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# Cloning of Genes *Sef1* and *Tup1* Encoding Transcriptional Activator and Global Repressor in the Flavinogenic Yeast *Meyerozyma* (*Candida*, *Pichia*) *guilliermondii*

**D. Fedorovych[a, *], V. Boretsky[a], Y. Pynyaha[a], I. Bohovych[b], Y. Boretsky[a, c], and A. Sibirny[a, d]**

[a] *Institute of Cell Biology National Academy of Sciences of Ukraine, Lviv, 79005 Ukraine*
[b] *University of Nebraska-Lincoln, Lincoln, NE 68588 United States*
[c] *Lviv State University of Physical Culture, Lviv, 79000 Ukraine*
[d] *University of Rzeszow, Rzeszow, 35-601 Poland*
**e-mail: fedorovych.d@gmail.com*

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**Abstract**—Two *Meyerozyma* (*Candida*, *Pichia*) *guilliermondii* genes coding for homologs of transcriptional factor Sef1p of *Candida famata* and Tup1p of *Candida albicans* were identified, cloned, and deleted. Deletion of a homologue of Sef1p transcriptional factor in *M.*(*P.*) *guilliermondii* completely blocked over-synthesis of riboflavin under conditions of iron deficiency. The results of genetic complementation analysis suggest that previously reported *rib83* mutants and newly constructed knock-out strains belong to the same complementation group and are defective in the same *SEF1* gene. Inactivation of the identified homolog of the *TUP1* gene in *M.*(*P.*) *guilliermondii* wild-type strain led to 1.5-fold increase in cellular iron content and 1.5–1.7-fold increase in riboflavin production. Introduction of a plasmid-borne copy of the *TUP1* gene did not restore metabolic defects of the riboflavin overproduction and iron accumulation in mutant strain *M.* (*P.*) *guilliermondii* m3, bearing the hit1 mutation. The obtained results suggest that both transcription factors Sef1p and Tup1p are involved in the regulation of iron acquisition and riboflavin biosynthesis by yeast belonging to the CUG-clade. The molecular mechanism of action Tup1p on riboflavin biosynthesis in *M.*(*P.*) *guilliermondii* required further elucidation.

*Keywords*: yeast, riboflavin, iron assimilation, transcriptional regulation
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## INTRODUCTION

*Candida guilliermondii* (teleomorph *Pichia guilliermondii*, *Meyerozyma guilliermondii* since 2010) is an ascomycete yeast widespread in the environment and is also part of the human saprophytic microflora [1]. *M.*(*P.*) *guilliermondii* belongs to the Crabtree-negative yeast, which cannot grow under strictly anaerobic conditions and has all three phosphorylation points in the respiratory chain [2]. *M.*(*P.*) *guilliermondii* and *Candida albicans*, like a majority of representatives of the so-called CUG clade, are capable of overproducing riboflavin (vitamin $B_2$) under insufficient iron supply conditions [1, 3]. Mutants of *M.*(*P.*) *guilliermondii* with impaired regulation of riboflavin biosynthesis were selected and it was shown that they also have impairments of iron supply regulation and changes in response to oxidative stress [4]. For example, the mutant *M.*(*P.*) *guilliermondii* hit1-1 (high iron transport), which constitutively overproduces riboflavin, is characterized by increased cell reductase activity, high iron content, hypersensitivity to copper ions, and some defects in response to oxidative stress [5]. In contrast, the mutant strain rib83-13, incapable of overproducing riboflavin under iron deficiency, has a damaged high-affinity iron absorption system [6]. However, these genes have not been identified due to the lack of relevant phenotypic traits of mutants suitable for the cloning of the corresponding genes.

Probably, *M.*(*P.*) *guilliermondii* and other yeasts capable of overproduction of riboflavin in response to iron deficiency have very similar iron-dependent regulatory mechanisms, which are significantly different from those of *Saccharomyces cerevisiae* [3, 7]. It was previously reported that the main regulator of riboflavin biosynthesis in another species of flavinogenic yeast, *Candida famata*, is the transcription activator Sef1p [8]. Sef1p of *C. albicans* (as well as Hap43p and Sfu1p proteins) were later identified as the key regulator of iron metabolism (but not riboflavin biosynthesis) [9–11].

