregation for both alleles (either $3^+:1^-$ accompanied by $1^+:3^-$, type 48 Class II, Table 3 or $4^+:0^-$ accompanied by $1^+:3^-$, types 62-64, 66, 73, 77, 80, 81, 84, 85, Class II, Table 3). Thus both sites appear to have undergone conversion. Moreover, the second pair of allele segregations ($4^+:0^-$ with $1^+:3^-$) implies that conversion may have occurred in two strands at the same site in some fraction

of these. The remainder of the 21 pedigrees are in Class III (types 91, 95, 97, Table 4; total 3). While the initial event cannot be unequivocally reconstructed, some information is obtained from the order in which the cells appear. Thus in type 91, the genotypes of the first two cell products are $++ / ++$, and $+1 / +1$. The third cell ($++ / +1$) derives from the prototrophic cell. In type 95, the first two division products are represented as $++ / +1$ and $+1 / +1$, while the third cell ($315+ / +1$) was subsequently isolated from the prototrophic cell. The exact order is unknown in the type 97 pedigree.

Thus as many as 40 or 16.9% of the 236 categorized pedigrees may display at least two discernible events at the his cistron.

Although parentally marked prototrophic and auxotrophic chromosomes characterized each strand in 22 (types 3, 10, 11, Class I-A, Table 1; types 25, 26, Class I-B, Table 2; types 32, 35, Class I-C, Table 2; types 51, 52, 54, 55, 60, 65, 67, Class II, Table 3) of the 32 uncategorized pedigrees, the nature of the prototrophic event cannot be precisely ascertained. With the exception of types 35, 51, 55 and 67 (total 5), which contain an accompanying double mutant strand, each pedigree displays a non-parental arrangement of the flanking

markers with an input his allele. Ten proximal convertants (types 10, 11, 26, 60, 65) 6 distal convertants (types 3, 25, 52, 54), 1 proximal-distal convertant (type 32) and 5 reciprocal recombinants (types 35, 51, 55, 67) are scored when the pedigrees are defined by the his allele distribution. Coincident exchanges involving the prototrophic chromosome are required. When the assignment is made from the prototrophic chromosome along, 8 proximal convertants (types 3, 32, 51, 52, 54, 55), 9 distal convertants (types 10, 11, 35, 65, 67) and 5 proximal-distal convertants (types 25, 26, 60) are obtained. If it is assumed that all cell products participating in prototroph formation have been recovered, then additional conversional events must be postulated. Alternatively, multiple exchanges between two auxotrophic strands, one of which is not recovered, is necessary. A similar discrepancy exists in the interpretation of 7 of the remaining 10 unclassified pedigrees (type 4, Class I-A, Table 1; types 23, 24, Class I-B, Table 2; types 56, 68, Class II, Table 3; types 94, 98, Class III, Table 4).

Analysis of Flanking Marker Arrays Among HIS Prototrophic Chromosomes

The distribution of prototrophic chromosomes within each HIS recombinant type among the three classes of categorized pedigrees is shown in Table 6. The results are both quantitatively and qualitatively similar in each

TABLE 6. Classification of prototroph chromosomes from the 236 categorized pedigrees of Tables 1-4.

+ his1-315 + arg6 thr3-1 + his1-1 +											
Class	HIS Origin	No.	Parental thr + and/or + arg	Recombinant							
				thr arg	++	+ arg thr arg	+ arg + +	thr arg + +	thr + + +	+ arg thr + thr arg	+ arg thr + thr arg + +
I-A	Cp	62	47	13	2	0	0	0	0	0	0
	Cd	45	35	6	4	0	0	0	0	0	0
	Cpd	4	3	0	0	0	0	1	0	0	0
	R *	4	0	4	0	0	0	0	0	0	0
I-B	Cp	8	0	5	0	2	1	0	0	0	0
	Cd	1	1	0	0	0	0	0	0	0	0
	Cpd	1	0	0	0	0	0	0	0	1	0
	R	2	0	1	0	1	0	0	0	0	0
I-C	Cp	0	0	0	0	0	0	0	0	0	0
	Cd	0	0	0	0	0	0	0	0	0	0
	Cpd	9	3	2	0	0	0	4	0	0	0
	R	3	0	3	0	0	0	0	0	0	0
I-D	Cp	0	0	0	0	0	0	0	0	0	0
	Cd	0	0	0	0	0	0	0	0	0	0
	Cpd	3	1	0	0	1	0	1	0	0	0
	R	0	0	0	0	0	0	0	0	0	0

TABLE 6. Continued

+ thr3-1 his1-3 + ... + arg6 +												
Class	HIS origin	No.	<u>Parental</u> thr + and/or + arg	Recombinant								
				thr arg	++	+ arg thr arg	+ arg + +	thr arg + +	thr + + +	+ arg thr + thr arg	+ arg thr + thr arg + +	
II	Cp	65	39	15	8	3	0	0	0	0	0	
	Cd	18	14	2	2	0	0	0	0	0	0	
	Cpd	1	0	0	0	0	0	0	1	0	0	
	R	1	0	1	0	0	0	0	0	0	0	
III	Cp	7	4	0	0	3	0	0	0	0	0	
	Cd	0	0	0	0	0	0	0	0	0	0	
	Cpd	1	0	0	0	0	0	0	0	0	1	
	R	1	0	1	0	0	0	0	0	0	0	
Total		236	147	53	16	10	1	6	1	1	1	

* See Table 5 for convention on notation

sample and they have been summed for this table. Homozygosis for unlinked segregating loci was detected in three pedigrees, at the ura3 locus, at the ade6 locus and at the ade6, leu1 loci. The reciprocal strand was recovered from the last two events.

