Siderophore-mediated iron uptake in *Saccharomyces cerevisiae*: the *SIT1* gene encodes a ferrioxamine B permease that belongs to the major facilitator superfamily

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Uptake of iron from various siderophores by a $\Delta fet3\Delta fet4$ strain of *Saccharomyces cerevisiae* was investigated. The catecholate enterobactin and the hydroxamate coprogen were taken up by the cells by passive diffusion, whereas the hydroxamates ferrioxamine B (FOB) and ferricrocin (FC) were taken up via a high-affinity energy-dependent mechanism. The kinetics of FOB and FC uptake showed reciprocal competitive inhibition. The transport was regulated by iron availability, but was independent of the Aft1p and Mac1p transcriptional activators. Mutants affected in the transport of FOB were isolated. The transport of FC was not impaired in these mutants. Functional complementation of one mutant allowed the identification of the *SIT1* gene (*S*iderophore *I*ron *T*ransport) encoding a putative permease belonging to the major facilitator superfamily. The Sit1 protein is probably a permease specific for the transport of ferrioxamine-type siderophores. The evidence suggests that the uptake of ferrichrome-type siderophores like FC involves other specific permease(s), although there seems to be a common handling of FOB and FC following their internalization by the cell.

Keywords: iron uptake, siderophore, yeast

INTRODUCTION

The principal mechanism of iron uptake in *Saccharomyces cerevisiae* begins with the reduction of ferric iron at the cell surface (Lesuisse *et al.*, 1987; Dancis *et al.*, 1990). The iron is then taken up by the cells, by either a low-affinity transport system involving the product of the *FET4* gene (Dix *et al.*, 1994) or a high-affinity transport system that depends on a permease–oxidase complex encoded by the *FET3* and *FTR1* genes (Askwith *et al.*, 1994; Stearman *et al.*, 1996). Another strategy commonly used by micro-organisms to take up iron involves the excretion of siderophores. These are chelating agents that form complexes with Fe^{3+} with very high complexation constants. Ferrisiderophore complexes are formed outside the cells and are then taken up by the micro-organisms via specific transport systems. Most known fungal siderophores are hydroxamates (reviewed by van der Helm & Winkelman, 1994). Unlike many other yeasts, *S. cerevisiae* excretes

no siderophore. However, we have shown (Lesuisse & Labbe, 1989) that it can use siderophores excreted by other micro-organisms and take up iron, at least from ferrioxamine B (FOB), via a non-reductive mechanism (which means that the iron–siderophore complex enters the cell without prior reduction). The ability of *S. cerevisiae* to use ‘xenosiderophores’ could constitute a selective advantage of great value for this yeast. Hydroxamate siderophores excreted by bacteria and actinomycetes are abundant in nature. Their concentrations in bulk soils can reach 0.1 μM (Powell *et al.*, 1980), and these concentrations can be substantially higher (5–10 μM) in rhizosphere soil or soil having high microbial activity (Crowley *et al.*, 1987). The opportunistic strategy that consists of developing specific uptake systems for xenosiderophores is not restricted to *S. cerevisiae*. Crowley *et al.* (1987) showed that several plants, such as monocotyledonous grasses, have direct mechanisms for acquiring iron from a variety of microbial siderophores. The molecular steps by which siderophores are taken up by bacteria are well-known, but those of eukaryotes have not yet been elucidated. Siderophore uptake by bacteria is often specific and

Abbreviations: (D)FC, (desferri)-ferricrocin; (D)FOB, (desferri)-ferrioxamine B.

involves a number of proteins, whose production is negatively regulated by iron, but no transport proteins have yet been identified in fungi. The yeast *S. cerevisiae* is a very good model system for investigating reductive iron assimilation in the eukaryotic cell (Askwith & Kaplan, 1998). We believe that it is also a good model system for studying non-reductive iron uptake from siderophores. This report describes the use of a $\Delta fet3\Delta fet4$ double mutant to investigate the mechanisms by which *S. cerevisiae* takes up iron-siderophore complexes. This mutant lacks both the high-affinity and low-affinity transport systems involved in reductive iron uptake.