In addition, the conservative transcription factor Tup1p is involved in the regulation of both of these metabolic processes in *C. albicans* [12, 13]. It should be noted that the global repressor Tup1p forms com-

**Table 1.** Strains used in the study

| Strain, auxotrophic marker | Genotype** | Source |
|---|---|---|
| R-66, ura3 hisX | WT | [16] |
| L2, hisX | WT | [1] |
| m3, ura3 hisX | *hit1* | [17] |
| sef1-1, hisX | *sef1Δ* | This study |
| sef2-2, hisX | *sef1Δ hit1* | This study |
| rib83-13, argX | *rib83* | [6] |
| rib81-131, hisX | *rib81* | [4] |
| sef1-1, hisX x L1 adeX | *sef1Δ/SEF1* (diploid) | This study |
| sef1-2, adeX x rib81-131, hisX | *sef1ΔRIB81/SEF1 rib81* (diploid) | This study |
| S181 | *sef1Δ rib81* | This study |
| DS1-83-LV251 | *sef1-1Δ* × *rib83* (diploid) | This study |
| S1 | *sef1Δ* | This study |
| S2 | *sef1Δ* | This study |
| S3 | *sef1Δ* | This study |
| tup1Δ38-2,hisX | *tup1Δ* | This study |
| tup1Δ53,hisX | *tup1Δ hit1* | This study |
| m3-R-pT1-11, hisX | *hit1* | This study |
| TUP1 | *TUPl hit1* | This study |

** Only mutations related to riboflavin and iron metabolism are listed.

plexes with Cyc8p and other specific proteins and regulates a number of other genes involved in such processes as meiosis, sporulation, flocculation, use of various carbon sources, and protection against osmotic stress [14, 15].

In this study, we describe the identification of the transcription factors Sef1p and Tup1p in the yeast *M.*(*P.*) *guilliermondii* and demonstrate that these proteins are involved in the regulation of both processes: iron supply and riboflavin biosynthesis.

## MATERIALS AND METHODS

**Strains, cultivation conditions, and media.** *M.*(*P.*) *guilliermondii* strains used in this study are listed in Table 1.

*Escherichia coli* strain DH5α (lac-ZDM15 recA1 endA1 gyrA96 thi-1hsdR17 ($rK^-$ $mK^+$) supE44 relA1 deoR Δ(lacZYA-argF)U169 was used for construction and amplification of plasmids. *E. coli* strains were grown on Luria-Bertani (LB) medium which contained, if it was necessary, ampicillin (100 μg $mL^{-1}$) at 37°C.

The yeast was grown on YPD rich medium (10 g yeast extract, 20 g peptone, 20 g sucrose, 20 g agar/L at 30°C or on Berkholder's synthetic medium with the addition of amino acids (40 mg/L) and uridine (400 mg/L) if it was necessary. The yeast was grown in Erlenmeyer flasks on a shaker (200 rpm) at 30°C. The hybridization of yeast strains and segregation analysis were performed as described earlier [1].

**Plasmid construction and gene deletion.** DNA manipulation and transformation of *E. coli* were performed according to previously published protocols [18]. 4.7 kb chromosomal DNA fragment of *M.* (*P.*) *guilliermondii* containing the gene PGUG_03868.1 (encoded by the Sef1p ortholog) together with the flanking regions (1 kb) was amplified by PCR using chromosomal DNA of *M.*(*P.*) *guilliermondii* ATCC6260 as a template and primers SEFd1 TGAATTCA-TATAGCTTAAC-TACTTC and SEF2r GAATTCGTTGATTTGTGT-GACCAC, bearing the introduced EcoRI sites. The PCR product was purified, treated with EcoRI restriction endonuclease, and cloned into the EcoRI site of pUC57 plasmid. The constructed plasmid pSEF1 was used for the substitution of the structural *SEF1* gene with a modified *URA3* gene of *S. cerevisiae* under the promoter of the phosphoglycerate kinase gene [19]. Almost the entire sequence of plasmid pSEF1, with the exception of the structural gene *SEF1*, was amplified using primers Bse1 AG-ATCTTTTTAGGGT-GAATTAGTG and RBS2 AG-ATCTAGTGATGAC-TTTTTGGGG containing BglII sites. The PCR product was purified, treated with BglII l restriction endonuclease, and ligated with a 1.5 kbp BamHI fragment of the plasmid pPGKURA3 containing the modified *URA3* gene of *S. cerevisiae*. The resulting plasmid pSEF1dURA3 contained a modified *URA3* gene of *S. cerevisiae* cloned between the 1.0 kbp promoter and the 1.0 kbp terminator sequence of the SEF1 gene of *M.*(*P.*) *guilliermondii*. Afterwards, plasmid pSEF1dU-RA3 was treated with restriction endonuclease EcoRI.

