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Siderophore-iron Uptake in *Saccharomyces Cerevisiae*

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Virtually every organism on earth requires iron as an essential nutrient. Although iron is the second most abundant metal in the crust of the earth, the bioavailability of iron can be extremely low. This poor bioavailability occurs because iron is rapidly oxidized in an aerobic environment to the ferric form (Fe(III)), which is poorly soluble in water and forms precipitates of oxyhydroxides. Microorganisms have the capacity to scavenge iron from insoluble precipitates by secreting and taking up siderophores, low molecular weight compounds that bind to Fe(III) with very high affinity and specificity. Siderophores are synthesized and secreted in the iron-free form, which then binds and solubilizes Fe(III) in the extracellular environment. The Fe(III)-siderophore complex is then recognized and selectively taken up by specific transport mechanisms. Many microorganisms synthesize one or a few types of siderophores, yet have the capacity to take up iron from a variety of siderophores secreted by other species of bacteria and fungi (1). Budding and fission yeast appear to be an exception; they neither synthesize nor secrete these compounds (2,3). Saccharomyces cerevisiae can, however, recognize and take up iron from a variety of structurally distinct siderophores (4-10).

S. cerevisiae has two genetically separable systems for the uptake of siderophore-bound iron. One system depends on a family of homologous transporters of the major facilitator superfamily that is expressed as part of the *AFT1* regulon and are termed *ARN1*, *ARN2* (also *TAF1*), *ARN3* (also *SIT1*), and *ARN4* (also *ENB1*) (6-11). These transporters are expressed in intracellular vesicles. The individual ARN transporters exhibit specificity for different siderophores of the hydroxamate and catecholate classes; however, some siderophores, such as rhodotorulic acid, are not substrates of the *ARN* transporters (9). A second system of uptake for siderophore-bound iron depends on the high affinity ferrous iron (Fe(II)) transport complex, which is encoded by *FET3* and *FTR1* and is located on the plasma membrane (12-15). A low affinity Fe(II) transporter encoded by *FET4* is also expressed on the plasma membrane (16). For the siderophore-bound Fe(III) to become a substrate for the Fe(II) transporter, the iron must be both reduced and dissociated from the siderophore. This is accomplished in a single step by the activity of plasma membrane reductase systems, which contain flavocytochromes and have the capacity to reduce siderophore-bound iron (4, 5, 17). *FRE1* and *FRE2* encode plasma membrane metalloreductases that can reduce oxidized forms of both iron and copper (18-23). Strains deleted for *FRE1* exhibit only 10% of the Fe(III)-citrate reductase activity that is inducible in wild-type strains. Deletion of both *FRE1* and *FRE2* results in cells that are completely lacking Fe(III)-citrate reductase activity and fail to grow on iron-poor media. The completed sequence of the *S. cerevisiae* genome revealed the presence of five additional genes with striking similarity to *FRE1* and especially to *FRE2*. Four of these (*FRE3*, *FRE4*, *FRE5*, and *FRE6*) are greater than 35% identical to *FRE2* and are regulated at the transcriptional level by Aft1p (24). The fifth homologue (*FRE7*) is regulated by exogenous copper ions through the Mac1p transcription factor. The functions of these new *FRE* family members have not been identified.

We have investigated the role of the *FRE* family of genes in the uptake of siderophore-bound iron.

Although the *FRE* genes appeared to have no role in the *ARN*-dependent uptake of siderophores, they were required for the uptake of siderophore-bound iron through the high affinity Fe(II) transport system. Although *FRE1* and *FRE2* encoded the majority of siderophore reductase activity, Fre3p could specifically facilitate reduction and uptake of iron bound to the trihydroxamate siderophores ferrioxamine B (FOB), ferrichrome (FC), and triacetylfusarinine C (TAFC) and to the dihydroxamate rhodotorulic acid (RA). Fre3p was expressed on the plasma membrane in a pattern consistent with its role in iron uptake through the plasma membrane Fe(II) transport system. Uptake of iron bound to the catecholate siderophore enterobactin (ENT) also occurred through the Fe(II) transport system and required either Fre1p or Fre2p. Expression of Fre4p was sufficient to facilitate the utilization of RA-bound iron when the siderophore was present in higher concentration (25).

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