ALLELIC RECOMBINATION AND ITS RELATION TO RECOMBINATION OF OUTSIDE MARKERS IN YEAST[1]

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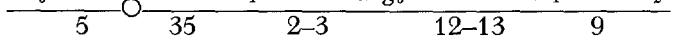
IN recent years a number of examples have been reported in which crosses between allelic mutants of *Saccharomyces cerevisiae* (ROMAN 1956a; ROMAN and JACOB 1958) produce nonmutant progeny with a frequency which is higher than is expected from reverse mutation. Such revertants can be shown to result from an event which does not have the characteristics of crossing over. ROMAN and JACOB (1958) have described one such case in mitotically dividing diploid yeast cells involving two allelic isoleucine-requiring mutants of different origin. This system has been examined further in the present study by including two more allelic mutants at this locus. The relationship between allelic recombination and the recombination of outside markers among spontaneous revertants has been examined in detail and the mutant sites of the four alleles have been ordered on the chromosome.

MATERIALS AND METHODS

Yeast strains and genetic markers: The four recessive allelic mutants requiring isoleucine were originally isolated by DR. R. K. MORTIMER of the University of California, Berkeley, from cultures irradiated with ultraviolet light. They were incorporated into the present stocks through further crosses and dissections. The four alleles have been designated is_{1-1}, is_{1-2}, is_{1-3}, and is_{1-4}, two of which (is_{1-1} and is_{1-2}, representing *ia* and *ib* respectively) were used by ROMAN and JACOB (1958).

Other genetic markers employed were the recessive mutants controlling nutritional requirement for uracil (ur_3), histidine (hi_1), arginine (arg_6) and tryptophan (tr_2). The genes ur_3, hi_1, is_1, and tr_2 are on chromosome V and are distributed as follows (HAWTHORNE and MORTIMER 1960)



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The gene arg_6 is linked as shown above (HAWTHORNE, personal communication).

The genotypes of various heteroallelic and the corresponding homoallelic diploids employed are given in Table 1. Pairs of heteroallelic diploids represent

TABLE 1

Genotypes of various homoallelic and heteroallelic diploids

Homoallelic diploids:	
7113Z	*HI* is_{1-1} *tr/hi* is_{1-1} *TR*
X-138	*ur HI* is_{1-1} *tr/UR hi* is_{1-1} *TR*
X-287	*UR HI ar* is_{1-1} *tr/ur hi AR* is_{1-1} *TR*
7581Z	*HI* is_{1-2} *tr/hi* is_{1-2} *TR*
X-139	*ur HI ar* is_{1-2} *tr/UR hi AR* is_{1-2} *TR*
X-275	*UR HI ar* is_{1-2} *tr/ur hi AR* is_{1-2} *TR*
X-305	*UR HI ar* is_{1-3} *tr/ur hi AR* is_{1-3} *TR*
Heteroallelic diploids:	
7110Z	*HI* is_{1-1} *tr/hi* is_{1-2} *TR*
7409Z	*hi* is_{1-1} *TR/HI* is_{1-2} *tr*
X-141	*ur HI* is_{1-1} *tr/UR hi* is_{1-2} *TR*
X-140	*UR hi AR* is_{1-1} *TR/ur HI ar* is_{1-2} *tr*
X-286	*UR HI ar* is_{1-1} *tr/ur hi AR* is_{1-2} *TR*
X-277	*ur hi AR* is_{1-1} *TR/UR HI ar* is_{1-2} *tr*
X-303	*UR HI ar* is_{1-1} *tr/ur hi AR* is_{1-3} *TR*
X-299	*ur hi Ar* is_{1-1} *TR/UR HI ar* is_{1-3} *tr*
X-304	*UR HI ar* is_{1-1} *tr/ur hi AR* is_{1-3} *TR*
X-300	*ur hi AR* is_{1-2} *TR/UR HI ar* is_{1-3} *tr*
X-296	*UR HI ar* is_{1-1} *tr/ur hi AR* is_{1-4} *TR*
X-297	*UR HI ar* is_{1-2} *tr/ur hi AR* is_{1-4} *TR*
X-298	*UR HI ar* is_{1-3} *tr/ur hi AR* is_{1-4} *TR*

reciprocal crosses and differ from each other with respect to the arrangement of outside markers around the isoleucine locus. A homoallelic diploid for is_{1-4} was not made for lack of a parent of appropriate genotype of opposite mating type. For this reason also, reciprocal crosses of the heteroallelic diploids involving is_{1-4} were not made. In the construction of the X-series of diploids, the haploid parents used for the homoallelic diploids were the same ones used in the construction of the heteroallelic diploids. Each of the diploids required only isoleucine plus minimal medium for growth.