A parental configuration characterizes each of the recovered prototrophic chromosomes in 147 of the 236 pedigrees (62.2%). The table does not distinguish the two possible arrangements. However, pedigrees with parentally marked prototrophic strands were categorized only when conversion occurred at the his site that was associated with that marker combination in the parent diploid. The number of each type is therefore available in Table 6 by summing proximal and proximal-distal convertants and distal and proximal-distal convertants. The respective values are 97 (+ arg) and 57 (thr +). Fourteen additional pedigrees included in Table 6 display both parental and non-parental prototroph chromosomes. The results are not altered when these are considered (+ arg 110: thr + 60).

As in a selected population of meiotic HIS prototrophs (Fogel and Hurst, 1967) the unequal recovery of parental genotypes is directly correlated with unequal conversion frequency of the two his sites. Twenty-four uncategorized pedigrees with parental arrays but a non-parental relation

of flanking markers and an input his allele are exceptional in that the prototrophic strand may not reflect the converted allele.

An asymmetric distribution of the two recombinant flanking marker combinations is likewise apparent in Table 6. Of the remaining 89 pedigrees 71 or 79.3% contain at least one prototrophic strand with the array thr arg. Forty-one of the 71 were defined as proximal his convertants, 8 as distal convertants, 11 as proximal-distal convertants and 11 as reciprocal recombinants. A prototrophic strand with the complementary ++ genotype is recovered in only 25 pedigrees (28.4%; 11 proximal convertants, 6 distal convertants, 8 proximal-distal convertants). While a reciprocally related type does not accompany the recombinant prototrophic strand in 20 of the 89 pedigrees, the direction of the inequality is maintained when these are excluded (thr arg, 57; ++, 20), as is the distribution with respect to HIS type (thr arg: 28 proximal convertants, 8 distal convertants, 10 proximal-distal convertants, 11 reciprocal recombinants; ++: 8 proximal convertants, 5 distal convertants, 7 proximal-distal convertants). Only one reciprocal exchange in the arg-try interval was observed.

Both parental configurations occur in equivalent numbers in Class I-A (+ arg, 50; thr +, 38). As in the summed data, the number with each parental configuration is obtainable from the converted allele(s) (first column Table 6). Thus an excess of one array is observed only in Class II. However, neither the proportion of pedigrees with recombinant prototroph strands (30/115 Class I-A; 32/86 Class II) nor the proportion of each recombinant type (thr arg 24/31, ++ 7/31 Class I-A; thr arg 21/32, ++ 11/32 Class II) occurs at a significantly different frequency in the two pedigree classes.

It may be concluded from these data that selection for HIS prototrophy results in a considerable number of recombinants for the adjacent unselected thr and arg markers. The absence of significant numbers of exchanges between the unlinked loci and their centromeres as well as for the linked try marker make it unlikely that the observed frequency is attributable to radiation induced enhancement of independent events. Thus mitotic prototroph formation at the his1 cistron appears to be closely associated with recombination only of the adjoining loci. Fogel and Hurst (1967) observed that about 50% (544/1081) of meiotically derived HIS prototrophic chromosomes display non-parental

arrays for the thr, arg markers. As in the mitotic population, recombination in the thr, arg interval resulted in the unequal occurrence of the two recombined configurations (thr arg, 429; ++, 115).

The data suggest that the diagnostic characteristics of a meiotic prototroph population are equally characteristic of an X-ray induced mitotic population.

The prototrophic chromosome participates in almost all of the coincident exchanges in a selected meiotic population (Fogel and Hurst, 1967). A similar analysis is not possible in the mitotic system where all relevant cell products may not have been recovered. However, a rough approximation to the frequency and distribution of mitotic recombination between accompanying auxotrophic strands can be obtained from the Class II and Class III pedigrees. Among these, 17% (16/94) show reciprocal exchanges which do not directly involve the prototrophic strand (types 49, 53, 70, 76, 77, 79, 80, 83, Class II, Table 3; types 90, 92, 95 Class III, Table 4). Equal numbers of exchanges are observed in the thr-his and his-arg regions in 13 of these (7 and 7, respectively: 12 single exchanges, 1 presumed 2-strand double exchange). The interval cannot be determined in three remaining pedigrees. The two reciprocally related auxotrophic strands occur with a non-parental prototroph strand and its complement in 12 of

the 16 pedigrees. In each of these, the prototroph strand displays the same recombinant array as one auxotrophic strand, while the remaining two show the complementary recombinant array. With the exception of pedigrees containing the double his mutant, the latter two chromosomes invariably show the same input his allele.

Irregular Segregations of Markers Flanking the HIS Cistron

The proportion of categorized HIS prototrophic pedigrees showing segregations other than $2^+:2^-$ at linked heterozygous loci is shown in Table 7. Only one pedigree displayed aberrancy for an unlinked marker. Clearly, irregular segregation for the thr3-1 site alone (total 40) is the prevalent type in the 47 such pedigrees. This results in 15 pedigrees with only the wild type or mutant allele in each recovered strand (3 and 12, respectively; types 9, 13, Class I-A, Table 1; type 28, Class I-B, Table 2; types 47, 72, 73, 85, Class II, Table 3). The remaining 25 show thr segregations of $3^+:1^-$ (types 46, 53, 63, 66, 75, 86, 87, Class II, Table 3; total 8) or $1^+:3^-$ (types 21, 27, 30, 31, Class I-B, Table 2; types 50, 59, 69, 84, Class II, Table 3; types 89, 90, 93 Class III, Table 4; total 17). Thus thr auxotrophic segregants (total 29) are generated among the 40 pedigrees at a significantly greater frequency than dominant homozygotes (total 11). Moreover, it may be seen in Table 7 that

TABLE 7. Distribution of irregular flanking marker segregations in 236 categorized HIS prototroph-containing pedigrees from Tables 1-4

