The ORF YBL042 of *Saccharomyces cerevisiae* encodes a uridine permease

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**Abstract**

The purpose of this work was to identify the function of an open reading frame called YBL042, found during the systematic sequencing of *Saccharomyces cerevisiae*’s chromosome II. The YBL042 gene product shows 70% similarity with the uracil permease and the allantoin permease encoded by FUR4 and DAL4, respectively. The mutation constructed by disruption of this ORF is allelic to the FUI1 gene previously described as encoding the uridine permease but not cloned yet. A strain carrying the disrupted allele and a full mutant exhibit the same phenotype as they do not grow on a medium containing uridine as the sole source of pyrimidines and as they are resistant to $10^{-3}$ M 5-fluorouridine (5FUI), a toxic analog of uridine. Even though the FUI1 gene has a multicopy suppressor effect on uracil transport, its product does not seem to be involved in this transport, in contrast to the FUR4 gene product which is involved in uridine transport. Moreover, the FUI1 gene product does not play any role in allantoin transport. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

**Keywords:** Uracil; Allantoin permease; Uridine permease; Saccharomyces cerevisiae; FUI1

1. **Introduction**

The complete sequence of *Saccharomyces cerevisiae*’s genome has revealed a large number of open reading frames (ORFs). However, only approximately 30% of them correspond to known genes and one of the challenges consists in characterizing the function of the unknown ORFs. This is the aim of this study for the YBL042 ORF, located on chromosome II [1], which encodes a putative 639-amino acid protein. This ORF exhibits a high percentage of similarity with the FUR4 and DAL4 genes of *S. cerevisiae*: 49.9% and 49.8% identity respectively at the nucleotide level, 70.0% and 69.9% similarity respectively at the peptide level. The DAL4 gene encodes a permease for allantoin which is a nitrogen source allowing growth of the yeast cell in unfavorable nitrogen source conditions [2]. The FUR4 gene encodes a uracil permease [3] which imports exogenous uracil molecules in order to satisfy the need for UMP, UDP or UTP of the cell in addition to the de novo pyrimidine pathway (Fig. 1). This permease can also transport the toxic analog 5-fluorouracil (5FU). Therefore a strain bearing a wild-type allele...
of the FUR4 gene is 5FU sensitive whereas a fur4 mutant is drug resistant.

Considering the similarities between YBL042 and DAL4 and FUR4, and since it has been shown that a sequence resemblance often involves a functional relationship (as for the PMA1 and PMA2 genes [4], the ENA1 and ENA2 genes [5] or the MAL61 and MAL31 genes [6]), we have constructed a disrupted allele and subcloned the YBL042 gene in multicopy and low copy number vectors in order to test the role of this unknown ORF in uracil or allantoin transport.

2. Materials and methods

2.1. Strains and media

All the S. cerevisiae strains (Table 1) were derived from (a) FL100 (ATCC 28383) or (a) S288C (ATCC 26108). The strains were grown in YPD (2% peptone, 1% yeast extract, 2% glucose) or in minimal medium YNB (6.7 g l\(^{-1}\) yeast nitrogen base, 2% glucose) appropriately supplemented (50 μg ml\(^{-1}\)). Escherichia coli strains used for amplification of recombinant DNA were DH5α {supE44 hsdR17 thi-1 ΔlacU169 recA1 gyrA96 endA1 [ΔF80 lacZΔM15]} and XL1-Blue {supE44 hsdR17 recA1 endA1 gyrA96 thi relA1 lac- [F proAB lacP1 lacZΔM15 Tn10]} and were grown on Luria broth (LB). When necessary, ampicillin was added at 100 μg ml\(^{-1}\).

2.2. Plasmids

The a1201 cosmid derives from the pYC3030 cosmid [1]: it possesses a 31-kb fragment of chromosome II containing the YBL042 gene, and the HIS3 gene as a marker. The pRS305 plasmid [7] is an integrative plasmid carrying the LEU2 gene. pFL39 and pFL45 are respectively centromeric and multicopy plasmids carrying the TRP1 marker [8].

2.3. Preparation and analyses of DNA

S. cerevisiae genomic DNA for Southern blot analysis was prepared as described by Winston et al. [9]. Restriction endonuclease digestions were carried out as described by the manufacturers. Standard published procedures were used for DNA molecule ligation, agarose gel electrophoresis, DNA transfer to nitrocellulose paper, nick translation, and hybridization [10].

2.4. Cell transformations

Transformations of E. coli and S. cerevisiae were performed by electroporation according to Dower et al. [11] and Becker and Guarente [12], respectively.

2.5. Yeast genetics methods

The phenotypes of haploid and diploid cells were analyzed on YNB media appropriately supplemented. Spore analyses were performed either randomly [13] or by tetrad analyses using a Singer MSM® micromanipulator [14].

