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ORIGINAL PAPER

Identification and analysis of iron transporters from the fission yeast *Schizosaccharomyces pombe*

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Abstract

Iron is an essential trace metal ion required for all living organisms, and is taken up by iron transporters. Here, we identified and characterized three-candidate high-affinity (Fio1, Frp1 and Frp2) and two-candidate low-affinity iron transporters (Fet4 and Pdt1) from the fission yeast *Schizosaccharomyces pombe*. Protein sequence analyses revealed that Fio1 is a multicopper oxidase that contains three cupredoxin domains with eleven candidate iron-binding ligands, whereas Frp1 harbors a ferric reductase domain with three-candidate heme-binding ligands. Protein sequence analyses also revealed that Fet4 and Pdt1 are integral membrane proteins with 10 and 11 transmembrane regions, respectively. Deletion of *fio1* and, to a lesser extent, *frp1* impaired growth under iron-depleted conditions, whereas deletion of *frp1* and, to a lesser extent, *frp2* inhibited growth under iron-replete conditions. Deletion of *fet4* and *pdt1* did not affect the growth of cells under iron-depleted and iron-replete conditions. Deletion of *fio1* or *frp1* also increased the sensitivity of cells to other transition metals. The copper sensitivity of Δ*fio1* cells could be rescued by iron, suggesting that the addition of iron might decrease the uptake of potentially toxic copper in Δ*fio1* cells. The copper sensitivity of Δ*fio1* cells could also be rescued by deletion of *frp1*, suggesting that Fio1 and Frp1 may function together in iron and copper uptakes in *S. pombe.* Our results revealed that iron and copper uptake systems may be partially overlapped in *S. pombe*.

Keywords *Schizosaccharomyces pombe* · Iron uptake · Multicopper oxidase · Ferric reductase · Metal sensitivity

Introduction

Iron is essential to nearly all organisms. Cellular iron exists mainly in the form of iron-containing cofactors heme and iron–sulfur clusters. Heme is involved in diverse biological processes, such as transport, cell respiration, drug metabolism, ion channel function and gene regulation (Liu et al. 2020; Fleischhacker et al. 2021). Iron–sulfur clusters are present in proteins involved in ATP production, metabolism, DNA synthesis and repair, gene expression regulation, protein translation, and the antiviral response (Rouault and Maio 2017; Lill and Freibert 2020).

In organisms including fungi (Bairwa et al. 2017, Misslinger et al. 2021), plants (Kobayashi et al. 2019), and bacteria (Bradley et al. 2020), iron is taken up by two basic strategies under iron-deficient conditions. One strategy involves the cell surface Fe^{3+} reductase and a high-affinity iron-uptake complex composed of a ferroxidase and a Fe^{2+} permease. Extracellular Fe^{3+} is first reduced to the more soluble Fe^{2+} form by a Fe^{3+} reductase such as Fre1 from *Saccharomyces cerevisiae* and Frp1 from *Schizosaccharomyces pombe*. Fe^{2+} is then re-oxidized by a ferroxidase such as Fet3 from *S. cerevisiae* and Fio1 from *S. pombe*, and transported by an iron permease such as Ftr1 from *S. cerevisiae* and Fip1 from *S. pombe*. Notably, the *S. cerevisiae* Fre1 requires heme for its activity because cells defective in heme biosynthesis lack ferrireductase activity (Lesuisse and Labbe 1989) and Fet3 is a multicopper oxidase (Askwith et al. 1994).

The second strategy involves siderophores, which are small Fe^{3+}-specific chelators. Siderophores are secreted to take up extracellular Fe^{3+}. The Fe^{3+}–siderophore complex is then taken up by the cell via specific transport systems.

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In *S. pombe*, the cell surface protein Str1 has been found to take up siderophore-bound iron (Pelletier et al. 2003). Unlike *S. cerevisiae*, which cannot synthesize siderophore and use siderophore synthesized by other microorganisms, *S. pombe* is able to synthesize the hydroxamate-type siderophore ferrichrome (Schrettl et al. 2004; Mercier and Labbe 2010). The siderophore synthetase Sib1 and the ornithine N^5-oxygenase Sib2 are two critical enzymes involved in ferrichrome biosynthesis in *S. pombe*.

In addition, *S. cerevisiae* employs the low-affinity iron-uptake system under iron-replete conditions. This system also uses cell surface ferric reductases to convert extracellular Fe^{3+} into Fe^{2+}. Fe^{2+} is then transported by the plasma membrane protein Fet4 (Dix et al. 1994). In addition to Fet4, another plasma membrane protein Smf1 from *S. cerevisiae* is also involved in low-affinity iron uptake (Chen et al. 1999). However, transports involved in the low-affinity iron uptake have not been identified in *S. pombe*. In addition to the basic iron-uptake strategy, *S. pombe* also makes use of extracellular heme acquisition to take up iron (Mourer et al. 2015, 2017, 2019). During this process, the cell surface membrane protein Shu1 is required for high-affinity uptake of heme, and heme is initially accumulated in vacuoles and then distributed in the cytoplasm. Besides Shu1, Str3 is involved in low-affinity heme acquisition in *S. pombe* (Normant et al. 2018).

In this study, we identified the homologs of *S. cerevisiae* two high-affinity iron transporter (Fre1 and Fet3) and two low-affinity iron transporters (Fet4 and Smf1) in fungi, and analyzed the protein domains of selective homologs. We also examined the effects of deletion of *S. pombe* homologous genes on the sensitivity of *S. pombe* cells to iron and other transition metals. Our studies suggested that *S. pombe* two high-affinity iron transporter Fio1 and Frp1 may be involved in the uptake of transition metals in addition to iron. Our studies also suggested that iron uptake and copper uptake may be coupled in *S. pombe*. The analysis provides a foundation for future studies aimed at elucidating the detailed coupling mechanism of iron and copper uptake in *S. pombe*.

Materials and methods

Strains, plasmids and media

Schizosaccharomyces pombe strains used in this study are described in Table 1. The PCR primers used in this study are described in Table S1. Gene deletions were made by the one-step gene replacement using a kanamycin resistance gene (kanMX6) or hygromycin resistance gene (hphMX6) as a selectable marker (Hentges et al. 2005). Constructs for the deletion mutants were generated as described below. Briefly, the 5′- and 3′-flanking regions of the target gene were amplified by PCR using the *S. pombe* genomic DNA as a template, and cloned into the Sal I/Bgl II and Sac I/Spe I sites of the plasmid pAF6a-kanMX6 (Bahler et al. 1998) or pAF6a-hphMX6 (Wang et al. 2017), respectively. To generate single deletion mutants, the deletion cassettes were amplified from corresponding deletion constructs by PCR and transformed into the wild-type strain yHL6381 using the lithium acetate method (Forsburg and Rhind 2006). The deletion cassettes were transformed into the Δ*fio1* strain to generate double deletion mutants. Transformants were selected on YES plates containing 100 µg/mL of geneticin (G418) or 100 µg/mL of hygromycin. All deletion mutants were verified by PCR.

