TRANSFER OF CHROMOSOME III DURING *kar* MEDIATED CYTODUCTION IN YEAST

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We describe the transfer, at low frequency, of a limited number of nuclear markers during *karl* mediated cytoduction of the RHO^+ factor. By selection for a chromosome III marker in *KAR1 HIS4* [RHO^+] $\times$ *kar1 his4* [rho^-] crosses, strains disomic for chromosome III were isolated. Markers carried on five other chromosomes in the *HIS4* donating strain could be shown to be absent from the disomic strains. When these disomic strains were force-mated to haploid tester strains the rare prototrophic products were sporulators with good spore viability. These observations suggest that only one or possibly a few chromosomes were transferred to the recipient strain during cytoduction of the RHO^+ factor.

1. INTRODUCTION

Mixing of haploid cells of opposite mating type in *Saccharomyces cerevisiae* results in pairwise cell fusion and the production of a short-lived heterokaryon. Normally karyogamy follows rapidly after cell fusion and a diploid zygote is formed. However, mutations affecting karyogamy have been described (1).

These mutations greatly increase the life time of the heterokaryons, and daughter cells usually have the nuclear genotype of one of the parents, while cytoplasmic markers are mixed. The transfer of a cytoplasmic marker from one nuclear genotype to another in this way has been called cytoduction (9).

Abbreviations: SD = synthetic minimal dextrose, YPD = yeast extract peptone dextrose.

Table I.

Yeast strains employed.

Strain	Genotype	Source
K5-5A [rho ⁻]	<i>MATα his4-15 ade2-1 can1 kar1-1</i> [rho ⁻]	CONDE & FINK (1) and KIELLAND-BRANDT et al. (7)
XXI-I-37	<i>MATa cyh2 ilv1 lys1 trp5</i>	NILSSON-TILLGREN
C78-I-23	<i>MATa</i>	NILSSON-TILLGREN
C79-C1	<i>MATa HIS4/MATα his4-15 ade2-1 can1 kar1-1</i>	This study
C79-C2	<i>MATa HIS4/MATα his4-15 ade2-1 can1 kar1-1</i>	This study
Tester strains:		
C79-992	<i>MATα his4-644 leu2 thr4 ura4</i>	NILSSON-TILLGREN
C79-993	<i>MATa his4-644 leu2 thr4 ura4</i>	NILSSON-TILLGREN
C79-994	<i>MATa leu2 thr4</i>	NILSSON-TILLGREN
C79-995	<i>MATα leu2 thr4</i>	NILSSON-TILLGREN

In studies of the inheritance of a pure yeast plasmid carrying the *HIS4* gene (7) we observed the transfer of a limited number of nuclear markers from one parent nucleus to the other during cytoduction of the non-mendelian character RHO⁺. In this communication we describe this phenomenon and show that the resulting strains are disomic for chromosome III.

2. MATERIALS AND METHODS

2.1. Media and strains

All media used in this study have been described by KIELLAND-BRANDT et al. (7). The strains employed are presented in Table I.

2.2. Cytoduction and crosses

Two different mating methods for cytoduction were used. Mating on filter is described (7). The other method was based on JAKOB's (6) procedure for mass mating: Two strains of different mating type were grown to late log phase in YPD. Equal numbers of cells were mixed and incubated for 30 minutes at room temperature, whereafter they were centrifuged. The pellet was further incubated for 4 hours, washed two times in water and resuspended in water.

Crosses for recovery of rare mating products (forced mating) were carried out as follows: Approximately 10⁸ cells of each parent were

mixed on a YPD plate and allowed to grow for two days at 30 °C. The cells were then replica plated to minimal medium (SD) and further incubated for 4 days after which isolated diploid colonies were picked.

Sporulation and dissection were performed according to standard procedures (4).

3. RESULTS

In a control experiment during our studies of the cytoduction of a *HIS4* carrying yeast plasmid (7) we observed what looked like a reversion of *his4-15*. Mutation *his4-15* is a deletion lacking approximately 500 base pairs in segment A of the *HIS4* gene and reversions have consequently never been observed (2, 5). In this experiment strain K5-5A [rho⁻] (*MAT α his4-15 ade2-1 kar1-1 can1* [rho⁻]) was mated to C78-I-23 (*MATa* [RHO⁺]) by the filter mating technique and the mating products were plated on minimal medium with adenine and canavanine. Several colonies appeared that were red (*ade2* [RHO⁺]), histidine prototrophic and canavanine resistant. The frequency of appearance of these colonies was 1.4 · 10⁻⁵ per K5-5A cell that by cytoduction had received a functional rho factor from the prototrophic strain. The red histidine prototrophic colonies did not mate or sporulate; i.e. they could not be simple revertants for the histidine requirement, neither could they be

diploids which had undergone somatic recombination in the *ade2* and *can1* loci. Rare prototrophic mating products could be obtained after mating to both *a* and α strains and subsequent tetrad analysis gave results which indicated that the aberrant colonies were disomic for chromosome III. Support to this notion was given by the observations that they segregated rare histidine auxotrophic colonies which were found to be efficient maters.

