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# The response to iron deprivation in *Saccharomyces cerevisiae*: expression of siderophore-based systems of iron uptake

**C. C. Philpott[1], O. Protchenko, Y. W. Kim, Y. Boretsky and M. Shakoury-Elizeh**

Liver Diseases Section, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Building 10, Room 9B-16, 10 Center Drive, Bethesda, MD 20892-1800, U.S.A.


## Abstract

The budding yeast *Saccharomyces cerevisiae* responds to growth in limiting amounts of iron by activating the transcription factor Aft1p and expressing a set of genes that ameliorate the effects of iron deprivation. Analysis of iron-regulated gene expression using cDNA microarrays has revealed the set of genes controlled by iron and Aft1p. Many of these genes are involved in the uptake of siderophore-bound iron from the environment. One family of genes, *FIT1*, *FIT2* and *FIT3*, codes for mannoproteins that are incorporated into the cell wall via glycosylphosphatidylinositol anchors. These genes are involved in the retention of siderophore–iron in the cell wall. Siderophore-bound iron can be taken up into the cell via two genetically separable systems. One system requires the reduction and release of the iron from the siderophore prior to uptake by members of the Fre family of plasma-membrane metalloreductases. Following reduction and release from the siderophore, the iron is then taken up via the high-affinity ferrous transport system. A set of transporters that specifically recognizes siderophore–iron chelates is also expressed under conditions of iron deprivation. These transporters, encoded by *ARN1*, *ARN2/TAF1*, *ARN3/SIT1* and *ARN4/ENB1*, facilitate the uptake of both hydroxamate- and catecholate-type siderophores. The Arn transporters are expressed in intracellular vesicles that correspond to the endosomal compartment, which suggests that intracellular trafficking of the siderophore and/or its transporter may be important for uptake.


Key words: cell wall, transport, yeast.

Abbreviations used: FOB, ferrioxamine B.

[1]To whom correspondence should be addressed (e-mail carolinep@intra.niddk.nih.gov).

## Introduction

Iron is an essential nutrient for virtually every organism and is the second most abundant metal in the Earth's crust. Despite this abundance, the acquisition and utilization of iron poses significant challenges for the cell. Iron is largely present in an aerobic environment as insoluble polymers of ferric oxyhydroxides; therefore, micro-organisms have developed sophisticated strategies to solubilize and take up iron. The budding yeast *Saccharomyces cerevisiae* responds to iron deprivation by increasing the expression levels of genes involved in the acquisition of iron. These genes are largely transcribed under the control of Aft1p, the major iron-dependent transcription factor in yeast [1,2]. Aft1p stimulates the transcription of genes encoding the components of the reductive system of elemental iron uptake. In this system, ferric iron is first reduced to the ferrous form by the plasma-membrane metalloreductases encoded by *FRE1* and *FRE2* [3,4]. Following reduction, the ferrous iron is taken up into the cell by the high-affinity ferrous iron transporter, which is composed of a multicopper oxidase, encoded by *FET3*, and a permease, encoded by *FTR1* [5,6]. Fet3p requires copper for activity, and the copper

chaperone Atx1p and the copper transporter Ccc2p are also required for Fet3p activity [1,7].

Reduction and uptake of elemental iron is not the only strategy used by yeast to accumulate iron. Most micro-organisms and some plants rely on siderophores, low-molecular-mass organic compounds that specifically bind (and thereby solubilize) ferric iron with exceptionally high affinity. Siderophores are synthesized and secreted in the iron-free form by micro-organisms. Secreted siderophores can bind iron and the iron–siderophore chelates are then captured by specific cellular transport systems. Although prokaryotic systems of siderophore–iron uptake are well described, eukaryotic systems of siderophore utilization have been identified more recently. Neither *S. cerevisiae* nor *Schizosaccharomyces pombe* secrete siderophores, yet both species are capable of taking up a variety of types of siderophore-bound iron [8].

Siderophores exhibit a variety of structures, but can be broadly categorized into two classes: the hydroxamates (secreted primarily by fungi) and the catecholates (secreted primarily by bacteria; Figure 1) [9,10]. Ferrichrome is a prototypical example of the tri-hydroxamate class (Figure 1A). It is a cyclic hexapeptide that co-ordinates iron through three bidentate hydroxamate side chains. Triacetylfusarinine C is also a cyclic tri-hydroxamate, but differs from ferrichrome in that the hydroxamic acid residues are joined by ester linkages (Figure 1B). Ferrioxamine B (FOB) is a tri-hydroxamate bacterial siderophore consisting of a linear chain of three peptide-linked amino acids, terminating in a free amino group (Figure 1C). The iron-free form of FOB, shown in Figure 1, is used clinically in the treatment of iron-overload disorders. Enterobactin is a prototypical example of a catecholate siderophore (Figure 1D) and consists of a tri-ester ring, from which extend three side chains of dihydroxybenzoyl serine [11]. Each of these siderophores shares the common feature of a hexadentate co-ordination site with iron.