Bacterial cells were grown in liquid or solid LB medium (1% tryptone, 0.5% yeast extract, 1% sodium chloride, pH 7.2) without or with 100 µg/mL ampicillin to select for plasmids at 37 °C. *S. pombe* cells were routinely grown at 30 °C in liquid or solid YES medium containing 0.5% yeast extract, 3% glucose and 225 mg/L adenine, leucine, histidine, and uracil.

Table 1 Strains used in the present study

Strain	Genotype	Source
yHL6381	*h+ his3-D1 leu1-32 ura4-D18 ade6-M210*	H. Levin
yHL1	*h+ his3-D1 leul-32 ura4-D18 ade6-M210 Δfio1::hphMX6*	This report
yHL2	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfrp1::hphMX6*	This report
yHL3	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfrp2::kanMX6*	This report
yHL4	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δpdt1::hphMX6*	This report
yHL5	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfet4::kanMX6*	This report
yHL6	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δpap1::kanMX6*	This report
yHL7	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfio1::hphMX6 Δfrp1::kanMX6*	This report
yHL9	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfio1::hphMX6 Δfrp2::kanMX6*	This report
yHL10	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfio1::kanMX6 Δfet4::hphMX6*	This report
yHL12	*h+ leu1-32 his3-D1 ura4-D18 ade6-M210 Δfrp1::kanMX6 Δfet4::hphMX6*	This report

Standard media and protocols for genetic manipulation of fission yeast were used as described (Forsburg and Rhind 2006).

Metal sensitivity assays

Schizosaccharomyces pombe cells were grown overnight at 30 °C in YES medium. Cells were diluted to an OD_{600} of 0.2 in fresh YES medium, and grown to early exponential phase. Cells were collected and adjusted to the same OD_{600} of 3.0. Serial tenfold dilutions were prepared and 2 µL of each dilution was spotted onto YES plates supplemented with 25 µM bathophenanthroline disulfonic acid (BPS), 2.75 mM $Fe_2(SO_4)_3$ (Fe^{3+}), 800 µM $CuSO_4$ (Cu^{2+}), 800 µM $ZnSO_4$ (Zn^{2+}), 800 µM $MnSO_4$ (Mn^{2+}), or 80 µM $CdCl_2$ (Cd^{2+}). The plates were incubated at 30 °C for 4–7 days and photographed. Initially, different concentrations of metal ions were tested based on the previous studies (see below). $CuSO_4$ was tested at 0.5, 0.8 and 1.5 mM (Li and Kaplan 1998). $ZnSO_4$ was tested at 0.5, 0.8, 2, and 3.5 mM (Li and Kaplan 1998). $MnSO_4$ was tested at 0.5, 0.8, 2 and 3.5 mM (Li and Kaplan 1998). $CdCl_2$ was tested at 80 and 100 µM (Kennedy et al. 2008). $Fe_2(SO_4)_3$ was used at 2.75 mM based on our previous study (Su et al. 2017). BPS was used at a concentration of 25 µM based on a previous study (Encinar del Dedo et al. 2015).

Bioinformatics analyses

Homologs of *S. cerevisiae* Fet3, Fre1, Fet4 and Smf1 were identified through BLAST (Basic local alignment search tool) searches of the NCBI non-redundant protein sequences database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with default parameters. The candidate proteins were then analyzed for the presence of the conserved domain using the Conserved Domain Database (CDD, https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Lu et al. 2020) and Simple Modular Architecture Research Tool (SMART, http://smart.embl-heidelberg.de/) (Letunic et al. 2021). Protein sequences were aligned using the MUSCLE algorithm included in the Molecular Evolutionary Genetics Analysis (MEGA) software (version 10.1.8) (Tamura et al. 2021). Sequence logos were generated using WebLogo 3 (http://weblogo.threeplusone.com/create.cgi).

Results

Schizosaccharomyces pombe high-affinity iron transporter Fio1 contains the cupredoxin-like domains

We first performed BLAST searches against protein databases using the *S. cerevisiae* multicopper ferroxidase Fet3 as a query. Fet3 homologs were identified in Ascomycota, including Pezizomycetes (792 species), Saccharomycotina (1021 species) and Taphrinomycotina (39 species), and Basidiomycota (735 species), as well as in the basal clades of fungi, including Zygomycota (375 species) and Chytridiomycota (79 species) (data not shown). This result suggested that Fet3 homologs are universally present in fungi. Analysis of the protein sequences using the CDD and SMART databases predicted that like *S. cerevisiae* Fet3, *S. pombe* Fio1 (a homolog of *S. cerevisiae* Fet3) and other representative fungal Fet3 homologs contain three cupredoxin-like domains linked by external interdomain (Fig. 1). The cupredoxin domain typically adopts a Greek-key, β-barrel structure, comprising two β-sheets formed by 7 or 8 β strands. This structure was initially found in small copper-containing electron-transfer proteins in bacteria, fungi, and plants termed blue-copper proteins or cupredoxins, and later found in multicopper oxidase (Sedlak et al. 2008).

Multiple sequence alignment revealed that *S. cerevisiae* Fet3 and its homologs contain ten absolutely conserved histidine residues and one absolutely conserved cysteine residue located in two cupredoxin domains, suggesting that these histidine and cysteine residues may be involved in copper binding in Fio1. Indeed, these residues have been shown to be important for the binding of four coppers. Fet3 contains three different copper centers involved in the redox reaction. It remains to be determined that Fio1 is a multicopper oxidase.