			+ his1-315		+ arg6		try2	
			thr3-1	+	his1-1	+	+	+
Number of pedigrees with irregular segregation for:								
Class	HIS origin	Number	thr	try	arg	thr	arg	thr
I-A	Cp	62	7	0	0	0	1	0
	Cd	45	1	1	0	0	0	0
	Cpd	4	0	0	0	0	0	0
	R *	4	0	0	0	0	0	0
I-B	Cp	8	7	0	0	0	0	0
	Cd	1	0	1	0	0	0	0
	Cpd	1	1	0	0	0	0	0
	R	2	1	0	0	0	0	0
I-C	Cp	0	0	0	0	0	0	0
	Cd	0	0	0	0	0	0	0
	Cpd	9	0	0	0	0	0	0
	R	3	0	0	0	0	0	0
I-D	Cp	0	0	0	0	0	0	0
	Cd	0	0	0	0	0	0	0
	Cpd	3	0	0	0	1	0	0
	R	0	0	0	0	0	0	0
II	Cp	65	15	0	0	0	0	0
	Cd	18	3	0	0	0	1	0
	Cpd	1	1	0	0	0	0	0
	R	1	0	0	0	0	0	0
III	Cp	7	4	0	0	0	0	1
	Cd	0	0	0	0	0	0	0
	Cpd	1	0	0	1	0	0	0
	R	1	0	0	0	0	0	0
Total		236	40	2	1	1	2	1

* See Table 5 for convention on notation.

irregular thr segregations occur predominantly among proximal his allele convertants (33/142 proximal convertants, 4/164 distal convertants, 2/19 proximal-distal convertants, 1/11 reciprocal recombinants).

While 17% (40/236) of the HIS prototrophs fail to show normal segregation of the thr3 marker, only 7 aberrant segregations of this same allele were observed among 1,674 unselected four spored asci, or 0.4%. Consequently, the high coincidence observed in the mitotic system is not a reflection of the characteristic meiotic conversion frequency of the thr3-1 site. Moreover, the absence of a high frequency of aberrant segregation for the less closely linked arg6 and try2 loci suggests that irregular segregation at the proximal thr locus may reflect its proximity to the his locus.

Although some fraction of irregular thr segregations might represent conversion, such a result is expected when the reciprocal product of mitotic recombination is not recovered. Lethal sectoring occurred in 12 or 30% of the 40 pedigrees. Since the number of occurrences of thr aberrancy is significantly lower in Class I-A than in Class II (8/115 and 19/96, Table 7), a greater proportion of undetected cell death is required in those pedigrees

with an his auxotrophic cell. However, the possibility that aberrant thr segregation is exclusively attributable to the loss of genetic material cannot be eliminated in the interpretation of these data.

X-RAY INDUCED PROTOTROPHIC PEDIGREES FROM Z3735

The nature of the events at the thr3 cistron was studied in a diploid doubly heteroallelic for his1 and thr3, Z3735. For direct comparison with Z2367, HIS prototrophic pedigrees rather than THR prototrophs were selected from a culture of uniformly sized cells treated with 6.6Kr X-rays. The genotypes of 101 HIS prototrophic pedigrees are shown in Tables 8 and 9. One polyploid and 10 pedigrees with petite cells which did not sporulate are not included. With the exception of two pedigrees (type 104, Table 8) normal $2^+:2^-$ segregations were observed for the various linked and unlinked heterozygous markers.

As in Z2367 the frequency of conversion to HIS prototrophy at 6.6Kr was 0.0003 ($277/10^6$). The frequency of THR prototrophic conversions in this same culture, as determined by simultaneous platings on threonineless media, was 0.0002 ($217/10^6$). These values are corrected for spontaneous his and thr events. Clearly, coincident conversions are not expected among the 101 pedigrees if the two events originate independently. However, 15.8% of the HIS pedigrees (16/101) contain irregular segregations for either or both thr alleles. These cannot be attributed to thr convertants preexisting in the culture. In this experiment the spontaneous THR prototroph titer was 5.7 per 10^6 cells. No convertants would

TABLE 8. Genotypes* of 6.6Kr derived pedigrees with one progeny type from diploid Z3735.

thr3-1 + his1-315 + arg6 try2			
+ thr3-2 + his1-1 + +			
Type	Parental		
	- + - + - -		
	+ - + - + +		
Type	Mother Cell	Number	Number with lethal sector**
99	- + (+) + - -	19	4
	+ - + - + +		
100	- + (+) + - -	1	0
	+ (+) + - + +		
101	- + (+) + - -	1	1
	+ - (- +) + +		
102	(+ - +) + - -	2	1
	(- +) + - + +		
103	(+ - +) + - -	4	0
	+ - + - + +		
104	- + - + - -	2	0
	+ - + (+ - -)		
105	(+ - +) + - -	1	0
	+ - (- +) + +		
106	- + - + (+ +)	3	0
	+ - + (+ - -)		
107	- + (+) + - -	2	0
	+ - + (+) + +		
108	- + - + - -	29	2
	+ - + (+) + +		
109	(+ -) - + - -	1	0
	+ - + (+) + +		
110	- + (+ -) - -	3	0
	+ - + (+) + +		
111	- + - + - -	1	0
	(- +) + (+) + +		
112	(+ -) - + - -	2	0
	(- +) + (+) + +		
113	(+) + (+) + - -	1	0
	+ - (- +) + +		
Total Class I-A		72	8

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands.

