MAPPING AND GENE CONVERSION STUDIES WITH THE STRUCTURAL GENE FOR ISO-1-CYTOCHROME *C* IN YEAST

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ABSTRACT

We have investigated the order of the four genes *cyc1*, *rad7*, *SUP4*, and *cdc8* which form a tightly linked cluster on the right arm of chromosome *X* in the yeast *Saccharomyces cerevisiae*. Crossing over and coconversion data from tetrad analysis established the gene order to be centromere–*cyc1*–*rad7*–*SUP4*. Also *cdc8* appeared to be distal to *SUP4* on the basis of crossovers that were associated with conversion of *SUP4*. The frequencies of recombination and the occurrence of coconversions suggest that these four genes are contiguous or at least nearly so. Gene-conversion frequencies for several *cyc1* alleles were studied, including *cyc1–1*, a deletion of the whole gene that extends into the *rad7* locus. The *cyc1–1* deletion was found to be capable of conversion, though at a frequency some fivefold less than the other alleles studied, and both 3:1 and 1:3 events were detected. In general 1:3 and 3:1 conversion events were equally frequent at all loci studied, and approximately 50% of conversions were accompanied by reciprocal recombination for flanking markers. The orientation of the *cyc1* gene could not be clearly deduced from the behavior of the distal marker *SUP4* in wild-type recombinants that arose from diploids heteroallelic for *cyc1* mutations.

DURING the course of investigation the action of nonsense suppressors on *cyc1* mutants (GILMORE, STEWART and SHERMAN 1971) and the influence of UV-sensitive (*rad*) mutations on *cyc1* reversion (LAWRENCE and CHRISTENSEN, manuscript in preparation), it was noted that there exists a close linkage between the *cyc1*, *SUP4*, and *rad7* loci. Since it is known that *SUP4*, along with a variety of other markers, is located on chromosome *X* and is closely linked to the temperature-sensitive cell-cycle mutation *cdc8* (MORTIMER and HAWTHORNE 1973; HARTWELL *et al.* 1973), we have investigated the order of the four genes in this cluster. We find the order, proximal to distal, to be *cyc1*, *rad7*, *SUP4* with *cdc8* probably distal to *SUP4*; our present data suggest that these may be contiguous genes. These data have also been examined for frequencies of gene conversion of markers on chromosome *X*, including several different alleles of the *cyc1* gene. Finally, attempts have been made to orient the *cyc1* gene from the

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behavior of a distal marker in recombinants from diploids heteroallelic for *cyc1* mutations.

MATERIALS AND METHODS

Origin and properties of mutant genes: The origin and some characteristics of the iso-1-cytochrome *c* deficient mutants *cyc1-1*, *cyc1-2*, *cyc1-9*, *cyc1-11*, *cyc1-13*, and *cyc1-123* have been tabulated by SHERMAN *et al.* (1975). The *cyc1-1* mutant contains a deletion of the entire *CYC1* locus; *cyc1-1* strains do not revert nor recombine with any point mutants (PARKER and SHERMAN 1969), including those that are situated at the extreme ends of the gene (SHERMAN *et al.* 1975). Recently SINGH and SHERMAN (manuscript in preparation) have shown that the *cyc1-1* deletion also encompasses at least part of the *rad7* locus. The *cyc1-2* (STEWART and SHERMAN, manuscript in preparation; see SHERMAN and STEWART 1974) and *cyc1-9* (STEWART *et al.* 1972) alleles contain ochre (UAA) codons corresponding, respectively, to amino acid positions 21 and 2. The *cyc1-13* mutant contains one of the isoleucine codons, AUU, AUC or AUA, instead of the normal initiation codon AUG (STEWART *et al.* 1971). Although the types and sites of the *cyc1-11* and *cyc1- 123* mutants have not been established from altered iso-1-cytochrome *c*, their approximate positions within the gene have been determined with deletion mapping (SHERMAN *et al.* 1975). The *cyc1-11* mutant, which was the first mutant found to map outside of the region corresponding to the amino-terminal portion of iso-1-cytochrome *c* (PARKER and SHERMAN 1969), appears to contain a lesion approximately at or near position 76. The site of the *cyc1-123* mutant corresponds to an amino acid position greater than 93 and probably near or at position 102, in the total of 108 amino acid positions in iso-1-cytochrome *c*. Both the *cyc1-11* and *cyc1-123* mutants did not appear suppressible in diploids that were heterozygous for suppressors and hemizygous for the *cyc1* alleles (SHERMAN *et al.* 1974); however in a more sensitive test, a low level of suppression was clearly observed with both of these mutations in haploid strains that contained an ochre-specific suppressor (SHERMAN *et al.* 1975). While it would appear from the action of the suppressors as if these *cyc1* mutants simply contain ochre (UAA) codons, the examination of abnormal iso-1-cytochrome *c* from intragenic revertants of *cyc1-11* indicates that neither the amino acid replacements nor the normal amino acids have codons that differ from the UAA codon by single base-pair substitutions (SHERMAN *et al.* 1970; STEWART and SHERMAN, unpublished results). Thus the *cyc1-11* mutant appears to be an exception to the mutants assigned nonsense codons on the basis of the pattern of both amino acid replacements and suppression (SHERMAN and STEWART 1974; also see PRAKASH and SHERMAN 1974). While the lesion of the *cyc1-11* mutant remains unclear, so far there is no reason to believe that the *cyc1-123* mutant does not contain a simple ochre codon near the end of the gene corresponding to the carboxy terminus of iso-1-cytochrome *c*.