Schizosaccharomyces pombe Frp1 and Frp2 contain the ferric reductase domain

A BLAST search using the *S. cerevisiae* Fre1 protein sequence revealed that Fre1 homologs are ubiquitously present in fungi. *S. pombe* contains two Fre1 homologs (Frp1 and Frp2). The two proteins share 47% identity and 63% similarity in the amino acid sequence over the entire length. Frp1 is localized to the cell surface, whereas Frp1 resides in the endoplasmic reticulum (ER) (Matsuyama et al. 2006). SMART database search predicted six transmembrane domains in both Frp1 and Frp2 proteins. CDD and SMART analyses revealed that both Frp1 and

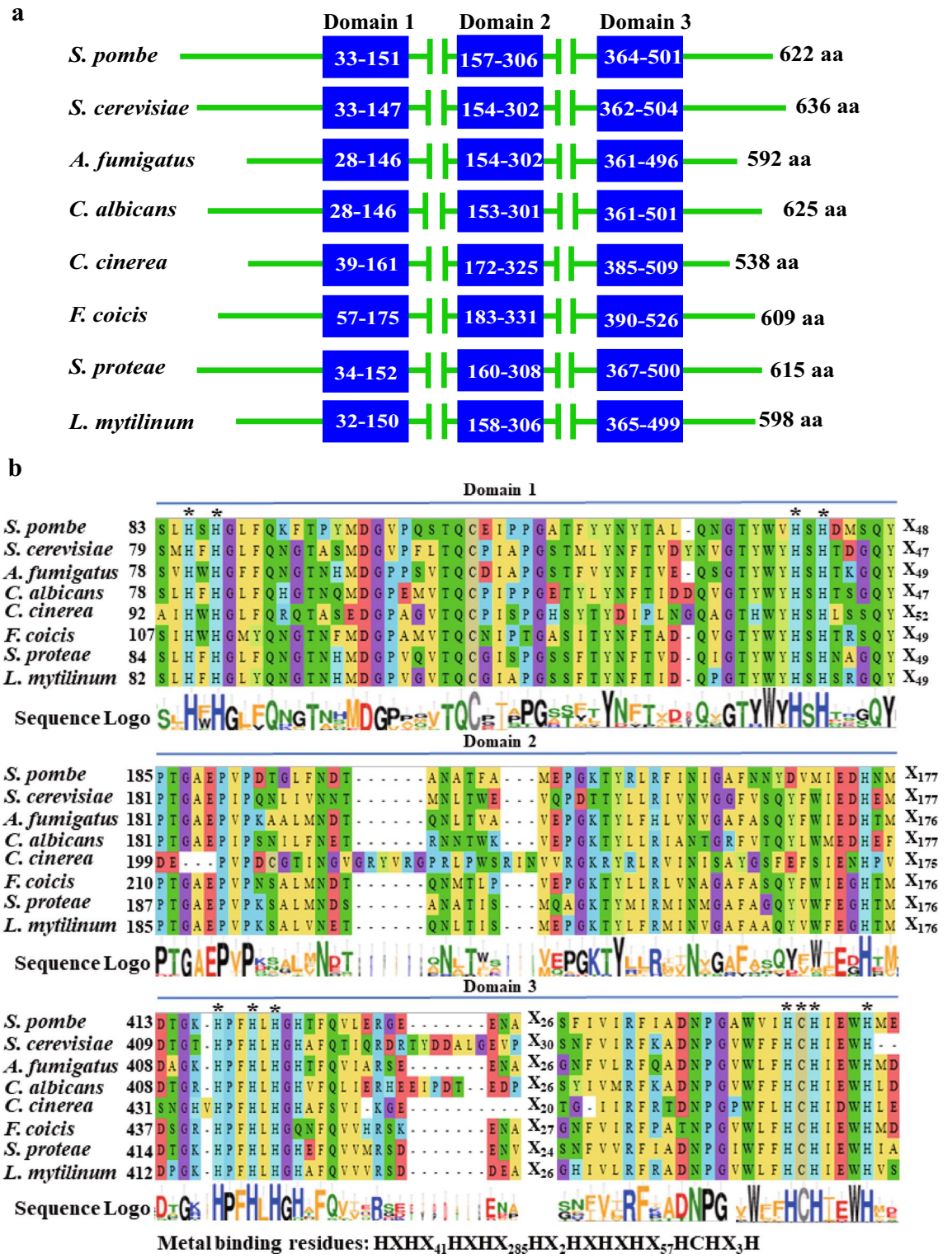

Fig. 1 *Saccharomyces cerevisiae* Fet3 and its homologs contain three cupredoxin-like domains. **a** Domain organization of representative Fet3 proteins from *S. pombe*, *S. cerevisiae*, *Aspergillus fumigates*, *Candida albicans*, *Coprinopsis cinerea*, *Fusarium coicis*, *Saccharata proteae*, and *Lophium mytilinum*. Domains were identified by CDD and SMART analyses. The cupredoxin-like domains are indicated in the blue boxes. **b** Multiple sequence alignment of the cupredoxin-like domain sequences of representative Fet3 proteins. The alignment was generated using MEGA_X_10.1.8. The copper-binding residues are marked with an asterisk above the alignment. Amino acid residues are highlighted according to the biochemical properties of the amino acid residues. The sequence logo was generated using WebLogo 3