The obtained deletion cassette sef1::URA3 was used to transform *M.* (*P.*) *guilliermondii* strain R-66 [16].

A 3.3-kbp chromosomal DNA fragment containing the target gene (encoded by the Tup1p ortholog), together with the flanking regions, was amplified by PCR using primers PgΔTup1F ATCTAGATTCTGTGCTCGAATAAG and PgΔTup1R TATCTAGATACTTTTCATCGTAACG and chromosomal DNA of *M.*(*P.*) *guilliermondii* ATCC6260 as a template. The amplified DNA fragment was treated with *Xba*I endonuclease and cloned into the same sites of the vectors pUC57(-BamHl) and pUC-ARS-URA3. The obtained plasmids pUC57TUP1 and pUC-ARS-URA3-TUP1 were used for the construction of the deletion cassette and complement analysis, respectively. Plasmid pUC-57TUP1 was treated with *BamH*I endonuclease for the removal of a 1.0 kb DNA fragment containing the central portion of the open reading frame *PgTUP1.* Its larger fragment (5.1 kbp) containing the rest of the open reading frame together with the 5' and 3' flanking regions of the gene was purified and used for cloning of the modified *URA3* gene of *S. cerevisiae* [19]. The resulting plasmid pdTU5 was treated with restriction endonuclease XbaI, and the obtained deletion cassette tup1::URA3-5 was used for the transformation of the recipient *M.*(*P.*) *guilliermondii* strain R-66 [16].

For the identification of the deletion strains, PCR analysis using total DNA purified from selected transformants as a template and primers located near the respective loci but outside the sequences used in the deletion cassette was performed. Yeast transformation and PCR analysis of transformants were performed as described previously [16].

Homology and sequence searches were performed with BLAST and ClustalW 1.8 using sequencing data provided by the Broad Institute (available at http://blast.ncbi.nlm.Nih.gov/Blast.cgi, http://www.ebi.ac.uk/Tools/msa/clustalw2/i http://www.broadinstitute.org/annotation/genome/candida_group/MultiHome.html, respectively).

**Other procedures.** Riboflavin was determined fluorimetrically, using synthetic riboflavin as standard, with a fluorimeter FM 109510-33 (excitation maximum 440 nm, emission maximum 535 nm). The cells were desrupted with 0.4–0.5 mm glass beads. Protein was determined after dialysis by the Lowry method. The activity of GTP-cyclohydrolase II, the first enzyme of the riboflavin biosynthesis pathway, was determined in cell-free extracts by the fluorimetric method [20]. The incubation mixture for the determination of GTP cyclohydrolase II contained 20 mM Tris-HCl buffer (pH 8.2), 2 mM $MgCl_2$, 0.5 mM GTP, and 2 mm dithiothreitol. The reaction was started by adding cell-free extract (0.5 mg protein). The iron content in the cells and the ferrireductase activity were determined with 2,2'-dipyridyl as described previously [5].