In a second experiment strain K5-5A [ρ^-] was mated to XXI-I-37 as well as to C78-I-23. The mass mating technique of JAKOB (6), modified as described in section 2.2, was used. Red (*ade2* [ρ^+]), histidine prototrophic and canavanine resistant colonies appeared after both types of mating with a frequency of $5.8 \cdot 10^{-7}$

(for XXI-I-37) and $1.9 \cdot 10^{-6}$ (for C78-I-23) per K5-5A cell that received a functional ρ factor by cytoduction. As in the first experiment all red, histidine prototrophic colonies were non-maters and did not sporulate. One colony from each cross, C79-C1 and C79-C2, was chosen for further analysis.

The two isolates were force-mated to *a* and α tester strains carrying different chromosome III markers. Individual prototrophic diploid colonies were isolated and sporulated. The results of tetrad analysis of these diploids are shown in Table II. It can be seen that *his4*, *leu2*, *MATa*, *MAT α* and *thr4* segregate abnormally when C79-C1 or C79-C2 are mated to strains carrying *his4-644* (C79-992 and C79-993). The segregation pattern of these chromosome III

Table II.

Tetrad analysis of rare mating products showing that C79-C1 and C79-C2 are disomic for chromosome III.

Analysed parent Prototrophic isolate No. Tester strain	C79-C1 1a C79-993 (<i>MATa his4</i> <i>ura4 leu2</i> <i>thr4</i>)	C79-C1 1b C79-993 (<i>MATa his4</i> <i>ura4 leu2</i> <i>thr4</i>)	C79-C1 1c C79-993 (<i>MATa his4</i> <i>ura4 leu2</i> <i>thr4</i>)	C79-C1 2 C79-994 (<i>MATa leu2</i> <i>thr4</i>)	C79-C1 3 C79-992 (<i>MATα his4</i> <i>ura4 leu2</i> <i>thr4</i>)	C79-C1 4 C79-995 (<i>MATα leu2</i> <i>thr4</i>)	C79-C2 5 C79-993 (<i>MATa his4</i> <i>ura4 leu2</i> <i>thr4</i>)
No. of tetrads	25	7	4	11	21	12	6
Segregation of markers:							
<i>HIS4/his4</i>	4:0	0	0	0	0	12	0
	2:2	23	6	4	11	19	0
	1:3	2	1	0	0	2	0
<i>LEU2/leu2</i>	4:0	10	7	0	0	11	0
	3:1	5	0	1	1 ^c	4	2 ^c
	2:2	10	0	3	10	6	10
<i>MATa/MATα</i>	2:2	25	0	1	11	5	12
other	0	7 ^a	3 ^a	0	16 ^b	0	6 ^a
<i>THR4/thr4</i>	4:0	0	4	1	0	8	0
	3:1	1 ^c	2	2	0	10	0
	2:2	24	1	1	11	3	12
<i>ADE2/ade2</i>	2:2	25	7	4	11	21	11
	1:3	0	0	0	0	1 ^c	0
<i>URA4/ura4</i>	2:2	25	7	4	—	21	—
							6

^a Tetrads with non-mating spores and excess of α spores.

^b Tetrads with non-mating spores and excess of *a* spores.

^c Interpreted to represent a meiotic gene conversion.

Table III.

Interpretations of the tetrad analysis presented in Table II.

