ROLE OF DNA SEQUENCES IN GENETIC RECOMBINATION IN THE ISO-1-CYTOCHROME *c* GENE OF YEAST. I. DISCREPANCIES BETWEEN PHYSICAL DISTANCES AND GENETIC DISTANCES DETERMINED BY FIVE MAPPING PROCEDURES

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ABSTRACT

Recombination rates have been examined in two-point crosses of various defined *cyc1* mutants using five mapping methods. Nucleotide sequences of mutant codons were identified in previous studies from alterations in functional iso-1-cytochromes *c* produced by intragenic revertants. Heteroallelic diploids were analyzed for rates of mitotic recombination that occurred spontaneously and that were induced with X-rays, ultraviolet light and the near-ultraviolet light emitted by sunlamps, as well as rates of meiotic recombination that occur after sporulation. Frequencies of both mitotic and meiotic recombination do not necessarily correspond with physical distances separating altered nucleotides. The most extreme discrepancy involved two adjacent intervals of thirteen base pairs which differed approximately thirtyfold in their spontaneous and X-ray-induced recombination rates. Marked disproportions between genetic and physical distances appear to be due to the interaction of the two nucleotide sequences in the heteroallelic combination and not to the sequences of the mutant codons alone. Recombination values that were obtained by all five methods could not be used to establish the correct order of mutant sites. Relationships of the recombination rates for the various pairwise crosses are different after mitosis from those after meiosis, suggesting that these two recombinational processes are to some extent different in their dependence on particular nucleotide configurations. On the other hand, the relationships of the rates induced by UV-, sunlamp- and X-irradiation were identical or very similar. In addition to the intrinsic properties of the alleles affecting frequencies of mitotic and meiotic recombination rates, two- to threefold variations in recombination rates could be attributed to genetic backgrounds.

RECOMBINATION frequencies derived from two-point crosses are not necessarily correlated with physical distances separating the mutant sites. Numerous studies with wide varieties of genetic systems have indicated disproportionalities of intragenic recombination rates that appear to be intrinsic properties of the mutant alleles. The molecular bases for these disparities are unknown.

Precise investigations of the roles of nucleotide sequences on recombination rates require the examination of intragenic recombination at loci for which the primary sequence analyses of the gene products are available. In this way one

can unequivocally determine the positions of the mutant sites in the structural gene, as well as uncover factors other than distance which might affect frequencies of genetic exchanges, e.g., the nature of the mutational change or particular nucleotide sequences surrounding the mutational lesion. Mutants of the gene determining iso-1-cytochrome *c* of the bakers' yeast *Saccharomyces cerevisiae*, therefore, are particularly suitable, since nucleotide sequences of a number of mutational lesions of *cyc1* mutants have been determined from alterations of revertant forms of iso-1-cytochromes *c*. In addition, yeast provides flexibility for investigating recombinational processes since one can determine the rates of recombination that occur both in meiosis and mitosis, as well as by induction with numerous agents.

We have initiated an extensive investigation designed to examine how rates of genetic exchanges are affected by the nature of mutant base pairs and by adjacent nucleotide sequences. In this paper, we report the rates of interallelic recombination obtained with five methods of mapping all possible pairwise combinations of four *cyc1* mutations. Four of the mapping procedures are based on the rates of mitotic recombination either that occur spontaneously or that are induced with each of the three recombinogens, X-rays, ultraviolet light and the near-ultraviolet radiation emitted by sunlamps. The fifth mapping procedure involved assaying the frequencies of allelic recombinants arising after meiosis in sporulated diploids. The results of these studies clearly demonstrate large disproportionalities between physical distances separating mutant sites and rates of recombination derived by all five mapping procedures. Not only do the data show the unreliability of translating map distances based on recombination frequencies into physical distances measurable in numbers of base pairs, but also these data could not be used to correctly assign the order of the *cyc1* mutant sites.

A comparison of the five mapping methods shows that the relationships of the recombination rates for the various pairwise crosses are different after meiosis than after mitosis. This finding suggests these two recombinational processes are to some extent different in their dependence on particular nucleotide configurations. Although the study of X-ray-induced recombination rates has been presented in a preliminary report (MOORE and SHERMAN 1974), it will be presented here along with results from the other four methods in order to make comparisons of intragenic recombination rates obtained by the various methods.

MATERIALS AND METHODS

Mutant codons: The mutational lesions of the four *cyc1* mutants used in this study have been identified from altered iso-1-cytochromes *c* in intragenic revertants. The *cyc1–13* mutant contains one of the isoleucine codons AUU, AUC or AUA, instead of the normal AUG initiation codon (STEWART *et al.* 1971). The *cyc1–179* (STEWART and SHERMAN 1972) and *cyc1–76* (STEWART and SHERMAN 1973) mutants contain amber (UAG) codons corresponding, respectively, to amino acid residue positions 9 and 71. The *cyc1–239* frameshift mutant has a deletion of a G:C base pair at the third position of the lysine 4 codon (see STEWART and SHERMAN 1974).

Yeast strains: The diploid strains constructed for this study were derived by crossing the haploid strains listed in Table 1. Three haploid strains, containing the *cyc1–13*, *cyc1–76* or *cyc1–179* alleles, were isolated directly from strain D311–3A (**a** *CYC1 lys2 his1 trp2*) (SHERMAN

TABLE 1

Haploid cyc1 *strains*

Strain no.	Genotype	Source
B–461	**a** *cyc1–13* ρ^- *lys2 his1 trp2*	Mutant from D311–3A
D375–2C	**a** *cyc1–13 lys2 his1 trp2*	Meiotic segregant from D–375
D375–5A	α *cyc1–13*	Meiotic segregant from D–375
D493–4A	α *cyc1–13 his2*	Meiotic segregant from D–493
D519–5C	**a** *cyc1–13 lys2*	Meiotic segregant from D–519
B–581	**a** *cyc1–76 lys2 his1 trp2*	Mutant from D311–3A
D546–2C	α *cyc1–76 lys2 leu1 trp5*	Meiotic segregant from D–546
B–699	**a** *cyc1–179 lys2 his1 trp2*	Mutant from D311–3A
SL76–4A	α *cyc1–179 tyr7 his5*	Meiotic segregant from SL–76
SL76–9A	α *cyc1–179 tyr7 trp1 his5 leu2*	Meiotic segregant from SL–76
B–2111	α *cyc1–239 arg4*	Recombinant from D–613
CM16–4A	**a** *cyc1–239 his5 arg4*	Meiotic segregant from CM–16
CM16–10B	α *cyc1–239 arg4*	Meiotic segregant from CM–16
CM16–11D	**a** *cyc1–239 trp5*	Meiotic segregant from CM–16

et al. 1968; SHERMAN *et al.* 1974) and are therefore closely related. The *cyc1–239* mutant was isolated as a recombinant from a frameshift revertant (see STEWART and SHERMAN 1974); as a result, it is not isogenic to the series of other *cyc1* haploid strains. Additional *cyc1* haploid strains are meiotic segregants from various related pedigrees, and were obtained by conventional techniques of crossing, sporulation, and dissection. The diploid strains having various pairwise combinations of the four *cyc1* alleles are listed in Table 2.