METHODS

Strains, media and iron compounds. The following strains of *S. cerevisiae* were used: DEY1455 (*MAT α ade2 can1 his3 leu2 trp1 ura3 gal*), DEY1433 (*MAT α ade2 his3 leu2 trp1 ura3 fet3::HIS3 fet4::LEU2*), DDY4 (*MAT α ade6 can1 his3 leu2 trp1 ura3 fet3-2::HIS3 fet4-1::LEU2*) (Dix *et al.*, 1997), S150-2B (*MAT α his3 leu2 trp1 ura3*), S150-2Baf1 and S150-2Bmac1. Unless otherwise stated, cells were grown in complete YPG medium (Difco) or in minimal YNB-glucose media (Difco) containing the required amino acids and various iron sources (as siderophores or ferric citrate). Desferri-compounds were obtained as described by Wiebe & Winkelmann (1975). When required, they were labelled with ^{55}Fe [50 mCi (1850 MBq) mg^{-1}]. Throughout this work, the name '(desferri)-ferrioxamine B' (DFOB) refers to the commercially available mesylate derivative Desferal (Ciba-Geigy).

Iron uptake experiments. Cells from an overnight culture in YPG (OD₆₀₀ 0.5) were used to inoculate fresh YPG medium containing 200 μM bathophenanthrolinedisulphonic acid (iron-deficient medium) or with no addition (iron-rich medium). The cells were grown for 6 h at 30 °C, washed with distilled water and suspended at a final OD₆₀₀ of 3 in 50 mM citrate buffer (pH 6.5) containing 4% (w/v) glucose. The cell suspension was incubated for 3 min at 30 °C and iron was added as ^{55}Fe -labelled siderophores (0.1–5 μM). Aliquots (1 ml) were withdrawn at intervals and added to a 200-fold excess of unlabelled siderophore. Aliquots were kept on ice until the cells were harvested by filtering through nitrocellulose filters. The cells were washed on the filter with about 20 ml 50 mM citrate buffer (pH 6.5) containing a 10-fold excess of unlabelled siderophore and 2% (w/v) EDTA. The iron taken up by the cells was measured by liquid scintillation counting.

Selection of mutants. Cells of the DEY1433 strain ($\Delta fet3\Delta fet4$) were subjected to EMS mutagenesis, with about 50% survival. The cells were then plated (about 500 colonies per plate) on YNB-glucose-agar medium containing 20 μM ferric citrate and incubated for 3 d. The colonies were replicated on YNB-glucose-agar medium containing 100 μM DFOB and on YNB-glucose-agar medium containing ferric citrate (20 μM). Colonies that grew on ferric-citrate-containing medium but not on DFOB-containing medium were selected for further analysis. The mutants were analysed genetically by classic methods.

Molecular biology techniques. Functional complementation of the mutants was carried out using a genomic library constructed from the $\Delta fet3\Delta fet4$ strain. The genomic DNA was digested with *Sau3A* to obtain fragments of mean size of 5–8 kb. The DNA fragments ranging from 3 to 15 kb were purified from the agarose gel after electrophoresis. The

fragments were then ligated to the multicopy vector YEp352 previously digested by *Bam*HI. The mutants were transformed by the resulting genomic library, and the cells were plated onto YNB-glucose-agar medium containing 0.1 mM DFOB. The colonies that grew on such medium were transferred to YPG-agar medium and replica-plated onto YPG-agar medium containing 5-fluoroorotic acid (100 mg l^{-1}) to select cells that had lost the YEp352 (URA3) plasmid. The colonies were then tested for recovery of the mutant phenotype (did not grow on DFOB-containing medium). This selection step on 5-fluoroorotic acid was important, since most of the clones that grew on DFOB-containing medium after transformation by the genomic library were revertants. The plasmid DNA was extracted from the clones of interest and amplified in *Escherichia coli*. The plasmid inserts were then analysed by sequencing and restriction studies. The YEL065w ORF (*SIT1* gene) was disrupted using a disruption plasmid constructed from the YEp352 vector bearing a 4 kb fragment including the *SIT1* ORF. A 300 bp fragment in the middle of the ORF was removed by *Xho*I/*Bst*XI digestion, and the *kanM* gene was ligated (blunt end) to the vector. The products of *Pvu*II digestion were used to transform the $\Delta fet3\Delta fet4$ strain and its wild-type counterpart. The disruptants were selected on YPG-agar plates containing 200 mg l^{-1} geneticin (G418). PCR methods were used to check the correct knock-out of the *SIT1* gene.