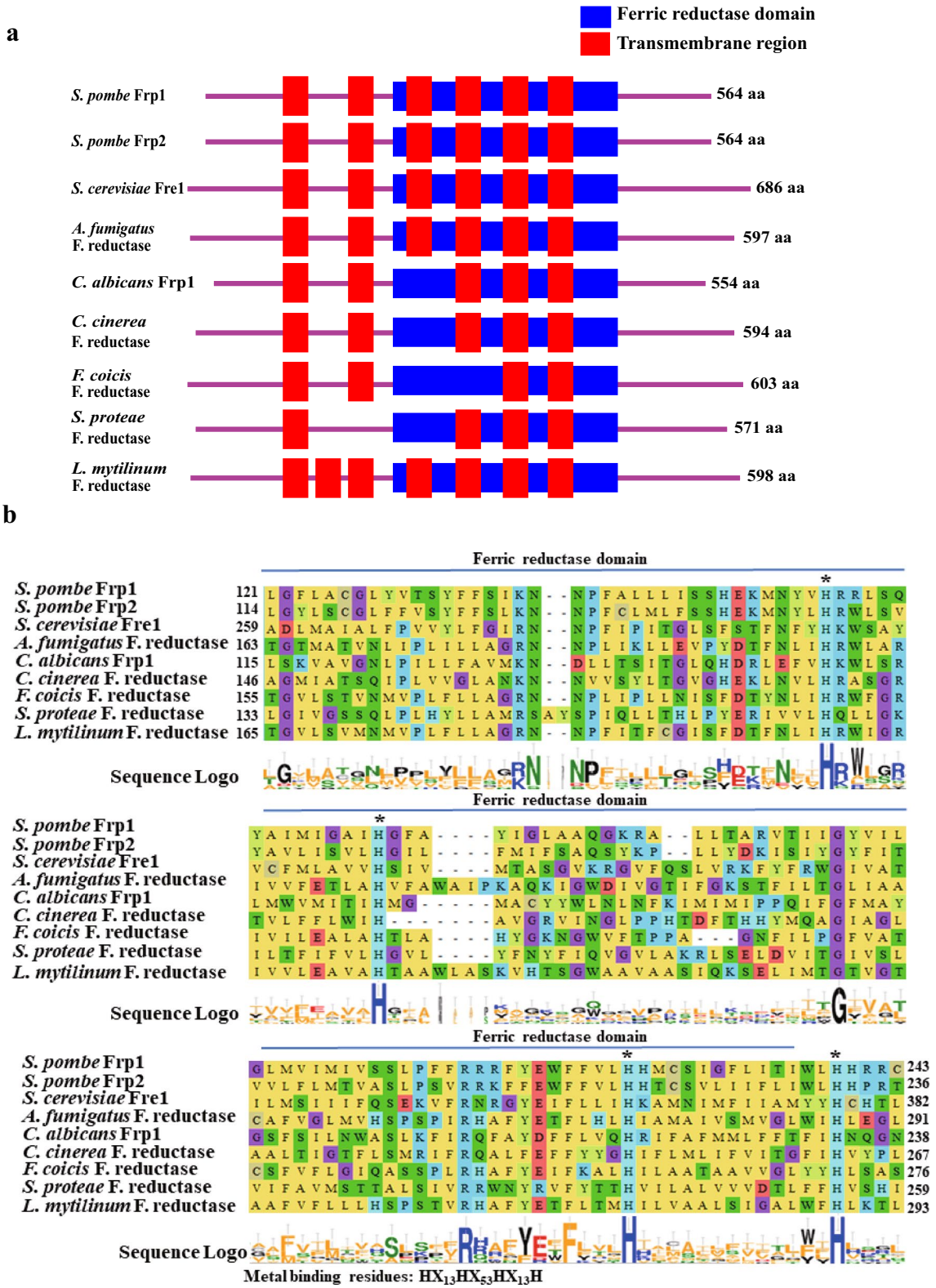

Fig. 2 *Saccharomyces cerevisiae* Fre1 and its homologs contain the ferric reductase and transmembrane domains. **a** Domain organization of *S. cerevisiae* Fre1 and its homologs from *S. pombe* (Frp1 and Frp2), *A. fumigates*, *C. albicans*, *C. cinerea*, *F. coicis*, *S. proteae*, and *L. mytilinum.* The ferric reductase and transmembrane domains identified by CDD and SMART are indicated in blue and red boxes, respectively. **b** Multiple sequence alignment of the ferric reductase domain sequences of representative Fre1 proteins. The conserved heme-coordinating histidines are indicated with asterisks. Amino acid residues are highlighted according to the biochemical properties of the amino acid residues. The alignment and the sequence logo were generated as described above

Frp2 and other representative Fre1 homologs contain a conserved ferric reductase domain (Fig. 2) (Zhang et al. 2013). This domain includes three canonical conserved heme-coordinating histidines (Fig. 2). Multiple sequence alignment of Fre1 and selected Fre1 homologs revealed two additional absolutely conserved histidine residues, which are likely involved in catalysis and/or heme-binding (Fig. S1).

Schizosaccharomyces pombe putative low iron affinity transporters Fet4 and Pdt1 are integral transmembrane proteins

We went on to identify homologs of two *S. cerevisiae* low-affinity iron transporters Fet4 and Smf1 using BLAST searches. Fet4 and Smf1 homologs are widely present in fungi. We selected the representative candidates of Fet4 and Smf1 for further analysis. CDD and SMART database searches showed that *S. cerevisiae* Fet4 and representative Fet4 homologs contain an iron-permease domain consisting of 9–11 transmembrane regions (Fig. S2). An examination of the amino acid sequence of the iron-permease domain from *S. cerevisiae* Fet4 and its homologs did not reveal any obvious candidate metal-binding ligands in the hydrophilic regions of the proteins. Such ligands have been found in *S. cerevisiae* Fet3 and its homologs (see above). The exact mechanism of how this iron-permease domain controls iron uptake into the cell remains to be determined.

CDD analyses revealed that *S. cerevisiae* Smf1 and its homologs, including *S. pombe* Pdt1, belong to the NRAMP metal ion transporter family, which is characterized by a conserved hydrophobic core of 10–12 transmembrane regions (Bozzi and Gaudet 2021). SMART analysis revealed the presence of 10 conserved transmembrane regions in the proteins (Fig. S3). Multiple sequence alignment revealed absolutely conserved aspartic acid and glutamic acid residues in the NRAMP domain, which are likely to be involved in catalysis and metal binding (Fig. S3).

Effect of deletion of iron mutants on the growth under iron-replete and iron-depleted conditions

To examine whether *S. pombe fio1*, *frp1*, *frp2*, *fet4* and *pdt1* are involved in iron uptake, we constructed deletion mutants for each of these genes by homologous recombination and analyzed the growth of these deletion mutants under iron-replete and iron-depleted conditions. To create iron-depleted conditions, 25 µM of iron chelator bathophenanthroline disulfonate (BPS) was added to YES media. For high-iron conditions, $Fe_2(SO_4)_3$ was added to YES media for a final concentration of 2.75 mM. The BPS and all metal ion concentrations used in the assays were optimized. Δ*fio1* cells, and to a much lesser extent, Δ*frp1* cells, exhibited a growth defect on iron-depleted media; whereas Δ*frp1* cells, and to a much lesser extent, Δ*frp2* cells showed a growth defect on iron-replete conditions (Fig. 3a). In contrast, Δ*fet4* and Δ*pdt1* cells did not exhibit a growth defect under iron-depleted and iron-replete conditions (Fig. 3a). These results suggest that Fio1 is essential for iron uptake under iron-deficient conditions and that additional proteins may be involved in ferric reduction under iron-deficient conditions in *S. pombe*. These results also suggest that Frp1 and Frp2 may be involved in iron uptake under iron-replete conditions.