## RESULTS AND DISCUSSION

**The role of the homologue Sef1p in the biosynthesis of riboflavin and the regulation of iron transport in *M.*(*P.*) *guilliermondii*.** Earlier, *C. famata* insertion mutant incapable of riboflavin overproduction was identified and its defect in a gene designated as *SEF1* was demonstrated [8]. This gene encodes a potential Zn2-Cys6 transcription factor, the homologue of which is involved in the regulation of iron supply in *C. albicans*. However, its role in the regulation of riboflavin biosynthesis in this yeast species has not been studied [10, 11]. We assumed that the transcription factor Sef1p is involved in the regulation of both of these metabolic pathways in representatives of the so-called "flavinogenic" yeast, which belong to the CUG clade. The gene PGUG_03868.1, which potentially encodes Sef1p, was identified in the genome of *M.*(*P.*) *guilliermondii* using the homology search for the sequence of this transcription factor of *C. famata*. This gene is located in the sequence 115630–1118095 bp of the fourth genomic supercontig of *M.*(*P.*) *guilliermondii*. It encodes a protein with a molecular weight of 92528 kDa, consisting of 821 amino acid residues, which contains a characteristic sequence capable of forming a binuclear Zn(2)-Cys(6) cluster domain, and shows 64% similarity to the corresponding analogues of *C. famata* and *C. albicans*. No other potential homologues of Sef1p of *C. famata* were found in *M.*(*P.*) *guilliermondii*. The *sef1::URA3* deletion cassette, the construction of which is described above, was used to transform two recipient strains: wild-type *M.*(*P.*) *guilliermondii* R-66 and strain m3, capable of overproduction of riboflavin. In both cases, several recombinant clones containing *sef1::URA3* deletion cassettes integrated into the genome by homologous recombination were selected. The integration of deletion cassette resulted in a deletion of the *SEF1* structural gene, which was confirmed by PCR analysis. The selected *sef1Δ* strains, which were derived from R-66 (wild type) and m3 (produces riboflavin) had a very similar phenotype, despite significant differences between the parent strains (Table 2).

The production of riboflavin and GTP-cyclohydrolase activity II of the constructed *sef1Δ* strains obtained from the wild-type strain and mutant m3, (capable to overproduction of the riboflavin) grown at a high iron content, were low, like in the wild-type strain R66 (Table 2). This suggests that inactivation of Sef1p suppresses the constitutive overproduction of riboflavin in strain m3, bearing the *hit1* mutation. In contrast to the corresponding parental strains, both Sef1p-deficient mutants had low GTP-cyclohydrolase II activity and did not have increased riboflavin synthesis under iron deficiency conditions. Under conditions of sufficient supply of iron, the iron content in cells and ferrireductase activity of *sef1Δ* mutants of *M.*(*P.*) *guilliermondii* were the same as in the recipient strains. Under conditions of iron deficiency, the intra-

**Table 2.** Production of riboflavin and the activity of GTP-cyclohydrolase II of *M.*(*P.*) *guilliermondii* strains with deleted PGUG_03868.1 gene and wild-type strains

| Strain | Genotype | Production of riboflavin, μg/mg dry cells | | GTP-cyclohydrolase II activity, U/min × mg protein | |
|---|---|---|---|---|---|
| | | 3.6 μM iron | 0.18 μM iron | 3.6 μM iron | 0.18 μM iron |
| R-66 | WT | 0.20 ± 0.03 | 7.2 ± 0.7 | 0.60 ± 0.05 | 8.20 ± 0.90 |
| L2 | WT | 0.25 ± 0.04 | 8.2 ± 0.7 | 0.72 ± 0.04 | 8.00 ± 0.81 |
| m3 | *hit1* | 1.40 ± 0.30 | 7.0 ± 0.7 | 2.20 ± 0.05 | 7.85 ± 0.65 |
| sef1-1 | *sef1Δ* | 0.31 ± 0.04 | 0.37 ± 0.04 | 0.66 ± 0.03 | 0.78 ± 0.05 |
| sef1-2 | *sef1Δ hit1* | 0.26 ± 0.03 | 0.35 ± 0.04 | 0.70 ± 0.06 | 0.81 ± 0.04 |
| rib83-13, | *rib83* | 0.30 ± 0.04 | 0.50 ± 0.05 | 0.35 ± 0.04 | 0.46 ± 0.06 |
| rib81-131 | *rib81* | 5.50 ± 0.60 | 8.00 ± 0.70 | 6.19 ± 0.60 | 10.19 ± 1.11 |
| sef1-1 × L1 | *sef1Δ/WT* | 0.28 ± 0.04 | 3.20 ± 0.30 | 0.70 ± 0.05 | 3.85 ± 0.08 |
| sef1-1 × rib81-131 | *sef1ΔRIB81/SEF1 rib81* (diploid) | 0.50 ± 0.05 | 3.30 ± 0.50 | 0.40 ± 0.06 | 1.70 ± 0.07 |
| S181 | *sef1Δ* rib81 | 0.21 ± 0.03 | 0.23 ± 0.03 | n/d | n/d |
| DS1-83-1 | *sef1-1Δ rib83* (diploid) | 0.09 ± 0.02 | 0.24 ± 0.03 | n/d | n/d |
| S1 | segregant from DS1-83-1 | 0.24 ± 0.03 | 0.30 ± 0.04 | n/d | n/d |
| S2 | segregant from DS1-83-1 | 0.22 ± 0.03 | 0.29 ± 0.04 | n/d | n/d |
| S3 | segregant from DS1-83-1 | 0.25 ± 0.04 | 0.31 ± 0.04 | n/d | n/d |