Media: The routine growth medium (YPD) contained 1% Bacto-yeast extract, 2% Bacto-peptone and 2% dextrose. Strains for meiotic analyses were grown in presporulation medium containing 0.8% Bacto-yeast extract, 0.3% Bacto-peptone and 10% dextrose before transfer to either a sporulation medium consisting of 1% potassium acetate, 0.1% Bacto-yeast extract and 0.05% dextrose, or a similar sporulation medium having 2% potassium acetate and 0.1% Bacto-yeast extract. The synthetic minimal medium used for scoring various auxotrophic markers contained 2% dextrose and 0.67% Bacto-yeast nitrogen base without amino acids, and various supplements such as L-lysine (30 mg/l), L-tryptophan (20 mg/l) and L-histidine-HCl (20 mg/l). The lactate medium used to select *CYC1* revertants and recombinants contained 0.67% Bacto-yeast nitrogen base without amino acids, 1% DL-lactate, 1% Ionagar no. 2 (Wilson Diagnostic,

TABLE 2

Homoallelic and heteroallelic cyc1 *diploid strains*

	a parents						
α parents	B–461 (*cyc1–13*)	D375–2C (*cyc1–13*)	D519–5C (*cyc1–13*)	CM16–4A (*cyc1–239*)	CM16–11D (*cyc1–239*)	B–699 (*cyc1–179*)	B–581 (*cyc1–76*)
D375–5A (*cyc1–13*)	—	—	—	—	—	—	CM–131
D493–4A (*cyc1–13*)	CM–102	CM–6	—	CM–103	CM–57	CM–101	CM–128
B–2111 (*cyc1–239*)	CM–97	CM–4	CM–18	—	CM–21	CM–105	CM–118
CM16–10B (*cyc1–239*)	CM–104	CM–19	CM–22	—	CM–122	CM–99	CM–126
SL76–4A (*cyc1–179*)	CM–54	CM–12	CM–15	CM–107	CM–100	—	CM–130
SL76–9A (*cyc1–179*)	CM–98	CM–1	CM–14	CM–106	CM–60	CM–3	CM–127
D546–2C (*cyc1–76*)	CM–135	CM–136	CM–137	CM–139	CM–138	CM–134	CM–133

Inc.), 0.05% Bacto-yeast extract and, in some cases, the supplements indicated above. The glycerol medium used for assaying viability and cell number in reversion and recombination studies contained 1% Bacto-yeast extract, 2% Bacto-peptone, 3% (v/v) glycerol and 1% Ionagar. It should be pointed out that ρ^- mutations (cytoplasmic petites), which can be induced by irradiation, do not grow on lactate or glycerol media, while *cyc1* mutants can grow on glycerol, but not lactate medium.

Preparation of cells for irradiation: Cell cultures to be irradiated were grown with shaking for three days at 30° in liquid YPD medium. Appropriate dilutions of washed cells were plated on lactate medium to determine the frequency of prototrophic revertants and recombinants, and on glycerol medium to determine the number of viable cells. At least three plates were used for each dose point. For most strains, survival was normally between 93% and 100% at the highest X-ray, sunlamp or UV dose, and was 100% at each of the two lower doses.

Plating densities: High cell densities were found to inhibit the expression of prototrophs on lactate medium. In addition, the presence of more than 150 recombinants per plate tended to decrease mapping estimates. Since some strains were more severely affected than others by one or both of these effects, as a matter of routine during the meiotic and induced mitotic mapping procedures described in this report, several dilutions were plated for every experiment or experimental point, the number of cells per plate was limited to between 5×10^5 and 5×10^7, and in some instances experiments were repeated up to six times.

X-irradiation procedure: Cells were X-irradiated on the surface of solid medium in open plastic petri dishes with a Machlett OEG-60–7 X-ray tube, powered by a custom-made X-ray generator (Picker Corp.). The unit was operated by 50 KVP and 25 ma, with only inherent filtration; the dose-rate at the surface of the plates was determined to be 28 kilorads/min by a Model 555 Radcon II ratemeter with a 555–100 LA probe (Victoreen Instrument Division). Cells on lactate medium and glycerol medium were irradiated at 2, 4 and 6 krad.

Sunlamp irradiation procedure: The fluorescent sunlamps (Westinghouse FS40T12) have been described by LAWRENCE and CHRISTENSEN (1974). Closed plastic petri plates were exposed for 2, 4 and 6 minutes to the near ultraviolet radiation emitted by the tubes, which gave a uniform illumination of about 25 J m^{-2} sec^{-1} over an area of 90×35 cm. Irradiated plates were manipulated under illumination from "gold" fluorescent lights to avoid photoreactivation.

Ultraviolet irradiation procedure: Cells in open petri plates were exposed to ultraviolet light in a custom-built irradiator described by LAWRENCE *et al.* (1974). The particular combination of germicidal lamps (General Electric G8T5) and wire mesh neutral filter employed in these experiments resulted in a fluence rate of 0.1 J m^{-2} sec^{-1}, with variation in energy fluence rate over individual plates no greater than ± 5%. All irradiations were carried out at room temperature and with "gold" fluorescent lamps the sole source of illumination to avoid photoreactivation.

Measurement of spontaneous mitotic recombination: Spontaneous rates of prototroph production were determined from the fraction of mutant-free cultures in a series of parallel cultures by the method of LURIA and DELBRUCK (1943). In order to accurately determine the rates of recombination with this procedure, the number of cell divisions must be adjusted for each cross. This was accomplished in these experiments by limiting the growth medium (YPD) so that strains with higher rates of recombination would be allowed to go through fewer divisions. For some crosses that were characterized by relatively high prototrophic frequencies, the growth medium consisted of only 0.00167% Bacto-yeast extract, 0.0033% Bacto-peptone and 0.0033% dextrose.

For each determination, fresh cells were suspended in the appropriately diluted YPD medium to a final concentration of 15–60 cells/ml. A Brewer Automatic Pipetting Machine, model No. 40 (Baltimore Biological Laboratory) was employed to dispense one ml aliquots of the suspension to each of 170–250 one-ml culture tubes. Following incubation with shaking at 30° until the maximum titer was reached, at least ten cultures were appropriately diluted and plated on glycerol medium for cell density determinations, and each remaining culture was plated in its entirety on a lactate plate. Plates were incubated 4 days before counting.

In this method, it is necessary to determine the fraction of cultures containing no prototrophs in the series of independent cultures. From this fraction, p_0, the average number of events, m,

which leads to prototrophs on lactate medium, can be estimated from the zero term of the Poisson distribution,

$$p_0 = e^{-m}.$$

Since inocula contain so few cells relative to the total number of cells produced per culture, any prototrophs present in an inoculum are negligible. Thus, the rate (R) of prototrophs per cell division would be given by

$$R = \frac{m}{d},$$

where d is the number of divisions or acts of duplication. Counting from cell one, d should be approximately equal to the mean final cell count per culture (N) less one. Therefore, the rate of prototrophs per cell per cell division is

$$R = \frac{m}{N}.$$

Since there is a small amount of residual growth on lactate medium, the values R may have been slightly overestimated.

Procedure for meiotic analysis: For meiotic recombination estimates, appropriate dilutions of 30–48-hour cultures aerobically grown in liquid presporulation medium were plated on lactate medium and glycerol medium to determine the fraction of prototrophs produced during mitotic growth before sporulation. Aliquots of washed cells were then suspended in various types of sporulation media at 2×10^6 to 5×10^7 cells/ml, and incubated aerobically three to five days at 30°. For each strain, cells were selected for analysis from the medium yielding the higher frequency of asci. Cultures containing less than 9% asci were not used.

To assay the frequency of allelic recombinants after meiosis, appropriate dilutions of the mixture of asci and unsporulated cells were plated on lactate medium and glycerol medium. The percentage of asci in each culture was determined with the aid of a counting chamber. Recombination and reversion frequencies (i.e., prototrophs) could be calculated:

$$\frac{\text{prototrophs}}{10^5 \text{ asci}} = \frac{\left[\frac{\text{prototrophs}}{10^5 \text{ plating units}} \text{ after sporulation}\right] - \left[\frac{\text{prototrophs}}{10^5 \text{ plating units}} \text{ before sporulation}\right]}{\text{fraction of asci}}$$

These values have not been corrected for possible recombination occurring in non-sporulated cells.