RESULTS AND DISCUSSION

A strain of *S. cerevisiae* with disrupted *FET3* and *FET4* genes cannot take up iron by either the high-affinity or low-affinity reductive transport systems (Dix *et al.*, 1997). This strain did not, therefore, grow on minimal medium (YNB-glucose, containing about 1 μM iron), unless a large excess of iron [20 μM Fe(III) citrate] was added to the medium (not shown). We have previously reported (Lesuisse & Labbe, 1989) that *S. cerevisiae* has a non-reductive transport system with high affinity for FOB. When present at low concentrations (micromolar) this siderophore was taken up by the cells via a high-affinity transport system without prior reductive dissociation of the complex. Haem was required for siderophore dissociation within the cell (Lesuisse & Labbe, 1989). Thus, we tested whether the addition of DFOB to a minimal medium would allow a $\Delta fet3\Delta fet4$ mutant to grow. We also tested the effect of two other hydroxamate-type siderophores [desferri-coprogen and desferri-ferricrocin (DFC)], and of one catecholate-type siderophore (desferri-enterobactin). All the siderophores tested led to growth of the cells on minimal medium (Fig. 1). Other iron chelators like salicyl-hydroxamic acid and dihydroxybenzoic acid did not restore cell growth (Fig. 1). The growth-promoting effect of the siderophores tested could be due to it being easier for iron to diffuse into the cells when in the form of ferrisiderophores, rather than 'free'; alternatively, the ferrisiderophores could be taken up actively by the cells. The active uptake possibility seems more probable, since an active, specific transport has already been shown for FOB (Lesuisse & Labbe, 1989). In addition, the greatest effect was obtained with DFC. This siderophore (belonging to the ferrichrome family) allowed full growth of the mutant ($\Delta fet3\Delta fet4$) cells at a

Fig. 1. Effect of various iron chelators on the growth of a $\Delta fet3\Delta fet4$ strain on minimal (YNB) medium. Cells were inoculated at an OD_{600} of 0.05 in YNB-glucose medium containing various concentrations of DFOB ($\blacktriangle$), desferri-enterobactin ($\circ$), DFC ($\bullet$), desferri-coprogen ($\square$), salicylhydroxamic acid ($\blacksquare$) or dihydroxybenzoic acid ($\triangle$). The optical density of the cultures was measured after 16 h growth and compared to the OD_{600} of a reference culture (wild-type cells on YNB-glucose without addition). The OD_{600} of the reference culture was 3. The mutant cells did not grow in YNB-glucose alone (not represented).

Table 1. Iron uptake from various iron sources by a $\Delta fet3\Delta fet4$ strain and its wild-type parent

Iron uptake (pmol min^{-1} per OD_{600} unit) by resting cells previously grown for 6 h in iron-deficient medium was measured. The concentration of iron in the assay was $1 \mu\text{M}$ in each case. Values represent means $\pm$ SE from three experiments.

Iron source	$\Delta fet3\Delta fet4$	Wild-type
Fe(III) citrate	0.05 ± 0.02	1.80 ± 0.20
Fe(II) ascorbate	0.07 ± 0.01	2.30 ± 0.30
FOB	2.20 ± 0.40	1.90 ± 0.10
Enterobactin	1.40 ± 0.30	2.50 ± 0.50
Coprogen	0.90 ± 0.10	1.00 ± 0.05
FC	6.30 ± 0.40	5.20 ± 0.30

concentration of only $0.1 \mu\text{M}$ (Fig. 1), suggesting that there is a very high affinity uptake mechanism. We measured the rate of iron uptake from various iron sources in resting wild-type and $\Delta fet3\Delta fet4$ cells, and found that FOB, ferricrocin (FC), enterobactin and coprogen were all taken up efficiently by cells of both strains (Table 1). As expected, ferric citrate and ferrous ascorbate were not taken up by the $\Delta fet3\Delta fet4$ strain (Table 1), demonstrating that the iron uptake from the siderophores was not due to a reductive mechanism.