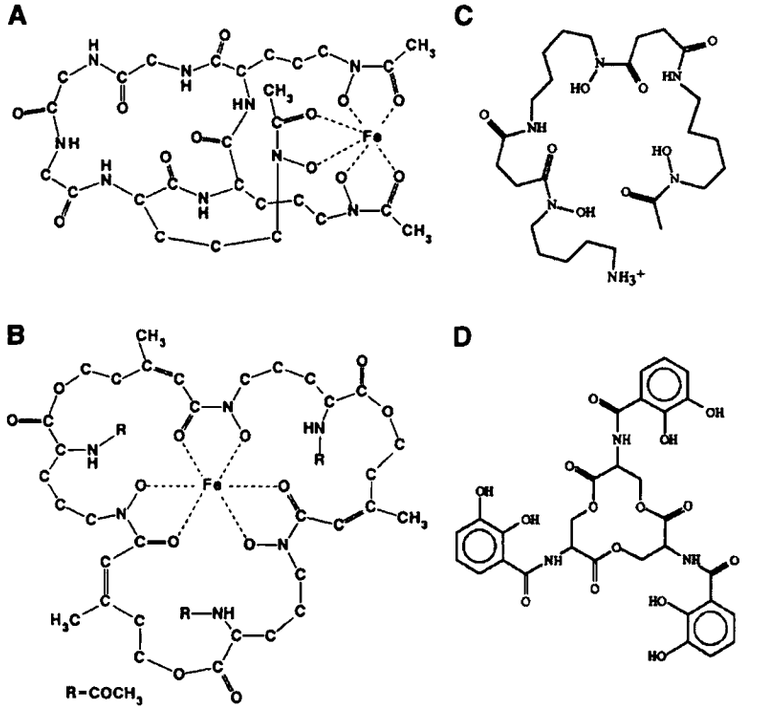
## Interactions of siderophores with the Fit family of cell-wall proteins

Analysis of iron- and Aft1p-regulated gene expression using cDNA microarrays identified three homologous genes that are very highly expressed

**Figure 1**

**Structures of siderophores of the hydroxamate and catecholate classes**

(**A**) Ferrichrome. (**B**) Triacetylfusarinine C. (**C**) Ferrioxamine B. (**D**) Enterobactin. Desferriforms are shown in (**C**) and (**D**). Modified from [9,10,24] by courtesy of Marcel Dekker Inc.



under conditions of iron deprivation and are components of the yeast cell wall [12–14]. These secreted proteins, Fit1p, Fit2p and Fit3p, are attached to the β-glucan scaffold of the cell wall via glycosylphosphatidylinositol anchors and serve to enhance the uptake of siderophore-bound iron. They are acidic serine- and threonine-rich mannoproteins and contain numerous repeated sequences. Deletion of the *FIT* genes results in impaired uptake of ferrichrome- and FOB-bound iron, despite an increased level of expression of iron-transport systems in these deletion strains. Significant amounts of iron can be retained in the cell wall of budding yeast and this iron can be released and detected after enzymic digestion of the cell wall. Strains carrying deletions of the *FIT* genes retain much less ferrichrome-bound iron in their cell wall than do wild-type strains, suggesting that the function of the Fit proteins is to trap siderophore-bound iron in the cell wall and facilitate the uptake of siderophore–iron through the plasma membrane.