RESULTS

This investigation was designed to examine the relationships of recombination frequencies to physical distances in two-point crosses of various defined *cyc1* mutants. The five mapping methods used to analyze the heteroallelic diploids involved mitotic recombination that occurred spontaneously and that was induced with X-rays, sunlamps, and ultraviolet light, as well as meiotic recombination that occurred after sporulation. The mutation lesions of three of the mutants, *cyc1-13*, *cyc1-179*, and *cyc1-239*, delimit two adjacent intervals of 13 base pairs each (Figure 1) at the beginning of the gene (SHERMAN and STEWART 1973; STEWART and SHERMAN 1974). The internal mutant selected for these analyses, *cyc1-76*, was the first mutant identified that was nearer the carboxyl terminus (STEWART and SHERMAN 1973); it contains an amber codon corresponding to amino acid position 71 in the total sequence of 108 residues (see Figure 4).

Haploid strains containing these four *cyc1* alleles were used to construct from 3 to 8 different diploid strains for each heteroallelic combination (see Table 2). The *cyc1-13*, *cyc1-76*, and *cyc1-179* haploid strains that were directly derived

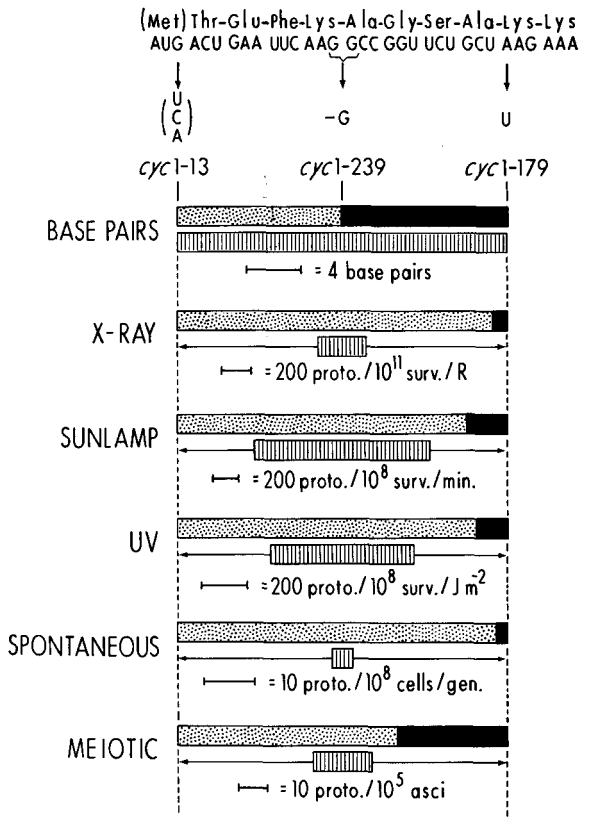

FIGURE 1.—The mutational locations and alterations in the messenger RNA sequence of the three mutants, *cyc1–13*, *cyc1–239* and *cyc1–179*, and the mean rates of recombination determined by the five mapping procedures for the three possible sets of heteroallelic crosses (data from Table 3). Shown on the top of the figure are the normal amino acid sequence of the amino-terminal region of iso-1-cytochrome *c* and the corresponding sequence of the mRNA (STEWART and SHERMAN 1974). Relative rates for the five methods are drawn to scale and are represented as follows: stippled bar, *cyc1–13* × *cyc1–239*; black bar, *cyc1–239* × *cyc1–179*; and vertically-lined bar, *cyc1–13* × *cyc1–179*.

from D311-3A should be closely related and these could be used to construct isogenic series of diploid crosses. Since the *cyc1-239* mutant is a recombinant from a frameshift revertant, neither this mutant nor the 10 additional haploid strains which include segregants of all four *cyc1* mutants from related pedigrees are isogenic to the other three *cyc1* haploid strains (see Table 1). The potential variability due to the influence of genetic background should be uncovered in the analyses of the non-isogenic diploids containing the same heteroallelic pairs. These results can be used to estimate the reliability of comparing non-isogenic diploids involving different heteroallelic pairs.

The first mapping procedure involved examining rates of X-ray-induced mitotic recombination. X-irradiation of all of the 39 diploid strains listed in Table

2 resulted in the induction of prototrophs with a frequency linearly proportional to dose, except for the two homoallelic *cyc1-239/cyc1-239* diploids which rarely gave rise to prototrophs. Typical dose response curves based on 0, 2, 4 and 6 kilorads are shown in Figure 2 for the three possible heteroallelic combinations among mutants *cyc1-13*, *cyc1-239* and *cyc1-179*, for crosses of each of these three mutants with *cyc1-76*, and for the four homoallelic strains. In this report these linear rates are expressed in X-ray mapping milliunits, defined as prototrophs per 10^{11} survivors per rad. The values for each strain, presented in Table 3, never varied more than 20% from one experiment to another. Since the values obtained with heteroallelic strains were generally two to three orders of magnitude higher than those for homoallelic strains, the increases above corresponding homoallelic values can be assumed to measure primarily interallelic recombination.

The near-ultraviolet radiation emitted by fluorescent sunlamps at sublethal doses has been shown recently by LAWRENCE and CHRISTENSEN (1974) to behave like ionizing radiation by giving approximately linear dose-responses in fine structure mapping studies of *his4* alleles in yeast. When heteroallelic *cyc1* diploids were sunlamp-irradiated at sublethal doses, their dose-response kinetics were also fairly linear (Figure 3), although some combinations appeared to give rise to curvilinear responses, more characteristic of UV (see below) and MMS (SNOW and KORCH 1970; KORCH and SNOW 1973) at low doses. This deviation

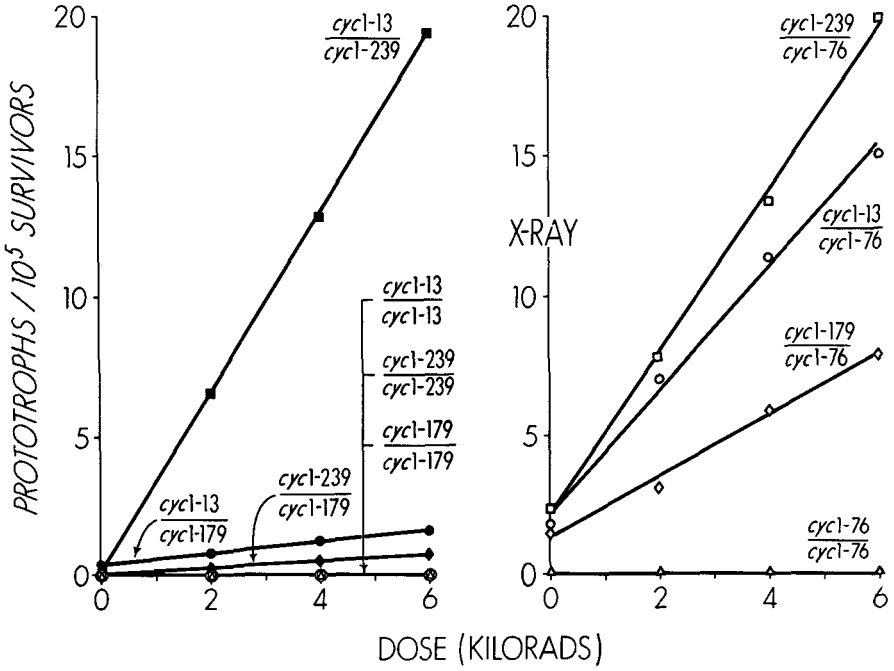

FIGURE 2.—Typical X-ray dose response curves for heteroallelic and homoallelic diploid strains. Slopes were determined from observed frequencies of prototrophic induction by the method of least squares.