** Includes lethal sectoring of any of the first four division products.

TABLE 9. Genotypes* of 6.6Kr induced HIS prototroph-containing pedigrees with two unique progeny cells from Z3735.

		thr3-1	+	his1-315	+	arg6	try2
		+	thr3-2	+	his1-1	+	+
		<u>Parental</u>					
		-	+	-	+	-	-
		+	-	+	-	+	+
Type	Mother cell	First bud				Number	Number with lethal sector**
114	<u>- +(+)+ - -</u>	<u>- +(+)+ - -</u>				1	0
	+ (+)+ - + +	+ - + - + +					
115	<u>- +(+)+ - -</u>	<u>(+)(+)+ - -</u>				1	0
	+ - + - + +	+ - + - + +					
116	<u>- + - +(+ +)</u>	<u>- + - + - -</u>				1	0
	+ - +(+)+ +	+ - +(+ - -)					
117	<u>- + - + - -</u>	<u>- +(+)(+ +)</u>				1	1
	+ - +(+ - -)	+ - (- +)+ +					
Total Class I-B						4	1
118	<u>- + - + - -</u>	<u>- +(+)+ - -</u>				1	0
	+ - +(+)+ +	+ - +(+)+ +					
119	<u>- +(+)+ - -</u>	<u>- +(+)+ - -</u>				1	0
	+ - (-) - + +	+ - + - + +					
120	<u>(+ -) - + - -</u>	<u>(+ - +)+ - -</u>				1	0
	(- +)+(+)+ +	(- +)+(+)+ +					
Total Class I-C						3	0
121	<u>- +(+)(+ +)</u>	<u>- + - + - -</u>				1	0
	+ - +(+)+ +	+ - +(+ - -)					
Total Class I-D						1	0
122	<u>- +(+)+ - -</u>	<u>- + - + - -</u>				7	1
	+ - + - + +	+ - + - + +					
123	<u>- +(+)+ - -</u>	<u>(+ -) - + - -</u>				1	0
	+ (+)+ - + +	+ - + - + +					
124	<u>- +(+)+ - -</u>	<u>+ - + - + +</u>				1	0
	- + - + - -	+ - + - + +					
125	<u>- + - + - -</u>	<u>- + - + - -</u>				2	0
	+ - +(+)+ +	+ - + - + +					
126	<u>- + - +(+ +)</u>	<u>- + - + - -</u>				1	0
	+ - +(+)+ +	+ - + - (- -)					
127	<u>(+)(+)+ - -</u>	<u>+ (+)+ - + +</u>				1	0
	(+)(+)+ - -	+ - + - + +					
128	<u>(+ - +)+ - -</u>	<u>(+ - + -) - -</u>				1	0
	+ - + - + +	+ - + - + +					

TABLE 9. Continued

		thr3-1	+	his1-315	+	arg6	try2
		+	thr3-2	+	his1-1	+	+
		Parental					
		-	+	-	+	-	-
Type	Mother	+	-	+	-	+	+
	cell	-	+	-	+	-	-
	First bud						
	Number	Number with					
		lethal sector**					
129	(+ - +) + - -	-	+	(+ - + +)	1	0	
	(- +) + - + +	+	-	+	-	(- -)	
130	- + - + (+ +)	-	+	-	+	(+ +)	0
	+ - + (+ - -)	+	-	+	-	(- -)	
131	- + - + (+ +)	-	+	-	+	- -	0
	+ - + (+ - -)	+	-	+	-	+	+
132	- + - + (+ +)	-	+	(+ - + +)	1	0	
	+ - + (+ - -)	+	-	(- + - -)			
133	(+ - +) + - -	+	-	+	-	+	+
	(+ - +) + - -	(- +) + -	+	+			
134	(+ - +) + - -	+	-	+	-	+	+
	(+ - +) + - -	+	-	+	-	+	+
135	- + (+) + (+ +)	-	+	(+ - + +)	1	0	
	+ - + - (+ -)	+	-	+	-	(- -)	
Total Class II		21					
		1					

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands.

** Includes lethal sectoring of any of the first four division products.

TABLE 10. Distribution of coincident thr and his events among 101 6.6Kr induced HIS prototrophic pedigrees from Tables 8,9.

		<u>thr3-1</u>	<u>+</u>	<u>his1-315</u>	<u>+</u>	<u>arg6</u>	<u>try2</u>	
		<u>+</u>	<u>thr3-2</u>	<u>+</u>	<u>his1-1</u>	<u>+</u>	<u>+</u>	
HIS		Number		Genotypes*				
Class	origin	Number	thr events					
I-A	Cp	26	5	<u>- + (+) + - -</u>	(1)			
				<u>+ (+) + - + +</u>				
				<u>(+ - +) + - -</u>	(4)			
				<u>+ - + - + +</u>				
	Cd	38	2	<u>(+ -) - + - -</u>	(1)			
				<u>+ - + (+) + +</u>				
				<u>- + - + - -</u>	(1)			
				<u>(- +) + (+) + +</u>				
	Cpd	2	0					
	R **	0	0					
I-B	Cp	2	2	<u>- + (+) + - -</u>	<u>- + (+) + - -</u>	(1)		
				<u>+ (+) + - + +</u>	<u>+ - + - + +</u>			
				<u>- + (+) + - -</u>	<u>(+) + (+) + - -</u>	(1)		
				<u>+ - + - + +</u>	<u>+ - + - + +</u>			
	Cd	2	0					
	Cpd	0	0					
	R	0	0					
	I-C	Cp	0	0				
		Cd	0	0				
		Cpd	2	0				
R		0	0					
I-D	Cp	0	0					
	Cd	0	0					
	Cpd	1	0					
	R	0	0					
II	Cp	15	5	<u>- + (+) + - -</u>	<u>(+ -) - + - -</u>	(1)		
				<u>+ (+) + - + +</u>	<u>+ - + - + +</u>			
				<u>(+) + (+) + - -</u>	<u>+ (+) + - + +</u>	(1)		
				<u>(+) + (+) + - -</u>	<u>+ - + - + +</u>			
				<u>(+ - +) + - -</u>	<u>(+ -) - + - -</u>	(1)		
				<u>+ - + - + +</u>	<u>+ - + - + +</u>			
				<u>(+ - +) + - -</u>	<u>+ - + - + +</u>	(1)		
				<u>(+ - +) + - -</u>	<u>(- +) + - + +</u>			
				<u>(+ - +) + - -</u>	<u>+ - + - + +</u>	(1)		
				<u>(+ - +) + - -</u>	<u>+ - + - + +</u>			
	Cd	6	0					
Cpd	0	0						
R	0	0						

TABLE 10. Continued

thr3-1 + his1-315 + arg6 try2					
+ thr3-2 + his1-1 + +					
HIS	Number				
Class	origin	Number	thr	events	Genotypes*
Uncategorized	7	2	(+ - +) + - -		
			+ - (- +) + +		
			(+) + (+) + - -		
			+ - (- +) + +		
Total		101		16	

* + is wild type allele, - is mutant allele, as determined by tetrad analysis and allele identification tests; () denotes differences from the two parental input strands.