* Cells were grown in medium with a high iron content of 3.6 μM (iron sufficient supply conditions) or with an iron content of 0.18 μM (iron deficiency conditions); n/d—not determined.

cellular iron content and ferrireductase activity of both constructed mutants were lower than in the recipient strains (data not shown). This phenotype resembles the key features of the previously described mutant rib83-13, which had both impaired regulation of the biosynthesis of riboflavin and the high-affinity iron uptake system (Table) [6]. It can be assumed that the mutations of *sef1Δ* and rib83 belong to the same complementary group and inactivate the same gene. In this case, a diploid strain containing both mutations should not produce riboflavin under iron deficiency conditions. It can also be suggested that the *sef1Δ* mutation may suppress the constitutive overproduction of riboflavin by *M.*(*P.*) *guilliermondii* strain which contains the *rib81* mutation, as previously reported for the *rib83* mutation [6]. In order to confirm these hypotheses, the corresponding diploid strains and their meiotic segregants were selected and their phenotype was studied.

Heterozygous diploid strains containing *rib83* or *Δsef1* did not lose the ability to produce riboflavin in response to iron deficiency. At the same time, the diploid strain *Δsef1*-1 X *rib83-LV251*, as well as all its meiotic segregants (e.g. S1, S2, S3), were incapable of overproduction of riboflavin under conditions of iron deficient (Table 2).

In addition, the inactivation of Sef1p suppressed the overproduction of riboflavin in haploid strains containing mutations *hit1* or *rib81* (strain *sefΔ* 1-2 and meiotic segregant S181, respectively). Therefore, it can be stated that both *sefΔ* and *rib83* mutations inactivate the same gene PGUG_03868.1, which encodes a transcriptional activator homologous to Sef1p involved in the regulation of riboflavin biosynthesis in *C. famata* [8]. Since the *rib83* mutation has been shown to block both riboflavin overproduction and activation of the high-affinity iron uptake system in *M.*(*P.*) *guilliermondii*, the identified transcription activator Sef1p is involved in the regulation the regulation of iron supply in this yeast species as it was described for *C. albicans* [11]. In general, it can be assumed that, in yeasts that belong to the CUG clade and overproduce riboflavin under conditions of iron deficiency, Sef1p homologues are involved in the regulation of both metabolic processes: the biosynthesis of riboflavin and iron transport.

**Potential role of the general transcriptional repressor Tup1p in the regulation of riboflavin biosynthesis and iron uptake in *M.*(*P.*) *guilliermondii*.** The gene PGUG_01096.1, which encodes a potential homologue of a general transcriptional repressor, was identified in the genome of *M.*(*P.*) *guilliermondii* by searching for homologies to the amino acid sequences of Tup1p of *C. albicans* and *S. cerevisiae* (data not shown). This gene of *M.*(*P.*) *guilliermondii*, designated *PgTUP1*, encodes a protein of 569 amino acid residues with a molecular weight of 63113 kDa. Despite the difference in polypeptide length (Tup1p of *C. albicans* and *S. cerevisiae* contain 512 and 713 amino acid residues, respectively), the amino acid sequence of *M.*(*P.*) *guilliermondii* has 85% similarity to Tup1p of *C. albicans* and contains the characteristic domain WD40.