TABLE 3

Recombination and reversion frequencies

Crosses	No. of base pairs	Diploid strain nos.	Prototrophic frequencies*				
			X-ray	Sunlamp	UV	Spontaneous	Meiotic
cyc1-13×cyc1-239	13	CM-104	3180 ±22	2290 ±210	1730 ±390	69	51
		CM-18	2450 ±120	2060 ±430	960 ±110	52	140
		CM-4	2060 ±61				43
		CM-19	2080 ±160				
		CM-22	2250 ±99				
		CM-57	1800 ±240				
		CM-97	1610 ±36				
		CM-103	2170 ±82				
		Mean	[2200]	[2175]	[1345]	[61]	[78]
cyc1-239×cyc1-179	13	CM-105	115 ±2	321 ±35	134 ±22	2.0	37
		CM-107	62 ±8	270 ±43	152 ±15		42
		CM-60	47 ±5			1.4	
		CM-99	125 ±9				
		CM-100	80 ±7				
		CM-106	72 ±7				
		Mean	[84]	[297]	[143]	[1.7]	[40]
cyc1-13×cyc1-179	25	CM-98	207 ±16	1470 ±330	627 ±69	3.3	11
		CM-101	280 ±24	1140 ±100	655 ±81		
		CM-1	218 ±41				29
		CM-54	503 ±31			4.0	22
		CM-12	326 ±26				
		CM-14	257 ±29				
		CM-15	587 ±74				
		Mean	[340]	[1309]	[641]	[3.7]	[21]
cyc1-179×cyc1-76	186	CM-130	1110 ±71	2780 ±99	1580 ±510	47	509
		CM-127	1510 ±210	2730 ±530	1470 ±270	70	475
		CM-134	1090 ±73			84	497
		Mean	[1237]	[2755]	[1525]	[67]	[494]
cyc1-239×cyc1-76	199	CM-126	2920 ±86	5450 ±490	2720 ±530	71	
		CM-118	4010 ±610	9110 ±1920	4890 ±650	100	
		CM-138	1600 ±120				259
		CM-139	3000 ±120			106	277
		Mean	[2882]	[7280]	[3805]	[92]	[268]
cyc1-13×cyc1-76	211	CM-136	2200 ±120	4000 ±740	1330 ±420	112	93
		CM-128	3980 ±100	4940 ±800	2610 ±360		
		CM-131	4080 ±240	6110 ±1460	3940 ±220		185
		CM-135	1550 ±300			134	152
		CM-137	1380 ±240				57
		Mean	[2638]	[5017]	[2627]	[123]	[122]
cyc1-13×cyc1-13	0	CM-6	3.3 ±1.9				0.083 <0.09
		CM-102	1.5 ±0.9				
		Mean	[2.4]				

TABLE 3—Continued

Crosses	No. of base pairs	Diploid strain nos.	Prototrophic frequencies* X-ray	Sunlamp	UV	Spontaneous	Meiotic
cyc1-239×cyc1-239	0	CM-21	0 ±0			0.025	
		CM-122	0 ±0				
cyc1-179×cyc1-179	0	CM-3	6.1 ±1.8				
cyc1-76×cyc1-76	0	CM-133	9.7 ±2.6				

Units for each of the mapping methods are as follows: X-ray mapping milliunits, prototrophs per 10^{11} survivors per rad; sunlamp, prototrophs per 10^8 survivors per minute sunlamp irradiation; UV, prototrophs per 10^8 survivors per J m^{-2}; spontaneous mitotic, prototrophs per 10^8 cells per generation; and spontaneous meiotic prototrophs per 10^5 asci. X-ray-, sunlamp- and UV-induced mitotic recombination values are presented with standard errors of the linear regression coefficients based on unirradiated controls and, respectively, 2, 4 and 6 kilorads, 2, 4 and 6 minutes and 10, 20 and 30 J m^{-2}. Bracketed values are means for the groups.

* Prototrophs are defined as all colonies growing on the selective medium, SL, and include both *CYC1* recombinants and revertants.

from linearity was observed with all strains carrying the physically more separated alleles, *cyc1-179/cyc1-76*, *cyc1-239/cyc1-76* and *cyc1-13/cyc1-76*, as well as the *cyc1-13/cyc1-239* strains, having the close heteroalleles which yield rates of recombination similar to strains involving *cyc1-76*. Nevertheless an approximately linear slope does characterize each pair of *cyc1* alleles, and this rate can be expressed in units defined as recombinants per 10^8 survivors per minute sunlamp irradiation. These values for each strain tested, presented in Table 3, were as reproducible from experiment to experiment as X-ray values.

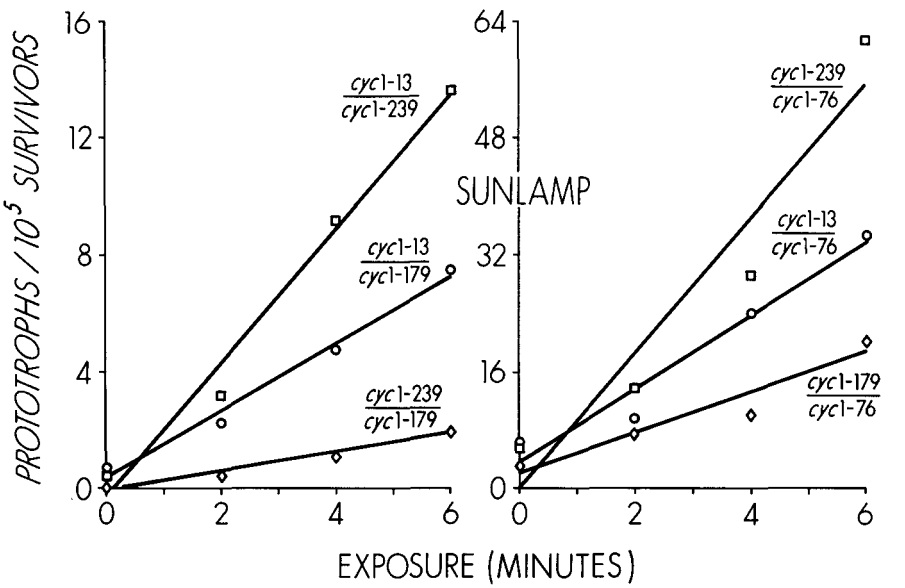

FIGURE 3.—Typical response curves following exposure to fluorescent sunlamps. Slopes were determined from observed frequencies of prototrophic induction by the method of least squares.

Although UV light usually has been reported to induce recombination with nonlinear kinetics (ROMAN and JACOB 1958; NAKIA and MORTIMER 1967; PARRY and Cox 1968; SNOW 1968; PARRY 1971; LAWRENCE and CHRISTENSEN 1974; see MANNEY and MORTIMER 1964), some dose-response curves appear to be approximately linear (PARRY 1971; PARRY and PARRY 1972). We observed with the dose ranges employed in these experiments that the frequencies of recombinants induced with UV were as linearly proportional to dose as those induced with sunlamps (Figure 4). Also, the rates with both sunlamps and UV were nearly as linear as with X-rays for *cyc1-239/cyc1-179* and *cyc1-13/cyc1-179* diploids. A comparison of the dose-response curves in Figures 3 and 4 indicates that the tendency toward nonlinearity noted in sunlamp experiments for *cyc1-13/cyc1-239* diploids and strains bearing alleles separated by about 200 nucleotides was also observed with UV mapping. Nevertheless, the linear regressions of the induced rates of recombination, expressed in numbers of prototrophs per 10^8 survivors per $J\ m^{-2}$, can be used as an arbitrary measure of relative response by the various strains (Table 3), and these frequencies of recombination tend to be as reproducible from experiment to experiment as values based on X-ray- and sunlamp-induced prototrophs.