** See Table 5 for convention on notation.

be expected among 101 cells even if this titer were doubled to correct for conversion to the mutant allele.

The number of occurrences of thr aberrancy among the four his recombinant types is included in Table 10. Twelve are associated with conversion of the proximal his allele (total 43) while only two accompany conversion of the distal allele (total 46). The respective frequencies of 27.9% (12/43) and 4.3% (2/46) are significantly different at the 0.05 level. Two other thr events occur among uncategorized pedigrees that may tentatively be interpreted as distal convertants (types 105, 113, Table 8). Significance is maintained when these two equivocal pedigrees are included.

The genotypes of the 16 relevant pedigrees are also shown in Table 10. Six prototrophic and 10 auxotrophic events were recovered. Three of these contain an his auxotrophic strand with recombinant configurations of the parental thr and his alleles (types 113, 123, 133). Interpretation of the 6 prototrophs from the recovered thr alleles suggests that three originate through thr3-1 conversion, two through thr3-2 conversion and one through conversion of both alleles. Threonine 3-1 is assumed to be the proximal site. Though this sequence is tentative, the sample size is too small to observe differential conversion

of one allele. Four of the six THR, HIS prototroph-containing pedigrees are in Class I (two, Class I-A; two, Class I-B). Specification of these conversional events are subject to the qualification that all participating strands may not be available for analysis.

If it is assumed that the remaining 10 pedigrees originate in a similar manner, namely from conversion, then two thr events must be postulated, perhaps at adjacent sites on one strand. Specifically, nine require conversion of the thr3-1 allele to wild type simultaneous with conversion of the thr3-2 allele to mutant. The remaining pedigree type would likewise arise through a double conversional event (thr3-1 to mutant with thr3-2 to wild type).

Double conversions are commonly observed in unselected yeast tetrads with certain allelic combinations (Fogel and Mortimer, 1969). Conceivably, the 10 thr auxotrophs represent analogous events. However, only three of these occur among Class II pedigrees. The particular segregations may therefore be the consequence of mitotic recombination followed by the loss of the reciprocal product. Although lethal cell sectoring was absent in each of the 16 relevant pedigrees, the loss of genetic material through some other

unspecified mechanism cannot be excluded. Thus conversion cannot clearly be established in the 10 pedigrees.

The six mitotic THR prototrophs represent the minimum number of coincident THR and HIS events. Only three conversions to THR prototrophy were observed in some 900 unselected meiotic tetrads from this diploid. It may therefore be concluded that selection for mitotic HIS convertants enhances conversion at the unselected closely adjacent thr cistron. However, the relationship between these two events cannot be ascertained in the small sample size.

DISCUSSION

Theories of conversion and recombination in mitotic diploid cells typically suppose a four-strand origin for these events. However, an adequately marked system permitting total recovery and analysis of all strands associated with prototroph formation has not been available. Consequently, the evidence for this assumption is largely indirect.

Formal genetic criteria may be applied in evaluating the relationship of heteroallelic conversion and reciprocal recombination to DNA replication. Three auxotrophic strands are expected to accompany a prototrophic strand when a single conversional event occurs at the time of, or subsequent to DNA duplication. In the absence of an exchange between the heteroallelic cistron and its centromere, normal mitotic centromere disjunction will yield a heteroallelic auxotrophic segregant and a prototrophic cell. When reciprocal products of recombination originate in a four-strand stage, two parental and two recombinant arrays are expected. Assuming random centromere assortment, this will generate recessive homozygosis in one-half the events.

The system used in the present study can clarify the timing of mitotic prototroph formation and flanking marker recombination. From the data in Tables 1-4 and Tables 8-9,

it is apparent that one-half to four-fifths of the pedigrees contain only HIS prototrophic progeny (Class I). With the exception of Class I-B and I-D types, this absence of an his auxotrophic cell is associated with the absence of two parentally marked strands in pedigrees displaying recombinant thr, arg configurations. Alternatively two parental strands are recovered with a pair of reciprocal non-parental types about one-half the time in pedigrees characterized by an his auxotrophic segregant (Class II and Class III).

These results are not entirely ascribable to clonally related prototrophic cells preexisting in the culture or to lethal cell sectoring. While the proportion of lethal sectors in Class I is relatively high (39/157 or 24.9% in Z2367; 9/80 or 11.5% in Z3735), it is comparable to the value observed in the remainder of the population and in unselected, 7.2Kr derived pedigrees from heterogeneous cultures of Z2367 (25%; C. Beam, personal communication). Thus cell death and X-ray induced prototrophy are probably independent.

The data indicate that X-ray induced conversion can occur in the absence of major DNA synthesis. Thus the Class I-A convertants are most simply understood if they mainly originate in a two-stranded prereplicative stage. Since partial strand recovery will result in a similar pattern, the frequency of such events is not directly ascertainable.

The recovery of a complementary flanking marker strand with 23 of the 32 non-parental prototroph strands in Class I-A (Z2367) suggests that reciprocal products of recombination may likewise arise prereplicatively. This distribution (23/32) is significantly different from the one expected when random centromere disjunction and partial recovery are assumed. However the data do not exclude a four-strand origin where the reciprocally related type is absent.