The *SUP4-1* gene suppresses the markers *trp5-48*, *his5-2*, *lys1-1* (GILMORE 1967) and *can1-100* (MORTIMER, unpublished results) and causes the insertion of residues of tyrosine at ochre (UAA) sites (GILMORE, STEWART and SHERMAN 1971). The *ilv3* gene was obtained from the strain E-313 that was kindly furnished by DR. R. K. MORTIMER; this allele was chosen from a number of independently derived *ilv3* mutants for its higher spore viability. Since *met3* mutants previously used in linkage studies were not available, we employed a *met3* mutant (strain no. S-30) that was recently isolated by SINGH and SHERMAN (1974) and that was identified by its linkage to *ilv3* and to its centromere (SINGH, unpublished). The *cdc8* and *cdc11* genes, which conditionally affect cell division (HARTWELL 1971; CULOTTI and HARTWELL 1971), were obtained, respectively, from strains X3258-28D and X3330-3B, kindly furnished by DR. R. K. MORTIMER. The UV-sensitive mutant *rad7* (GAME and COX 1971), kindly supplied by DR. B. S. COX, was isolated by COX and PARRY (1968). The remaining mutants, *leu1-12*, *ura4-11*, *tyr7-1* and *met1-1*, are from our stock collection.

Media: General types of media and media used specifically with the iso-1-cytochrome *c* system have been described in detail by SHERMAN *et al.* (1974). Canavanine sulfate (60 mg/l) was added to synthetic medium for scoring *can1* mutants.

General genetic methods: Conventional yeast genetic procedures of crossing, sporulation and

tetrad analysis were used to construct strains with desired markers and for the meiotic analyses (see SHERMAN and LAWRENCE 1974). Ascospore viability was high, generally in excess of 90%.

Scoring methods: Nutritional markers and the canavanine-resistant marker (*can1*) were scored by transferring drops of cell suspension onto appropriate media with a replicator containing an array of inoculating rods; this procedure, which is used routinely in our laboratories, results in clearer discrimination of growth differences in comparison to the usual procedure of replica plating with velveteen. Mating types were determined by crossing each of the segregants to tester strains and either spotting the mating mixture on minimal medium or examining the mating mixture for zygotes. The *SUP4-1* gene was scored by the suppression of two or more suppressible genes previously described (GILMORE 1967; GILMORE, STEWART and SHERMAN 1971). The *cyc1* genes were scored by the level of cytochrome *c* in intact cells, using spectroscopic examinations at room temperature or in special cases at low temperature (—190°) (SHERMAN and SLONIMSKI 1964). The spectroscopic examinations at room temperature were carried out with strains grown on the surface of nutrient medium, using the apparatus previously described for use at low temperature (SHERMAN 1964). Since levels of cytochrome *c* cannot be accurately estimated by the method employed at room temperature, further spectroscopic examinations at low temperature were made of all strains not clearly diagnosed and all tetrads exhibiting irregular segregation of the *cyc1* gene or of the closely linked markers. The *rad7* gene, causing UV sensitivity, was scored with spots of cell suspensions irradiated with 120 J m^{-2}. Since there is some variation among haploid strains having the same *rad7* genotype, UV sensitivity was usually determined with diploid strains that were made by crossing *rad7* testers to each of the unknown segregants. The *cdc4* and *cdc11* genes were scored with spots of cell suspensions incubated on nutrient medium at the permissive (23°) and restrictive (36°) temperatures; in some instances the budding patterns of cells incubated at the restrictive temperature were used to verify the *cdc4* and *cdc11* genotype (HARTWELL 1971; HARTWELL *et al.* 1973).

In the gene conversion studies, it was of particular importance to exclude data from asci in which aberrant segregation arose from false tetrads, aneuploidy or similar artifacts. Conversion was most convincingly demonstrated to be the cause of aberrant segregation when the alleles at a closely linked locus showed a normal 2:2 distribution. The simultaneous occurrence of 2:2 segregation for alleles at a variety of unlinked loci is a much less satisfactory criterion, since independent conversion events at one or more of these loci is in any case expected in large pedigrees. The mating type locus shows unusually low conversion frequencies (FOGEL, HURST and MORTIMER 1971), however, and the simultaneous occurrence of aberrant segregation at this and other loci provides grounds for suspecting false tetrads. Tetrads with non-mating spores were also discarded. It should be noted that the method used does not allow the detection of post-meiotic segregation.