The fourth mapping procedure involved assaying the rates of allelic recombinants that arise spontaneously. The spontaneous rates of mitotic formation of prototrophic revertants and recombinants were computed from fractions of prototroph-free cultures that were produced in a series of parallel cultures, where

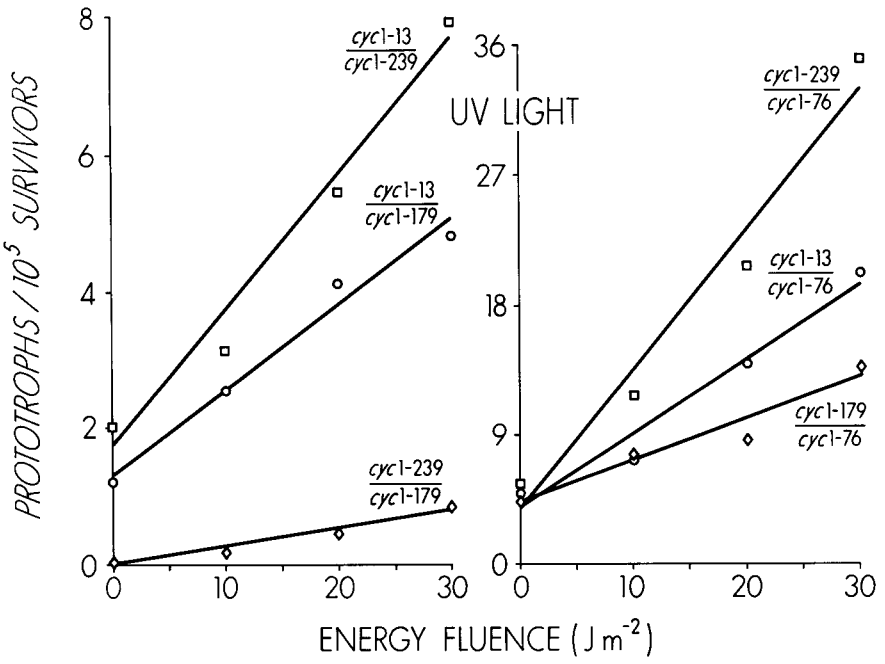

FIGURE 4.—Typical UV response curves. Slopes were fitted by the method of least squares.

TABLE 4

Representative measurements of spontaneous mitotic recombination rates

Diploid strain		Growth medium*	No. of cells per ml†	Total no. of cultures	No. of cultures lacking prototrophs	Reversion and recombination frequencies‡
Genotype	No.					
cyc1-13 × *cyc1-239*	CM-104	0.0075	2.9×10^6	226	29	69×10^{-8}
cyc1-239 × *cyc1-179*	CM-105	0.33	6.4×10^7	208	54	2.0×10^{-8}
cyc1-13 × *cyc1-179*	CM-98	0.067	2.8×10^7	171	64	3.3×10^{-8}
cyc1-179 × *cyc1-76*	CM-130	0.0063	5.4×10^6	236	20	47×10^{-8}
cyc1-239 × *cyc1-76*	CM-126	0.006	3.4×10^6	211	18	71×10^{-8}
cyc1-13 × *cyc1-76*	CM-136	0.005	2.0×10^6	212	16	112×10^{-8}
cyc1-13 × *cyc1-13*	CM-6	0.50	1.2×10^8	228	197	$.083 \times 10^{-8}$
cyc1-239 × *cyc1-239*	CM-122	0.50	1.2×10^8	234	218	$.025 \times 10^{-8}$

* The liquid YPD growth medium consisting of 1% yeast extract, 2% peptone and 2% dextrose was diluted by the amount indicated.

† At least 10 cultures were appropriately diluted and plated on glycerol medium for cell density determinations.

‡ Values are numbers of prototrophs per 10^8 cells per generation.

each culture was started from a small inoculum (LURIA and DELBRUCK 1943). In determining these values, the standard errors of the recombination rates were minimized by adjusting the medium for each strain so that the fraction of cultures free of prototrophs would approximate 20% as nearly as possible (Table 4; see LEA and COULSON 1949). As shown in Table 4 for strains representing each of the heteroallelic combinations and two of the homoallelic crosses, diploids with higher rates of recombination were permitted to complete fewer divisions by limiting the growth medium.

A primary advantage of this method is that estimates of rates of recombination are not biased by possibly differing growth rates of *CYC1* and *cyc1* cells since computations are based only upon the presence or absence of prototrophy in a culture. For unknown reasons, stationary phase cell populations of a few strains occasionally varied significantly from culture to culture in experiments where extremely low nutrient medium was used (see MATERIALS AND METHODS and Table 4). Since this would obviously indicate that parallel cultures were not identical in their growth rates, the results from such experiments were excluded from this report.

The spontaneous rates of mitotic recombination for all strains tested are given in Table 3, where the values are expressed as numbers of prototrophs per 10^{-8} cells per generation. These rates were found to be highly reproductive when experiments were repeated using the same strain. It can be seen that values for heteroallelic strain were two to four orders of magnitude higher than those for homoallelic stocks.

The fifth recombinational analysis, which involved assaying frequencies of allelic recombinants arising following meiosis, was hampered by low frequencies of sporulation of several diploid strains. While most of the diploids analyzed produced between 25% and 60% asci, four (CM-105, CM-107, CM-131 and CM-

134) yielded only from 9% to 15% sporulated cells. In this investigation we have reported the results of only those strains that produced at least 9% asci (Table 3). A low production of asci could inflate an estimate (see MATERIALS AND METHODS) since sporulating cultures can contain cells that underwent recombination and yet that did not form asci. In fact, some strains having very low degrees of sporulation gave rise to recombination frequencies that were drastically inconsistent with frequencies observed with higher-sporulating strains. The rates of allelic recombination followed meiosis, expressed in numbers of prototrophs produced per 10^5 asci (see MATERIALS AND METHODS), are presented in Table 3. For any given strain the meiotic recombination rates were usually reproducible from one experiment to another within 20%.

DISCUSSION

Influence of strain variability on recombination rates: The series of diploid strains containing the same heteroallelic *cyc1* pairs allow the examination of the influences of undefined genetic background on the rates of recombination. The knowledge of the degrees of consistencies between different strains having the same heteroallelic pairs are required for comparisons of different heteroallelic pairs that are not isogenic. The rates of recombination determined by all five mapping methods, presented in Table 3, indicate that independent crosses containing the same heteroallelic *cyc1* pair gave rise to similar rates of recombination, in spite of the fact that parental haploid strains were not isogenic. As shown in Table 2, some diploid strains have one parent in common, but others do not. The consistencies between isogenic strains and only partially related strains suggests that major differencies in recombination values are not the result of modifying genes peculiar to genetically identical strains, but are properties of the particular combinations of *cyc1* alleles.

