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Expression of the cloned uracil permease gene of *Saccharomyces cerevisiae* in a heterologous membrane

M.R. Chevallier* and F. Lacroute

Laboratoire de Génétique Physiologique, I.B.M.C. du C.N.R.S., 15, rue R. Descartes, 67084 Strasbourg Cedex, France

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A piece of DNA of the yeast *Saccharomyces cerevisiae* complementing the uracil permease gene was introduced into a plasmid able to replicate autonomously in *Schizosaccharomyces pombe*. A strain of *S. pombe* lacking uracil transport activity was transformed with this new plasmid carrying the gene of *S. cerevisiae*. The behaviour of the transformant shows not only an expression of the uracil permease gene in the heterologous membrane but also that the transport of uracil is active and coupled to the energy furnishing system of the heterologous host.

Key words: cloning/membrane/*Saccharomyces cerevisiae*/*Schizosaccharomyces pombe*/uracil permease

Introduction

In the yeast *Saccharomyces cerevisiae*, the transport of uracil is mediated by a specific permease (Lacroute, 1966; Grenson, 1969; Jund and Lacroute, 1970; Jund *et al.*, 1977). Accumulation of uracil occurs against its concentration gradient and does not require the participation of the uracil phosphoribosyltransferase, unlike in the bacterial system (Jund *et al.*, 1977). Uracil uptake is inhibited by the action of 2,4-dinitrophenol (DNP) and by other uncoupling agents. We were able to clone this permease by complementation in *S. cerevisiae* (Chevallier, in preparation). Hybrid plasmids able to replicate in *Schizosaccharomyces pombe* have recently been constructed (Lacroute and Losson, in preparation). Using such a plasmid, into which the uracil permease gene was inserted, we have shown that the cloned uracil permease of *S. cerevisiae* is functional and coupled to the energy furnishing device in a heterologous membrane. The use of *Schizosaccharomyces* as a heterologous receptor is especially interesting because this yeast genus is evolutionarily distant from *Saccharomyces* (Egel *et al.*, 1980; Mao *et al.*, 1980).

Results

A *Bam*HI-*Bgl*II DNA fragment of 2.3 kb that complements uracil permease deficiency in *S. cerevisiae* was introduced in both orientations at the unique *Bam*HI site of the *S. pombe* multicopy plasmid pFL20. The new plasmids, which are depicted in Figure 1, have been named pFL20-T1 and pFL20-T2.

A strain of *S. pombe* lacking OMP decarboxylase was used to select a mutant lacking uracil transport activity. Since hardly any cytosine transport occurs in *S. pombe*, this strain lacking uracil permease could only be grown by supplementing the media with uridine. The new strain *ura4 fur4* was used as a recipient in transformation with the plasmids pFL20-T1 and pFL20-T2 and prototrophic strains were selected. Prototrophic strains were then tested for uracil transport activity and the results are given in Table I. The transport activity found in these strains clearly exceeds the wild-type level, suggesting the existence of a multicopy vector. Moreover, the regained transport activity was inhibited by DNP as shown in Table I.

The amount of RNA transcripts made in the *S. pombe* strains and hybridizing to the OMP decarboxylase and to the uracil permease genes of *S. cerevisiae* were also measured. The *S. pombe* transformed strains, as well as the recipient strain, were labelled for 3 min with tritiated adenine. RNAs were then extracted and hybridized to specific DNA probes. The probes were single-stranded DNA of phage M13mp7 or of phage fd106 containing, respectively, each of the complementary strands of the 2.3-kb *Bam*HI-*Bgl*II DNA frag-