The dependence on an energy source for transport and

the kinetic characteristics of the transport were determined for each siderophore (Figs 2 and 3). The uptake of enterobactin was glucose-independent (not shown). This siderophore did not inhibit the uptake of FOB (Fig. 2a), and concentration-dependent uptake yielded a straight line (Fig. 3b). These data indicate that simple diffusion is the underlying principle of enterobactin iron transport. The same was true for coprogen (Figs 2b and 3d). As previously shown (Lesuisse & Labbe, 1989), the uptake of FOB was glucose-dependent with saturation kinetics enabling the determination of K_m (about $0.5 \mu\text{M}$) and $V_{\max}$ (about 2 pmol min^{-1} per OD_{600} unit) (Fig. 3a). The uptake of FC was also highly dependent on glucose (not shown) and showed saturation kinetics ($K_m = 0.2 \mu\text{M}$; $V_{\max} = 7 \text{ pmol min}^{-1}$ per OD_{600} unit; Fig. 3c). FC strongly and competitively inhibited the uptake of FOB (Fig. 2c). Our data clearly indicate that both FOB and FC are taken up by the cells via energy-dependent, high-affinity mechanisms. The finding of competition for the transport of these two siderophores could mean that: (i) both siderophores are taken up by the same transport system, including recognition and translocation; (ii) each siderophore is recognized by a separate receptor before their common transport; (iii) both siderophores are recognized by the same receptor protein prior to their separate transport; (iv) there is a common step in the handling of both siderophores downstream from their separate recognition and translocation (Fig. 4). A shared transport system functioning with two different siderophore receptors has been proposed for the uptake of coprogens and ferrichromes by *Neurospora crassa* (Winkelmann & Huschka, 1987).

We investigated whether the siderophore-mediated iron uptake system was regulated in the same way as the reductive transport system. Aft1p and Mac1p mediate the transcriptional control of the genes involved in reductive iron uptake in response to iron (Aft1p) and copper (Mac1p) availability (Yamaguchi-Iwai *et al.*, 1996; Georgatsou & Alexandraki, 1994). The reductive transport system is also regulated to some extent at the post-transcriptional level, because the copy number of YAP genes, which are involved in the cell response to oxidative stress, affects ferrireductase activity but not the transcription of *FRE* genes (Lesuisse *et al.*, 1995). Cd^{2+} ions also strongly inhibit ferrireductase activity (Lesuisse *et al.*, 1995) by promoting degradation of Fre1p (unpublished data). The siderophore-mediated transport system of *S. cerevisiae* was also regulated by iron availability (Table 2), but it did not depend on the Aft1p transcription factor. The *aft1* mutant had very low rates of ferrous iron uptake in all the growth conditions tested, whereas the rate of FC uptake by the same mutant was higher than that of the wild-type (Table 2). This difference is probably due to the *aft1* mutant being constitutively iron-deficient, because the reductive iron uptake pathway is repressed in this mutant. The independent siderophore uptake pathway, which is also regulated by iron, was presumably induced to some extent in the *aft1* mutant. Similarly, the

Fig. 2. Effect of enterobactin (a), coprogen (b) and FC (c) on the uptake rate of FOB at various concentrations. We measured FOB uptake by resting cells of the $\Delta fet3\Delta fet4$ strain previously grown for 6 h in iron-deficient medium. The uptake of ^{55}Fe -labelled FOB was measured in the presence or absence of unlabelled siderophores at various concentrations. (a) $\bullet$, No addition; $\square$, 1 μM enterobactin; $\blacktriangle$, 5 μM enterobactin. (b) $\bullet$, No addition; $\circ$, 1 μM coprogen. (c) $\bullet$, No addition; $\square$, 0.5 μM FC; $\blacktriangle$, 2 μM FC.