2.6. PCR amplification

PCR experiments were performed for 30 cycles using an Eppendorf Mastercycler 5330® thermocycler. In each reaction, 10–100 pg of DNA template and 1 unit of Taq DNA polymerase were used in a total reaction volume of 100 μl.

3. Results

3.1. Subcloning of the YBL042 gene and disruption of the chromosomal locus

A 2.75-kb SacII/SacI fragment of the a1201 cosmid containing the entire YBL042 gene was introduced into the SacI site of the multicopy pFL45 vector [8] and of the low copy number pFL39 vector [8]. These two plasmids were named pRW45 and pRW39 respectively.

In order to interrupt the YBL042 gene, a 495-bp internal fragment called L0 (obtained by PCR experiments using the a1201 cosmid as a template and specific oligonucleotides internal to the YBL042 gene) was introduced into the SacI site of the integrative vector pRS305 [7]. The resulting plasmid, named pRW305, linearized by BglII was used to direct the integration at the chromosomal YBL042
locus of the FY1679-5C strain (his3Δ200, leu2Δ1, ura3-52) [15]. Twelve Leu⁺ transformants were retained to check the disruption by Southern blotting. NsiI was chosen to digest the genomic DNA because there are no NsiI sites within the pRW305 vector or in the YBL042 coding sequence. Thus, an internal fragment used as a probe might reveal a unique band in a wild-type strain and a unique band of higher molecular mass in a disrupted strain. As expected, the internal L0 fragment revealed a 5.6-kb band in the reference strain FY1679-5C and a 11.6-kb band in the transformed strains; the difference of 6 kb corresponds to the size of the pRW305 plasmid integrated into the YBL042 locus (data not shown). These results confirmed that the Leu⁺ transformants contained a disrupted ybl042 allele. One of these transformants, called RW301, was retained for the functional analyses.

3.2. The YBL042 ORF is involved in neither uracil transport nor allantoin transport

Considering that in a fur4 mutant uracil still enters the cell [16] and considering the similarity between the YBL042 and FUR4 gene sequences, we first tested a possible role in uracil transport. For this purpose, the YBL042 gene of the FY1679-5C strain (his3Δ200, leu2Δ1, ura3-52) was disrupted. In this strain (Fig. 1) the de novo pyrimidine pathway is interrupted due to a ura3 mutation, so that an efficient salvage pathway and in particular efficient uracil transport is necessary to satisfy the need for pyrimidines. In other words, if YBL042 is involved in uracil transport, a double mutant in the de novo pathway and in the salvage pathway should be more resistant to 5FU than a wild-type strain. In contrast, the disrupted RW301 strain (K his3Δ200 leu2Δ1 ura3-52 ybl042::pRW305) showed the same resistance to 5FU as the FY1679-5C strain. Moreover, the YBL042 gene carried on a low copy number plasmid (pRW39) did not complement a fur4 mutant (Table 2); indeed, the RW127 strain (fur4Δ his3Δ trp1Δ dal4Δ::HIS3) exhibited the same resistance to 5FU when transformed with the pFL39 or pRW39 vec-

![Fig. 1. Pyrimidine metabolic pathway in strain FY1679-5C (leu2Δ1 his3Δ200 ura3-52) used for YBL042 gene disruption. UP, uracil permease; UIP, uridine permease; 5FU, 5-fluorouracil; 5FUI, 5-fluorouridine.](image-url)
tors. Nevertheless, it must be noted that YBL042 exerts a multicopy suppressor effect on uracil transport since a fur4 mutant transformed by pRW45 is more sensitive to 5FU than a fur4 mutant transformed by the cloning pFL45 vector (Table 2). All these results strongly suggest that the YBL042 gene may have a role in uracil transport but it is clearly not its main role.

Taking into account the similarity between YBL042 and DAL4, the same approaches were used to test a possible role in allantoin transport. First, it was necessary to construct a dal4 mutant in the FL100 background. Thus, the complete coding sequence of the DAL4 gene of the RW105 strain (fur4 trp1 his3Δ200) was replaced by the HIS3 marker following the method described by Baudin et al. [17]. All the His⁺ transformants were unable to grow on allantoin used as the sole nitrogen source. The DAL4 locus of these transformants was checked by PCR amplification using two sets of oligonucleotides hybridizing in the 5' and 3' ends of the DAL4 locus. The fragment amplified from His⁺ transformant DNA was 1.2 kb smaller than the control fragment amplified from RW105 DNA. Since the DAL4 coding sequence is 1908 bp long and the HIS3 fragment 660 bp, these results confirm the DAL4 gene replacement. One of these transformants, RW127 (fur4Δ trp1Δ his3Δ200 dal4Δ::HIS3), was transformed with the low copy number pRW39 and the multicopy pRW45 plasmids. These plasmids did not restore the ability of the transformants to grow on allantoin as sole nitrogen source (Table 2). In addition, the strain containing the disrupted ybl042 allele was able to grow on allantoin. These results show that the YBL042 gene product is definitely not involved in allantoin transport even when it is overexpressed.

