YEAST VOL. 12: 1279–1283 (1996)

Yeast Mapping Reports

Mapping of Gene Controlling Thiamine Transport in *Saccharomyces cerevisiae*

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Received 1 March 1996; accepted 10 May 1996

A recessive mutation leading to complete loss of thiamine uptake in *Saccharomyces cerevisiae* was mapped on the left arm of chromosome VII, approximately 56 cM centromere-distal to *trp5*. As the analysed locus is relatively distant from its centromere and from the markers used, its attachment to chromosome VII was confirmed by chromosome loss methods.

KEY WORDS — *Saccharomyces cerevisiae*; thiamine transport; recessive allele; chromosome VII

INTRODUCTION

Thiamine is transported into cells of *Saccharomyces cerevisiae* by a very efficient active transport (Iwashima *et al.*, 1973) leading to 1000-fold accumulation of thiamine over the external levels. This process is irreversible (Ruml *et al.*, 1988) and is completely inhibited by pyrithiamine and other thiamine analogues but not by oxythiamine (Iwashima *et al.*, 1973). Two mutants with practically no thiamine uptake were found on the basis of their resistance to pyrithiamine (Iwashima *et al.*, 1975) but they were not characterized genetically. The impaired gene controlling thiamine uptake is one of the complementary mutated genes leading to the over-production of thiamine and to its excretion from yeast cells (Ruml and Šilhánková, submitted for publication).

In the present paper, the gene controlling thiamine transport (*thp1*) in *S. cerevisiae* was localized on the chromosome map. Tetrad analysis combined with autoradiographic thiamine estimation and chromosome loss methods were used for this purpose. Excretion of thiamine appearing in the combination of the studied recessive allele with the dominant resistance to oxythiamine (Ruml and Šilhánková, submitted for publication) was exploited in the chromosome loss methods.

MATERIALS AND METHODS

Strains

Genotypes of strains of *S. cerevisiae* used are shown in Table 1. *Rhodotorula mucilaginosa* DBM19 was used for cross-feeding tests.

Genetic methods

Basic genetic methods, i.e. hybridization, sporulation, dissection of asci and tetrad analysis were done as described previously (Šilhánková, 1972). Replica plating for crosses of auxotrophs with complementary markers was also used. The map distance was calculated by the equation of Perkins (1979): $x = 100(TT + 6NPD)/2(PD + NPD + TT)$cM. Corrections for long distances according to Snow (1979a, b) and King and Mortimer (1991) were calculated from graphs presented by Mortimer and Schild (1981) and King and Mortimer (1991).

The use of 2 μ tester strains for the estimation of assignment to certain centromeres was based on the findings of Falco *et al.* (1982, 1983) and Falco and Botstein (1983). Because of the instability of the integration of 2 μ DNA into chromosomes, cir^0 strains were cultivated on media without uracil, as *URA3* is present only in the integrated part of 2 μ DNA. Diploids cir^+/cir^0 obtained by

CCC 0749–503X/96/121279–05

Table 1. Genotypes and phenotypes of strains of *S. cerevisiae* used in this study.

Strain	Genotype	Thiamine transport
TN 31	α *OXT1 thi3 thp1*	Thiamine excretion, absence of thiamine uptake
21/16	**a** *thp1*	Absence of thiamine uptake
X-2928-3D	α *ade1 gal1 leu1 his2 ura3 trp1 met14*	Normal
2/38	α *thp1 met14*	Absence of thiamine uptake
XS144-S19	**a** *met13 leu1 trp5 cyh2 aro2 lys5 ade5*	Normal
XS144-S22	α *met13 leu1 trp5 cyh2 aro2 lys5 ade5*	Normal
XB-103-17	**a** *trp1 aro7 ade5 ura4 met4 gal1*	Normal
XS-122-57D	**a** *rad52-1 ura3*	Normal
XS-122-49C	α *rad52-1 leu2*	Normal
XS-214-1B	**a** *rad52-1 leu2 trp5 arg4 his6 ilv3 ura1 lys9 met2 ade2*	Normal
X-1986-16C	**a** *trp5-48 his5-2 ade2-1 lys1-1 arg4-17 leu1-12*	Normal
27/20	α *rad52-1 his6 trp5 OXT1 thi3 thp1 thi5*	Thiamine excretion, absence of thiamine uptake
28/21	α *rad52-1 his6 ade2 arg4 OXT1 thi3 thp1 thi5*	Thiamine excretion, absence of thiamine uptake
B-7588	chrI **a** *cir^o ura3-52 leu2-3,112 trp1-289 met2 HIS3+*	Normal
B-7100	chrII **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7171	chrII **a** *cir^o ura3-52 LEU2+ trp1-289 his3-D1 met2 cyh^r*	Normal
B-7590	chrV **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7591	chrVI **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2*	Normal
B-7173	chrVII **a** *cir^0 ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7174	chrVIII **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7175	chrIX **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7593	chrX **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2*	Normal
B-7178	chrXI **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7595	chrXII **a** *cir^o ura3-52 leu2-3,112 trp1-289 met2 cyh^r HIS3+*	Normal
B-7255	chrXIII **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7596	chrXIV **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7180	chrXV **a** *cir^o ura3-52 leu2-3,112 trp1-289 his3-D1 met2 cyh^r*	Normal
B-7598	chrXVI **a** *cir^o ura3-52 leu2-3,112 trp1-289 met2 cyh^r*	Normal