It is believed, however, that at least some of the two- to threefold variation among strains of the same *cyc1* genotype may be strain-dependent. For example, CM-54, CM-12, and CM-15 were constructed using the same *cyc1-179* parent and three different *cyc1-13* parents (see Table 2) and these three diploid strains as a group exhibited twofold higher frequencies of X-ray-induced mitotic recombination than CM-98, CM-1, and CM-14, which share in common another *cyc1-179* parent (Table 3). The CM-54 strain also exhibited higher frequencies of spontaneous mitotic and meiotic recombination than the CM-98 strain. Similarly, CM-128 and CM-131 were constructed using the same *cyc1-76* strain and two *cyc1-13* strains; their X-ray-induced prototrophic recombination frequencies were nearly identical, but were two to three times higher than those of CM-135, CM-136, and CM-137, which were constructed using a common *cyc1-76* parent and different *cyc1-13* strains. The CM-128 and CM-131 strains also gave rise to higher frequencies of sunlamp- and UV-induced recombination, and, in fact, the CM-131 strain yielded the highest values of all *cyc1-13/cyc1-76* diploids in meiotic and induced mitotic analyses. A fourth example of possible strain-dependent variation among recombination values involves two strains which share

in common their *cyc1-76* parent, but whose *cyc1-239* parental strains differ; the four mapping procedures based on mitotic recombination resulted in 1.4 to 1.7 times higher values for CM-118 in comparison to CM-126.

It is apparent that among the diploids examined by the various methods, particular strains did not necessarily give rise to higher values with more than one mode of recombination. For example, a comparison of two *cyc1-13/cyc1-239* diploids shows that CM-18 exhibited threefold higher recombination frequencies than CM-104 in meiotic analyses but not with mitotic mapping procedures. Similarly, the CM-105 strain (*cyc1-239/cyc1-179*) yielded twice the recombination rate of the CM-107 strain (*cyc1-239/cyc1-179*) with the X-ray procedure but not with other mapping methods (Table 3). It can be noted that each of the CM-18, CM-104, CM-105, and CM-107 diploids were derived from different haploid strains (see Table 2), unlike the diploid groups compared above which involved crossing the same haploid strain to various haploid strains carrying the same *cyc1* allele.

Variations in interallelic recombination frequencies attributed to genetic background have previously been reported for Neurospora (CATCHESIDE 1968; RADFORD 1968), Coprinus (MOORE 1973), and *Zea mays* (NELSON 1968; MOORE and CREECH 1972). Although particular genes which alter frequencies of intragenic recombination have been described in Neurospora (JESSOP and CATCHESIDE 1965; SMITH 1965; JHA 1967) and Ascobolus (GIRARD and ROSSIGNOL 1974), the specific factors responsible for the variation among our strains are unknown at this time.

Relationships between physical distances and recombination rates: The lack of correspondence between physical distances and recombination rates derived from two-point crosses is clearly indicated when rates of prototroph production by the five mapping procedures are considered in relation to the number of base pairs separating the mutational sites. The use of *cyc1* mutants with defined nucleotide changes permits calculations of recombination frequencies as a function of the number of base pairs that separate the mutant sites. These normalized values based on recombination frequencies obtained from the five procedures of mapping all pairwise crosses of the four mutants, *cyc1-13*, *cyc1-76*, *cyc1-179*, and *cyc1-239*, listed in Table 5, clearly demonstrate, first of all, the gross disparity in rates of mitotic recombination for the heteroallelic combination *cyc1-13/cyc1-239* in comparison to rates for the other five heteroallelic combinations. For X-ray-, sunlamp-, and UV-induced, as well as spontaneous methods, the rates for *cyc1-13/cyc1-239* diploids are, respectively, 5 to 69, 3 to 12, 3 to 21, and 6 to 48 times greater than rates for all other heteroallelic strains. Like mitotic results, rates for *cyc1-13/cyc1-239* diploids in meiotic analyses were found to be quite different from rates for *cyc1-13/cyc1-179*, *cyc1-13/cyc1-76*, and *cyc1-239/cyc1-76* diploids; however, the normalized meiotic frequencies for *cyc1-13/cyc1-239* and *cyc1-239/cyc1-179* strains as well as *cyc1-13/cyc1-239* and *cyc1-179/cyc1-76* are more similar. The high rate of recombination in *cyc1-13/cyc1-239* strains cannot be attributed simply to high rates of recombination peculiar to the *cyc1-13–cyc1-239* region or peculiar to the end of the gene; gross disparities of recom-

TABLE 5

Genetic distance as a function of physical distance

Cross	Base pairs	Frequencies of recombination* per base pair				
		X-ray	Sunlamp	UV	Spontaneous	Meiotic
cyc1-13 × *cyc1-239*	13	124 to 245 [169]	158; 176 [167]	74; 133 [103]	4.0; 5.3 [4.7]	3.3 to 11 [6.0]
cyc1-239 × *cyc1-179*	13	3.6 to 9.6 [6.5]	21; 25 [23]	10; 12 [11]	0.11; 0.15 [0.13]	2.8; 3.2 [3.0]
cyc1-13 × *cyc1-179*	25	8.3 to 23 [13.6]	46; 59 [52]	25; 26 [26]	0.13; 0.16 [0.15]	0.44 to 1.2 [0.84]
cyc1-179 × *cyc1-76*	186	5.9 to 8.1 [6.7]	15; 15 [15]	7.9; 8.5 [8.2]	0.25 to 0.45 [0.36]	2.6 to 2.7 [2.65]
cyc1-239 × *cyc1-76*	199	8.0 to 20 [14.5]	27; 46 [36.6]	14; 25 [19]	0.36 to 0.53 [0.46]	1.3; 1.4 [1.3]
cyc1-13 × *cyc1-76*	211	6.5 to 19 [13]	19 to 29 [24]	6.3 to 19 [12]	0.53; 0.64 [0.58]	0.27 to 0.88 [0.58]

* Derived from prototrophic frequencies for the series of *cyc1* pairwise crosses listed in Table 3. Mean values are indicated in brackets.

bination were not observed in other strains encompassing this region, *cyc1-13/cyc1-179* and *cyc1-13/cyc1-76*, nor were they observed in the strains encompassing the adjacent region, *cyc1-239/cyc1-179*. Instead, the extensive disproportion between genetic and physical distances presumably is due to the combination of nucleotide sequences in the *cyc1-13/cyc1-239* diploids since neither *cyc1-13* nor *cyc1-239*, in combination with the other mutants, *cyc1-76* or *cyc1-179*, gave rise to as disproportionately high rates of recombination.

The interpretation of the relationships of recombination to physical distance for the five additional heteroallelic pairs was hampered by the undefined differences in genetic backgrounds which led to variability in recombination rates with different strains having the same heteroallelic pairs. The differences in normalized recombination rates among the various heteroallelic pairs become more apparent, however, when one considers a more isogenic series, where differences in genetic backgrounds should be minimal. For example, the two diploid strains CM-105 (*cyc1-239/cyc1-179*) and CM-118 (*cyc1-239/cyc1-76*) differ from each other by presumably only the mutational events of the *cyc1* alleles (Tables 1 and 2), yet the recombination rates for CM-105 are always two or three times higher than those for CM-118 (Table 6). The differences of these two strains are particularly relevant since the ranges of normalized values of the *cyc1-239/cyc1-179* and *cyc1-239/cyc1-76* strains overlap with at least one mapping method (Table 5).