Media: The various types of media used have been described by ROMAN (1956b). Isoleucine was used in the synthetic medium at 60 mg per liter. The cultures were sporulated in liquid 0.02 percent raffinose and 0.3 percent sodium acetate medium.

Techniques: The cultures were grown to stationary phase in 5 ml of yeast extract peptone medium (YEP) on a shaker. They were stored in the refrigerator when not in use. The different crosses were made by the prototroph-recovery procedure of POMPER and BURKHOLDER (1949). Dissections were made with the aid of snail enzymes (JOHNSTON and MORTIMER 1959). The various nutritional

requirement tests were carried out on synthetic media using the replica-plating technique of LEDERBERG and LEDERBERG (1952). The incubation temperature was 30°C.

RESULTS

Outside-marker recombination among the isoleucine revertants: In order to obtain information on the relation of outside marker recombination to reversion at the isoleucine locus, it was important to insure that the isoleucine revertants were of independent origin. To achieve this, approximately 200 cells were plated on a synthetic complete plate and incubated for seven to eight days. During this period isoleucine revertants arose and accumulated in the growing colonies. The revertant was isolated from the colony by streaking a portion of the latter on an isoleucineless plate. On the average, 20 to 30 revertant colonies appeared per plate. One colony was picked up and transferred to a YEP plate for further test of nutritional requirement by the replica-plating technique. The remaining portion of the colony in which the revertant arose was also transferred to a YEP plate for the purpose of comparing the nutritional requirement of the cell mixture in the colony with that of the revertant.

Outside-marker recombination was detected in the diploids by the occurrence of a nutritional requirement, such as histidine or tryptophan, due to homozygosity for the recessive gene in question. Outside marker recombination which resulted in dominant homozygosis or which retained the heterozygous condition was not detectable in the diploid but was identifiable in the meiotic products of the diploid.

Table 2 indicates the extent of outside-marker recombination among non-revertant cells. Little recombination of outside markers is evident among the homo- and heteroallelic diploids prior to reversion. The histidine recombinant of X-275, the uracil recombinant of X-286 (which probably arose early and was therefore found relatively frequently) and the tryptophan and arginine recombinant of X-140 could have resulted from mitotic crossing over in the regions between the centromere and histidine, uracil and histidine respectively, while the cultures were growing in YEP.

The extent of outside-marker recombination among revertant cells is shown in Table 3. Revertants occur with a very low frequency among homoallelic diploids and were not found in the samples tested in certain of the combinations. Among those that were obtained, the frequency of outside-marker recombination was no higher than that found among nonrevertant cells.

In the heteroallelic diploids, by contrast, a significant amount of recombination is seen among the revertants. The reciprocal crosses show homozygosis for the distal marker tryptophan to a different extent. Thus, X-140, X-277 and X-299 gave a higher frequency of *tr* homozygosis than did the corresponding reciprocal diploids X-141, X-286 and X-303 respectively. Also, more recombinants are obtained in crosses of is_{1-4} with is_{1-2} and is_{1-9} than with is_{1-1}.