replica plating on minimal agar were cultivated under shaking in minimal medium without thiamine for 36 h. After that, approximately 200 cells were spread on minimal agar without thiamine, previously inoculated with 10^6 cells of *R. mucilaginosa*. As auxotrophic requirements coded on the chromosome homologous to that bearing the part of 2 μm DNA are phenotypically expressed during chromosome loss, corresponding nutrients were supplemented to minimal medium for shaken cultures and for cross-feeding tests. Cross-feeding resulting from the loss of the homologous chromosome was evaluated after 3–5 days of incubation at 28°C. It appeared as a zone of growth of the test organism around the colony of the tested one.

Mitotic chromosome loss in *rad52/rad52* diploids (Mortimer *et al.*, 1981) was followed after irradiation of the suspension of washed cells (10^5–10^6 cells/ml) by γ rays (Gammacell 220, Atomic Energy of Canada) at the dose of 100 Gy. After that, cells were diluted, plated on complete agar and incubated for 3–4 days at 28°C. Individual colonies were then tested for cross-feeding of thiamine and for amino acid auxotrophy.

Analytical methods

Autoradiographic method for testing the presence of thiamine uptake 20 μl spots of washed suspensions of cells harvested from thiamine-free medium were placed on a Millipore membrane laid on agar medium containing [*thiazole*-2-^{14}C]thiamine (6 nmol/ml) and glucose (100 μmol/ml) in citrate-phosphate buffer (50 μmol/ml) at pH 5·0 and incubated for 3 h at 28°C. After that, the bottom side of the membrane was washed with ice-cold

Table 2. Tetrad analysis of the products of the cross thp1 × X-2928-3D.

Interval tested*	Number of asci			λ^2 (PD:NPD)
	PD	NPD	TT	
thp1–ade1 (I)	19	12	41	1·58
thp1–trp1 (IV)	15	18	38	0·27
thp1–ura3 (V)	12	16	38	0·57
thp1–his2 (VI)	7	12	49	1·32
thp1–leu1 (VII)	22	8	43	6·53
thp1–met14 (XI)	17	16	38	0·03

*Numbers in parentheses indicate the chromosome of the auxotrophic marker.

water, dried and exposed to Fortepan film (23 DIN) for 48 h. The exposed film was developed with Fomadon N developer. Black spots indicated cell suspensions with thiamine uptake, while only lightly greyish spots appeared in the absence of thiamine uptake.

Quantitative estimation of thiamine uptake This was as previously described (Ruml *et al.*, 1988) using [*thiazole*-2-^{14}C]thiamine (6 µmol/ml) and Aquasol (NEN Chemicals, Boston) as scintillant. Samples were counted in a Packard Tricarb 300 for estimation of the radioactivity of washed cells.

RESULTS AND DISCUSSION

Dissected tetrads of the cross of mutant *thp1* to auxotroph X-2928-3D yielded monofactorial segregation of thiamine uptake and of all auxotrophic markers. Tetrad analysis showed a high probability of *thp1* localization on chromosome VII (Table 2). A χ^2 test for PD:NPD confirmed this localization with greater than 95% probability (χ^2 value should be >3·84) but with lower than 99% probability (χ^2>6·64). Crosses of the *thp1* segregants to strains XS144-S19 and XS144-S22, containing markers on chromosome VII (Table 1), yielded complete tetrads regularly segregating spores that did not grow on minimal agar with all requirements. This phenomenon was connected with allele *aro2* and also appeared in the crosses of the *thp1* strains to strain XB103-17 containing allele *aro7*. Strains with these *aro* alleles cannot, therefore, be used for mapping allele *thp1*.

Using the results of the cross of mutant *thp1* to strain X-2928-3D (Table 2) for the calculation of the distance of *thp1* from centromere-linked allele *leu1* by means of the Perkins equation led to the value of 62·3 cM. This is very inaccurate because triple and even higher-order cross-overs appear in distances longer than 40 cM. With the correction for long distances according to Snow (1979a, b), the approximate distance would be 78 cM. The mathematical model of King and Mortimer (1991) for long distances in *S. cerevisiae*, which assumes chiasma interference, gives the value of 70 cM for the approximate distance *leu1–thp1*. Using the frequencies of TT of *thp1* in relation to markers very close to their centromeres such as *trp1* and *met14* (Table 1) for the evaluation of second-division segregation (Mortimer and Schild, 1981), we obtained the value of 53% in both cases, which is not too far from the limit for the linkage to a centromere, i.e. 66·7%. For these reasons, methods based on mitotic chromosome loss were used to confirm the assignment of the *thp1* allele to chromosome VII. Crosses of thiamine-excreting strains 27/20 and 28/21 to the set of mapping cir^o strains yielded non-excreting diploids. After cultivation in minimal thiamine-free medium, a significant number of thiamine-excreting cells appeared in the crosses to the cir^o strains with the segment of 2 µm DNA incorporated near the centromeres of chromosomes II, VII and IX, respectively (Table 3).