## The reductive system of siderophore–iron uptake

Siderophore-bound iron that reaches the plasma membrane can be taken up into the cell by a process that involves the reduction of iron from the ferric to the ferrous state and the release of the iron from the siderophore prior to transport through the high-affinity ferrous iron system (Fet3p and Ftr1p) [15–17]. This reduction and release of iron is accomplished in a single step by the activities of the Fre family of plasma-membrane metalloreductases. Fre1p and Fre2p are flavocytochromes that have the capacity to reduce oxidized forms of both iron and copper, and they are required for growth on media containing low concentrations of simple iron salts [3,4,18–21]. The yeast genome contains five additional genes with homology to *FRE1* and especially to *FRE2*, termed *FRE3*, *FRE4*, *FRE5*, *FRE6* and *FRE7* [22]. Four of these genes (*FRE3–FRE6*) are regulated by iron through Aft1p, and two of them (*FRE3* and *FRE4*) are siderophore–iron reductases [23]. Fre1p and Fre2p have been shown to catalyse the reduction of iron bound to siderophores of both the hydroxamate and catecholate classes. Fre3p has also been shown to be a plasma-membrane reductase with specificity for siderophores of the hydroxamate class. Rhodotorulic acid, a siderophore of the di-hydroxamate class, is a substrate for Fre4p, as well as for Fre1, Fre2p and Fre3p. The differences in specificity of the Fre reductases for different siderophore substrates may be based on differences in the reduction potential of the siderophores rather than recognition of the structure of the siderophore–iron chelate [23,24]. Fre1p and Fre2p exhibit the broadest range of substrate specificity as well as the capacity to reduce enterobactin, the siderophore with the most negative reduction potential. Fre4p exhibits the most restricted range of specificity and the capacity to reduce only rhodotorulic acid, a siderophore with a much less negative reduction potential. Whether the remaining *FRE* genes exhibit specificity for other siderophores remains to be tested.

## The Arn family of siderophore transporters

In addition to the Fit family of cell-wall proteins, cDNA microarrays identified four iron- and Aft1p-regulated genes that are part of the major facilitator superfamily of transporters [17]. These genes form the *ARN* family and are termed *ARN1*, *ARN2/TAF1*, *ARN3/SIT1* and *ARN4/ENB1*. *ARN3* is identical to *SIT1*, a gene that confers FOB–iron-uptake activity to a strain deleted for *FET3* and *FET4*, a low-affinity iron transporter [25]. Each of the *ARN* genes has subsequently been shown to function as a siderophore–iron transporter and the substrate specificity of each transporter is shown in Table 1. Arn1p, Arn2p and Arn3p transport hydroxamate siderophores with Arn1p exhibiting specificity for the ferrichromes [26–28], Arn2p for the fusarinines [28,29] and Arn3p for both the ferrioxamines and the ferrichromes [17,25,27]. Surprisingly, although Arn1p and Arn2p exhibit the great-

**Table 1**

**Specificity and kinetics of Arn transporters**

Arn1p and Arn3p exhibit specificity for multiple members of the ferrichrome family of siderophores.

| Transporter | Siderophore substrate | $K_m$ for transport (μM) |
|---|---|---|
| Arn1 | Ferrichrome (triacetylfusarinine C) | 0.9 |
| Arn2 | Triacetylfusarinine C | 1.6 |
| Arn3 | FOB | 0.5 |
| | Ferrichrome (triacetylfusarinine C) | 2.3 |
| Arn4 | Enterobactin | 1.9 |