Although a prereplicative origin is suggested for many of the remaining Class I pedigrees, this is not compatible with the recovery of two parental and two reciprocally related strands in Class I-B and I-D, nor with three prototrophic strands or two prototrophic and one double mutant strand in Class I-C and I-D types. These probably do not have a single mode of origin. Speculatively, the latter types may be analogous to the post-meiotic segregants observed in other fungi (Kitani, Olive and El-Ani, 1962; Lissouba, Mousseau, Rizet and Rossignol, 1962). It would be assumed that the two identical prototroph chromosomes in such pedigrees are related through replication, and that the his genotypes of both remaining strands are likewise generated, although not resolved, in a two-stranded stage. The high frequency of non-parental flanking marker configurations (about 66%) is consistent with this interpretation.

The relationship of prototroph formation to DNA duplication cannot be unequivocally established for Class II and Class III pedigrees. These may tentatively be interpreted as occurring at the four-strand stage. Alternatively some component may occur prior to DNA replication. This fraction is unidentifiable where a single parentally marked prototrophic chromosome is observed or where a reciprocal flanking marker array is not recovered. Among the remaining Class II and Class III pedigrees, a prereplicative origin can probably be assigned where two prototrophic strands and a homoallelic auxotrophic segregant is observed (11/121 in Z2367; 3/21 in Z3725). This timing is indicated as well for about 50% of those Class II and Class III pedigrees with flanking markers reciprocally recombined. Here, two parentally marked strands do not accompany a reciprocally related pair. Instead, a prototrophic and auxotrophic strand display the same recombined outside marker configurations while two auxotrophic strands show the complementary array. The his allele distribution implies that the latter strands are frequently sister chromosomes. Conceivably, such pedigrees originate in a two-stranded stage but require major DNA synthesis for resolution of the prototrophic genotype. Genotype expression as a consequence of DNA doubling finds support in the Class III pedigrees,

particularly where the first two division products of a prototrophic cell are both his auxotrophic.

Theories of genetic recombination in diploid organisms have been proposed by Whitehouse (1963), Holliday (1963), Meselson (1964), Whitehouse and Hastings (1965) and Howard-Flanders and Boyce (1966). These postulate that breakage and reunion is mediated through pairing between complementary chains of different parental origin so that hybrid DNA segments are produced. Conversion will occur when heterozygous sites are generated and corrected within such biparental regions. Recently, Whitehouse (1966) and Fogel and Mortimer (1969) have suggested that polarization of conversion reflects the preferential inclusion of one allele in a hybrid DNA segment, and consequently the existence of preferred breakage points.

In this study the diagnostic characteristics of X-ray induced histidine-1 prototrophs were compared with prototrophs originating at this same cistron during meiosis (Fogel and Hurst, 1967). The evidence for the similarity between the two populations is as follows: 1. the predominance of non-reciprocal products of recombination, or conversions, at the heteroallelic cistron 2. proximal polarization of conversion 3. correlation of conversion with non-parental flanking

marker arrangements. Thus the fundamental mechanisms in meiosis and mitosis appear similar.

The meiotic data largely derive from events during or subsequent to DNA replication. Minimally, the Class I-A mitotic HIS pedigrees originate at the two-stranded stage. Unlike Class II types, these characteristically display equal conversion frequencies at the two allelic sites (Table 5). This is apparent as well when parental and non-parental types are separately considered (47 proximal convertants: 35 distal convertants: 3 proximal-distal convertants and 15 proximal convertants: 10 distal convertants: 1 proximal-distal convertant, respectively). While a proximal polarization is observed in the combined Class I group when proximal-distal events are not included, the degree of polarity is significantly less than in Class II. However, a considerable number of Class II pedigrees may originate prior to DNA replication. This would imply that polarity is not obligatorily precluded in presynthetic cells. Moreover, the absence of a significantly different proportion of parentally marked prototrophic strands in Class I-A and Class II pedigrees (Table 6) is compatible with a common mechanism of prototroph formation. Nevertheless, the data indicate that polarity is expressed only among conversional events that are resolved during, or initiated after, DNA replication.

The view that conversion is often the consequence of excision and resynthesis of a single-stranded DNA segment finds support in the genetical data of Fogel and Mortimer (1969). The occasional occurrence of post-meiotic segregation and of phage heterozygosis (Hershey and Chase, 1951) imply that this removal is neither initiated at the site of, nor induced by, incorrectly paired bases. Rather excision probably proceeds from a free single-stranded end, so that in the absence of DNA replication, proper base pairing is restored only by the chance inclusion of a heterozygous site in an excised region. The differences in polarity between Class I-A and Class II pedigrees, as well as the apparent correlation of this phenomenon with DNA duplication, might be explained with the assumptions that the length of hybrid DNA exceeds the number of nucleotides removed and that the probability of repair at the heterozygous site is related to the distance of the site from the breakage point. Since the production of single-strand lesions is the primary effect of non-lethal X-ray doses on DNA (Freifelder, 1966), the absence of polarity in Class I-A types suggests they arise from X-ray induced breaks distributed at random throughout the cistron. Each allele will therefore have the same probability of being included in a hybrid DNA region. The proximity of either allele to the excision initiation point will allow resolution

of mispairing without major DNA synthesis. Polarity among the remaining pedigrees might be a consequence of localized breakage in a region beyond the proximal end of the histidine cistron. While hybrid DNA could originate from this area to include the proximal his allele at a two-strand stage, removal might rarely extend this distance. Proper pairing would be restored with DNA duplication to produce seemingly polarized post-replicative events. Such lesions would more effectively promote conversion in prototrophs of genuine post-replicative origin if hybrid DNA was of greater total length at this stage and if the number of nucleotides removed was more extensive.

