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Molecular strategies of microbial iron assimilation: from high-affinity complexes to cofactor assembly systems

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Microorganisms have to cope with restricted iron bioavailability in most environmental habitats as well as during host colonization. The continuous struggle for iron has brought forth a plethora of acquisition and assimilation strategies that share several functional and mechanistic principles. One common theme is the utilization of high-affinity chelators for extracellular iron mobilization, generally known as siderophore-dependent iron acquisition. This basic strategy is related with another central aspect of microbial iron acquisition, which is the release of the mobilized iron from extracellular sources to allow its transfer and incorporation into metabolically active proteins. A variety of mechanisms which are often coupled with high-affinity uptake have evolved to facilitate the removal of iron from siderophore ligands; however, they differ in many key aspects including substrate specificities and release efficiencies. The most sophisticated iron release pathways comprise processes of specific hydrolysis and reduction of ferric siderophores, especially in the case of high-affinity iron complexes with greatly negative redox potentials that often represent crucial factors for virulence development in bacterial and fungal pathogens. During the following steps of iron utilization, the acquired metal is transferred through intracellular trafficking pathways which may include diverse storage compartments in order to be directed to cofactor assembly systems and to final protein targeting. Several of these iron channeling routes have been described recently and provide first insights into the later steps of iron assimilation that characterize an essential part of the cellular iron homeostasis network.

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1 Introduction

Iron is a mineral nutrient with an outstanding physiological relevance for most forms of life. It is the fourth most abundant element in the earth's crust and exists in a wide range of oxidation states, though +2 and +3 are the most common and form a redox couple that has a standard reduction potential of +0.77 V in water, which is close to that of the O_2/H_2O redox pair. In this context, iron was most important during the early stages of evolution to drive dissimilatory processes, and it still fulfills this function during microbial ferric iron reduction, representing a key factor of many biogeochemical circuits.[1] Beyond its role as an alternative terminal electron acceptor, iron has been established as a versatile redox cofactor in a vast number of primary and secondary metabolic pathways. To drive these iron-related processes efficiently, several mechanisms for extracellular iron mobilization, uptake and intracellular assimilation had to be developed.[2] This is in particular the case as the non-limited uptake of soluble ferrous iron is only feasible in a rather small number of anoxic and strongly acidic habitats. In contrast, the vast majority of microorganisms live under conditions that provide soluble iron concentrations below 10^{-9} M, which is generally the case in aerobic and non-acidic environments as well as in association with many eukaryotic host organisms.[3,4] Under such conditions, the utilization of all potential organic and inorganic iron sources is a key determinant of microbial fitness. Iron mobilization includes the secretion of iron cofactor scavenging proteins like hemophores,[5] or small molecule chelators called siderophores that are able to sequester ferric iron with enormous affinities from numerous primary sources.[6,7] Siderophores are secreted by a vast number of microbes in broad structural variations including scaffold backbones and ligand donor groups and display iron formation constants (K_f) in a range of $\sim 10^{20}$ to $\sim 10^{50}$ M^{-1}.[8,9] They are hence compellingly designed to compete very effectively

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for low-soluble iron pools within a wide range of environmental conditions as well as in tightly iron-regulated host organisms.

Ferric iron that is captured by a high-affinity siderophore scaffold is shielded from external ligands and is exchanged very slowly with the environment at physiological pH.[10] The extracellularly formed iron–siderophore complexes are delivered to the microbial cell where the iron has to be released to become metabolically available. The release of iron from its external sources is a key process that allows its passage into the so-called labile cellular iron pool,[11] from where intracellularly or extracellularly directed trafficking can take place. The main cellular trafficking routes of iron include its long-term storage in proteins or vacuolar compartments, assembly of redox cofactors like iron–sulfur (Fe/S) clusters or heme variants, and its utilization for various informational pathways. However, the limiting external or internal iron release rates from siderophores with moderate ($K_f \sim 10^{30}$ M^{-1}) to extremely high ($K_f \sim 10^{50}$ M^{-1}) affinities mainly define the flow of iron through these assimilatory pathways.

The release from siderophores can occur either at the cell surface in association with free iron uptake, or after cellular uptake of the ferric siderophore complex by ligand-specific transport systems. In each case, the following basic strategies are utilized to facilitate the removal of iron from its extracellular chelator: (i) the competition of iron coordination with ligand protonation (pH-dependent release), (ii) the hydrolysis of the siderophore backbone (hydrolytic release), and (iii) the reduction of the ferric ion center (reductive release). Both ligand protonation and hydrolytic release are only feasible in a minority of cases due to limitations in changing the intra- and extracellular pH milieus and because of structural requirements such as the introduction of rather unstable ester bonds into the siderophore scaffold. Thus, the majority of iron release processes are reductive, which bears the further advantage of re-utilization of the siderophore scaffold in contrast to a single utilization cycle during a destructive release. However, since the redox potentials of ferric siderophores correlate reciprocally to their formation constants in general (Fig. 1), a combination of release mechanisms is often of vital importance to allow an efficient reduction of high-affinity ferric siderophores. For these cases, highly adapted kinetic mechanisms are required that allow a short-distance electron transfer to the scaffolded ferric iron on the one hand, and they may be coupled on the other hand with an increase of the iron redox potential within the protein–siderophore complex or by additional means of iron complex destabilization. A number of siderophores which are associated with such advanced release mechanisms are produced by important human, animal and plant pathogens as key factors for their virulence development. Hence, a deeper understanding of the molecular mechanisms of iron release and assimilation mechanisms is not only essential for the general characterization of iron acquisition pathways, but also for the development of novel potent therapeutics for iron-dependent pathogen control.