Heteroallelic recombination: Wild-type mitotic and meiotic recombinants from *cyc1* heteroallelic strains were selected on lactate medium (see SHERMAN *et al.* 1974). Spontaneous mitotic recombinants, or those induced by X-irradiating cells on the surface of plates using the apparatus and procedure described by SHERMAN *et al.* (1974), were subcloned and the markers of one subclone from each recombinant were scored by low-temperature spectroscopic examinations and by the other procedures described above. Only a single spontaneous recombinant was taken from any one culture to ensure that each was of independent origin.

RESULTS

Genetic map of the right arm of chromosome X: Previous studies have established the order and genetic distances of the markers *met3*, *ilv3*, *SUP4*, and *cdc11* on chromosome *X* (MORTIMER and HAWTHORNE 1973; HARTWELL *et al.* 1974). These studies also demonstrated a very close linkage but not the order of the *cdc8* and *SUP4* mutant genes. Genetic analyses were undertaken with a series of diploid strains, listed in Table 1, that were heterozygous for a number of markers on chromosome *X*, including several in the gene cluster comprising *cyc1*, *rad7*,

TABLE 1

Basic diploid strains

D-672	**a**	*ilv3*	+	*SUP4-1*	+		*his5-2*	*trp5-48*	*can1-100*	+													
	α	+	*cyc1-1*	+	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*	*lys1-1*													
D-683	**a**	*ilv3*	+	*SUP4-1*	+		*his5-2*	*trp5-48*	*can1-100*														
	α	+	*cyc1-11*	+	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*														
D-684	**a**	*ilv3*	+	*SUP4-1*	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*														
	α	+	*cyc1-13*	+	+		*his5-2*	*trp5-48*	*can1-100*														
D-687	**a**	*ilv3*	+	*SUP4-1*	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*	*met1-1*													
	α	+	*cyc1-13*	+	+		*his5-2*	*trp5-48*	*can1-100*	+													
D-691	**a**	*ilv3*	*cyc1-13*	*SUP4-1*	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*														
	α	+	*cyc1-123*	+	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*														
D-703	α	*ilv3*	*cyc1-123*	*SUP4-1*	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*														
	a	+	*cyc1-13*	+	*leu1-12*		*his5-2*	*trp5-48*	*can1-100*														
D-704	α	*ilv3*	+	*SUP4-1*	+		*leu1-12*		*his5-2*		*trp5-48*		*can1-100*										
	a	+	*cyc1-13*	+	*cdc11*		+		*his5-2*		*trp5-48*		*can1-100*										
D-705	**a**	*ilv3*	+	+	*cdc8*		+		*his5-2*		*trp5-48*		*can1-100*										
	α	+	*cyc1-13*	*SUP4-1*	+		*leu1-12*		*his5-2*		*trp5-48*		*can1-100*										
CL-180	α	*met3*	+	+	*rad7*	+		+		*his5-2*		*trp5-48*		*can1-100*		*ura4-11*		*tyr7-1*		+			
	a	+	*ilv3*	*cyc1-13*	+	*SUP4-1*		*leu1-12*		*his5-2*		*trp5-48*		*can1-100*		+		+		*met1-1*			

The suppressible markers *his5-2*, *trp-548* and *can1-100* were used to score the *SUP4-1* gene; the centromere-linked marker *leu1-12* was used for either determination of the frequencies of second-division segregation, or for selection purposes in heteroallelic tests of *cyc1-13* and *cyc1-123*.

SUP4, and *cdc8*, as well as for markers on other chromosomes. Genetic analysis of complete tetrads, presented in Table 2, verified the previous genetic map of the *met3*, *ilv3*, *SUP4*, and *cdc11* markers and established a very close linkage between the *cyc1*, *rad7*, *SUP4*, and *cdc8* markers. While there was only a low number of exchanges within this region, the configuration of the flanking markers was used to deduce the order of *cyc1*, *rad7*, and *SUP4* genes. This order was confirmed by the types of coconversion observed, as described below. Since no reciprocal exchanges were observed in the *SUP4–cdc8* interval, the order could only be inferred from the pattern of conversion and associated crossing over that will be described below.