**Table 3.** Production of riboflavin and iron content in cells of *M.*(*P.*) *guilliermondii* strains deficient in the PGUG_01096.1 (*PgTUP1*) gene

| Strain | Production of riboflavin, μg/mg dry cells | | Iron content, μg/g dry cells | |
|---|---|---|---|---|
| | 3.6 μM iron* | 0.18 μM iron* | 0.9 mM $CoCl_2$* | 3.6 μM iron |
| R-66 | 0.20 ± 0.03 | 6.10 ± 0.70 | 8.32 ± 0.82 | 78.37 ± 3.46 |
| m3 | 1.40 ± 0.31 | 7.00 ± 0.72 | n/d | 166.08 ± 7.64 |
| tup1Δ38-2,h | 0.46 ± 0.05 | 10.32 ± 1.16 | 17.75 ± 1.90 | 117.55 ± 5.64 |
| tup1Δ53,h1 | 2.40 ± 0.32 | 12.80 ± 0.86 | n/d | 282.31 ± 13.55 |

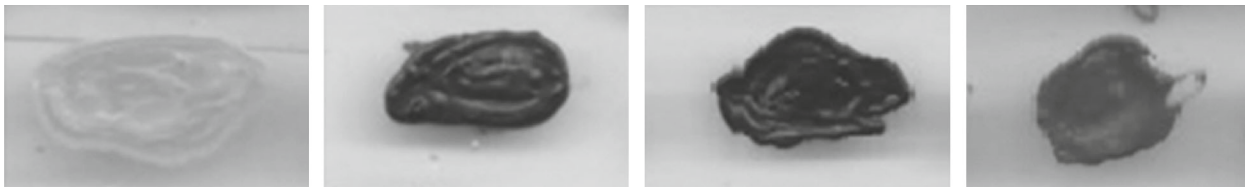
* Cells were grown in media with an iron content of 3.6 μm, 0.18 μM, and media containing 0.9 mm $CoCl_2$, conditions imitating iron deficiency.

For the elucidation of the role of Tup1p in the regulation of riboflavin biosynthesis and iron supply in *M.*(*P.*) *guilliermondii*, the *PgTUP1* gene was cloned along with flanking regions and used to construct a deletion cassette (see above). The constructed deletion cassette *tup1::URA3-5* was introduced into both recipient strains (WT) and m3 (constitutive riboflavin overproducer). The deletion of *PgTUP1* was confirmed by PCR analysis.

The constructed knockout mutants tup1Δ-38 and tup1Δ–53-h1 showed slightly increased (approximately twofold) production of riboflavin compared to the parent strains R-66 and m3, respectively (Table 3). This difference was more significant on a medium containing 0.9 mm cobalt chloride, that mimics iron deficiency [21]. Tup1p-deficient mutants also contained a higher amount of iron in cells (from 1.5 to 1.7 times) than the corresponding parental strains grown on the iron-containing medium. The parent (riboflavin-producing) strain m3 and its derivative tup1Δ53,h1 possess high nonspecific reductase activity of cells: their colonies were stained dark red on medium containing 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) due to the reduction of this compound into intensely colored formazan (Fig. 1).

Transformants of the parent strain m3, which contained an additional copy of the *PgTUP1* gene (obtained using plasmid pUC-ARS-URA3-TUP1) were characterized by significantly lower riboflavin production than described for the typical *M.*(*P.*) *guilliermondii* strain m3-R-pT1-11 (Fig. 2). In addition, they showed a much weaker color on the medium containing TTC (Fig. 1).