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2 Extracellular reduction of mobilized iron

The assimilatory reduction of ferric iron complexes in the extracellular environment is common to the majority of microorganisms and is often coupled with high-affinity uptake of the released ferrous iron species. In general, microbes have established two main strategies for extracellular ferric siderophore reduction: (i) reduction mediated by cell surface associated metalloreductases in fungi, and (ii) reduction mediated by secreted redox compounds mainly in bacteria, but also in fungi.

Iron reduction at the fungal plasma membrane has been best studied in *S. cerevisiae* that possesses a series of membrane-integrated ferric reductases (Fre) of the flavocytochrome superfamily. The four reductases Fre1p–Fre4p catalyze ferric siderophore reduction with broadly different substrate specificities and catalytic efficiencies, while the function of two additional homologs, Fre5p and Fre6p, has not been elucidated yet.[12] The main reductase activities are provided by Fre1p and Fre2p that reduce Fe(III) bound to scaffolding ligands like citrate, desferrioxamine B, desferrichrome, desferri-triacetyl fusarinine C (TAFC) and rhodotorulic acid. The Fre3p reductase activity for these substrates was found to be ~40-fold lower, and the Fre4p reductase only facilitated the utilization of iron bound to rhodotorulic acid.[12–13] Furthermore, Fre1p and Fre2p were shown to reduce ferric enterobactin, which is remarkable due to the extremely low redox potential of −0.75 V that is associated with one of the highest biological K_f of 10^{49} M^{-1} of the free complex at pH 7.0.[14,15] However, the reduction is supposed to be coupled with an extracellular acidification that is mediated by the Fre reductases at the same time.[16] This in turn leads to a partial destabilization of the ferric triscatecholate complex due to an increased ligand protonation as well as an induced intrinsic switch from the catecholate to the salicylate

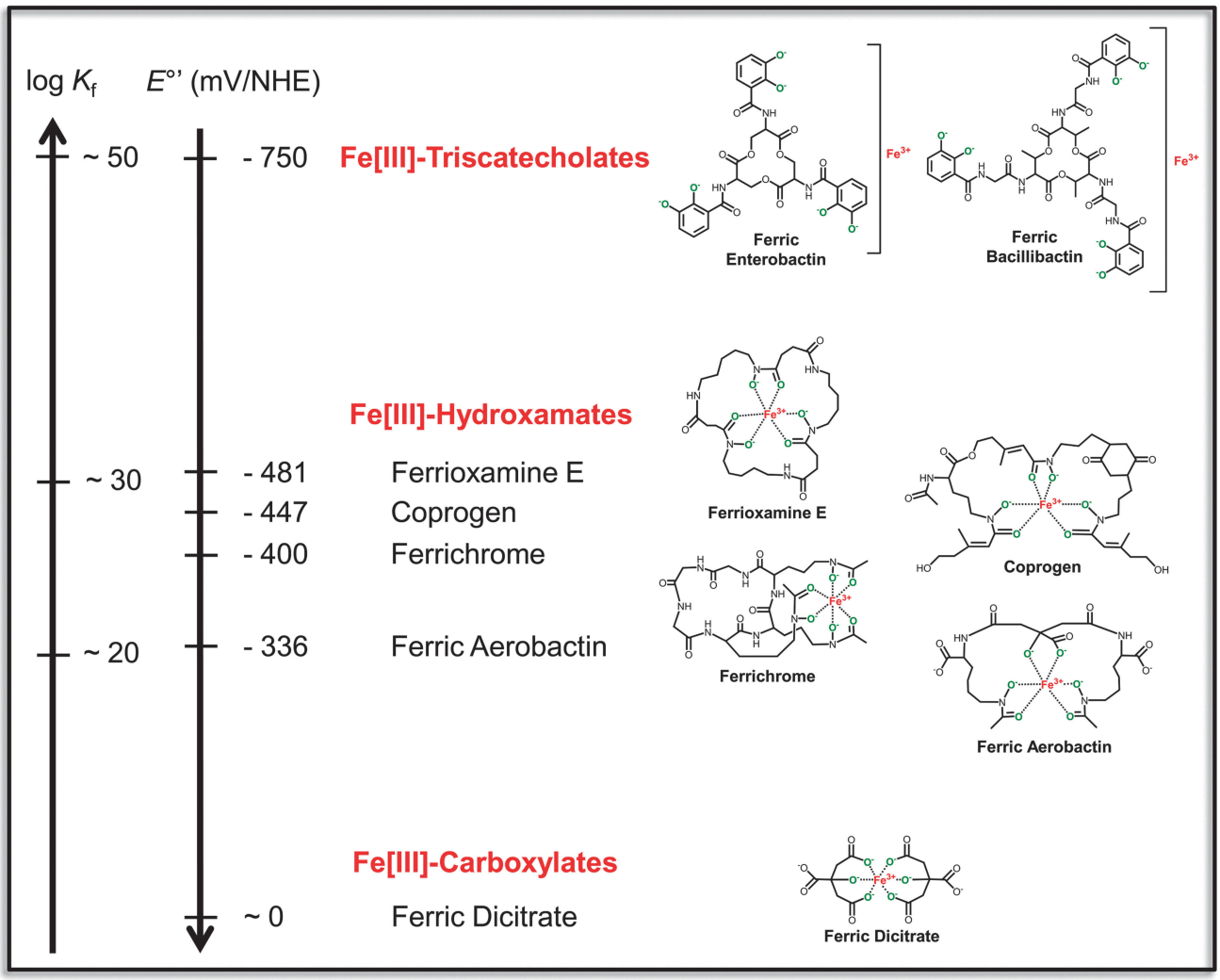

Fig. 1 Reciprocally correlated scales of formation constants (K_f) and standard redox potentials at pH 7.0 ($E°'$) for representative ferric siderophores of different structural classes (indicated in red). The ferric complexes are shown in their hexa-liganded coordination modes with fully deprotonated donor atoms in green. The triscatecholate scaffolds contain representative trilactone tri-L-serine and tri-L-threonine backbones which are accessible to enzymatic hydrolysis. Redox potentials of the ferric complexes are given *versus* the normal hydrogen electrode (NHE). Affinity constants and redox potentials are taken from the indicated references.[13,14,167–171]