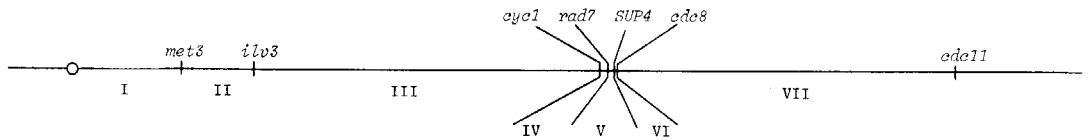
Frequencies and patterns of conversion: While the vast majority of the tetrads exhibited a normal 2:2 segregation for heterozygous markers, the expected low frequencies of aberrant segregation attributed to gene conversion were also observed (Table 3). A striking feature of these data is the great variation in conversion frequencies ranging from 14% for *ilv3* and *met3* to 0.9% for *leu1–12*, a result in keeping with those of FOGEL, HURST and MORTIMER (1971) who also observed a range of conversion frequencies for other loci. Also in keeping with the results of FOGEL, HURST and MORTIMER (1971), the frequencies of 1:3 and 3:1 conversion events are similar and statistically homogeneous, with the exception of the data for the *met3* locus where conversions to wild type predominate.

A considerable proportion of this variation in conversion frequency may reflect allele- or even locus-specific frequencies, but it should be pointed out that such frequencies are substantially dependent on the background genotype. Thus, the frequency of conversion at the *ilv3* site ranges from 3% in the D-683 pedigree to 21% in the CL-180 pedigree, a variation that is highly significant [$\chi^2(6) = 25.35$, $P<0.001$]. Because of this, and because only a single *ilv3* and *met3* allele were examined, it is premature to conclude that this region of chromosome *X* exhibits unusually high conversion frequencies, though the results would appear to indicate that this may be the case.

Table 3 also reveals that there is much variation between the various diploid strains in the frequency of gene conversion at the *cyc1* locus, though in this case, unlike the other genes listed in the table, the strains are heterozygous for different *cyc1* alleles. Among pedigrees of sufficient size for the numbers to me meaningful, it is of particular interest to note the low frequency of conversion in D-672, a strain heterozygous for the *cyc1–1* allele. This mutation comprises a deletion that encompasses the whole of the *cyc1* locus (PARKER and SHERMAN 1969; SHERMAN *et al.* 1975) and that extends into the *rad7* locus (SINGH and SHERMAN, manuscript in preparation). It is perhaps surprising to find any gene conversion for such a gross lesion. But to examine this further, and also to examine the possibility that particular conversion frequencies are associated with specific alleles, the results for the *cyc1* locus given in Table 3 have been supplemented by data collected from many pedigrees over a period of several years in our laboratories; these are presented in Table 4. Data for different alleles are tabulated only when a total of more than a hundred complete asci were dissected and adequate markers existed to detect false tetrads. The conversion frequencies listed in Table 4,

TABLE 2

Tetrad analysis of markers on chromosome X



	I*				II				I + II*				III				IV				V				IV + V				VI				VI + VII			
Strains	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM	PD	NPD	T	cM
D-672	..	..	..	..	..	..	..	..	75	75	64	14	113	3	96	27	..	..	..	..	..	..	..	..	228	0	1	0.2	..	..	..	..	..	..	..	..
D-683	..	..	..	..	..	..	..	..	30	26	22	13	51	0	26	17	..	..	..	..	..	..	..	..	77	0	3	2	..	..	..	..	..	..	..	..
D-684	..	..	..	..	..	..	..	..	29	34	23	12	51	1	26	21	..	..	..	..	..	..	..	..	86	0	2	1	..	..	..	..	..	..	..	..
D-687	..	..	..	..	..	..	..	..	16	8	9	12	20	0	13	20	..	..	..	..	..	..	..	..	38	0	1	1	..	..	..	..	..	..	..	..
D-704	..	..	..	..	..	..	..	..	19	23	14	11	33	1	22	25	..	..	..	..	..	..	..	..	62	0	4	3	..	..	..	..	37	0	22	19
D-705	..	..	..	..	..	..	..	..	75	49	67	17	111	0	71	20	..	..	..	..	..	..	..	..	198	0	12	3	219	0	0	<0.2	..	..	..	..
CL-180	100	86	43	8	153	0	32	9	..	..	..	..	106	6	88	31	246	0	6	1.2	247	0	1	0.2	..	..	..	..	..	..	..	..	..	..	..	..
Totals	100	86	43	8	153	0	32	9	244	215	199	14	485	11	342	24	246	0	6	1.2	247	0	1	0.2	689	0	23	1.6	219	0	0	<0.2	37	0	22	19

Abbreviation: PD, parental ditype tetrads; NPD, nonparental ditype tetrads; T, tetratype tetrads; cM, map distance in centimorgans, calculated using $cM = \frac{(T + 6NPD)100}{2(PD+NPD+T)}$.

* PD, NPD and T refers to *leu1*, which is assumed to be 2 cM from its centromere (MORTIMER and HAWTHORNE 1966), and map distances were calculated using $T_{ab} = T_a + T_b - 3/2\, T_a T_b$.