It is assumed that hybrid DNA may extend from a point outside of the histidine cistron to include the proximal his allele with high probability. The recovery of 14 categorized pedigrees from Z2367 (14/236 or 5.9%) in which conversion at adjacent proximal and distal sites may have occurred indicates that hybrid DNA may extend at least the length of the histidine cistron, as defined by the allele pair present. This interpretation may likewise be appropriate for 24 uncategorized types.

There is evidence that the unreplicated recombinant phage molecule consists of parts of parental duplexes joined by hybrid DNA segments (Meselson, 1964; Tomizawa and Anraku, 1964).

The occurrence of limited amounts of excision and replacement during recombination has been demonstrated as well (Meselson, 1964). Recently, Meselson (1967) has presented genetic data indicating that such recombinant structures are probably formed in pairs. This would formally account for reciprocal recombination in diploid organisms. Moreover these patterns of events, namely, heteroduplex formation, single-strand excision and resynthesis along a complementary chain of opposite parentage, are those postulated for conversion. A reciprocal exchange of parental DNA might therefore result in either reciprocal or non-reciprocal genotypes, according to the distribution of single-strand lesions and the resultant hybrid DNA lengths.

While the present results do not critically distinguish alternate mechanisms, a model is preferred for X-ray induced prototrophy in which genetic exchange is requisite to the formation of seemingly non-reciprocal products. Certain aspects of the data are particularly compatible with this view. About 17% of the categorized Z2367 pedigrees exhibit a second or third strand in which conversion was detected. This fraction comprises as much as 23% when a prototrophic chromosome is used to specify 24 uncategorized Z2367 pedigrees with a non-parental relation of flanking markers and an input his allele. The frequent occurrence of such events is

suggestive of the formation of a related pair of hybrid DNA segments involving the four strands of two chromosomes. That these may be generated by a single exchange is especially indicated where the pedigree displays reciprocally recombined flanking markers (27%). Thus in all but four such types, the two identifiable histidine events are in the same strands that participated in the exchange.

If it is supposed that reciprocal exchange is obligatorily required for conversion then a second recombinant event must be postulated when parental configurations occur. A minimum of two exchanges would therefore characterize approximately 63% of the mitotic HIS prototrophs (Table 6, first column). The absence of a single flanking marker type among prototrophs with an accompanying double mutant strand suggests that such coincident events may occur with high probability in the X-ray treated population. Thus parentally marked prototrophic and double mutant strands are observed as often as the recombinant arrays expected from reciprocal exchange between the his alleles (6, 5 of which are uncategorized, and 11, respectively). It is possible that reciprocity in the former types is a result of simultaneous conversion in opposite directions. However, pedigree type 22 (Table 2) is most simply explained as a prereplicative exchange between the his alleles. Parental markers would subsequently be restored in two of four replicated strands by an event in the thr, his interval.

The percentage of categorized HIS pedigrees containing segregations other than $2^+ : 2^-$ at the thr3 cistron was 19.9% in Z2367 and 15.8% in Z3735. All other linked and unlinked heterozygous loci segregated normally in these pedigrees. From Tables 7 and 10 it is apparent that the number of irregular thr segregations among proximal his allele convertants (33/143, Z2367; 12/43, Z3735) is significantly greater in both diploids than the number among distal allele convertants (4/164, Z2367; 4/48, Z3735).

If it is supposed that all of the irregular thr segregations represent conversion, then the non-random distribution among proximal and distal his convertants indicates that the two events do not originate from entirely independent lesions. Coincidence might result from inclusion of both sites in a single hybrid DNA segment. An alternative possibility is that the two events share a common single-stranded lesion between the proximal his and thr alleles, and from which two heteroduplex regions are initiated.

The number of nucleotides equivalent to a meiotic map unit is unknown. Moreover, if genetic exchange occurs in localized regions, and if the distribution or extent of such regions is variable, then recombination frequency may not accurately reflect physical distance. The thr and his loci might therefore be much closer than the meiotic exchange

frequency would suggest. Nevertheless, it is clear that hybrid DNA must extend a considerable distance in X-irradiated mitotic cells if all of the thr events actually are conversional. The recovery of apparent prereplicative his and post-replicative thr conversions in single pedigrees would imply that excision does not include this entire segment.

Similar thr segregations are expected when exchange occurs entirely in the thr, his interval and the reciprocally related product is not recovered. It was believed that this alternative might be distinguished in a double heteroallelic diploid. If conversion is genuinely occurring, then single-site events should predominate when the allele pair is sufficiently separated (Fogel and Mortimer, 1969). However 10 of the 16 presumptive thr convertants from Z3735 require double events at adjacent sites on one strand. Seven of these are in Class I-A.

Thus the data do not allow more than a minimum coincidence estimate for thr and his conversions. On the assumption that only the six THR prototrophs from Z3735 represent conversion, it is still clear that selection for HIS prototrophy results in an enhanced conversion frequency at the thr cistron. Coincidence could then reflect independently occurring events derived through selection for effective pairing

(Pritchard, 1960) in the thr, his region. Thus in selecting for HIS prototrophy, a selection for pairing in the proximal interval may be achieved. A significant coincidence of thr and his conversions has not been observed in unselected meiotic asci (Fogel and Hurst, unpublished data). The mitotic thr conversion frequency (6/101) might represent the normal meiotic frequency increased by X-rays.

That simultaneous events are genuinely occurring at a higher frequency than the number of single-site thr conversions suggests, is indirectly indicated in the data. Perhaps most convincing is the complete absence of detected lethal sectoring in each of the 16 thr pedigrees from Z3735. Moreover, since the total number of irregular thr segregations is significantly greater in Class II than in Class I-A (19/96 and 8/115, respectively, Z2367; 5/15 and 9/72, respectively, Z3735), a greater proportion of cell death is required in those pedigrees containing an his auxotrophic cell. However, the magnitude of the contribution of double-site conversions to irregular thr segregations cannot be determined with certainty.