The results indicate that the global repressor Tup1p may be involved in the regulation of riboflavin biosynthesis and iron assimilation in (*P.*) *guilliermondii*, but it plays a secondary, additional role compared to the transcriptional activator Sef1p. The involvement of Tup1p in the regulation of riboflavin biosynthesis is unlikely to be direct. *TUP1* is a global regulator of morphology and metabolism. It is known that, in *S. cerevisiae*, the Cyc8-Tup1 complex is required for repression of many gene families' transcription. Approximately 3% of yeast genes are derepressed in *tup1* mutants. Target genes include genes subjected to glucose repression, genes induced by osmotic stress, genes encoding enzymes of fatty acid metabolism, and genes associated with flocculation, sporulation, and meiosis [13, 14, 22]. *TUP1p* also plays an important role in iron-dependent gene regulation [12]. The deletion of this regulator affects the homeostatic control of the reductive absorption of iron. As already was mentioned, iron deficiency has been reported to induce flavin production, and this regulation may be altered in the absence of *TCP1* control. In *P. guilliermondii*, iron deficiency leads to the derepression of almost all



*M.*(*P.*) *guilliermondii* R66 | *M.*(*P.*) *guilliermondii* m3 | *M.*(*P.*) *guilliermondii* Δtupl-53-h1 | *M.*(*P.*) *guilliermondii* m3 with pPUC-RS-URA3-TUP1

**Fig. 1.** Staining of *M.*(*P.*) *guilliermondii* wild type and recombinant strains on medium containing TTC. All tested strains were grown aerobically on solid YPD medium for 48 h. The cells were then transferred to the same medium containing 40 mg/L TTC. The plates were incubated at 30°C for 3–4 days. The results of a typical experiment are shown.

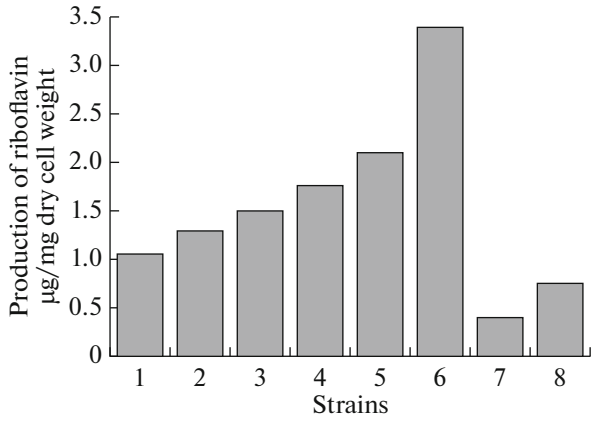



**Fig. 2.** Production of riboflavin by recombinant strains containing an additional copy of the *PgTUP1* gene and control *M.*(*P.*) *guilliermondii* strains (1–5), (6) m3, (7) R-66, (8) ATCC 6260.

enzymes involved in the biosynthesis of riboflavin [3, 4]. A number of *P. guilliermondii* mutants with damaged iron-dependent repression of riboflavin synthesis have been selected. Most of these mutants overproducing riboflavin also have derepressed iron transport and increased content of non-heme iron in cells [3, 4]. These data indicate coordinated regulation of riboflavin biosynthesis and iron supply in *P. guilliermondii*. It has been shown that iron regulates the biosynthesis of riboflavin at the transcriptional level, repressing the formation of *RIB1* and *RIB7* mRNA, and that such transcriptional repression is damaged in riboflavin overproducing mutants rib81 and red6 [23]. In addition, the overproduction of riboflavin in *P. guilliermondii* can be caused by $Co^{2+}$ ions [24] and oxidative stress [17]. Later, it was shown that $Co^{2+}$ ions, iron deficiency, and *rib80*, *rib81*, and *hit1* mutations cause oxidative stress [25]. The Cyc8-Tup1 complex in *S. cerevisiae* probably does not directly regulate the expression of other genes but controls the level of transcription of activators or repressors specifically acting on these other genes [26]. At the same time, in *C. albicans*, Ssn6 p is not important for *Tyr1p*-mediated repression of many genes [14]. The molecular mechanism of Tyr1p action on riboflavin biosynthesis in *M.*(*P.*) *guilliermondii* remains unclear.

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## COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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