### 3.3. The YBL042 gene corresponds to FUI1 encoding a uridine permease

We tested the ability to transport uridine consid-

<table>
<thead>
<tr>
<th>Strain</th>
<th>YNB+allantoin (mg ml⁻¹)</th>
<th>YNB+5FU (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL100</td>
<td>0  0.5  0.75  1  1.5</td>
<td>10⁻⁶ 2×10⁻⁶ 4×10⁻⁶ 6×10⁻⁶ 8×10⁻⁶ 10⁻⁵</td>
</tr>
<tr>
<td>RW127</td>
<td>+pFL39</td>
<td>-- -- -- -- --</td>
</tr>
<tr>
<td>RW127</td>
<td>+pRW39</td>
<td>-- -- -- -- --</td>
</tr>
<tr>
<td>RW127</td>
<td>+pFL45</td>
<td>-- -- -- -- --</td>
</tr>
<tr>
<td>RW127</td>
<td>+pRW45</td>
<td>-- -- -- -- --</td>
</tr>
</tbody>
</table>

Table 2
Growth of different strains on minimal media containing allantoin or a 5-fluorouracil concentration range

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We tested the ability to transport uridine consid-

<table>
<thead>
<tr>
<th>Strain</th>
<th>YNB+uridine (µg ml⁻¹)</th>
<th>YNB+5FU (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY1679-5C</td>
<td>0  5  10  20  40</td>
<td>6×10⁻⁵ 10⁻⁴ 3×10⁻⁴ 6×10⁻⁴ 10⁻³</td>
</tr>
<tr>
<td>NC243-sp4</td>
<td>-- -- -- -- --</td>
<td>+ + + -- --</td>
</tr>
<tr>
<td>RW301</td>
<td>-- -- -- -- --</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>NC243-sp4</td>
<td>+pFL39</td>
<td>-- -- -- -- --</td>
</tr>
<tr>
<td>NC243-sp4</td>
<td>+pRW39</td>
<td>-- + -- -- --</td>
</tr>
<tr>
<td>NC243-sp4</td>
<td>+pFL45</td>
<td>-- -- -- -- --</td>
</tr>
<tr>
<td>NC243-sp4</td>
<td>+pRW45</td>
<td>-- + -- -- --</td>
</tr>
</tbody>
</table>

Table 3
Growth of different strains on uridine and on a 5-fluorouridine concentration range

*pRW39 is the pFL39 single copy plasmid [10] carrying the FUI1 gene; pRW45 is the corresponding multicopy plasmid.

†A concentration of 5 µg ml⁻¹ uracil was added to these media as a source of pyrimidine.

+, cells grow; −, cells do not grow.
ering the similarity between uracil and uridine molecules and the multicopy suppressor effect on the uracil transport. Moreover, an active uridine transport was previously described in \textit{S. cerevisiae} [16] and the responsible gene has not yet been cloned. This gene, called \textit{FUI1}, clearly identified as deficient in uridine transport, has been characterized by the isolation of a monogenic mutant unable to grow on media containing uridine as the only pyrimidine source and resistant to $10^{-3}$ M of 5FUI, a toxic analog of uridine, whereas a wild-type strain is drug sensitive [16]. Since the RW301 strain exhibits the same phenotypes (Table 3) and since the \textit{YBL042} fragment carried by multicopy or low copy number plasmids (pRW45 and pRW39) complements the RW301 disrupted strain as well as the previous \textit{fui1-2} mutant (Table 3), it is clear that the \textit{YBL042} gene product is involved in the uridine transport.

Finally, we performed a complementation test by crossing the recessive disrupted \textit{ybl042} mutant with a strain containing the previous recessive \textit{fui1-2} mutation. The subsequent diploid is resistant to 5FUI showing that the mutations are allelic. This result confirmed that the \textit{YBL042} ORF corresponds to the \textit{FUI1} gene which encodes uridine permease.