Fio1 and Frp1 are likely to be involved in the uptake of transition metals other than iron

Because iron metal transporters may be involved in the transport of metal ions other than iron, we examined the sensitivity of Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* cells on transition metals Cu^{2+}, Zn^{2+}, Mn^{2+}, and Cd^{2+}. The concentrations of transition metals used in the assay were optimized. We also included Δ*pap1* cells as a control, because *pap1* encodes an oxidative stress-responsive basic leucine zipper (bZIP) transcription factor required for cell survival under metal ion stress. We found that only Δ*fio1* cells were sensitive to Cu^{2+}. In addition, Δ*fio1* and Δ*frp1* cells were sensitive to Zn^{2+}, Mn^{2+}. In contrast, Δ*frp2*, Δ*fet4* and Δ*pdt1* did not exhibit sensitivity to all metal ion tested. Δ*pap1* cells were only modestly sensitive Cd^{2+} (Fig. 3b), suggesting that the addition of Cd^{2+} activated Pap1, whereas the addition other transition metals did not. These results suggest that Fio1 and Frp1 may be involved in the uptake of transition metals in addition to iron.

Addition of iron can suppress the sensitivity of Δ*fio1* and Δ*frp1* to transition metals

Since Fio1 and Frp1 appear to be involved in the uptake of transition metals in addition to iron, we tested whether the addition of iron affected the growth of wild-type strain and Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* mutants on media supplemented with transition metal Cu^{2+}, Zn^{2+}, Mn^{2+}, or Cd^{2+}. We found that the addition of iron could suppress the sensitivity of Δ*fio1* cells to Cu^{2+}, Zn^{2+}, Mn^{2+}, and Cd^{2+}. In addition, the addition of iron could suppress the sensitivity of Δ*frp1* cells to Zn^{2+}, Mn^{2+}, and Cd^{2+} (Fig. 4).

Deletion of *frp1* can rescue the sensitivity of Δ*fio1* cells to copper

We genetically tested whether *S. pombe* Fio1 functions together with Frp1, Frp2 and Fet4 in the uptake of transition metals. To do so, we generated double mutants of Δ*fio1*Δ*frp1*, Δ*fio1*Δ*frp2* and Δ*fio1*Δ*fet4*. We also

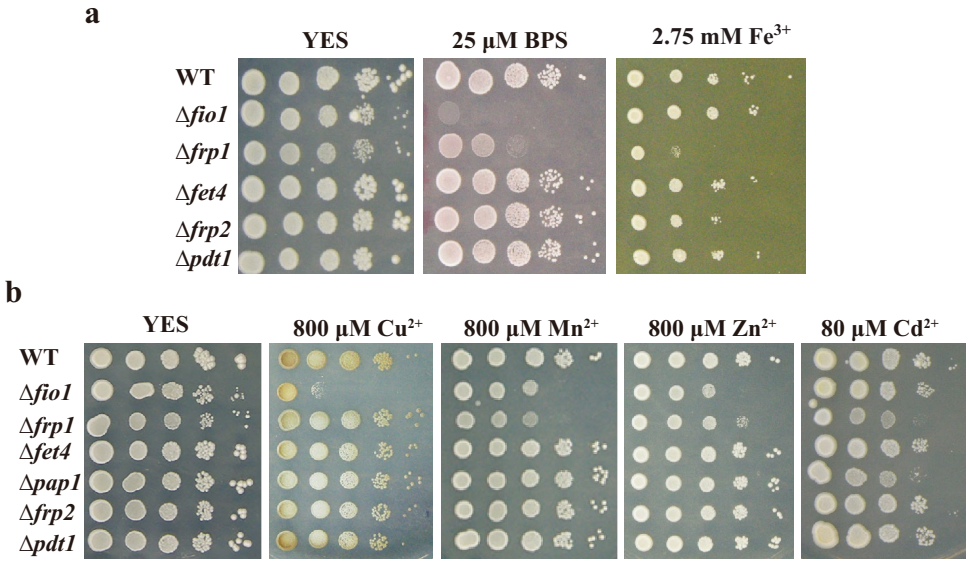

Fig. 3 The sensitivity of wild-type, Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* cells to iron, copper, zinc, manganese, and cadmium. **a** The effect of iron on the growth of wild-type, Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* cells. Cells were grown in YES medium to exponential phase and adjusted to an OD_{600} of 3.0. Tenfold serial dilutions were prepared, and 2.5 μL aliquots of each dilution were spotted on YES plates, and YES plates containing 25 μM BPS to generate the iron-depleted growth condition or 2.75 mM $Fe_2(SO_4)_3$ to generate the iron-replete growth condition. **b** The sensitivity of wild-type, Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* cells to copper, zinc, manganese, and cadmium. Cells were prepared as described above, and were spotted on YES plates, and YES plates containing 800 μM $CuSO_4$ (Cu^{2+}), 800 μM $MnSO_4$ (Mn^{2+}), 800 μM $ZnSO_4$ (Zn^{2+}), or 80 μM $CdCl_2$ (Cd^{2+})

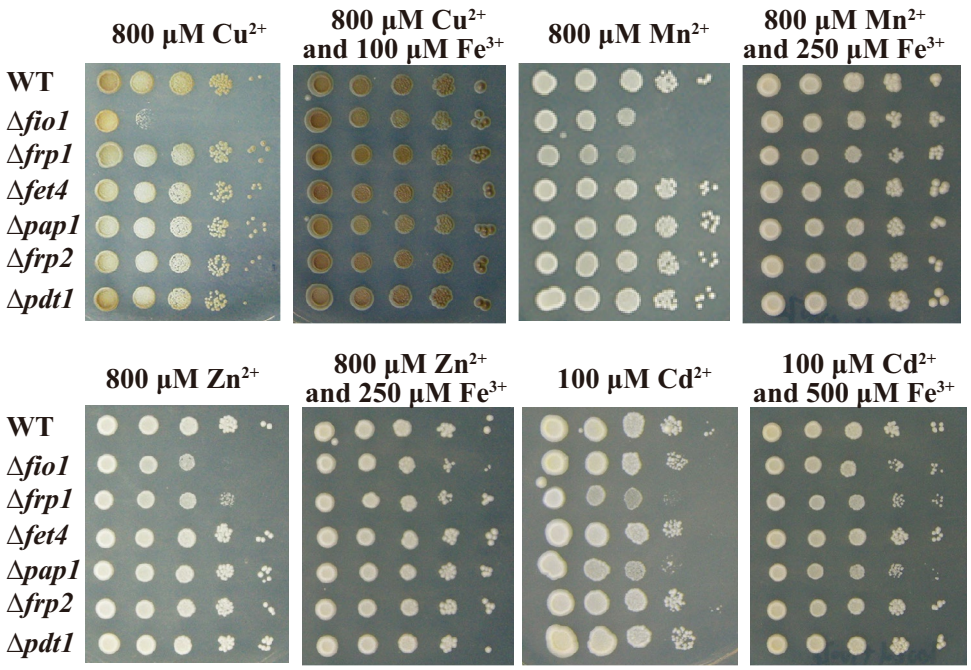

Fig. 4 The effect of iron additions on the sensitivity of wild-type, Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* cells to transition metals. Cells were grown in YES medium to exponential phase, adjusted to an OD_{600} of 3.0. Tenfold serial dilutions were prepared, and 2.5 μL aliquots of each dilution were spotted on YES plates containing designated concentrations of transition metals with or without indicated concentrations of $Fe_2(SO_4)_3$