Prototrophic isolate No.	Chromosome constitution of		Mitotic event giving rise to mating cell
	rare mating product	mating cell of the analysed parent	
1a	2n + 1	n + 1	Loss of <i>MATa</i> and <i>THR4</i> by a deletion
1b	2n + 1	n + 1	Recombination giving homozygosis for <i>MATα</i>
1c	2n + 1	n + 1	Recombination giving homozygosis for <i>MATα</i>
2	2n	n	Non-disjunction
3	2n + 1	n + 1	Recombination giving homozygosis for <i>MATa</i>
4	2n	n	Non-disjunction
5	2n + 1	n + 1	Recombination giving homozygosis for <i>MATα</i>

markers shows that C79-C1 and C79-C2 are disomic for this chromosome. The markers *ade2* (chromosome XV) and *ura4* (chromosome XII) segregate 2:2, demonstrating that these two chromosomes are present only in one copy in C79-C1 and C79-C2. Strain XXI-I-37, which was the chromosome III donor in the construction of C79-C1, also carries *cyh2*, *trp5* (both chromosome VII), *ilv1* (chromosome V) and *lys1* (chromosome IX). None of these alleles appear in spores from diploids where C79-C1 is a parent. From the last two observations it can be concluded that in the case of C79-C1 chromosome III, but not chromosomes V, VII, IX, XII and XV, was transferred to the recipient strain during cytoduction. The high spore viability (80–95%) found in crosses between C79-C1 or C79-C2 and the tester strains further support the notion that C79-C1 and C79-C2 are disomic only for chromosome III or a limited number of chromosomes.

Several different mitotic events in C79-C1 can lead to the formation of a cell capable of mating with the tester strains. These events are summarized in Table III. In the mating with strain C79-994 carrying *HIS4* a 2:2 segregation of all the chromosome III markers was observed, indicating that the mating cell of C79-C1 was formed by non-disjunction of the *MATa HIS4* carrying chromosome. The other possibility for non-disjunction, namely involving the *MATα his4-15* chromosome, occurred with the formation of the mating cell in the cross with C79-995: No segregation is found for the *HIS4*

marker but a 2:2 segregation for all other chromosome III markers. Mitotic recombination in chromosome III producing *MATa/MATa* or *MATα/MATα* cells, was detected in four prototrophic isolates (No. 1b, 1c, 3 and 5) from crosses to *his4* strains (C79-993 and C79-992) by the presence of asci with an excess of *a* or *α* spores in addition to non-mating spores. In the phototrophic isolate No. 1a aberrant segregation of *his4* and *leu2* was observed, while mating type and *thr4* segregated 2:2. The mitotic event giving rise to the mating C79-C1 cell in this isolate most likely involved a deletion of *MATa* and *THR4*, which made the cell become *α*. The two chromosomes without this deletion must have disjoined preferentially during the first meiotic division, since normal spore viability was observed.

4. DISCUSSION

The data reported here show that transfer of one or possibly a few chromosomes occasionally takes place from one nucleus to another in the transient heterokaryons produced in crosses involving the *kar1-1* mutation. S. DUTCHER and L. HARTWELL (personal communication) have made similar observations. In different experiments we have seen the frequency of transfer of *HIS4* relative to the transfer of *RHO*⁺ to vary from $6 \cdot 10^{-7}$ to $1.4 \cdot 10^{-5}$. It is possible that these variations depend on differences in the two methods for mating employed, but a genetic

variation in the frequency of transfer cannot be excluded.

The cells studied were disomic for chromosome III and thus heterozygous for the mating type gene *MATa/MATα*. The genetic analysis depended on a mitotic event to yield a cell competent to mate. In four cases this could be traced to result from mitotic recombination resulting in homozygosis for one mating type allele. In two cases mitotic non-disjunction led to a competent mater. In the remaining case a deletion covering the *MATa* gene and the *THR4* gene has to be postulated. HAWTHORNE (3) and STRATHERN et al. (8) have reported that the rare mating type interconversions in heterothallic strains are frequently associated with big deletions in chromosome III. The big deletion observed in the prototrophic isolate 1a of C79-C1 is different from mating type interconversions, since it has yielded a chromosome without any functional *MAT* allele.

The transfer of a single chromosome provides a means for the transfer of specific genes from one nucleus to another. Subsequent incorporation of one or several of these genes into the homologous chromosome of the recipient is simplified by the absence of genetic variation in the rest of the genotype.

We therefore believe that this *kar* mediated chromosome transfer will become a valuable tool in the genetic improvement of industrial *Saccharomyces* strains. In principle, it requires only mating and direct selection for the desired genotype and thus does not depend on good sporulation and spore viability, characteristics which are often absent in the diploid hybrid strains. In addition, the genetic constitution of poorly sporulating and/or distantly related *Saccharomyces* strains may be determined by the transfer of single chromosomes from these strains into the nuclei of appropriately marked laboratory strains. Using for instance multiple markers in a chromosome of the laboratory strain it may be possible to determine if there is a homologous chromosome in the strain to be analysed.

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