Fig. 3. Concentration-dependent uptake of FOB (a), enterobactin (b), FC (c) and coprogen (d) by cells of the $\Delta fet3\Delta fet4$ strain. Uptake kinetics were measured with resting cells previously grown for 6 h in iron-deficient medium. Results are from one representative experiment.

Fig. 4. Models of siderophore uptake in *S. cerevisiae*. Two types of siderophore (ferrioxamine-type and ferrichrome-type) are shown by squares and triangles. (1) Both siderophores are taken up by the same transport system, including recognition and translocation. (2) Both siderophores are recognized by separate receptors before common transport. (3) Both siderophores are recognized by the same receptor protein prior to separate transport. (4) There is a common step in the handling of both siderophores downstream to their separate recognition and translocation.

chelation of copper by bathocuproin sulfonate during growth resulted in lower rates of ferrous iron uptake but not of FC uptake by resting cells (Table 2). Thus, although copper is required for ferrous iron uptake (see

the role of Fet3p, Stearman *et al.*, 1996), it does not seem to affect the uptake of siderophores. The presence of non-toxic concentrations of Cd^{2+} ions in the growth medium inhibited the uptake of ferrous iron and

Table 2. Iron uptake by an *aft1* strain and its wild-type parental strain grown under various conditions

Measurements were made of iron uptake (pmol min⁻¹ per OD₆₀₀ unit) in either 0.5 µM FC or 0.5 µM ferrous ascorbate (Fe²⁺) by resting cells previously grown for 6 h in YPG medium containing: (1) no additions; (2) 200 µM bathophenanthrolinedisulphonic acid (BPS); (3) 200 µM bathocuproin disulphonic acid (BCS); (4) 200 µM ferrozine; (5) 70 µM Fe(III) citrate; (6) 25 µM FC; (7) 5 µM Cd²⁺. The strains used were S150-2B and S150-2B*aft1*. ND, Not determined. These data are from one representative experiment.

Growth conditions	Wild-type		<i>aft1</i> mutant	
	FC	Fe ²⁺	FC	Fe ²⁺
YPG	0.70	0.40	1.00	0.10
YPG + BPS	2.20	1.40	3.20	0.20
YPG + BCS	0.70	0.10	1.10	0.05
YPG + ferrozine	1.40	1.90	2.90	0.30
YPG + Fe(III) citrate	0.40	0.05	ND	ND
YPG + FC	0.60	0.05	ND	ND
YPG + Cd ²⁺	1.80	0.10	ND	ND

stimulated the uptake of FC by the resting cells (Table 2). The uptake of FC was also independent of the *MAC1* and *YAP1/YAP2* gene products (data not shown). Thus, *S. cerevisiae* clearly has at least two high-affinity iron uptake systems that are regulated independently. The Fet3p/Ftr1p-dependent system is mostly regulated via transcriptional control mediated by Aft1p (Yamaguchi-Iwai *et al.*, 1996), whereas the mechanisms by which the siderophore uptake system is regulated by iron availability are unknown. Siderophore uptake was inhibited less than reductive uptake by excess iron in the growth medium (in the form of ferric citrate or FC) (Table 2).

We obtained two mutants (from about 50000 colonies screened) by EMS mutagenesis of the *Δfet3Δfet4* strain that could not grow on YNB-glucose medium containing DFOB (Fig. 5). These mutants were deficient in both reductive iron transport and FOB uptake. Both mutants had the same K_m value as the parental strain (about 0.5 µM) for the uptake of FOB, but V_{max} values 10 times lower (0.2–0.3 pmol min⁻¹ per OD₆₀₀ unit). However, uptake of FC by the mutants was similar to that for the parental strain (data not shown). The uptake of both FOB and FC by the mutant strains was still regulated by iron availability during growth, as in the parental strain. Thus, both mutants probably lack some component of the translocation system rather than of the regulation of transport.