TABLE 3

Percent gene conversion

	D-672	D-683	D-684	D-687	D-704	D-705	CL-180	Total
+/*met3*								
3:1	—	—	—	—	—	—	30	30
2:2	—	—	—	—	—	—	234	234
1:3	—	—	—	—	—	—	8	8
% conversion	—	—	—	—	—	—	14	14
+/*ilv3*								
3:1	9	1	11	2	8	20	29	80
2:2	214	79	87	33	57	191	217	878
1:3	10	1	2	4	3	15	26	61
% conversion	8	3	13	15	16	16	21	14
+/*cyc1*								
3:1	1	1	4	0	1	1	11	19
2:2	232	81	90	39	67	215	253	977
1:3	0	0	6	0	0	10	8	24
% conversion	0.4	1.2	10.0	0.0	1.5	4.9	7.0	4.2
+/*rad7*								
3:1	—	—	—	—	—	—	5	5
2:2	—	—	—	—	—	—	262	262
1:3	—	—	—	—	—	—	5	5
% conversion	—	—	—	—	—	—	4	4
+/*SUP4*								
3:1	2	1	1	0	1	2	0	7
2:2	230	81	98	39	67	222	269	1006
1:3	1	0	1	0	0	2	4	9
% conversion	1.3	1.2	2.0	0.0	1.5	1.8	1.5	1.6
+/*cdc8*								
3:1	—	—	—	—	—	0	—	0
2:2	—	—	—	—	—	223	—	223
1:3	—	—	—	—	—	3	—	3
% conversion	—	—	—	—	—	1.3	—	1.3
+/*cdc11*								
3:1	—	—	—	—	5	—	—	5
2:2	—	—	—	—	60	—	—	60
1:3	—	—	—	—	3	—	—	3
% conversion	—	—	—	—	12	—	—	12
+/*leu1*								
3:1	0	1	0	0	0	1	4	6
2:2	234	82	100	39	67	224	268	1014
1:3	1	0	0	0	1	1	0	3
% conversion	0.4	1.2	0.0	0.0	1.5	0.9	1.5	0.9

ranging from 1.0% to 5.8%, are significantly heterogeneous [$\chi^2(4) = 13.75$, $P = 0.01$–0.001] but this heterogeneity can be shown to depend entirely on the difference between results for the deletion, *cyc1–1*, on the one hand and the point

TABLE 4

Conversion frequencies of different cyc1 *alleles*

Mutant allele	Number of hybrids	Number of asci	*CYC1:cyc1* 4:0	3:1	2:2	1:3	0:4	% gene conversion
cyc1-1	36	297	0	2	294	1	0	1.0
cyc1-2	21	245	1	7	233	4	0	4.9
clc1-9	21	251	0	4	238	9	0	5.2
cyc1-11	6	129	0	0	127	2	0	1.5
cyc1-13	5	705	0	17	664	24	0	5.8

mutations on the other; the results for *cyc1-2*, *cyc1-9*, *cyc1-11*, and *cyc1-13* are homogeneous [$\chi^2(3) = 4.12$, $P = 0.3$–0.2], but on average differ significantly from those for *cyc1-1* [$\chi^2(1) = 9.01$, $P = 0.01$–0.001]. It should be noted that despite the gross nature of the *cyc1-1* lesion, both 1:3 and 3:1 conversions were detected.

The concomitant conversions of two sites, i.e., double-site co-conversions, were observed for mutations within the four loci, suggesting that the cluster is composed of adjacent genes. With a number of the diploids listed in Table 1, it has been difficult to distinguish between co-conversion and false tetrads because there was heterozygosity for only two loci within the cluster and because there were too few unlinked markers in these strains. We have therefore confined our attention to only two of the pedigrees which were chosen because of their size, because the diploids were heterozygous at three loci within the cluster, and because several other segregating markers were present. In these circumstances one can be confident that artifacts due to false tetrads can be detected. In the pedigree obtained from the diploid CL-180 (Table 5), three double-site co-conversions of

TABLE 5

Recombinational events involving the cyc1, rad7, SUP4 *gene cluster in diploid CL-180*

	cyc1-13		*rad7*	*SUP4-1*	Total tetrads
Crossovers		6	1		} 244
Single-site conversions	16		6	3	} 272
Double-site conversions		3			
Double-site conversions			1		
Triple-site conversions		0			
% conversion	7.0		3.7	1.5	

cyc1–13 and *rad7* and one double-site co-conversion of *rad7* and *SUP4–1* were found in 272 tetrads. In all, there were 26 single-site conversions and no triple-site events. In the pedigree from D-705 (Table 6), a total of 18 single-site conversions, but no co-conversions of any kind, were found in 226 tetrads. Thus, although co-conversions for markers in different loci can be detected, it is clear that they represent a relatively small proportion of all conversion events, no doubt in part a consequence of the large distance between the mutant sites. The *cyc1–13* and *rad7* sites are over 1 cM apart, though the other sites are apparently much closer.