Genetic analysis of revertants from diploids heteroallelic for is_{1-1}/is_{1-2}: In order to further analyse the revertants genetically, ten revertants of 7110Z (some of which were obtained from a separate experiment not included here), four of

TABLE 2

Outside-marker recombination among nonrevertant cells

Diploid		No. of colonies tested	No. of colonies requiring:				
			ur	*hi*	*ar*	*tr*	*ar tr*
Homoallelic							
7113Z	(is_{1-1}/is_{1-1})	..	..	..	..	..	..
X-138	(is_{1-1}/is_{1-1})	120	0	0	..	0	..
X-287	(is_{1-1}/is_{1-1})	500	0	0	0	0	0
7581Z	(is_{1-2}/is_{1-2})	..	..	..	..	..	..
X-139	(is_{1-2}/is_{1-2})	120	0	0	0	0	0
X-275	(is_{1-2}/is_{1-2})	225	0	1	0	0	0
X-305	(is_{1-3}/is_{1-3})	225	0	0	0	0	0
Heteroallelic							
7110Z	(is_{1-1}/is_{1-2})	..	..	..	..	..	..
7409Z	(is_{1-1}/is_{1-2})	..	..	..	..	..	..
X-141	(is_{1-1}/is_{1-2})	120	0	0	..	0	..
X-140	(is_{1-1}/is_{1-2})	120	0	0	0	0	1
X-286	(is_{1-1}/is_{1-2})	225	8	0	0	0	0
X-277	(is_{1-1}/is_{1-2})	225	0	0	0	0	0
X-303	(is_{1-1}/is_{1-3})	225	0	0	0	0	0
X-299	(is_{1-1}/is_{1-3})	225	0	0	0	0	0
X-304	(is_{1-2}/is_{1-3})	225	0	0	0	0	0
X-300	(is_{1-2}/is_{1-3})	225	0	0	0	0	0
X-296	(is_{1-1}/is_{1-4})	225	0	0	0	0	0
X-297	(is_{1-2}/is_{1-4})	225	0	0	0	0	0
X-298	(is_{1-3}/is_{1-4})	225	0	0	0	0	0

X-286 and thirteen of X-277 which were tryptophan auxotrophs were dissected. Four to eight asci of each revertant were analysed.

To ascertain the genotype of a revertant diploid, the asci were classified into parental ditype, nonparental ditype and tetratype asci (PERKINS 1953) with respect to the histidine and isoleucine or histidine and tryptophan markers. For two linked markers the parental ditype asci are expected in excess of nonparental ditype asci since the formation of the latter requires a four strand double crossover. Thus the majority ditype class indicates the distribution of marker genes on the chromosomes of the revertant diploids.

The isoleucine-dependent segregants in the asci were tested to identify the isoleucine allele present in each case. The segregants were screened for is_{1-1} by crosses with haploid isoleucine-requiring strains which carried a dominant suppressor of the isoleucine requirement due to is_{1-1} and was specific for this allele (KAKAR 1963). The cross *s*-is_{1-1} is_{1-1} × *S*-is_{1-1} is_{1-2}, gave diploids which were prototrophic. Those segregants not carrying is_{1-1} were crossed with known is_{1-1} and is_{1-2}

TABLE 3

Outside-marker recombination among revertant cells

Diploid		No. colonies streaked	No. colonies from which revertants obtained	No. of colonies requiring: ur	hi	ar	tr	ar tr
Homoallelic								
7113Z	(is_{1-1}/is_{1-1})	..	101	..	0	..	0	..
X-138	(is_{1-1}/is_{1-1})	120	109	0	2	..	0	..
X-287	(is_{1-1}/is_{1-1})	500	0	..	..	..	..	..
7581Z	(is_{1-2}/is_{1-2})	..	41	..	0	..	0	..
X-139	(is_{1-2}/is_{1-2})	120	0	..	..	..	..	..
X-275	(is_{1-2}/is_{1-2})	225	105	0	1*	0	0	0
X-305	(is_{1-3}/is_{1-3})	225	24	0	0	0	0	0
Heteroallelic								
7110Z	(is_{1-1}/is_{1-2})	..	258	..	1	..	2	..
7490Z	(is_{1-1}/is_{1-2})	..	160	..	0	..	0	..
X-141	(is_{1-1}/is_{1-2})	120	108	1	0	..	11	..
X-140	(is_{1-1}/is_{1-2})	120	117	0	2	0	21	1*
X-286	(is_{1-1}/is_{1-2})	225	225	8*	0	0	5	0
X-277	(is_{1-1}/is_{1-2})	225	225	3	4	1	29	0
X-303	(is_{1-1}/is_{1-3})	225	222	0	0	2	5	2
X-299	(is_{1-1}/is_{1-3})	225	212	0	3	0	21	0
X-304	(is_{1-2}/is_{1-3})	225	97	0	7	1	6	1
X-300	(is_{1-2}/is_{1-3})	225	218	0	1	0	8	0
X-296	(is_{1-1}/is_{1-4})	225	225	0	0	0	4	0
X-297	(is_{1-2}/is_{1-4})	225	202	1	0	0	14	0
X-298	(is_{1-3}/is_{1-4})	225	202	0	0	0	16	0