We then focused on a single mutant, which had the most severely affected FOB transport. The mutant was crossed with a *Δfet3Δfet4* strain of the opposite mating type, and the phenotype of the diploid strain and of haploid segregants was determined (Table 3). The

mutation segregated 2:2, which strongly suggests that a single mutation was involved. We called the mutation responsible for the transport deficiency *sit1-1* (siderophore iron transport). The rate of FOB transport by the *SIT1/sit1-1* heterozygote was only 25 % of that observed for the *SIT1* parental haploid strain (Table 3). The K_m value for transport was, however, similar to that of the *SIT1* and *sit1-1* haploid strains (not shown). However, the slow rate of transport made it possible for the heterozygote to grow as well as the parental wild strain on minimal medium containing DFOB (not shown). This characteristic made it possible to screen a genomic library by functional complementation of the mutant. After transformation of the *sit1-1* mutant by the *Δfet3Δfet4* genomic library, we identified three independent clones that had recovered plasmid-dependent transport of FOB. A unique ORF, YEL065w, was common to the three plasmid inserts (size 3–8 kb). The product of the YEL065w ORF belongs to the major facilitator superfamily (MFS), and has been classified among the multidrug permease homologues for which no substrate is known (Nelissen *et al.*, 1997). The protein has 628 amino acids and 12 predicted transmembrane spans and is very similar to the products of YHLO40c and YHLO47c, two other putative permeases for which there is no known substrate (Nelissen *et al.*, 1997). No significant similarity was found between the product of YEL065w and known bacterial siderophore transport protein (not shown). The gene promoter does not show the consensus sequence for Aft1p transcriptional regulation. The YEL065w ORF was disrupted in both the wild-type and the *Δfet3Δfet4* strain. As shown in Table 4, the *Δfet3Δfet4* strain disrupted for the YEL065w ORF had no FOB transport activity, while the uptake of FC was only slightly affected. There was still residual iron uptake from FOB in the disrupted wild-type strain, which was probably due to reductive iron uptake. As YEL065w encodes a putative permease, it is reasonable to assume that its product, Sit1, is the major protein involved in the trans-plasma membrane translocation of FOB. Since there was competitive inhibition for the uptake of FOB and FC (Fig. 2), and since disruption of the *SIT1* gene led to defective transport only of FOB (Table 4), there cannot be a common recognition and transfer process for both FOB and FC (Fig. 4). To determine whether the competitive effect occurred upstream or downstream to translocation, the competitive effect of FOB on the uptake of FC was measured in the *Δfet3Δfet4* strain and in the *Δfet3Δfet4 Δsit1* strain. FC uptake was inhibited by increasing concentrations of FOB in the *SIT1* strain, but not in the *sit1* mutant (Fig. 6). These data strongly suggest that the competitive effect in the uptake of FOB and FC occurred downstream to the translocation of the siderophores (see the model 4 in Fig. 4). This would mean that after a separate and specific recognition/translocation process through the plasma membrane, the ferrioxamine-type and the ferrichrome-type siderophores could share a common step in their intracellular processing. For example, both siderophores could be bound to the same chaperone protein after their translocation into the cell.

Fig. 5. Growth on YNB-glucose-agar medium of the wild-type strain, of the $\Delta fet3\Delta fet4$ strain and of two mutant strains obtained by EMS mutagenesis of the $\Delta fet3\Delta fet4$ strain. Additions to the medium: (1) none; (2) 25 μM Fe(III) citrate; (3) 50 μM Fe(III) citrate; (4) 50 μM DFOB. WT, Wild-type.

Table 3. Uptake of FOB by various $\Delta fet3\Delta fet4$ strains

Iron uptake (pmol min^{-1} per OD_{600} unit) by resting cells previously grown for 6 h in iron-deficient medium was measured. The FOB concentration was 1 μM in each case.