The results given in Tables 5 and 6 also suggest that polarity, or a gradient in gene conversion frequencies, exists within the gene cluster reminiscent of a similar phenomenon observed for mutant sites *within* a given locus (FOGEL, HURST and MORTIMER 1971). Such a suggestion must remain tentative, however, pending further work using a greater variety of crosses and different alleles at each of the loci.

The coconversion events in CL-180 confirm the order of the *cyc1*, *rad7*, and *SUP4* loci, but in the absence of co-conversion in the D-705 pedigree, or of crossovers between *SUP4* and *cdc8*, it was not possible to order the last two markers unambiguously. A tentative ordering is, however, suggested by an examination of the four tetrads from D-705 in which *SUP4* had undergone single-site conversion. In three of the four tetrads, a crossover presumably associated with the conversion event had led to recombination between *cyc1* and *cdc8*. Examination of the association between conversions and crossovers in tetrads derived from crosses heterozygous for three or four mutant sites within the *arg4* locus (HURST, FOGEL and MORTIMER 1972; MORTIMER and FOGEL 1974) has shown that the majority of crossovers are in an adjacent region and very close to the conversion sites. If this is so, *cdc8* would appear to be distal to *SUP4*, since if it was proximal

TABLE 6

Recombinational events involving the cyc1, SUP4, cdc8 *gene cluster in diploid D-705*

	cyc1–13		*SUP4–1*	*cdc8*	Total tetrads
Crossovers		12	0		} 210
Single-site conversions	11		4	3	} 226
Double-site conversions		0			} 226
Double-site conversions			0		} 226
Triple-site conversions		0			} 226
% conversion	4.9		1.8	1.3	} 226

to this locus, the *cdc8* and *cyc1* alleles would have been expected to remain in a parental configuration. It should be pointed out, however, that the data presented by MORTIMER and FOGEL (1974, Table 2) also show that a minority of associated crossovers, possibly some 10%–20%, occur in regions not adjacent to the site of conversion. Because of this minority class, the ordering of the *SUP4* and *cdc8* loci could not be definitively established.

Data given in the papers cited above (HURST, FOGEL and MORTIMER 1972; MORTIMER and FOGEL 1974) show that approximately 50% of the conversions were reciprocally recombined for flanking markers. The results from each of the pedigrees have been analyzed from this point of view (Table 7), and although the distance between the flanking markers is often rather greater than is desirable, there is good overall agreement with the 50% rule.

Orientation of the cyc1 *locus*: Unequal frequencies of combinations of flanking markers are usually observed among selected intragenic recombinants and several workers have used such results to map mutant sites within locus (see DISCUSSION). Likewise, we have attempted to orient the *cyc1* locus relative to *SUP4* by examining the behavior of this distal marker in crosses heteroallelic for *cyc1–13* and *cyc1–123*. The *cyc1–13* allele contains an isoleucine codon in place of the normal initiation codon AUG (STEWART *et al.* 1971) and is therefore at a position in the gene corresponding to the amino terminus of the protein, while the mutant codon in *cyc1–123* occupies a site corresponding to the carboxy terminal region of the protein (SHERMAN *et al.* 1975).

Wild-type recombinants from the *cyc1–13/cyc1–123* heteroallelic crosses were obtained by the following three procedures: recombinants from ascospores (meiotic method); independently derived spontaneous recombinants from vegetative diploids (mitotic method); and X-ray-induced recombinants from vegetative diploids (X-ray method). The recombinants from the meiotic method were verified to be haploids by the mating reaction and the genotype was determined

TABLE 7

Frequency of crossing over associated with conversion

Strain	Allele converted	Flanking markers	Interval length (cM)	Number of conversions	Number of conversions with crossovers
D-672	*cyc1–1*	*ilv3–SUP4*	27	1	0
D-683	*cyc1–11*	*ilv3–SUP4*	18	1	0
D-684	*cyc1–13*	*ilv3–SUP4*	20	8	5
D-704	*cyc1–13*	*ilv3–SUP4*	28	1	1
D-704	*SUP4–1*	*cyc1–cdc11*	22	1	0
D-705	*cyc1–13*	*ilv3–SUP4*	20	8	7
D-705	*SUP4–1*	*cyc1–cdc8*	3	4	3
CL-180	*ilv3*	*met3–cyc1*	44	47	32
CL-180	*cyc1–13*	*ilv3–rad7*	31	14	9
CL-180	*cyc1–13, rad7*	*ilv3–SUP4*	31	2	2
CL-180	*rad7*	*cyc1–SUP4*	1.4	6	0
Totals				93	59

from growth tests on appropriate media. The diploid recombinants from the mitotic and X-ray methods were examined for the homozygosity of the *SUP4–1*/+ alleles, using the properties of the ochre-suppressible markers *trp5–48*, *his5–2*, and *can1–100* as indicated in Table 8.