* Also in nonrevertant cells from which this revertant was derived (see Table 2).

haploids. If is_{1-2} is present in the segregant, heteroallelic reversion should occur in the cross with the known is_{1-1} strain and not with the is_{1-2} strain (ROMAN 1956a). If the allele is doubly defective, $is_{1-1,2}$, as would be expected from reciprocal recombination between the defective sites is_{1-1} and is_{1-2}, neither cross should give heteroallelic reversion.

Results of such an analysis for the isoleucine revertants which were tryptophan auxotrophs are shown in Table 4. It is to be seen that among the revertants of this kind from 7110Z and X-286, the allele is_{1-1} is recovered more frequently (11 out of 14 cases), while among the revertants from X-277, the reciprocal cross, the allele is_{1-2} is more prevalent (11 out of 13 cases).

In addition, 17 revertants of 7110Z, 12 of X-286 and 13 of X-277, which were prototrophic for the nutritional requirements, were dissected to detect dominant homozygosis or heterozygosis for the marker genes. Six of the revertants of 7110Z, four of X-286 and two of X-277 proved to be homozygous for *TR* (Table 5). As is

TABLE 4

Dissection results of tr tr *homozygotes*

7110Z, X-286 = $\frac{HI\ is_{1-1}\ tr}{hi\ is_{1-2}\ TR}$ X-277 = $\frac{HI\ is_{1-2}\ tr}{hi\ is_{1-1}\ TR}$

Culture	Genotype			No. obtained
7110Z and X-286	*hi*	*IS*	*tr*	8
	HI	is_{1-1}	*tr*	
	HI	*IS*	*tr*	3
	hi	is_{1-1}	*tr*	
	hi	*IS*	*tr*	2
	HI	is_{1-2}	*tr*	
	HI	*IS*	*tr*	1
	hi	is_{1-2}	*tr*	
X-277	*hi*	*IS*	*tr*	1
	HI	is_{1-1}	*tr*	
	HI	*IS*	*tr*	1
	hi	is_{1-1}	*tr*	
	hi	*IS*	*tr*	11
	HI	is_{1-2}	*tr*	

seen in Table 5, among the *TR* homozygous revertants of 7110Z and X-286 it is the allele is_{1-2} which is more frequent (eight out of ten cases) in contrast to the allele is_{1-1} which was more prevalent among the *tr* homozygous revertants from these diploids. These results then indicate that there exists some kind of a pattern

TABLE 5

Dissection results of TR TR *homozygotes*

7110Z, X-286 = $\frac{HI\ is_{1-1}\ tr}{hi\ is_{1-2}\ TR}$ X-277 = $\frac{HI\ is_{1-2}\ tr}{hi\ is_{1-1}\ TR}$

Culture	Genotype			No. obtained
7110Z	*HI*	*IS*	*TR*	5
	hi	is_{1-2}	*TR*	
and	*hi*	*IS*	*TR*	3
	HI	is_{1-2}	*TR*	
X-286	*hi*	*IS*	*TR*	2
	HI	is_{1-1}	*TR*	
X-277	*HI*	*IS*	*TR*	1
	hi	*IS*	*TR*	
	hi	*IS*	*TR*	1
	HI	is_{1-2}	*TR*	

with respect to the allele present among the two kinds of tryptophan homozygotes.