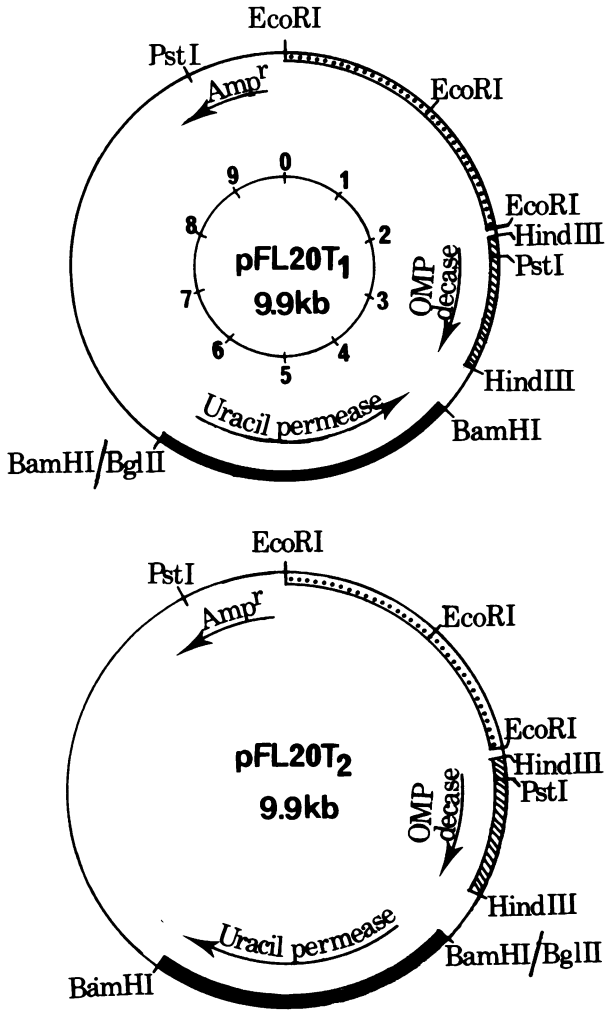

Fig. 1. Plasmids pFL20-T1 and pFL20-T2. : *S. pombe* DNA carrying an origin of replication. —— : pBR322 DNA. : *S. cerevisiae* DNA: *ura3* gene coding for OMP decarboxylase. : *S. cerevisiae* DNA:DNA sequence coding for the uracil permease. The direction of transcription, where known, is indicated (ampicillin: Sutcliffe, 1979; *ura3*: Rose *et al.*, 1981; uracil permease: Chevallier, in preparation).

*To whom reprint requests should be sent

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Table I. Uptake of uracil in *S. pombe* carrying the *S. cerevisiae* permease gene[a]

Uracil concentration	Strains				
	S. pombe wild-type	*S. pombe ura4 fur4*	*S. pombe ura4 fur4* [pFL20-T1]	*S. pombe ura4 fur4* [pFL20-T2]	*S. cerevisiae* wild-type
2.2 μM	0.108	[0.005][c]	1.060[b]	–	0.151
1.8 μM	0.075	–	0.584[b]	0.582[b]	0.093
2.2 μM + DNP 1 mM	[0.007][c]	–	0.070	–	[0.009][c]

[a]Initial velocities of uptake of uracil are expressed in nmols taken up by 1 ml of cells at 1 OD unit (Zeiss PM2D, 700 nm)/30 s of incubation with the radioactive substrate (30°C).
[b]These values are underestimated since ~30% of the substrate was taken up after 30 s incubation.
[c]Values at the limit of the sensitivity of the technique.

Table II. Transcription of the *S. cerevisiae* genes introduced in *S. pombe*

Strain *S. pombe*	Relative hybridization of [^{3}H]RNA of *S. pombe*[a] Single-stranded DNA probes of phage with insert:			
	uracil permease[b]		OMP decarboxylase[c]	
	coding strand (M13-1)	transcribed strand (M13-3)	coding strand (fd6)	transcribed strand (fd8)
ura4 fur4	0	0	0	0
ura4 fur4 [pFL20-T1]	3.6×10^{-4}	2.5×10^{-3}	6.1×10^{-4}	2.7×10^{-5}
ura4 fur4 [pFL20-T2]	2.7×10^{-4}	3.5×10^{-3}	3.4×10^{-4}	2.9×10^{-5}

[a]c.p.m. specifically retained by the probe *versus* input c.p.m. Labelling time: 3 min. All hybridizations were made in triplicate.
[b]Chevallier (in preparation).
[c]Hubert *et al.* (1980).

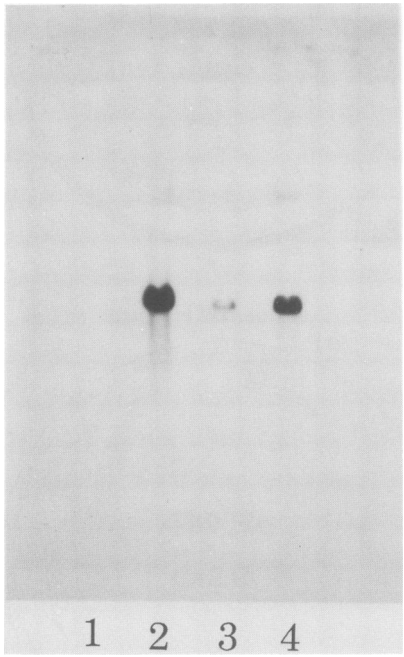

Fig. 2. Southern blot hybridization autoradiograph. Hybridization of total *S. pombe* DNA (**lane 1**), total *S. cerevisiae* DNA (**lanes 2** and **4**) and DNA of the probe to the nick-translated DNA of the RF of phage M13-3. **Lanes 1, 2** and **4** were loaded with 5 μg DNA, **lane 3** was loaded with 0.1 ng unlabelled DNA of M13-3 RF. The DNAs digested with the restriction enzymes *Bam*HI and *Bgl*II were loaded on 1% agarose gel, electrophoresed, and transferred to nitrocellulose paper (Southern, 1975). Nick-translated RF of phage M13-3 was hybridized to the transferred DNAs. The specific activity of the labelled DNA was $\sim 2 \times 10^7$ c.p.m./μg DNA.

ment coding for the uracil permease, and of the *Hind*III DNA fragment coding for the OMP decarboxylase (M13-1, M13-3 and fd6, fd8, respectively). These probes have already been described (Chevallier, in preparation; Hubert *et al.*, 1980). The results are summarized in Table II. They show that: (i) the recipient strains of *S. pombe* do not, before transformation, synthesize RNA sequences able to hybridize to the *S. cerevisiae* probes; (ii) the cloning of the permease gene in either direction does not change the amount of the corresponding transcripts; and (iii) the coding RNA transcripts of the uracil permease are ~10 times more abundant than those of the non-coding RNA strand. By contrast, for the OMP decarboxylase gene the hybridization to the phage fd6 indicates that the non-coding strand is produced in much higher amounts than the coding RNA strand.