coordination mode in the lower pH regime.[17,18] Thus, the combination of reduction with cell surface acidification likely enhances the electron transfer efficiency to the iron complex. On the other hand, altered iron binding modes including pH-dependent coordination shifts can be of general physiological relevance with respect to complex recognition during further transport and release processes. The fungal cell surface reduction of ferric siderophores is tightly coupled with a high-affinity uptake of ferrous iron by the Fet3p–Ftr1p system. The high-affinity uptake efficiently removes the liberated Fe(II) species from the extracellular equilibrium and hence helps to increase the actual redox potentials of the reductively converted ferric complexes. For the purpose of uptake, the yeast transporter employs the oxygen-dependent multicopper ferroxidase Fet3p, which converts Fe(II) to Fe(III) prior to membrane translocation by the iron permease Ftr1p.[19] Functionally related iron uptake systems in bacteria are EfeUOB and FetMP, which either oxidize or reduce free iron prior to its translocation; however, it is not known if they are also closely associated with extracellular ferric siderophore reduction.[20–22]

Bacteria, in contrast, have developed primarily indirect enzymatic strategies for extracellular ferric siderophore reduction by utilizing mobile electron carriers as redox compounds (Fig. 2). Especially the roles of reduced phenazines and flavins have been addressed in this context.[23,24] In soil-dwelling bacteria like *Pseudomonas chlororaphis* or *Shewanella oneidensis*, phenazines were shown to mediate electron shuttling to alternative terminal acceptors such as mineralized ferric iron or organic ferric iron chelates, enabling iron mobilization in the reduced form.[25,26] Thus, dissimilatory ferric iron reduction can be directly coupled with reductive iron assimilation by increasing the local bioavailability of ferrous iron. Further studies showed that this mechanism is also of importance for opportunistic pathogens such as *P. aeruginosa*, which produces phenazine derivatives such as phenazine-1-carboxylic acid (PCA) and 5-*N*-methyl-1-hydroxyphenazine (pyocyanin, PYO).