The proportion of *TR* homozygotes seems higher than expected in comparison with *tr* homozygotes recovered. In a comparison of X-277 and X-286 and 7110Z, for example, we find 29/225 *tr* homozygotes from X-277 and would therefore expect the same proportion of *TR* homozygotes from X-286 and 7110Z. The number observed, 10/29, is higher than expected and suggests that tryptophan prototrophs, whether homozygous or heterozygous, are selected for at the expense of the tryptophan auxotrophs. That the *TR* homozygous cells were not pre-existing prior to the reversion event was shown by dissections of asci of nonrevertant cells from 7110Z, X-286 and X-277, all of which were found to be heterozygous at this locus. If selection is responsible, it is likely to have occurred on the synthetic complete plates on which the original colonies were grown for reversion, and to have been aggravated by the loss of tryptophan from plates on standing, a fact not realized at the time these experiments were in progress.

The results of revertants which were heterozygous for tryptophan gene are shown in Table 6. The frequency of the two types of alleles present among the revertants of this kind is very nearly the same, the allele is_{1-1} being only slightly more abundant among the revertants from 7110Z and X-286 and the allele is_{1-2} among the revertants from X-277. The convertant strands of 7110Z and X-286 that are *hi IS TR* are all accompanied by the allele is_{1-1} whereas the same strands from X-277 are accompanied by is_{1-2}. Similarly, the convertant strand *HI IS tr* which is recovered slightly less abundantly than the strand *hi IS TR* is accom-

TABLE 6

Dissection results of TR tr *heterozygotes*

7110Z, X-286 $= \dfrac{HI\ is_{1-1}\ tr}{hi\ is_{1-2}\ TR}$ X-277 $= \dfrac{HI\ is_{1-2}\ tr}{hi\ is_{1-1}\ TR}$

Culture	Genotype			No. obtained
7110Z	*hi*	*IS*	*TR*	10
	HI	is_{1-1}	*tr*	
and	*HI*	*IS*	*TR*	2
	hi	is_{1-1}	*tr*	
X-286	*HI*	*IS*	*TR*	2
	hi	is_{1-2}	*tr*	
	hi	*IS*	*tr*	1
	HI	is_{1-2}	*TR*	
	HI	*IS*	*tr*	4
	hi	is_{1-2}	*TR*	
X-277	*hi*	*IS*	*TR*	7
	HI	is_{1-2}	*tr*	
	HI	*IS*	*tr*	4
	hi	is_{1-1}	*TR*	

panied by the allele is_{1-2} among the revertants from 7110Z and X-286 and with the allele is_{1-1} among the revertants from X-277, thus indicating a pattern in these kinds of revertants as well.

The results of Table 6 further show that in a majority of cases (7/11 in 7110Z, 7/8 in X-286 and 11/11 in X-277) the wild-type strand has the parental combination of outside markers. Also, the nonrevertant strands carry either the allele is_{1-1} or the allele is_{1-2} and in no case carry the doubly defective allele $is_{1-1,2}$. Among five cases of 7110Z and X-286, where the wild-type strand is recombinant for outside markers, it is accompanied in three cases with the strand carrying the allele is_{1-2}, and in two cases with the strand carrying the allele is_{1-1}.

It must be pointed out that in four more revertants of 7110Z which were prototrophic for various nutritional markers, irregular segregations for the isoleucine marker were encountered. Reversion here was due to dominant suppressors specific for the allele is_{1-1}. These cases are described elsewhere (KAKAR 1963).

Genetic analysis of revertants from a homoallelic diploid: In order to find out the mode of reversion in homoallelic diploids, five independent revertants of 7113Z (is_{1-1}/is_{1-1}) were dissected. In all of these the reversion was due to a mutation at a suppressor locus. These results are discussed in detail elsewhere (KAKAR 1963).

DISCUSSION

The study of allelic recombination through mitotic analysis has the limitation that one recovers only half of the products of the recombinational event. In the present analysis, for example, we are selecting for at least one strand which is a wild-type recombinant for the two isoleucine mutant alleles accompanied by another strand carrying one or another of these alleles. Still, such an analysis has revealed the complexity of the problem that we are concerned with.