### 3.4. Functional relationship between the \textit{FUI1} and \textit{FUR4} genes

When carried on a multicopy plasmid, the \textit{FUI1} gene is able to suppress the deficiency in uracil transport of a \textit{fur4} mutant (Table 2). We then asked the reverse question of a possible role of \textit{FUR4} in uridine transport. Therefore, 5FU and 5FUI resistance cross-tests were performed with different strains in order to study complementation at the physiological level between the \textit{FUI1} and \textit{FUR4} genes. Therefore, a \textit{fui1} mutation was introduced in a \textit{fur4Δ his3Δ trp1Δ} strain (RW105) by replacement of the \textit{FUI1} coding sequence by the \textit{HIS3} gene [22]. The replacement was checked by PCR experiments using a set of two oligonucleotides hybridizing in the 5’ and 3’ ends of the \textit{FUI1} locus as described before. This strain, RW128 (\textit{fur4Δ his3Δ trp1Δ fui1Δ::HIS3}), was tested on 5FU and 5FUI containing media (Table 4). The results show that the \textit{fur4Δ} strains share the same 5FU resistance level whether the \textit{FUI1} gene is deleted or not, confirming that \textit{FUI1} is not involved in uracil transport. In contrast, the \textit{fui1Δ} strain is less resistant to 5FUI than the \textit{fur4Δ fui1Δ} strain, meaning that the lack of uracil permease leads to less uridine transport. In other words, in a wild-type cell, the uracil permease is able to transport uridine molecules.

### 4. Discussion

The systematic sequencing of \textit{S. cerevisiae}’s genome has revealed 5800 different putative ORFs of more than 100 amino acids which may be classified into three main groups: ORFs corresponding to known genes, ORFs completely unknown and ORFs showing high percentages of identity with known genes. \textit{YBL042} belongs to the third category since it presents significant sequence similarities with the uracil permease encoded by \textit{FUR4} and the allantoin permease encoded by \textit{DAL4}. This study clearly demonstrates that \textit{YBL042} is the \textit{FUI1} gene described 30 years ago, by studying kinetic parameters of the uridine transport of monogenic mutants resistant to a toxic analog, 5FUI [16]. However, the corresponding gene has never been cloned by complementation.

| Table 4 |

| Resistance of different strains to 5-fluorouracil and 5-fluorouridine concentration ranges |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Strain         | YNB+5FU (M)    | YNB+5FUI (M)   |
|                | $10^{-6}$      | $5 \times 10^{-6}$ | $5 \times 10^{-5}$ | $5 \times 10^{-4}$ | $5 \times 10^{-6}$ | $5 \times 10^{-5}$ | $5 \times 10^{-4}$ | $5 \times 10^{-4}$ | $10^{-3}$ |
| FL100 (\textit{FUI1} \textit{FUR4}) | +              | +/−             | −               | −               | +              | +/−             | −               | −               | −               |
| NC243-sp5 (\textit{fui1} \textit{FUR4}) | +              | +/−             | −               | −               | +              | +/−             | −               | −               | −               |
| RW105 (\textit{FUI1} \textit{fur4}) | +              | +/−             | +               | +/−             | +              | +/−             | −               | −               | −               |
| RW128 (\textit{fui1} \textit{fur4}) | +              | +/−             | +               | +/−             | +              | +/−             | −               | −               | −               |

For each strain, the \textit{FUI1} and \textit{FUR4} alleles (mutated or wild-type) are indicated in parentheses. +, cells grow; −, cells do not grow.
The FUII gene product shows about 70% similarity with the FUR4 and DAL4 genes products; besides, these two latter proteins share about 80% similarity. These scores allow us to consider that these three genes belong to the same permease family and are probably derived from a common precursor. Therefore, we investigated the question of functional relationship between FUII and the other genes. We first pointed out that the FUII gene product is able to transport uracil when it is overexpressed. Other experiments showed that the overexpressed FUR4 gene allowed the complementation of the fui2-2 mutant (M.R. Chevallier, personal communication) and of a dal4 mutant [18]. In other words, at an expression level higher than the physiological level, these genes are able to carry out each other’s roles; then we can imagine that their products must possess conserved domains imparting common functions and specific features allowing the substrate discrimination.

In contrast, overexpression of the FUII gene does not complement the allantoin permease deficiency under the conditions we used. This could mean that these two genes have diverged to a higher substrate specificity.

Regarding the cross-complementation relation between the FUR4 and FUII genes when overexpressed, we investigated this relation at a physiological expression level, testing the 5FU and 5FUI resistance of single or double fui1 and fur4 deletion strains. The results revealed that 5FU transport is affected in the absence of uracil permease, meaning that this protein is involved in uridine transport. In contrast, there is no significant effect on 5FU resistance in the absence of uridine permease.

These experiments indicated that the FUR4 gene is able to have a pleiotropic effect and to supply allantoin or uridine transporters, suggesting that the FUR4 gene could be closer to an ancestral gene which encoded a non-specialized protein. Then, duplication and evolution could have led to a higher degree of substrate specificity. Indeed, the level of similarity between the DAL4, FUR4 and FUII genes can be compared with that of the TDH genes [19] or with that of another permease gene family such as HXT [20]; for each of these families, the genes show redundant functions. In contrast, the DAL4, FUR4 and FUII genes seem to be the result of an evolution to a specialized function.

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