Also, two- to threefold differences in spontaneous-, sunlamp-, and UV-induced rates are indicated between CM-98 (*cyc1-13/cyc1-179*) and CM-127 (*cyc1-179/cyc1-76*). The CM-54 (*cyc1-13/cyc1-179*) and CM-130 (*cyc1-179/cyc1-76*) strains similarly exhibit 1.6-fold to more than threefold differences in X-ray,

TABLE 6

The relationships between physical distances and genetic distances of isogenic strains

Cross	Strain no.	Frequencies of recombination* per base pair				
		X-ray	Sunlamp	UV	Spontaneous	Meiotic
cyc1-239 × *cyc1-13*	CM-104	245	176	133	5.3	
cyc1-239 × *cyc1-179*	CM-99	9.6				
cyc1-239 × *cyc1-76*	CM-126	14.7	27	13.7	0.36	
cyc1-239 × *cyc1-13*	CM-97	124				
cyc1-239 × *cyc1-179*	CM-105	8.8	25	10	0.15	
cyc1-239 × *cyc1-76*	CM-118	20	46	25	0.50	
cyc1-13 × *cyc1-179*	CM-101	11	46	26		
cyc1-13 × *cyc1-76*	CM-128	19	23.4	12		
cyc1-179 × *cyc1-13*	CM-54	20			0.16	0.88
cyc1-179 × *cyc1-76*	CM-130	6.0			0.25	2.7
cyc1-179 × *cyc1-13*	CM-98	8.3	59	25	0.13	0.44
cyc1-179 × *cyc1-76*	CM-127	8.1	15	7.9	0.38	2.6
cyc1-76 × *cyc1-179*	CM-134	5.9			0.45	2.7
cyc1-76 × *cyc1-13*	CM-135	7.3			0.64	0.72

* Derived from the frequencies presented in Table 3.

spontaneous, and meiotic recombination rates. The normalized values of various modes of recombination of the series of isogenic crosses listed in Table 6, therefore, not only establish the marked disproportionality of the *cyc1-13–cyc1-239* combination, but also indicate that there are differences between *cyc1-239–cyc1-179* and *cyc1-239–cyc1-76* combinations, between *cyc1-13–cyc1-179* and *cyc1-13–cyc1-76* combinations and between *cyc1-76–cyc1-179* and *cyc1-76–cyc1-13* combinations. In addition, there is little or no overlap of the values of the groups of *cyc1-179/cyc1-76* strains in comparison to the groups of *cyc1-239/cyc1-76* strains (Table 5), suggesting different rates of recombination. While it is unreliable to conclude from these results whether the normalized rates for any two different heteroallelic combinations are truly equivalent, some appear to be at least very similar, such as the *cyc1-239/cyc1-179* diploids compared to the *cyc1-179/cyc1-76* diploids, and the *cyc1-13/cyc1-76* strains compared to either the *cyc1-179/cyc1-76* or *cyc1-239/cyc1-76* diploids.

Comparisons of the five mapping methods: The mean frequencies of prototroph production for each of the heteroallelic classes are useful for comparing physical distances and relative rates among the five methods. Since the mutational lesions of the three mutants, *cyc1-13*, *cyc1-239*, and *cyc1-179*, delimit two adjacent intervals of 13 base pairs each, the two segments would be comparable in their recombination rates if frequencies of genetic exchanges directly correlated with distances separating mutational sites. In Figure 1, the mean rates of X-ray-, sunlamp-, and UV-induced, as well as spontaneous mitotic recombination for the intervals, *cyc1-13–cyc1-239*, *cyc1-239–cyc1-179* and the entire *cyc1-*

13–cyc1-179 (see Table 3) are drawn to related scales. X-ray- induced and spontaneous rates of mitotic recombination for the *cyc1-13/cyc1-239* diploids, on the average, were disproportional by twentysixfold and thirtyfold, respectively, with rates for the *cyc1-239/cyc1-179* diploids. The average rates of recombination induced by sunlamps and UV in these two intervals containing an equal number of nucleotide base pairs were also extremely disproportionate, the two intervals differing sevenfold and ninefold, respectivey, by the two methods.

While the rates of meiotic recombination also exhibited discrepancies with physical distances, their patterns among the three amino-terminal mutants are clearly different from those characterizing mitotic rates. Mitotic measurements more closely reflected the equality between the *cyc1-13–cyc1-239* and *cyc1-239–cyc1-179* intervals, exhibiting only a twofold difference between means for the two corresponding heteroallelic classes. Since the ranges of frequencies for the two classes overlapped to some extent, this difference is probably minor, especially when compared to seven- to thirtyfold disproportions obtained in mitotic mapping methods.

On the basis of known nucleotide distances, one would expect rates for the *cyc1-13/cyc1-179* diploids to be about twice the values for the *cyc1-239/cyc1-179* diploids. While mean mitotic measurements for *cyc1-13/cyc1-179* diploids were always at least twice the *cyc1-239/cyc1-179* frequencies, meiotic measurements for the *cyc1-13/cyc1-179* diploids were *less* than frequencies for either the *cyc1-13/cyc1-239* or *cyc1-239/cyc1-179* heteroallelic classes (Figure 1). Thus meiotic recombination frequencies constitute a different pattern in comparison to the patterns observed with mitotic mapping methods.

It is interesting to note that mitotic frequencies for heteroallelic strains carrying the *cyc1-13* and *cyc1-239* markers, which are separated by only 13 base pairs, fell into the same range as those values obtained when each of these two mutants was crossed to the distal *cyc1-76* marker which is, respectively, 211 and 199 base pairs away. The comparisons involving crosses with *cyc1-76* are illustrated in Figure 5. With all mitotic mapping methods, the *cyc1-179/cyc1-76* values are grossly inconsistent with the *cyc1-13/cyc1-76* and *cyc1-239/cyc1-76* values, which on the basis of physical distances separating the mutational sites should be similar to each other. Moreover, the induced mitotic mapping methods resulted in frequencies for the *cyc1-179/cyc1-76* diploids which were one-half those for the *cyc1-13/cyc1-239* strains whose mutational sites define a 13-base-pair interval. On the other hand, crosses of each of the amino-terminal mutants to the distal *cyc1-76* mutant resulted in relative rates of spontaneous mitotic recombination different from those obtained in the three induced mitotic mapping procedures (Figure 5). While it is possible to establish a correct order of the three *cyc1-13*, *cyc1-239* and *cyc1-179* mutants on the basis of mean spontaneous recombination rates from these three heteroallelic classes alone, there are disproportionalities in the relative genetic distances separating the *cyc1-76* site and each of the three proximal mutant sites. From the graphic presentation of the meiotic data representing the nine crosses involving the *cyc1-76* internal mutant, it is obvious that on the basis of these comparative frequencies alone, *cyc1-13*, *cyc1-*

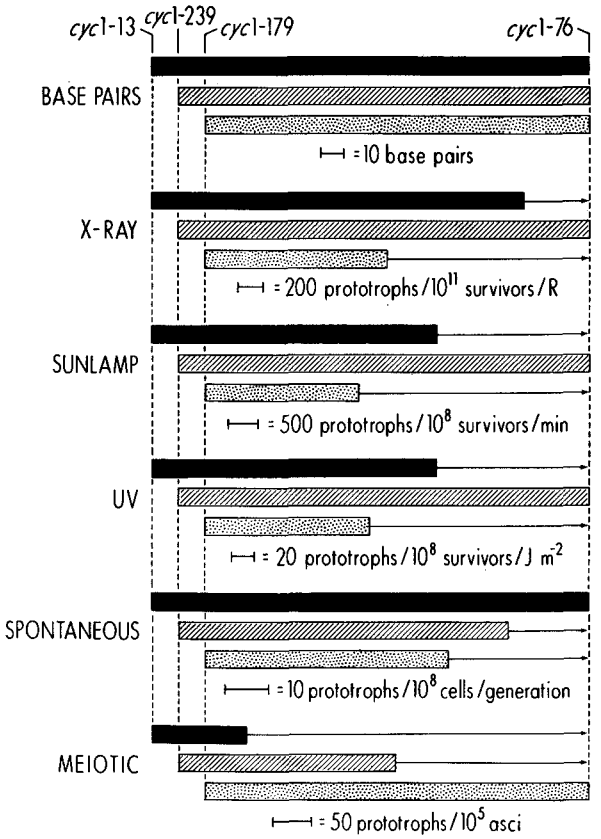

FIGURE 5.—Diagram comparing the relative physical distances separating *cyc1–76* and each of the amino-terminal mutants, and the mean rates of recombination derived from the three sets of crosses. The relative locations of the mutational alterations of the *cyc1–13*, *cyc1–239*, *cyc1–179* and *cyc1–76* mutants are shown at the top, and mean rates of recombination in crosses of each of the amino-terminal mutants to *cyc1–76* for all methods are shown below. The relative rates are drawn to scale and are represented as follows: black bar, *cyc1–13* × *cyc1–76*; diagonally-lined bar, *cyc1–239* × *cyc1–76*; and stippled bar, *cyc1–179* × *cyc1–76*.