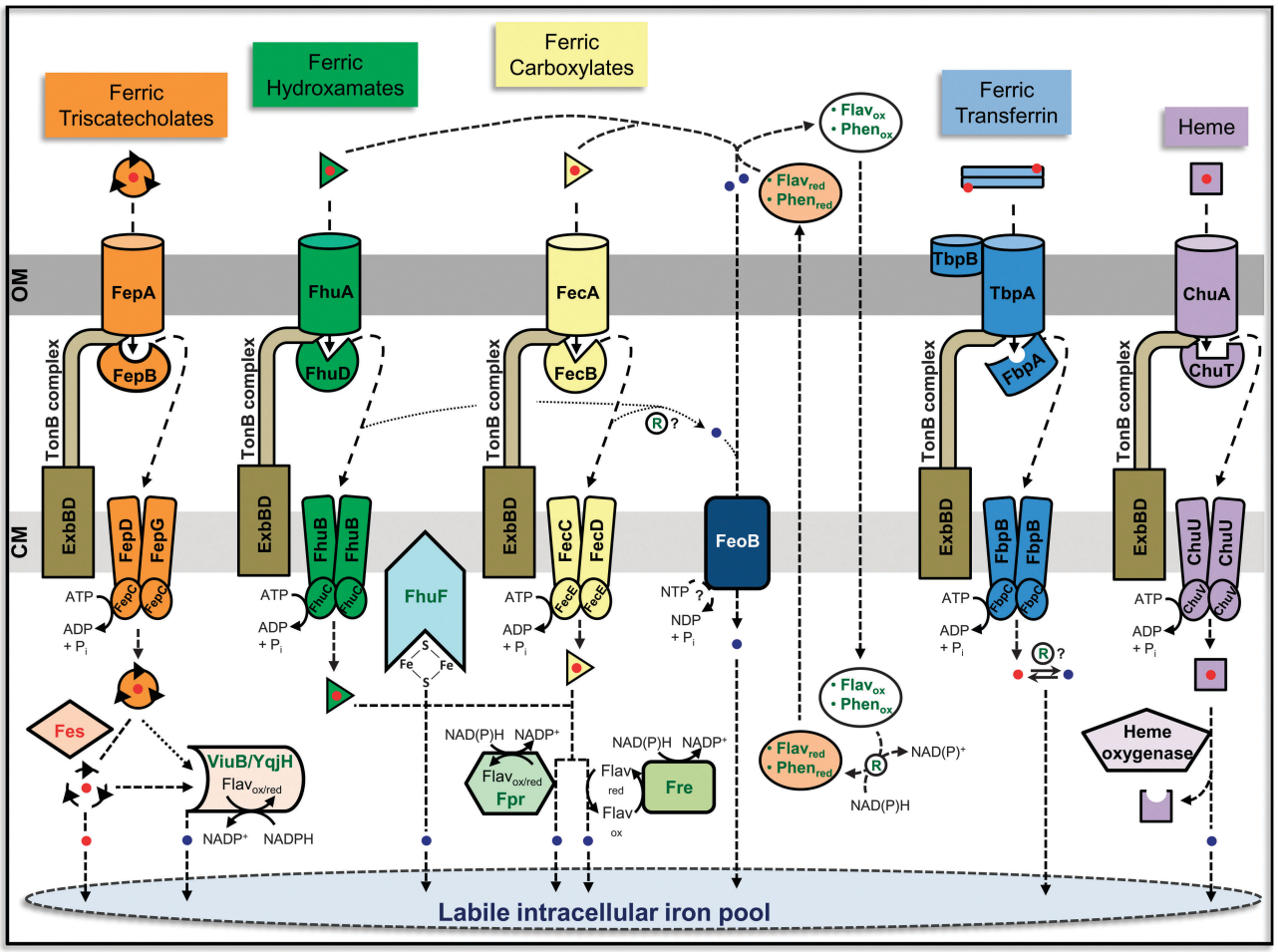

Fig. 2 Summary of basic iron transport and release pathways in Gram-negative bacteria. Fundamental transport systems for the main ferric siderophore classes as well as for transferrin- and heme-delivered iron are shown, with emphasis on the *E. coli* reference model regarding protein nomenclature. The iron transport routes consist of TonB/ExbBD-energized outer membrane receptors that deliver either the intact iron complexes or, as in the case of the transferrin-interacting system, extracellularly abstracted ferric iron into the periplasm, where specific binding proteins are in close association with the receptor complexes for rapid iron–ligand scavenging. The iron sources are delivered by the binding proteins to their specific cytosolic uptake systems, which mainly belong to the class of ABC-type transporters comprising integral membrane permease units that are associated with intracellular nucleotide binding domains that catalyze ATP hydrolysis. Alternatively, some complexes may undergo a periplasmic processing, especially in the case of ferric siderophores with lower iron-binding affinities such as hydroxamates and carboxylates, which can be substrates of periplasmic reductases (indicated by ''®?''). Ferrous iron that has been liberated in the periplasm as well as during extracellular reduction by mobile electron carriers such as flavins or phenazines can be taken up by high-affinity cytosolic transporters like FeoB that putatively employs GTPase activity as an energy source. On the other hand, ferric siderophore complexes which have been imported into the cytosol are subjected to diverse iron release pathways according to their intrinsic redox potentials. Ferric triscatecholates can either be directly reduced by triscatecholate-specific flavoenzymes such as YqjH or ViuB, belonging to the SIP (siderophore-interacting protein) family of ferric reductases, or can be hydrolyzed prior to reduction by specific esterases such as Fes if they contain cleavable trilactone backbones such as ferric enterobactin. Several ferric hydroxamates can be reduced according to their redox potentials by the loosely membrane-associated FhuF reductase that binds a [2Fe–2S] redox cofactor. Rather unspecific reductases that accept a broader spectrum of substrates including high-potential ferric chelates are represented by flavoenzymes such as Fpr. Exogenous flavin reductases like Fre primarily mediate the reduction of free diffusible flavins, which in turn can react inside or outside the cell with a high number of different substrates including ferric complexes with feasible redox potentials. In contrast, intact heme is cytosolically degraded by heme oxygenases, leading to biliverdin formation and ferrous iron release. The released iron species from all different sources may be further redox converted by yet unknown processes, and are supposed to interact with the labile intracellular iron pool comprising low affinity ligand interactions before they continue to pass through directed cellular trafficking routes for further assimilation.

PYO was shown to acquire iron from the human iron chelator transferrin, especially under low oxygen conditions.[27] PCA was found to be important for virulence-associated biofilm formation during later stages of infection by neutralizing the activity of Fe(III)-binding host proteins such as conalbumin.[28] Remarkably, the redox activity of PCA was found to be tightly coupled with FeoB-mediated high-affinity uptake of Fe(II). A recent study also showed metabolic transformations of *P. aeruginosa* phenazines by *A. fumigatus*, resulting in derivatives with alternative properties such as enhanced toxicities and the capability to induce fungal siderophore production.[29]

Flavins represent another important group of electron-shuttling compounds in microbes and also higher plants and can also fulfill a functional double role in dissimilatory and assimilatory iron reduction. They form unusually stable complexes with iron in remarkable contrast to further

transition metals,[30,31] which could support the possibility of a metal-selective inner-sphere electron transfer by these redox-active ligands. Flavin-dependent iron acquisition was shown in *Helicobacter pylori* that secretes riboflavin to reduce Fe(III) stored in ferritins.[32] Similar processes of extracellular iron reduction and the possibility of enhanced iron mobilization by flavins were observed in *Shewanella*,[23] *Pichia*,[33] as well as sugar beet and sunflower roots.[34] Notably, a great number of so-called ''extracellular ferric reductases'' in bacteria, which are either secreted into the extracellular milieu or are membrane-bound, belong to the class of NAD(P)H:flavin oxidoreductases (or commonly ''flavin reductases'') that can generate either $FADH_2$ or $FMNH_2$ as agents for single electron transfer.[13,35] The reduced flavins dissociate in most of the reported cases from the enzymes and hence fulfill an indirect role in unspecific ferric iron reduction. With standard midpoint potentials around −0.2 V, the free flavins can reduce most iron oxides, soluble forms of Fe(III) as well as organic ferric iron chelates including ferric siderophores whose redox potentials are within this range (see Fig. 1).

Another remarkable and wide-spread mechanism of extracellular ferric siderophore reduction is associated with photoreactive siderophores that contain α-hydroxy carboxylates, which are produced by a number of marine bacteria.[36,37] These chemical groups permit a sunlight-driven photoreduction of the ferric iron center through the ligand-to-metal charge transfer in ferric siderophores.