Table 3. Mitotic chromosome loss in the crosses of *thp1*, *thi3*, *thi5* strains to cir^o mapping strains.

Chromosome with 2 µm DNA fragment	Cross-feeding colonies (%) in the cross with:	
	28/21 α	27/20 α
I	17	2
III	16[a]	16[a]
IV	0	0
V	2	8
VI	0	0
VII	18[a]	33[a]
VIII	0	0
IX	23[a]	33[a]
X	4	2
XI	9	0
XII	6	0
XIII	0	4
XIV	0	0
XV	0	7
XVI	4	10

[a]Many very small cross-feeding colonies were also present.

Table 4. Mitotic chromosome loss after the irradiation (100 Gy) of *rad52/rad52* strains.

Cross: XS-122-57D x	Survival (%)	Number of colonies								
		Cross-feeding				Non-cross-feeding				Double auxotr.
		tested	prototr.	*his6*	trp5	his6	trp5	ade2	arg4	
27/20	5·0	436	16 (3·7%)	3 (0·7%)	3 (0·7%)	2 (0·7%)	1 (0·2%)	—	—	0
28/21	7·2	785	13 (1·7%)	7 (0·9%)	—	2 (0·3%)	—	9 (1·2%)	7 (0·9%)	0

This is in agreement with the fact that both cross-feeding parents contained dominant mutation *OXT1* and recessive mutations *thp1*, *thi3* and *thi5*. One of these recessive alleles is, therefore, situated on chromosome VII and the others on chromosomes II and IX.

When strains 27/20 and 28/21 were crossed to strain XS-122-57D (*rad52-1*, *ura3*) and the diploid progeny exposed to γ rays, most auxotrophs requiring tryptophan or histidine were cross-feeders (Table 4), confirming the involvement of chromosomes VII and IX in this process. Cross-feeding prototrophs (Table 4) are most probably due to the *thi* allele located on chromosome II in the cross to strain 27/20 and on chromosomes II and IX in the cross to strain 28/21, for no auxotrophic markers were available in these chromosomes in the mentioned crosses. The loss of chromosome XV (marked by *ade2*) and chromosome VIII (marked by *arg4*) did not lead to any cross-feeding, as was found by the appearance of no cross-feeding auxotrophs requiring adenine and arginine, respectively (Table 4). No appearance of double auxotrophs in both crosses of Table 4 indicates that cross-feeding auxotrophs were not the result of double chromosome losses. Transport experiments with *trp5* aneuploids confirmed the absence of thiamine uptake in all of them and thus confirmed the assignment of *thp1* to chromosome VII.

In order to obtain more precise information about the position of the *thp1* locus, segregant 2/38 (α *thp1, met14*) from the cross TN31 × X-2928-3D was crossed to auxotroph X-1936-16C containing markers *trp5* and *leu1* on the left arm of chromosome VII. All tetrads showed the monofactorial segregation pattern for individual markers. The χ^2 test for markers *trp5–thp1* confirmed the position of *thp1* on chromosome VII (Table 5) but results with *leu1* were less conclusive (Table 5), which might have been due to the different genetic background of strain X-1936-16C. The calculated distance of *thp1* from *trp5* was 52·3 cM according to Perkins' equation. Correction for multiple cross-overs according to Snow (1979a, b) gives an approximate value of 60 cM. Using the mathematical model of King and Mortimer (1991), the approximate value of 56 cM was obtained. Perkins' equation yielded the value of 65·6 cM for the distance of *thp1* from *leu1* in the cross, which led to 87 cM with the correction proposed by Snow (1979a, b) and 75 cM according to the model of King and Mortimer (1991). Taking into account the known interval between *trp5* and *leu1*, i.e. 17·5 cM, good agreement was achieved by the comparison of the distance *leu1–thp1* with the sum of distances *leu1–trp5* and *trp5–thp1* when the model of King and Mortimer was used (i.e. 75 cM and 73·5 cM), in contrast to the results obtained by the method of Snow (i.e. 87 cM and 77·5 cM).

In spite of the eventual effects of different genetic backgrounds in the crosses of Tables 2 and 5, better agreement was obtained using the model of King and Mortimer (1991), i.e. 70 cM and 75 cM, in comparison with 78 cM and 87 cM obtained by the method of Snow (1979a, b) for the distance *thp1–leu1*.

Table 5. Tetrad analysis of the products of the cross *thp1* × X-1986-16C.

Interval tested	Numbers of asci of			χ^2 (PD:NPD)
	PD	NPD	TT	
thp1–trp5 (VII)	17	4	45	8·05
thp1–leu1 (VII)	15	7	42	2·9
thp1–lys1 (IX)	11	11	42	0·0

Thus we may conclude that allele *thp1* is located on the left arm of chromosome VII approximately 56 cM centromere-distal to *trp5*.

ACKNOWLEDGEMENT

This work was supported by Czech Grant Agency grant 204/93/0700.

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