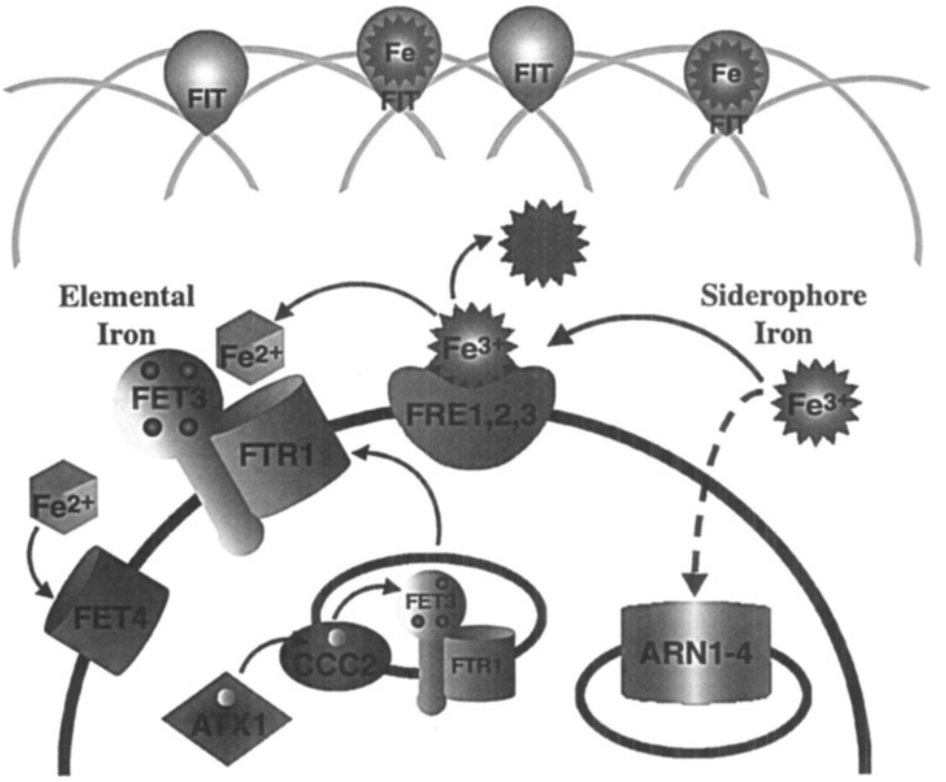
est sequence similarity, the substrate specificity of Arn1p is most similar to that of Arn3p. Both Arn1p and Arn3p exhibit specificity for multiple members of the ferrichrome family as well as a small degree of activity for triacetylfusarinine C (as measured in growth-promotion tests). The pathogenic fungus *Candida albicans* expresses a single siderophore transporter that is 46% identical to Arn1p and exhibits specificity for ferrichrome [30]. Arn4p exhibits no transport activity of hydroxamate siderophores and appears to transport enterobactin exclusively. There are some discrepancies in the literature regarding the specificity of the Arn transporters for different siderophores, and these discrepancies may be due to both strain variation and different methods for assessing specificity. The reported $K_m$ values for each transporter are very similar and in the low micromolar range. These data, coupled with previously reported data [16], suggest that at low siderophore concentrations siderophore-bound iron is taken up largely through the Arn transporters, while at higher siderophore concentrations both the reductive and Arn-dependent transport systems are active. A model summarizing the systems of siderophore–iron uptake in *S. cerevisiae* is shown in Figure 2.

The localization of Arn1p and Arn3p within the cell has been studied by indirect immunofluorescence and by biochemical fractionation methods and both transporters are found to reside in intracellular vesicles that resemble the late endosomal compartment [17,28]. Very little, if any, of these proteins is detected on the cell surface. These data suggest that the intracellular trafficking of the Arn transporters and/or their siderophore substrates may be important in the uptake of siderophore-bound iron. Whether the iron enters the cell as an intact siderophore–iron chelate, how the iron is separated from the

**Figure 2**

**Model of siderophore iron uptake in *S. cerevisiae***

The Fit mannoproteins of the cell wall facilitate retention of siderophore–iron in the cell wall and periplasmic space. Siderophore-bound iron can be reduced and released from the siderophore by the Fre reductases, then taken up through either the high-affinity ferrous iron transporter (Fet3p and Ftr1p) or the low-affinity transporter (Fet4). Fet3p is loaded with copper intracellularly through the activities of Atx1p and Ccc2p. Alternatively, the intact siderophore–iron chelate can be taken up via an Arn transporter with specificity for the particular siderophore. Whether the Arn transporter relocalizes to the plasma membrane and/or the siderophore undergoes endocytosis prior to translocation of the siderophore–iron chelate across the membrane is currently being investigated.



siderophore intracellularly, and the fate of intracellular siderophore are issues that have not been resolved and await further study.

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# A new mechanism for membrane iron transport in *Pseudomonas aeruginosa*

**I. J. Schalk[1], M. A. Abdallah and F. Pattus**

Département des Récepteurs et Protéines Membranaires UPR 9050 CNRS, ESBS, Bld Sébastien Brant, F-67400 Illkirch, Strasbourg, France

## Abstract

Various biochemical and biophysical studies have demonstrated the existence of a novel iron-uptake mechanism in *Pseudomonas aeruginosa*, different from that generally described for ferrichrome and ferric-enterobactin in *Escherichia coli*. This new iron-uptake mechanism involves all the proteins generally reported to be involved in the uptake of ferric-siderophore complexes in Gram-negative bacteria (i.e. the outer membrane receptor, periplasmic binding protein and ATP-binding-cassette transporter), but differs in the behaviour of the siderophore. One of the key features of this process is the binding of iron-free pyoverdin to the outer membrane receptor FpvA in conditions of iron deficiency.

Key words: fluorescence resonance energy transfer (FRET), iron uptake, outer membrane protein, siderophore.

Abbreviations used: $K_{dapp}$, apparent dissociation constant; PaA, pyoverdin.

[1]To whom correspondence should be addressed (e-mail schalk@esbs.u-strasbg.fr).

## Introduction

*Pseudomonas aeruginosa* is considered to be an important opportunistic pathogen, highly patho-