239 and *cyc1-179* would be erroneously mapped in reverse order relative to *cyc1-76*.

Fine structure maps and equivalencies to physical distances: Since frequencies of recombination of heteroallelic diploid strains increase linearly with doses of ionizing radiation, and since different heteroallelic combinations sometimes give rise to apparently additive rates, it is initially suggested that these rates were proportionally related to the physical distances between the mutational sites (MANNEY and MORTIMER 1964). The recombination rates acquired with ionizing irradiation have been used for mapping alleles of the *arg4*, *trp5* (MANNEY and MORTIMER 1964; MANNEY 1964), *his4* (FINK 1966), *ade8* (ESPOSITO 1968), *leu1* (NAKAI and MORTIMER 1967), *cyc1* (PARKER and SHERMAN 1969), *ilv1* (THURIAUX *et al.* 1971), *asp1* (JONES 1973), *his1* (KORCH and SNOW 1973), *ura2*

(DENIS-DUPHI, unpublished; see AITKEN, BHATTI and KAPLAN 1973), and *fas1* (TAURO *et al.* 1974) genes in yeast. However, extreme cases of nonadditivity are often observed and many sites cannot be unambiguously ordered. The results from this study as well as other studies with defined *cyc1* mutants (SHERMAN *et al.* 1974) clearly reveal the unreliability of equating map distances to physical distances and bring to light the erroneous order of mutational sites that could be deduced from genetic maps. An approximate equivalence was previously suggested from the comparison of the first X-ray map of 15 *cyc1* mutants (PARKER and SHERMAN 1969) and the related amino acid replacements in revertant forms of iso-1-cytochrome *c* (SHERMAN *et al.* 1970). While the map was based on the best additivity of intervals, some values, especially those involving the *cyc1-9* and *cyc1-15* alleles, drastically deviated from additivity. In fact, recent results with deletion mapping (SHERMAN *et al.* in preparation) indicated that the position of the *cyc1-15* site was incorrectly assigned. Also it now appears as if amino acid replacements in the revertants from the *cyc1-15* mutant did not correspond to the original mutation site (see PUTTERMAN, MARGOLIASH and SHERMAN 1974). Inconsistencies in ordering mutational sites by X-ray mapping and deletion mapping have also been observed for some alleles of the *his4* locus (FINK and STYLES 1974). Thus, as previously pointed out, "one should be reluctant to equate X-ray-mapping units with lengths of DNA or protein" (SHERMAN *et al.* 1970; SHERMAN and STEWART 1971) or to use X-ray maps for assigning the order of mutational sites.

Nevertheless, if one excludes the results from certain heteroallelic strains, X-ray-induced recombination frequencies provide a crude measure of physical distances. The values in Table 3 for all except the *cyc1-13/cyc1-239* strains correspond to 14 to 92 amino acid residues per X-ray mapping unit (prototrophs per 10^8 survivors per rad), with mean values falling between 23 and 52 amino acid residues. Thus approximate relationships to physical distances may be revealed when X-ray maps are constructed for large numbers of two-point crosses. It is reasonable to think that total map lengths determined with numerous combinations of alleles, including those at ends of genes, may be used with knowledge of total molecular weights of polypeptide chains to establish equivalences. The average equivalence deduced by relating the X-ray map of the *cyc1* gene to iso-1-cytochrome *c* is in surprisingly good agreement to the correspondence deduced by relating the X-ray maps of the *trp5* gene to tryptophan synthetase from *Neurospora crassa* (MANNEY and MORTIMER 1964), of the *his1* gene to phosphoribosyltransferase from *Salmonella typhimurium* (KORCH and SNOW 1973), and of the *ura2A* gene to aspartate carbamoyltransferase from yeast (AITKIN, BHATTI and KAPLAN 1973). However when only a few heteroallelic combinations are measured or when influencing factors extrinsic to the heteroallelic pair are not evaluated, the estimates of nucleotide distances and molecular weights of polypeptide chains by X-ray mapping are too precarious to be of much value. Clearly, the additional four mapping methods also have not proved to be reliable for measuring physical distances from recombination rates of limited numbers of two-point crosses.

Numerous studies with other organisms also have indicated the lack of strict correspondence between physical distances and recombination frequencies. Like the investigations with yeast discussed above, such disparities can be inferred where the order of the markers does not correspond to the order determined by deletion mapping (NORKIN 1970) or by purely genetic studies, where there are ambiguities in ordering markers (HOTCHKISS and EVANS 1958; HELINSKI and YANOFSKY 1962; KAPLAN, SUYAMA and BONNER 1962; RAVIN and IYER 1962; LOPER *et al.* 1964; YANOFSKY *et al.* 1964; TESSMAN 1965; LACKS 1966; MOUSSEAU 1966; FIELDS and OLIVE 1967; GRAY and EPHRUSSI-TAYLOR 1967; KRUSZEWSKA and GAJEWSKI 1967; MORSE and LERMAN 1969; PASZEWSKI and PRAZMO 1969; ROSSIGNOL 1969; GUTZ 1971; RONEN and SALTS 1971; JONES 1972; LEBLON and ROSSIGNOL 1973; MOORE 1973; STADLER and KARIYA 1973; and others). RONEN and SALTS (1971) found that frequencies of recombination between adjacent nucleotides varied 1000-fold among 12 different sites in the bacteriophage T4*rII* gene. In the studies of conjugation crosses reported by NORKIN (1970), where mutant sites in the *lacZ* gene of *E. coli* were ordered unambiguously by deletion mapping, distances separating allelic markers were shown to be of negligible importance in recombination between them. Further, the lack of correspondence between genetic and physical distances has been demonstrated clearly in recent studies of *E. coli* mutants, whose nucleotide sequence changes were deduced from amino acid alterations of tryptophan synthetase (STADLER and KARIYA 1973). As in our study with *cyc1* mutants of yeast, the marked disparities between recombination frequencies and physical distances could not be simply related to a particular base-pair change. However, studies of spore-color mutants of *Ascobolus immersus* indicated that frameshift mutants, in contrast to base substitution mutants, consistently showed an excess of conversion to the mutant type and no postmeiotic segregation (LEBLON 1972a, b; LEBLON and ROSSIGNOL 1973). While the results with Ascobolus suggest that frameshift mutants should act differently in recombination, it is difficult to evaluate the relationships between patterns of conversion determined in their studies and the rates of recombination determined in our studies with *cyc1* mutants. It is apparent from our investigation that disproportionalities in recombination rates cannot be simply explained by the types of mutational changes, but it appears as if combinations of certain nucleotide sequences in some way influence the rate of recombination. Additional studies with numerous other defined *cyc1* mutants may reveal combinations of nucleotide sequences that markedly influence recombination rates.

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