3 Periplasmic iron trafficking

Dependent on the nature of the utilized iron source, several specific pathways in Gram-negative bacteria allow trafficking or processing of iron in the periplasm prior to its transport into the cytosol (Fig. 2). Iron acquisition from non-heme iron sources like lactoferrin or transferrin by several Gram-negative pathogens such as *Neisseria* or *Haemophilus* is essentially dependent on the periplasmic ferric ion binding protein FbpA, a so-called ''bacterial transferrin'' due to remarkable structural and functional similarities to mammalian transferrin.[38,39] The TonB-dependent surface transferrin receptor complex TbpAB binds transferrin, removes the iron and transports it across the outer membrane to the periplasmic side, where it is bound by the FbpA protein.[40–42] The TbpA receptor preferentially interacts with apo-FbpA at the inner side of the outer membrane and releases holo-FbpA,[43] which shuttles the iron to the inner membrane transporter FbpBC for cytosolic uptake.[44] The iron binding affinity of FbpA is comparable to that of transferrin with a log K_f of about 20 at physiological pH.[45] The FbpABC transport system is also involved in TonB-independent iron acquisition from xenosiderophores in *Neisseria*.[46] The initial anchoring of ferric iron in the FbpA binding site depends on a twin-tyrosine motif, whose double mutation is deleterious to the iron sequestration activity.[47] Binding of a ''synergistic'' phosphate anion that completes the iron co-ordination shell of FbpA was further shown to play a crucial role in iron sequestration and release *in vitro*;[48,49] however, mutants of the anion binding site were still capable of mediating iron acquisition *in vivo*.[50]

Iron-charged siderophores which are transported across the outer membrane through TonB-energized receptors are usually readily captured by their cognate periplasmic binding proteins with dissociation constants (K_D's) in the micro- to nanomolar range. Binding proteins like FhuD or BtuF were shown to interact with the TonB part of the outer membrane receptor/transporter complex, their binding sites supposedly facing the periplasmic side of the receptor lumen.[51,52] These observations led to the hypothesis of an unidirectional ligand transport through the periplasm to the respective inner membrane ATP-binding-cassette (ABC) transporter that interacts *via* salt bridges with the holo-form of the binding protein for ligand transfer.[53,54] Such a quasi-chaperoned trafficking of most ferric siderophores through the periplasm may reflect that rather few processing mechanisms of siderophores seem to have developed in this compartment. One example of such a periplasmic processing activity is the trilactone esterase IroE, which hydrolyzes triscatecholate siderophores like salmochelin and enterobactin.[55,56] Similar to cytosolic trilactone hydrolases, IroE belongs to the α/β-hydrolase superfamily, but it lacks an N-terminal lid region and possesses an atypical catalytic diad comprising only the reactive serine and a conserved histidine.[57] In contrast to its cytosolic counterparts, its activity has been mainly related with the single hydrolysis (linearization) of apo-siderophores prior to their secretion, rather than with the complete hydrolysis of ferric siderophores.[58–60] However, some groups of proteobacteria like the epsilon subdivision possess only IroE-like hydrolases, whose precise role in the corresponding iron acquisition pathway(s) has not been addressed yet. In addition to hydrolysis, only a marginal number of reports exist about periplasmic ferric siderophore reduction. Such activities have been detected for substrates like ferric citrate in *Legionella pneumophila* and *Vibrio vulnificus*,[61,62] and for ferripyochelin in *Pseudomonas aeruginosa*.[63] The involved enzymes prefer NAD(P)H or glutathione as reductants.

Further, no evidence about iron release from heme iron sources in the periplasm exists. In contrast to a report about putative activities of periplasmic and cytoplasmic heme dechelation,[64] it was shown that the addressed enzymes EfeB and YfeX are rather heme-dependent peroxidases involved in iron transport and protoporphyrinogen oxidation, respectively.[65] Heme from extracellular sources is usually not processed in the periplasm, but transported into the cytosol *via* specific systems that are functionally similar to those of the ferric siderophore transport routes described above.[66] If heme is abstracted from host hemoproteins such as hemopexin or methemoglobin, specific uptake pathways are required involving hemophore-mediated transport in many Gram-negative pathogens,[5] or cell surface-anchored components such as in the Isd systems of Gram-positive pathogens, that contain high-affinity NEAT domains for peripheral protein binding, heme capturing and directed transfer to the cytosolic importer.[67–69]

4 Cytosolic iron release

Iron-charged siderophores which have passed the cytoplasmic membrane undergo enzymatic conversions that are essential for iron removal, especially from high-affinity complexes. These reactions are essential in order to allow a subsequent intracellular iron trafficking. The internalized complexes are in part subjected to scaffold backbone hydrolysis or are reduced by different types of oxidoreductases, either directly or after the hydrolytic processing has taken place (Fig. 2).

4.1 Ferric siderophore hydrolases

Enzymes that cleave the trilactone rings of ferric siderophores generally belong to the superfamily of α/β-hydrolases. They generate ferric ligand species of higher molecular stoichiometry, which decreases entropy and hence stability of these iron complexes. The release of iron may then occur by a direct competition with ligands of ferric iron binding sites, and can be further enhanced by subsequent complex reductions as discussed below.