We have investigated the two strains D-691 and D-703, which are both heteroallelic for *cyc1–13* and *cyc1–123* and which differ principally with respect to the coupling of the *cyc1* and *SUP4* markers, as shown in Table 1. The results of the mitotic and meiotic analysis with these two strains are shown in Table 9. In the meiotic data, the *SUP4* allele coupled to *cyc1–13* was recovered substantially more frequently than the other, suggesting that *cyc1–13* is the proximal site. However, the mitotic results are much less satisfactory, especially with strain D-691, which gave rise to nearly equal proportions of both types of homozygotes. If one assumes that only the meiotic method is valid for mapping with outside markers, then the results indicate that the order is centromere–*cyc1–13*–*cyc1–123*–*SUP4*. However we believe this suggestion to be inconclusive because of uncertainty with respect to the recombinational process (see DISCUSSION).

DISCUSSION

The aim of this work was to order the four genes—*cyc1*, *rad7*, *SUP4*, and *cdc8*—which form a tightly linked cluster on the right arm of chromosome *X*, to examine the frequencies and patterns of gene conversion of *cyc1* alleles and other markers on this chromosome, and to attempt to orient, relative to the centromere, the two alleles *cyc1–13* and *cyc1–123* whose mutant sites occupy positions in the gene corresponding, respectively, to the amino and carboxy terminal regions of the protein.

The order (proximal to distal) of *cyc1–rad7–SUP4* was established from the results of crossing over and co-conversion; *cdc8*, which is very closely linked to *SUP4*, could not be located in this way because of the absence of suitable conversion events or crossovers. However, crossovers associated with the conversion of *SUP4* tentatively suggest that *cdc8* is distal to this marker. The observation

TABLE 8

Phenotypes associated with haploid and diploid strains containing SUP4–1, cyc1 *or their wild-type alleles, and the suppressible markers* trp5–48, his5–2, *and* can1–100

Cell type			*SUP4–1*			*cyc1*	
				Growth on:			
Ploidy	Mating	Sporul.*	Genotype	minus tryp. hist.	plus canav.	Genotype	Approx. cyto. *c* content
Haploid	+	0	*SUP4–1*	+	0	*cyc1*	5%
Haploid	+	0	+	0	+	+	100%
Diploid	0	+	*SUP4–1/SUP4–1*	+	0	*cyc1/cyc1*	5%
Diploid	0	+	+/+	0	+	+/+	100%
Diploid	0	+	*SUP4–1*/+	+	+	*cyc1*/+	50%

* Some *SUP4–1/SUP4–1* diploids sporulate poorly.

TABLE 9

The frequencies of SUP4 *genotypes of* CYC1 *recombinants from heteroallelic strains*

Method	*SUP4* genotype	Frequencies D-691	Frequencies D-703
Meiotic	*SUP4*	34 (*81%*)	1 (*2%*)
	+	8 (*19%*)	53 (*98%*)
Spontaneous Mitotic	*SUP4/+*	111 (*90%*)	125 (*86%*)
	SUP4/SUP4	5 (*4%*)	1 (*1%*)
	+/+	7 (*6%*)	19 (*13%*)
X-ray-induced Mitotic	*SUP4/+*	183 (*70%*)	133 (*69%*)
	SUP4/SUP4	50 (*19%*)	7 (*4%*)
	+/+	28 (*11%*)	51 (*27%*)

The complete genotypes of strains D-691 $\left[\frac{cyc1\text{-}13 \quad SUP4}{cyc1\text{-}123 \quad +}\right]$ and D-703 $\left[\frac{cyc1\text{-}13 \quad +}{cyc1\text{-}123 \quad SUP4}\right]$ are presented in Table 1.

that mutant sites within the *cyc1* and *rad7* loci, and also in *rad7* and *SUP4*, can be co-converted, as well as the extremely tight linkage in the cluster, suggest that these may well be contiguous genes. Results for other chromosome *X* markers, namely *met3*, *ilv3* and *cdc11*, as well as for *SUP4* and *cdc8*, confirm previous data concerning the order and map distances for these loci (MORTIMER and HAWTHORNE 1973; HARTWELL *et al.* 1974).