To see if there was some homology between the DNAs coding for the uracil permease in *S. pombe* and in *S. cerevisiae*, DNA-DNA hybridization was carried out using the method of Southern (1975). Total DNA from *S. pombe* wild-type 972h$^-$ and total DNA of *S. cerevisiae* wild-type FL100 were digested with the enzymes *Bam*HI and *Bgl*II, separated by electrophoresis, transferred to nitrocellulose paper, and hybridized with ^{32}P-labelled DNA probe. This probe was the replicative form (RF) of phage M13-3 containing the *S. cerevisiae* uracil permease fragment labelled by nick translation (Rigby *et al.*, 1977). The nitrocellulose paper was then autoradiographed (see Figure 2). No hybridization at all was detected in track 1 where the *S. pombe* DNA was loaded whereas one sees clear hybridization on track 2 and 4 where the same amount of *S. cerevisiae* DNA was loaded. This proves that there is no homology at the level of DNA sequence for the uracil uptake systems of *S. pombe* and *S. cerevisiae*.

Discussion

Our results show that a specific transport system of the yeast *S. cerevisiae* expressed in *S. pombe* is able to function in a heterologous membrane. The fact that there is no homology at the level of the DNA sequence between the transport systems of the two species suggests that the corresponding proteins are distinct even if they have the same function. Nevertheless, the permease of *S. cerevisiae* is able to make use of the energy available in the membrane of *S. pombe* since accumulation occurs against the concentration gradient and DNP acts as an inhibitor. As the permease is active in a heterologous membrane it is very unlikely that the necessary energy derives from a stereospecific interaction between the permease and the energy furnishing system. This suggests that the permease is making use of the existing proton gradient in accordance with the chemiosmotic theory of Mitchell (1970). In *Escherichia coli*, it was found that the uracil permease gene of similar plasmids is transcribed into RNA, but no translation product was detected in the maxicell system described by Sancar *et al.* (1979). Therefore, we could not test the functioning of the eucaryotic permease in a procaryotic membrane. To do so, a more sophisticated approach must be used, namely the *in vitro* recombination of the *S. cerevisiae* structural sequence with procaryotic initiation and termination signals. We are also interested in seeing whether the *S. cerevisiae* gene will be expressed in mammalian cells.

Materials and methods

Strains and media

Strains of *S. cerevisiae* were FL100 (a, wild-type) or isogenic derivatives of it. The strains of *S. pombe* were 972h$^-$ (wild-type) and an isogenic mutant strain *ura4* (a kind gift from H. Heslot and U. Leupold). Using the *ura4* mutant, the strain *ura4 fur4* was selected on a mixture of 5-fluorouracil and uridine. We confirmed that the mutant lacked uptake activity for uracil (see Table I). Yeast was grown in YEPD (1% yeast extract, 2% peptone, 2% glucose) or in YNB (yeast nitrogen base Difco 6.7 g/l, 2% glucose). YNB was adequately supplemented for the growth of auxotrophs.

Plasmid construction

The restriction enzymes used were supplied by Biolabs Inc. (Beverly, USA) and Boehringer Mannheim (Mannheim, FRG). After digestion with the appropriate enzyme, the DNA was electrophoresed on horizontal agarose gels as previously described (Chevallier *et al.*, 1980). The DNA fragments of interest were then cut out, electro-eluted, and precipitated with tRNA as a carrier. The DNA pellet was resuspended in ligation buffer, incubated with T_4 DNA ligase for 20 h at 14°C and used to transform *E. coli* recipient cells.

Transformation procedures

E. coli strain BJ5183, F$^-$, recBC, sbcB, endoI$^-$, Gal$^-$, met$^-$, hsdR, strR, B_1^-, Bio$^-$ grown in L-broth was transformed according to the method of Cohen *et al.* (1972). The transformation procedure for *S. pombe* was as described by Hinnen *et al.* (1978) for *S. cerevisiae* with minor modifications.

Uracil uptake measurements

To determine uptake levels of uracil, the cells were incubated for 30 s in [^{14}C]uracil, filtered, and washed. Then the radioactivity of the dried filters was measured. The procedure has been fully described (Jund *et al.*, 1977).

Labelling of cells and DNA-RNA hybridization

Cells growing in minimal medium were labelled by incubation in [^{3}H]adenine 20 μCi/ml for 3 min. The specific radioactivity was 50 Ci/mmol. Cellular metabolism was stopped by the addition of 2 volumes of ethanol. RNA extraction and hybridization to the DNA probes was carried out as described by Losson and Lacroute (1979).

DNA-DNA hybridization

The DNA probes were labelled with ^{32}P by "nick-translation" as described by Rigby *et al.* (1977). The procedure of Southern (1975) was used to transfer DNA from agarose to nitrocellulose and to carry out hybridization to the labelled DNA probe.

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