E. coli and its close relatives possess the best studied cytosolic siderophore esterases, Fes and IroD, which preferentially hydrolyze the cyclotrimeric scaffolds of ferric enterobactin and its glucosylated derivatives called salmochelins, respectively.[55,56] IroD is specifically associated with the *iroA* gene cluster that contributes to virulence development.[70] A further triscatecholate–trilactone hydrolase is the ferric bacillibactin esterase BesA that is conserved in *Bacillus* spp. and in paenibactin-producing *Paenibacillus*.[71] BesA was found to hydrolyze both tri-L-threonine and tri-L-serine backbones which are present in bacillibactin and enterobactin, respectively, while the Fes esterase cleaves only the tri-L-serine backbone.[72,73] The trilactone hydrolases usually possess a conserved GxSxG motif that is characteristic for serine esterases and part of a catalytic triad consisting of serine-histidine-glutamate/aspartate. The crystal structures of *Shigella flexneri* and *Salmonella typhimurium* Fes (PDB entries 3C87 and 3MGA), and *Bacillus cereus* BesA (PDB entry 2QM0) further show prominent N-terminal lid domains. At the interfaces of the lid domains and the catalytic domains that bear the reactive serine, several conserved aromatic residues seem to form binding sites that are arranged as a mirror image to the circular plane of the ligand aryl subunits and may hence contribute to hydrophobic interactions. However, the exact molecular mechanisms for substrate recognition as well as for the substrate-specific formations of partial or complete hydrolysis products remain unclear so far. Due to protein sequence analyses, IroD was proposed to be structurally very similar to Fes including the presence of an N-terminal lid domain as well as a catalytic triad.[57]

Further biosynthesis gene clusters for putative triscatecholate–trilactone siderophores such as griseobactin from *Streptomyces*[74] or trichrysobactin from *Dickeya*[75] have been identified recently, which are in close association with genes encoding α/β-hydrolases like GriB or CbsH. These hydrolases are closely related to Fes, but they have not been shown yet to cleave trilactones and may possess further hydrolytic activities as in the case of CbsH.[74,76] Trivanchrobactin from *Vibrio* has been isolated as a linear trimer, which could potentially originate from a cyclic trilactone scaffold.[77] Also in this case, Fes-homologs such as VabH have been identified and found to be involved in vanchrobactin-mediated iron assimilation.[78]

Trilactone siderophores with non-catecholic ligands are represented by several trishydroxamates such as fusarinine C (fusigen), TAFC, or neurosporin which are produced and utilized as iron sources by many fungi.[79] The IroE-like trilactone esterase EstB was recently identified and found to hydrolyze ferric fusarinine complexes in *Aspergillus*.[80] After EstB-cleavage of fusigen or TAFC, a part of the released iron is usually transferred into the cell vacuole or sequestered by intracellular siderophore compounds for further storage.[81–82] Similar hydrolytic release mechanisms can be expected for ferric neurosporin and its bacterial counterpart ferric vicibactin that is produced by *Rhizobia*.[83]

4.2 Ferric siderophore reductases

In contrast to siderophore hydrolases, ferric siderophore reductases generate low-affinity ferrous iron complexes without changing the complex stoichiometry. The release of ferrous iron is facilitated by an increased kinetic exchange with the aqueous environment and with iron binding sites of equal or higher affinities. While siderophore hydrolases are rather substrate specific, there are broad variations of substrate specificities among ferric siderophore reductases. Generally, a higher specificity can be associated with an increasing efficiency of iron reduction in low-potential (high-affinity) ferric siderophore complexes.

4.2.1 Unspecific ferric complex reduction. In this case, the reduction is generally independent of the structural nature of the ferric siderophore and may only be limited by its redox potential and possible steric hindrances during ligand-mediated electron transfer. Comparable to unspecific extracellular ferric siderophore reduction, intracellular flavin reductases are usually involved in electron transfer from NAD(P)H toward FMN, FAD or riboflavin (Fig. 2). The reduced flavins are released as mobile electron carriers as observed for the *E. coli* NAD(P)H:flavin oxidoreductase Fre and its homologs in *Vibrio*,[84] the sulfite reductase SiR,[85] the *P. aeruginosa* ferripyoverdine reductase,[86,87] or the flavin reductases FerA and FeR in *Paracoccus denitrificans* and *Magnetospirillum gryphiswaldense*, respectively.[88,89] The reduced flavins can transfer electrons in their hydroquinone and semiquinone forms, and their different redox potentials may lead to the reduction of diverse ferric siderophore species. The kinetic mechanism of *E. coli* Fre was found to be of the ordered sequential type, in which NADPH binding is followed by flavin binding to yield ternary complex formation.[90] The reduced flavin leaves the complex first, then $NADP^+$ dissociates to allow another reaction cycle. The reductive mechanism at the iron substrate complex might in turn include an inner-sphere electron transfer, since the flavin isoalloxazine ring is capable of strong iron complex formation and eventually occupies iron coordination positions by replacing siderophore donor atoms

or external ligands such as water molecules that often take part in complex formation.[31,91] An outer-sphere transfer could in turn be preferred in the presence of further cellular reductants like glutathione or ascorbate, which can reduce ternary ferric siderophore complexes if they carry an inner-sphere redox-active ligand.[91]

Other types of ferric reductases are independent of exogenous flavin substrates, as they transfer electrons *via* intrinsically bound flavin cofactors such as *E. coli* flavohemoglobin Hmp,[92] nitroreductase NfnB and ferredoxin–$NADP^+$ reductase Fpr,[93] *Paracoccus* FerB,[88] or the archaeal ferric reductase FeR from *Archaeoglobus fulgidus*.[94] Generally, these flavoenzymes can act on a broad set of substrates and are often not specifically associated with assimilatory iron reduction. The reaction mechanism for these ferric reductases is assumed to be rather of the nonsequential ("Ping-Pong") type,[13] which has also been suggested for the iron cofactor-directed flow of electrons in ferredoxin–$NADP^+$ reductases.[95]

4.2.2 Ferric siderophore reductases with redox-controlled specificities for ferric hydroxamates and carboxylates. There are a few ferric reductases that are iron-dependently regulated and serve as direct reductants for a set of structurally related ferric siderophore substrates including hydroxamates and/or carboxylates. One prominent example that belongs to this category is the *E. coli* ferric hydroxamate reductase FhuF (Fig. 2). This enzyme binds a [2Fe–2S] cluster *via* an unusual C-terminal C–C–x_{10}–C–x–x–C motif for electron transfer.[96] The midpoint redox potential of the cluster is ~ -0.31 V, which is within or near the effective range for electron transfer onto complexes like ferrichrome, coprogen or ferrioxamine B which belong to the set of substrates reduced by FhuF.[97] Ferric enterobactin as a low-potential triscatecholate siderophore does not belong to the substrate spectrum of FhuF.