generated Δ*frp1*Δ*fet4* as a control. The results showed that deletion of *frp1* but not *frp2* and *fet4* could rescue the sensitivity of Δ*fio1* cells on Cu^{2+}. In addition, deletion of *frp1*, *frp2* and *fet4* could not rescue the sensitivity of Δ*fio1* cells to Zn^{2+} and Mn^{2+} (Fig. 5). These results suggested

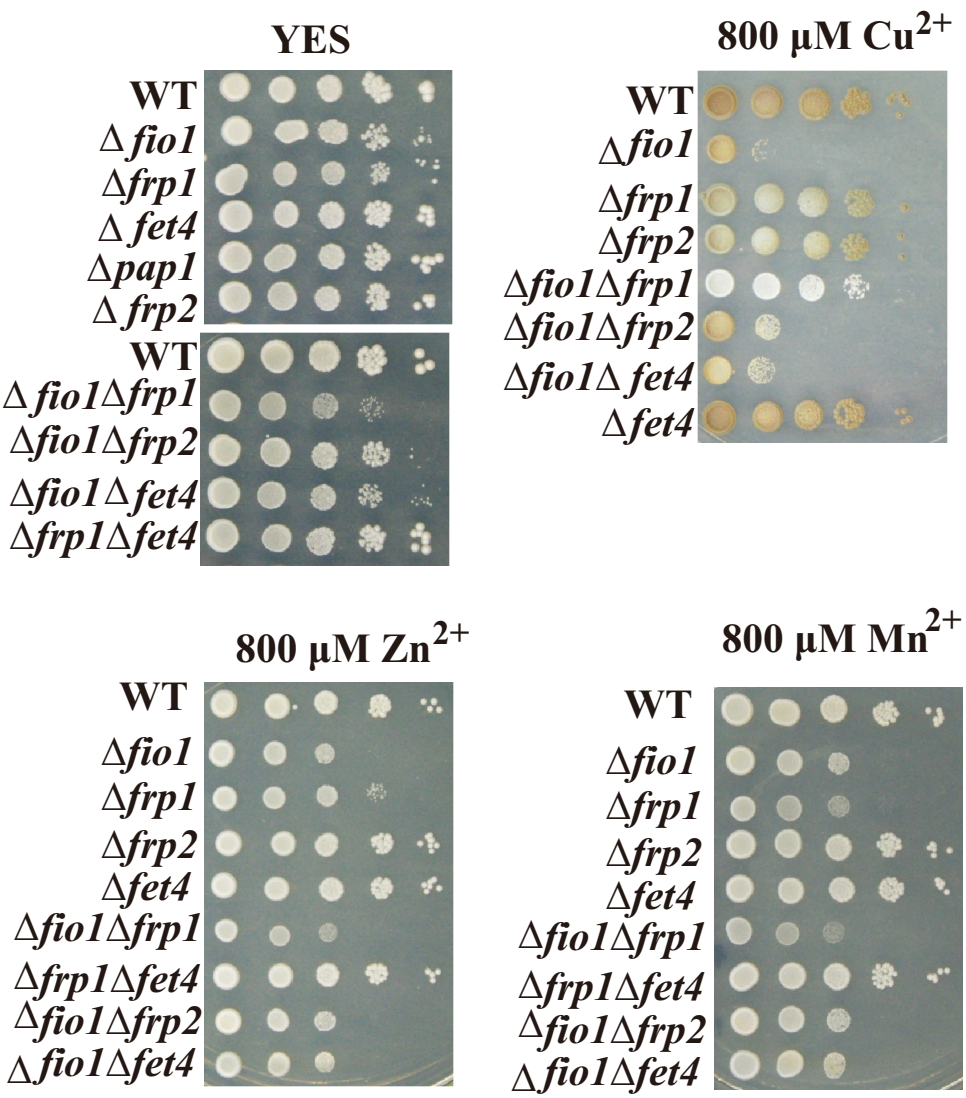

Fig. 5 Deletion of *frp1*, but not *frp2* or *fet4*, can rescue the sensitivity of Δ*fio1* cells to copper. Cells were grown in YES medium to exponential phase, and adjusted to an OD_{600} of 3.0. Tenfold serial dilutions were prepared, and 2.5 µL aliquots of each dilution were spotted on YES plates, and plates supplemented with transition metals at the indicated concentrations

that *S. pombe* Fio1 and Frp1 function together in copper uptake.

Discussion

In this study, we investigated the role of *S. pombe* Fio1, Frp1, Frp2, Fet4 and Pdt1 in the uptake of iron and other transition metals. Our iron sensitivity assays suggested that ferroxidase Fio1 and, to a much lesser extent, ferric reductase Frp1 are involved in high-affinity iron uptake, which is required for cell survival under iron-deficient conditions, consistent with the previous finding (Kwok et al. 2006). In addition, our results suggest that Frp1 is likely involved in low-affinity iron uptake. It remains to be determined whether Fet4 and Pdt1, like their *S. cerevisiae* counterparts, are involved in low-affinity iron uptake.