The number of X-ray induced HIS prototrophs declines to a constant minimal level as a function of preincubation time in YEPD. This pattern is altered when the culture is prein-

cubated in starvation medium supplemented with histidine. The resultant curve shows an initial increase in the number of prototrophs followed by a decline, and resembles the curves obtained by Esposito (1967) at all of the loci investigated other than the tyrosine-1 locus. Esposito proposed that the variation in prototroph number is related to the DNA cycle. However, DNA replication does not occur in starvation medium supplemented with histidine, nor is it necessary for the resolution of a large fraction of the induced prototrophs.

Kirby, Jacob and Goldthwait (1967) have suggested that filament formation and induction in irradiated E. coli B and phage lambda, respectively, occur through inactivation of repressor by an accumulated adenine derivative. In these systems, pre-irradiation incubation in nutrient medium as well as post-treatment in a non-nutrient medium enhance these effects (reviewed by Witkin, 1967).

An analogous system may be operative in yeast. It is proposed that the variation in the number of prototrophs in nutrient medium during the cell cycle reflects a variation in the synthesis of relevant recombinational enzymes. Esposito (1967) observed that under FUDR inhibition of DNA synthesis the prototroph frequency increases to a maximum and remains at this level. Thus the relationship between the DNA cycle

and the recombination cycle may reside in the accumulation of intermediates with inducer-like properties. The induction pattern in starvation medium supplemented with histidine may similarly be explained.

Departures from this pattern have been observed at the histidine-1 cistron in this study, and at the tyrosine-1 cistron (Esposito, 1967). The correlation in time of low prototroph frequencies and X-ray resistance suggests that prototroph number may decline when repair is efficient. Thus efficient enzymatic activity may be characterized by a low recombination frequency if primary lesions are repaired prior to the formation of hybrid associations. It is possible that differences in the available pool nutrients as well as differential activity or activation of particular enzymes may determine the particular prototroph induction pattern observed.

If it is accepted that the fluctuation in prototroph number during the cell cycle is related to a variation in enzyme activity, then the absence of genotypic differences among pedigrees at various cellular stages suggests that differences in enzyme levels do not result in differential genetic effects.

The curve of X-ray induced prototrophy with dose is linear until 3.3Kr in Z2367 (Hurst, Fogel and Weinstock, 1967). If the non-linear portion of the curve reflects a non-linear relationship between the production of lesions and the amount of recombinational enzymes, then the absence of observable genotypic differences among pedigrees derived from 3.3Kr and 6.6Kr X-rays again indicates the absence of differential events. However this distinction may be obscured by the relatively high X-ray doses employed.

SUMMARY AND CONCLUSIONS

Prototrophic pedigrees at the histidine-1 cistron were isolated from synchronous yeast cultures, thereby ensuring the complete recovery of all viable cells associated with prototroph formation. Prereplicative cultures were either treated directly with non-lethal X-ray doses or preincubated in different liquid media.

A total of 268 pedigrees were analyzed for the primary patterns observed in meiotically arising histidine-1 prototrophs. A double mutant strand expected of conventional exchange was recovered rarely, the proximal allele usually converted more frequently than the distal allele and the prototrophic strand was frequently associated with non-parental flanking marker arrays. Additionally, a minimum of about 15% of the mitotic pedigrees contained a second strand in which conversion was discernible.

At least one-half of the total X-ray derived prototrophs were characterized by the absence of a histidine auxotrophic cell. Two parentally marked strands were generally absent as well when reciprocal recombination occurred. These results were not ascribable to lethal sectoring or clonal organization. Alternatively, two parental strands accompanied

two recombinant arrays about one-half the time in those pedigrees with a histidine auxotrophic segregant. Although the proportion of non-parentally marked prototroph strands was statistically similar in these two classes, polarity was apparent only in the latter types. A small percentage of the pedigrees contained progeny cells with three unique histidine genotypes.

Irregular segregation of the closely linked proximal threonine-3 site characterized about 19% of the prototroph-containing pedigrees. These were predominantly associated with proximal histidine allele conversion. Since similar results are expected when the reciprocal product of an exchange in the threonine-histidine interval is not recovered, a double heteroallelic diploid was constructed. Sixteen of the 101 pedigrees selected for histidine prototrophy displayed threonine aberrancy. However only six of these could unambiguously be interpreted as conversional in origin.

X-ray induced prototrophy was studied as a function of preincubation in liquid media. The prototroph frequency declined to a constant minimal level with time in YEPD. In contrast, a variation in prototroph number was observed with continued incubation in starvation medium supplemented with histidine. This was apparent in the absence of a DNA doubling.

No significant genotypic differences were observed between prototrophs selected at different times in the cell cycle prior to major DNA synthesis or between prototrophs induced with different non-lethal X-ray doses.

The following conclusions are indicated: (1) The mechanism of prototroph formation in heteroallelic diploids is fundamentally similar in selected meiotic and X-ray treated mitotic cells. (2) On the assumption that conversion reflects the formation of mispaired bases in hybrid DNA regions, such segments often occur coincidentally in both homologous chromosomes. (3) Mitotic conversion can occur in the absence of major DNA synthesis in the two-strand stage. It is probable that such prototrophs originate through an interaction between homologous strands. (4) Proper base pairing may be corrected with DNA replication in a certain fraction of the prototrophs. (5) Selection for histidine prototrophy results in coincident threonine conversions at significantly higher frequencies than expected on the assumption of independence of events at the two loci. The possibility that the two events merely reflect selection for effectively paired regions could not be excluded in this study. (6) DNA replication does not appear to be obligatorily associated with the variation in prototroph number during the cell cycle.

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