A second example is the homologous FchR reductase that was found in the Gram-positive extremophile *Bacillus halodurans* where it is associated with a ferric schizokinen uptake system, but accepts several further ferric hydroxamates as well as ferric dicitrate as its substrates.[98] The midpoint potential of the FchR cluster is ~ -0.35 V, and its close relationship with FhuF suggests a similar mechanistic mode of action. Electron transfer onto FchR *in vitro* is possible *via* a regenerative ferredoxin transfer system. Determination of K_m and K_D values for several ferric complexes revealed the highest substrate binding affinities for low-potential ferric hydroxamates, while ferric dicitrate was bound with the lowest affinity to the enzyme. The low-potential substrates showed saturated reaction kinetics already at very low concentrations, likely due to rate limitations of electron transfer. Thus, the effective range of electron transfer through the intrinsic Fe/S cofactor basically determines the substrate-dependent turnover rate, hence leading to redox-controlled substrate spectrum. However, the tight binding of several low-potential substrates suggested a mechanism which allows a significant increase of the electron transfer efficiency even onto substrates whose free redox potentials are well below the effective transfer range of the enzyme cofactor. Thus, the actual potentials of both the cofactor and the ferric substrates in the enzyme–substrate complex are of critical importance in these cases.

Regarding the reductive mechanism, an inner-sphere electron transfer from the FchR cofactor to the ferric iron center of the substrate ligand was found to be unlikely since no symmetry changes of the reduced cofactor occurred in the presence of preferred substrates. The general question if a direct inner-sphere electron transfer between cofactor and metal or an outer-sphere transfer *via* redox-active ligand(s) is favored during enzymatic ferric siderophore reductions has to be addressed in more detail. An outer-sphere transfer might generally be more likely due to the slow ligand exchange rates at the metal center,[10] which would cause strong catalytic rate limitations in addition to those limitations that are caused by very low substrate redox potentials. An enzymatic outer-sphere electron transfer may possibly exploit the siderophore-specific ligand-to-metal charge-transfer that is mediated *via* the inner shell of ligand donor atoms.

A further question that was addressed while studying the FchR kinetic was the influence of product scavengers such as ferrous iron binding apo-proteins. According to the Nernst equation, the actual standard redox potentials of the ferric siderophore substrates would increase in the presence of efficient Fe(II) scavengers which remove the reduced metal ion from the equilibrium by sequestration.[99] An increased catalytic efficiency of FchR was indeed observed in the presence of a transition metal binding Fe/S scaffold protein.[98] Thus, redox potential shifts due to the presence of efficient iron sinks can be seen as a further indirect mechanism to overcome rate limitations and hence to increase the redox capacities of ferric siderophore reductases.

With respect to the development of counteracting strategies against microbial ferric iron assimilation, siderophores charged with redox-inert metals such as Ga(III)-desferrioxamine were found to be specific inhibitors of FchR and reduced its catalytic activity significantly with inhibition constants in the lower micromolar range.[98] The inhibition effect was also observed in bacterial culture and was dependent on the presence of the reductase.

4.2.3 Ferric siderophore reductases with specificity for ferric triscatecholate complexes. It has long been assumed that reductases are also associated with an intracellular release of iron from high-affinity triscatecholate scaffolds.[100,101] However, the investigation of the involved enzymes and their mechanisms started only recently. The bacterial enzymes that are commonly associated with ferric triscatecholate reduction are called Siderophore Interacting Proteins (SIPs) and belong to the NAD(P)H:flavin oxidoreductase superfamily. SIPs are conserved in various Gram-negative and Gram-positive phyla, especially in Proteobacteria, and can be further divided into two structural subfamilies, depending on the presence (group I) or absence (group II) of a prominent C-terminal α-helix domain.[102] The first described SIP was the group I-type ViuB, which is required for ferric vibriobactin utilization in *Vibrio cholerae*.[103] Interestingly, ViuB complements the function of Fes in *E. coli*, but Fes does not complement for a ViuB deficiency in *V. cholerae*, revealing that the capacity of ViuB to release iron by reduction