Gene conversion frequencies of several *cyc1* alleles were studied, including *cyc1–1*, a deletion of the whole of the structural portion of the gene (PARKER and SHERMAN 1969; SHERMAN *et al.* 1975) that extends into the *rad7* locus (SINGH and SHERMAN, manuscript in preparation). This deletion, which must encompass a region well in excess of 300 nucleotides long, was found to be capable of gene conversion, though at a rate some fivefold lower than most of the other *cyc1* alleles, which had conversion frequencies around 5% (Table 4). Both 1:3 and 3:1 events were seen for the *cyc1–1* allele. Conversions, both of the 1:3 and 3:1 type, have also been seen for deletions in the *his4* region of yeast (FINK and STYLES 1974), though in these cases the frequencies appeared to be similar to those for non-deletion mutations.

Overall, gene-conversion frequencies for the different markers studied on chromosome *X* varied from 14% for *ilv3* and *met3* down to about 1% for *cdc8*. In part this variation may depend on the particular allele, locus, or genetic region examined—it is suggestive that the linked markers *ilv3* and *met3* convert at frequencies well above the rest—but it is also likely that much of the variability depends on genetic background. In different strains the *ilv3* marker converts at frequencies ranging from 3% to 21%, results which are significantly heterogeneous. In general, conversion data for these chromosome *X* markers support the findings of MORTIMER and FOGEL (FOGEL, HURST and MORTIMER and FOGEL 1974) concerning the *his1* and *arg4* regions of yeast. Thus 1:3 and 3:1 conversion events are approximately equal, except for the *met3* locus where con-

version to wild type predominates. Moreover, about 50% of the conversions are accompanied by reciprocal recombination for flanking markers. Finally, the conversion frequencies of the four genes within the cluster appear to show polarity in a manner reminiscent of sites within a single gene. While this suggestion must, of course, be substantiated by further work using a greater variety of alleles and genetic backgrounds, if proved to be true it would lend support to the notion that a single recombination structure can cover a region much larger than a single gene.

We have attempted to orient the *cyc1* locus relative to the centromere by examining the behavior of a distal gene, *SUP4*, in intragenic recombinants from crosses that were heteroallelic for mutant sites located at the extreme ends of the structural gene. Such relationships of heteroallelic sites and outside markers have been extensively studied in intragenic recombinants from yeast and other fungi (for examples of studies with yeast see KAKAR 1963; HURST and FOGEL 1964; FOGEL, HURST and MORTIMER 1971; HURST, FOGEL and MORTIMER 1972; MORTIMER and FOGEL 1974; HASTINGS 1975). Some of these and other findings have been discussed by HASTINGS (1975), who has suggested that the behavior of outside markers in heteroallelic crosses can be used to map mutant sites within a locus only if most recombinants originate in one of two hypothetical ways. According to the model of FINCHAM and HOLLIDAY (1970) intragenic recombination of the kind used in mapping can be initiated either when only one of the mutant sites lies within a length of heteroduplex DNA or when both fall within such a heteroduplex region but are subject to independent correction. It was suggested that independent correction may be greatly and unpredictably influenced by the nature of the mutant sites, so that if recombination does not arise by the former method, mapping may be impossible.

If it is assumed in our present study that the vast majority of wild-type recombinants arose as a consequence of conversion, and moreover from single-site conversion in which only one of the two sites, *cyc1–13* or *cyc1–123*, were located in heteroduplex DNA, then it is expected that conversion of the site proximal to the centromere will lead to the recovery of a wild-type chromosome carrying the *SUP4* allele which was coupled to this proximal site. Conversion of the distal site might, in many cases, be expected to be accompanied by co-conversion at the *SUP4* site, thus leading again to the recovery of a wild-type chromosome carrying the *SUP4* allele that was coupled to the proximal site. Only if the converted segment is sufficiently short not to cover the *SUP4* locus will the *SUP4* allele coupled to the distal *cyc1* site be recovered. Moreover, it should be noted that the occurrence of crossovers associated with the ends of converted segments should increase the proportion of *CYC1* chromosomes carrying the *SUP4* allele coupled to the proximal site. In summary, a preponderance of wild-type (*CYC1*) recombinants should carry the *SUP4* allele that was originally coupled to the proximal *cyc1* marker, and in the meiotic method this inequality can be detected directly. In the mitotic method, the inequality is recognized by the occurrence of diploids homozygous for the preponderent *SUP4* allele, together with heterozygotes, and the absence of the other homozygote.

The order, centromere–*cyc1–13–cyc1–123–SUP4*, is suggested from the results of the meiotic recombinants (Table 9), but it should be emphasized that this order has not been definitively established. As discussed above, loci have been reported to be both mappable and immappable by this procedure (see HASTINGS 1975). Moreover, the suggested order is uncertain because we have examined only one heteroallelic pair, *cyc1–13/clc1–123*. No consistent order could be deduced from the results concerning spontaneous or X-ray-induced recombinants in diploid cells (Table 9). However, it is unclear to us whether or not one can determine the orientations of genes by the behavior of outside markers in selected mitotic recombinants; the recombinational process in mitotic cells is far from understood and the fundamental properties of mitotic gene conversion have not been established.

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