We tested the sensitivity of Δ*fio1*, Δ*frp1*, Δ*frp2*, Δ*fet4* and Δ*pdt1* deletion mutants to transition metals such as copper, zinc, manganese, and cadmium. The growth of Δ*fio1* cells was inhibited at 0.8 mM $CuSO_4$ (Fig. 3). The growth of Δ*fio1* cells was inhibited even at a lower $CuSO_4$ concentration (0.5 mM) (Fig. S4). A similar result was obtained with a higher concentration of $CuSO_4$ (1.5 mM) (Fig. S4). The growth of Δ*fio1* and Δ*frp1* cells was impaired at 0.8 mM $MnSO_4$ and 0.8 mM $ZnSO_4$. Similar results were obtained with 0.5 and 2 mM $MnSO_4$, and 0.5 mM $ZnSO_4$ (Fig. S4). At high concentrations of $MnSO_4$ (3.5 mM) and $ZnSO_4$ (2 and 3.5 mM), the growth of both wild-type and mutant cells was impaired (Fig. S4). Δ*frp1* cells were more sensitive to $CdCl_2$ than wild-type cells at concentrations of 80 and 100 µM (Fig. 3 and Fig. S4). One possible explanation for how deletion of *fio1* or *frp1* increases sensitivity to transition metals is that defective iron uptake caused by deletion of *fio1* or *frp1* may increase the uptake of other transition

metals, resulting in the elevated levels of transition metals, which are toxic to the cells. These results suggested that like in *S. cerevisiae*, in *S. pombe* iron uptake may be coupled to other transition metal uptake. Interestingly, our results revealed that the sensitivity of Δ*fio1* and Δ*frp1* cells to other transition metals could be rescued by iron addition. A similar observation has been made in *S. cerevisiae* (Li and Kaplan 1998). It is likely that iron addition to Δ*fio1* and Δ*frp1* cells may lead to an increased cellular iron level and a decreased uptake of potentially toxic transition metals.

Our results revealed that the sensitivity of Δ*fio1* cells to copper can also be rescued by deletion of *frp1*. This observation may be explained as follows. The ferric reductase Frp1 may also be involved in the reductive uptake of copper in *S. pombe*. In the copper uptake, Frp1 may produce toxic Cu^{1+} that may be re-oxidized to Cu^{2+} by the putative multicopper oxidase Fio1 and the Fio1-generated Cu^{2+} is then transported into the cell by a permease. Thus, it is likely that failure of re-oxidization of Frp1-produced Cu^{1+} to Cu^{2+} by Fio1 results in the sensitivity of Δ*fio1* cells to copper. In *S. cerevisiae*, deletion of *fre1* suppresses the copper sensitivity of Δ*fet3* cells (Shi et al. 2003). It is likely that a similar mechanism for coupling iron uptake to copper uptake may operate in *S. cerevisiae*. Further work is required to validate the proposed coupling mechanism.

We also observed that the sensitivity of Δ*fio1* cells to Cu^{2+} cannot be rescued by deletion of *frp2*. Frp2 is homologous to Frp1, and the two proteins share 47% amino acid sequence identity and 63% similarity. One possible explanation for this is that although the two proteins may perform similar functions, the two proteins have distinct subcellular localization in *S. pombe*. Like Fio1, Frp1 is distributed on the cell surface, whereas Frp2 is localized to the ER (Matsuyama et al. 2006). It seemed likely that the ER-resident Frp2, unlike the cell surface Frp1, is involved in iron uptake into the ER.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02683-y.

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Author contributions HY and YZ designed and performed the experiments. FA and YL conducted bioinformatics analyses. YH, FA, HY and YZ analyzed the data. YH, FA and YL prepared the manuscript. All authors read, reviewed and approved the final version of the manuscript.

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Data availability The data sets generated and analyzed during the current study are available on the request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication All authors read and approved the final version of the manuscript for publication.

References

Askwith C, Eide D, Van Ho A, Bernard PS, Li L, Davis-Kaplan S, Sipe DM, Kaplan J (1994) The FET3 gene of *S. cerevisiae* encodes a multicopper oxidase required for ferrous iron uptake. Cell 76(2):403–410. https://doi.org/10.1016/0092-8674(94)90346-8

Bähler J, Wu JQ, Longtine MS, Shah NG, McKenzie A 3rd, Steever AB, Wach A, Philippsen P, Pringle JR (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. Yeast 14(10):943–951. https://doi.org/10.1002/(sici)1097-0061(199807)14:10%3C943::aid-yea292%3E3.0.co;2-y

Bairwa G, Hee Jung W, Kronstad JW (2017) Iron acquisition in fungal pathogens of humans. Metallomics 9(3):215–227. https://doi.org/10.1039/c6mt00301j

Bozzi AT, Gaudet R (2021) Molecular mechanism of Nramp-family transition metal transport. J Mol Biol 433(16):166991. https://doi.org/10.1016/j.jmb.2021.166991

Bradley JM, Svistunenko DA, Wilson MT, Hemmings AM, Moore GR, Le Brun NE (2020) Bacterial iron detoxification at the molecular level. J Biol Chem 295(51):17602–17623. https://doi.org/10.1074/jbc.rev120.007746

Chen XZ, Peng JB, Cohen A, Nelson H, Nelson N, Hediger MA (1999) Yeast SMF1 mediates H(+)-coupled iron uptake with concomitant uncoupled cation currents. J Biol Chem 274(49):35089–35094. https://doi.org/10.1074/jbc.274.49.35089

Dix DR, Bridgham JT, Broderius MA, Byersdorfer CA, Eide DJ (1994) The FET4 gene encodes the low affinity Fe(II) transport protein of *Saccharomyces cerevisiae*. J Biol Chem 269(42):26092–26099

Encinar del Dedo J, Gabrielli N, Carmona M, Ayté J, Hidalgo E (2015) A cascade of iron-containing proteins governs the genetic iron starvation response to promote iron uptake and inhibit iron storage in fission yeast. PLoS Genet 11(3):e1005106. https://doi.org/10.1371/journal.pgen.1005106

Fleischhacker AS, Sarkar A, Liu L, Ragsdale SW (2021) Regulation of protein function and degradation by heme, heme responsive motifs, and CO. Crit Rev Biochem Mol Biol. https://doi.org/10.1080/10409238.2021.1961674

Forsburg SL, Rhind N (2006) Basic methods for fission yeast. Yeast 23(3):173–183. https://doi.org/10.1002/yea.1347

Hentges P, Van Driessche B, Tafforeau L, Vandenhaute J, Carr AM (2005) Three novel antibiotic marker cassettes for gene disruption and marker switching in *Schizosaccharomyces pombe*. Yeast 22(13):1013–1019. https://doi.org/10.1002/yea.1291

Kennedy PJ, Vashisht AA, Hoe KL, Kim DU, Park HO, Hayles J, Russell P (2008) A genome-wide screen of genes involved in cadmium tolerance in *Schizosaccharomyces pombe*. Toxicol Sci 106(1):124–139. https://doi.org/10.1093/toxsci/kfn153