Strain/tetrad	Rate of FOB uptake
<i>SIT1</i> haploid strain	1.80
<i>sit1-1</i> mutant	0.12
<i>SIT1/sit1-1</i> heterozygote	0.47
Tetrad 1	0.12/0.15/1.20/1.40
Tetrad 2	0.18/0.09/1.80/1.30
Tetrad 3	0.16/0.15/1.40/1.70
Tetrad 4	0.13/0.12/1.80/1.80

Table 4. Uptake of FOB and FC by various strains

Iron uptake (pmol min^{-1} per OD_{600} unit) by resting cells previously grown for 6 h in iron-deficient medium was measured. The FOB concentration was 1 μM in each case. Values represent means $\pm$ SE from three experiments.

Strain/genotype	Rate of iron uptake	
	FOB	FC
<i>SIT1</i> (wild-type)	1.40 ± 0.20	2.80 ± 0.20
<i>SIT1</i> ($\Delta fet3\Delta fet4$)	1.90 ± 0.20	3.50 ± 0.40
$\Delta sit1$ (wild-type)	0.05	2.40 ± 0.30
$\Delta sit1$ ($\Delta fet3\Delta fet4$)	0.00	2.90 ± 0.40
YEp352- <i>SIT1</i> ($\Delta fet3\Delta fet4 \Delta sit1$)	3.60 ± 0.40	3.40 ± 0.20

Fig. 6. Effect of FOB concentration on the rate of FC uptake by a $\Delta fet3\Delta fet4$ strain (●) and a $\Delta fet3\Delta fet4 \Delta sit1$ strain (○). FC concentration was 0.25 μM . Results are expressed as percentage of the rate of FC uptake measured in the $\Delta fet3\Delta fet4$ strain without added FOB (100% = 2.1 pmol min^{-1} per OD_{600} unit).

General conclusions

In addition to the reductive mechanism of iron uptake, *S. cerevisiae* has at least two other high-affinity transport systems for non-reductive siderophore assimilation, one for ferrioxamine-type siderophores and another for ferrichrome-type siderophores. Sit1 was identified as the ferrioxamine permease. It is tempting to speculate that the Sit1 homologues could be permeases involved in the transport of other siderophore families. On the basis of their great similarity, Nelissen *et al.* (1997) suggested

that these proteins should have similar functions. The energetics of siderophore transport in *S. cerevisiae* remain unclear. The Sit1 protein and its homologues have no ATP-binding cassette motif. On the basis of bacterial protein homologies, Goffeau *et al.* (1997) suggested that the members of this MFS cluster could function as $H^+_{in}/DRUG_{out}$ antiports. The Sit1 protein is responsible for importing of FOB; we are currently trying to determine whether the import of FOB could depend on ATPase-dependent proton excretion by the cells.

Despite the fact that *S. cerevisiae* does not itself produce any siderophore, the non-reductive uptake system for xenosiderophores in this yeast has the same characteristics as the siderophore uptake systems of siderophore-producing micro-organisms: regulation by iron, energy-dependence, high specificity, high affinity, complex processing of the internalized siderophores. We previously showed that haem-deficient cells are still able to take up FOB, but can no longer dissociate iron from the ferrisiderophore (Lesuisse & Labbe, 1989). This strongly suggests that the haem-dependent reduction of the siderophore is required for the dissociation of iron from the complex and its subsequent use. Many yeast-like organisms could have two types of high-affinity iron transport systems, one reductive and one non-reductive. Inducible reductive uptake systems have now been recognized in numerous fungi, including siderophore producers, such as species of the following genera: *Schizosaccharomyces*, *Pichia*, *Hyphopichia*, *Candida*, *Kluyveromyces*, *Endomyces*, *Yarrowia*, *Ustilago*, *Rhodotorula* (Emery, 1987; Roman *et al.*, 1993; Lesuisse *et al.*, 1995; Morrissey *et al.*, 1996; E. Lesuisse, unpublished results). In *S. cerevisiae*, the non-reductive and the reductive transport systems seem to be regulated independently. The physiological significance of such a dual regulatory mechanism affecting iron uptake is questionable. We are currently trying to determine whether the reductive and non-reductive uptake systems can each be induced/repressed under definite physiological conditions that cannot be separated under standard laboratory growth conditions.

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