The present results show a coincidence of occurrence of reversion at the isoleucine locus in the heteroallelic diploids with recombination of outside markers, more often so for the distal marker tryptophan than for the proximal marker histidine. This is further revealed by the dissections of the revertant diploids from one of the heteroallelic crosses. The fact that we do not get much recombination for histidine is not unexpected, since in the present analysis we are selecting for an event between the isoleucine alleles which presumably involved pairing in this region and any kind of event (events) taking place in this region will produce recombinants for distal markers only. These results are, therefore, different from the ones reported for an adenine locus in yeast (ROMAN 1956a) where the recombination for outside markers was not observed among the revertants and may be a reflection of the allelic pair in question.

A noteworthy feature of the results is the difference with respect to amount of homozygosity obtained for tryptophan among the isoleucine revertants from reciprocal heteroallelic crosses. Thus the diploids which were *hi* is_{1-1} *TR/HI* is_{1-2} *tr* (X-140 and X-277), *hi* is_{1-1} *TR/HI* is_{1-3} (X-299) and *hi* is_{1-3} *TR/HI* is_{1-2} *tr* (X-304) in constitution showed *tr* homozygosis with a higher frequency than did

the corresponding diploids of the reciprocal genotype. This suggests the following order of the alleles on the chromosome:

$$\circ\ \underline{hi \quad is_{1-2} \quad is_{1-3} \quad is_{1-4} \quad is_{1-1} \quad tr}$$

The allele is_{1-4} will have a position between is_{1-2} and is_{1-1} and has been tentatively placed between is_{1-3} and is_{1-1}.

The dissection results indicate that there exists some kind of a pattern with respect to the allele present among the two kinds of trypophan homozygotes. The dissections of revertants heterozygous for various markers also emphasize the evidence of a pattern and the dependence of the pattern on the starting genotype inasmuch as there is the correlation of is_{1-1} and is_{1-2} accompaniment with the parental genotypes. The reciprocal crosses show this pattern in reverse and this is further suggestive of a linear order of the alleles along the length of the chromosome.

The above order of the alleles is consistent with the fact that the marker relationship of the majority genotype class among the *tr* homozygous recombinants of X-277 (*hi IS tr/HI* is_{1-2} *tr*) and *TR* homozygous recombinants of 7110Z and X-286 (*HI IS TR/hi* is_{1-2} *TR*) can be simply accounted for on the basis of a single exchange between the two mutant alleles to produce the wild type strand. Whether such an exchange is accompanied by a reciprocal exchange as expected from a classical crossover type of event could not be determined by the experiment. The evidence from the analysis of the revertants heterozygous for the linked markers can be used to argue for the view that crossing over in the conventional sense is not involved in the recombination between the two alleles and that the revertant strand originates by a nonreciprocal recombination event, since the double defective strand $is_{1-1,2}$ is not recovered among the isoleucine prototrophs. Such an interpretation will also follow from the fact that among most of the heterozygous cases (25 out of 30) of 7110Z, X-286 and X-277 the wild-type strand for isoleucine is not recombinant for the outside markers. Also, it is accompanied with the alleles is_{1-1} or is_{1-2} with almost equal frequency (Table 6). The explanation of these results otherwise would require a tremendous amount of multiple crossing over. Considering the low frequency of back mutation of the two alleles as determined by reversion rate among the homoallelic diploids (most of which involve suppressor mutations [KAKAR 1963]), such an interpretation is also not sufficient to account for the results. Since we are dealing with a complex situation, it is very likely that both nonreciprocal recombination between the alleles and to some extent inertallelic crossing over are responsible for the production of isoleucine independent strands. The cases of back mutation of one or the other allele will not be distinguishable from that of nonreciprocal event. The yeast results for this locus might then be different from the ones reported for an adenine locus in Aspergillus, where in a similar kind of mitotic analysis the wild-type strand has been shown to originate by a crossover type of event between the mutant alleles (PRITCHARD 1960).

SUMMARY

Reversion to isoleucine prototrophy takes place quite frequently among hetero-

allelic diploids. The results show a coincidence of occurrence of the reversional event with the recombination of outside markers among heteroallelic diploids and not among homoallelic diploids. The reciprocal heteroallelic diploids exhibit recombination for the distal marker tryptophan to a different extent than for the proximal marker histidine, which permits arranging the alleles in a linear order on the chromosome. Other aspects of allelic recombination are discussed.

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