Kobayashi T, Nozoye T, Nishizawa NK (2019) Iron transport and its regulation in plants. Free Radic Biol Med 133:11–20. https://doi.org/10.1016/j.freeradbiomed.2018.10.439

Kwok EY, Severance S, Kosman DJ (2006) Evidence for iron channeling in the Fet3p–Ftr1p high-affinity iron uptake complex in the

yeast plasma membrane. Biochemistry 45(20):6317–6327. https://doi.org/10.1021/bi052173c

Lesuisse E, Labbe P (1989) Reductive and non-reductive mechanisms of iron assimilation by the yeast *Saccharomyces cerevisiae*. J Gen Microbiol 135(2):257–263. https://doi.org/10.1099/00221287-135-2-257

Letunic I, Khedkar S, Bork P (2021) SMART: recent updates, new developments and status in 2020. Nucleic Acids Res 49(D1):D458–D460. https://doi.org/10.1093/nar/gkaa937

Li L, Kaplan J (1998) Defects in the yeast high affinity iron transport system result in increased metal sensitivity because of the increased expression of transporters with a broad transition metal specificity. J Biol Chem 273(35):22181–22187. https://doi.org/10.1074/jbc.273.35.22181

Lill R, Freibert SA (2020) Mechanisms of mitochondrial iron-sulfur protein biogenesis. Annu Rev Biochem. https://doi.org/10.1146/annurev-biochem-013118-111540

Liu G, Sil D, Maio N, Tong WH, Bollinger JM Jr, Krebs C, Rouault TA (2020) Heme biosynthesis depends on previously unrecognized acquisition of iron-sulfur cofactors in human amino-levulinic acid dehydratase. Nat Commun 11(1):6310. https://doi.org/10.1038/s41467-020-20145-9

Lu S, Wang J, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Marchler GH, Song JS, Thanki N, Yamashita RA, Yang M, Zhang D, Zheng C, Lanczycki CJ, Marchler-Bauer A (2020) CDD/SPARCLE: the conserved domain database in 2020. Nucleic Acids Res 48(D1):D265–D268. https://doi.org/10.1093/nar/gkz991

Matsuyama A, Arai R, Yashiroda Y, Shirai A, Kamata A, Sekido S, Kobayashi Y, Hashimoto A, Hamamoto M, Hiraoka Y, Horinouchi S, Yoshida M (2006) ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. Nat Biotechnol 24(7):841–847. https://doi.org/10.1038/nbt1222

Mercier A, Labbé S (2010) Iron-dependent remodeling of fungal metabolic pathways associated with ferrichrome biosynthesis. Appl Environ Microbiol 76(12):3806–3817. https://doi.org/10.1128/aem.00659-10

Misslinger M, Hortschansky P, Brakhage AA, Haas H (2021) Fungal iron homeostasis with a focus on *Aspergillus fumigatus*. Biochim Biophys Acta Mol Cell Res 1868(1):118885. https://doi.org/10.1016/j.bbamcr.2020.118885

Mourer T, Jacques JF, Brault A, Bisaillon M, Labbé S (2015) Shu1 is a cell-surface protein involved in iron acquisition from heme in *Schizosaccharomyces pombe*. J Biol Chem 290(16):10176–10190. https://doi.org/10.1074/jbc.m115.642058

Mourer T, Normant V, Labbé S (2017) Heme assimilation in *Schizosaccharomyces pombe* requires cell-surface-anchored protein Shu1 and vacuolar transporter Abc3. J Biol Chem 292(12):4898–4912. https://doi.org/10.1074/jbc.m117.776807

Mourer T, Brault A, Labbé S (2019) Heme acquisition by Shu1 requires Nbr1 and proteins of the ESCRT complex in *Schizosaccharomyces pombe*. Mol Microbiol 112(5):1499–1518. https://doi.org/10.1111/mmi.14374

Normant V, Mourer T, Labbé S (2018) The major facilitator transporter Str3 is required for low-affinity heme acquisition in *Schizosaccharomyces pombe*. J Biol Chem 293(17):6349–6362. https://doi.org/10.1074/jbc.ra118.002132

Pelletier B, Beaudoin J, Philpott CC, Labbé S (2003) Fep1 represses expression of the fission yeast *Schizosaccharomyces pombe* siderophore-iron transport system. Nucleic Acids Res 31(15):4332–4344. https://doi.org/10.1093/nar/gkg647

Rouault TA, Maio N (2017) Biogenesis and functions of mammalian iron-sulfur proteins in the regulation of iron homeostasis and pivotal metabolic pathways. J Biol Chem 292(31):12744–12753. https://doi.org/10.1074/jbc.r117.789537

Schrettl M, Winkelmann G, Haas H (2004) Ferrichrome in *Schizosaccharomyces pombe*—An iron transport and iron storage compound. Biometals 17(6):647–654. https://doi.org/10.1007/s10534-004-1230-z

Sedlák E, Ziegler L, Kosman DJ, Wittung-Stafshede P (2008) In vitro unfolding of yeast multicopper oxidase Fet3p variants reveals unique role of each metal site. Proc Natl Acad Sci U S A 105(49):19258–19263. https://doi.org/10.1073/pnas.0806431105

Shi X, Stoj C, Romeo A, Kosman DJ, Zhu Z (2003) Fre1p Cu2+ reduction and Fet3p Cu1+ oxidation modulate copper toxicity in *Saccharomyces cerevisiae*. J Biol Chem 278(50):50309–50315. https://doi.org/10.1074/jbc.m307019200

Su Y, Yang Y, Huang Y (2017) Loss of *ppr3*, *ppr4*, *ppr6*, or *ppr10* perturbs iron homeostasis and leads to apoptotic cell death in *Schizosaccharomyces pombe*. FEBS J 284(2):324–337. https://doi.org/10.1111/febs.13978

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38(7):3022–3027. https://doi.org/10.1093/molbev/msab120

Wang Y, Yan J, Zhang Q, Ma X, Zhang J, Su M, Wang X, Huang Y (2017) The *Schizosaccharomyces pombe* PPR protein Ppr10 associates with a novel protein Mpa1 and acts as a mitochondrial translational activator. Nucleic Acids Res 45(6):3323–3340. https://doi.org/10.1093/nar/gkx127

Zhang X, Krause KH, Xenarios I, Soldati T, Boeckmann B (2013) Evolution of the ferric reductase domain (FRD) superfamily: modularity, functional diversification, and signature motifs. PLoS One 8(3):e58126. https://doi.org/10.1371/journal.pone.0058126