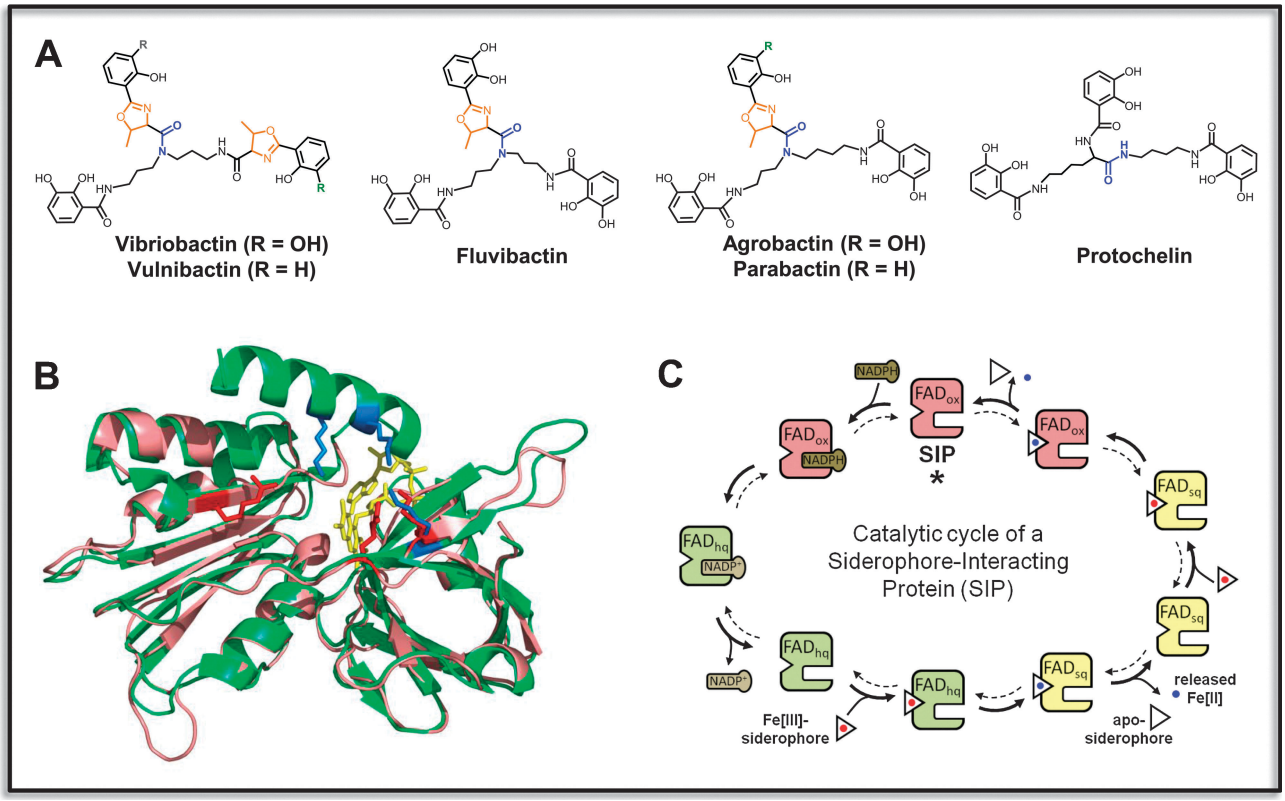

Fig. 3 Specific reductases for ferric triscatecholate siderophores. (A) Examples of aryl-capped siderophores with non-hydrolyzable backbones that contain stable amide-linkages (indicated in blue), and frequent oxazoline units adjacent to the aryl groups (shown in orange). Vibriobactin, vulnibactin and fluvibactin are produced by human-pathogenic *Vibrio* species *V. cholerae*, *V. vulnificus* and *V. fluvialis*, respectively, agrobactin is produced by the plant pathogen *Agrobacterium tumefaciens*, parabactin by the ecologically important soil denitrifier *Paracoccus denitrificans*, and protochelin by nitrogen-fixing *Azotobacter vinelandii*. (B) Superposition of a structural model of ferric triscatecholate reductase YqjH from *E. coli* (salmon) and the ViuB structure (PDB entry 2GPJ) from *S. putrefaciens* (green). Conserved basic residues forming part of the ligand binding sites are depicted in red for YqjH and in blue for ViuB. The FAD redox cofactor is shown in yellow. (C) Kinetic mechanism of the YqjH reductase. The enzyme undergoes a double-displacement-type (''Ping-Pong'') reaction, during which the oxidized FAD cofactor (FAD_{ox}) is at first reduced by NADPH, leading to an enzyme-bound flavohydroquinone (FAD_{hq}). The electron-charged enzymes then reduce successively two ferric siderophore species, resulting in the formation of ferrous iron products and the enzyme-bound flavosemiquinone (FAD_{sq}) and oxidized FAD species of the resting state, which concludes the cycle. Illustrations are partly adopted from the indicated reference.[102]

is superior if the siderophore does not contain intrinsic ester linkages, which is the case for many pathogen-related aryl-capped siderophores (Fig. 3A). Hence, ViuB and its homologs seem to provide a key activity in the iron assimilation routes in many microbes including various pathogens that utilize high-affinity catecholate siderophores, and therefore this currently investigated reductase family may represent an important missing link in numerous iron homeostasis networks. The crystallographic investigations of a ViuB homolog from *Shewanella putrefaciens* as well as the group II-type YqjH from *E. coli* revealed that a tightly bound FAD cofactor is located directly beneath the substrate binding site (Fig. 3B).[102,104] In both subfamilies, generally three conserved basic residues are scattered around this bilobate binding cleft that resembles the high-affinity binding sites of extracytosolic ferric triscatecholate binding proteins.[105] Indeed, the combination of high-affinity substrate binding with K_D's in the low micromolar to nanomolar range and the possibility of very short-distance electron transfer in the substrate binding site of SIPs allows the reduction of complexes such as ferric enterobactin whose free redox potentials are much below those of common intracellular reductants.[102,106] In the case of YqjH, this seems to be achieved by an increase in the ferric siderophore redox potential in the high-affinity binding site, possibly due to competitive effects of iron coordination and iron-complex association at the oxo-ligand center. Coincidentally, different types of ferric substrates that are also reduced by YqjH show much lower binding affinities than ferric triscatecholate complexes, either in their hydrolyzed or non-hydrolyzed form. The kinetic mechanism of YqjH was found to be a double-displacement (''Ping-Pong'') type, during which preferably NADPH delivers its electrons to the enzyme-bound flavin to generate the hydroquinone which in turn can reduce consecutively two ferric siderophore substrates (Fig. 3C). The development of a relatively stable flavosemiquinone species during this process indicated that this catalytic intermediate is indeed capable of preserving the unpaired electron for a second reduction of another substrate complex. Such a kinetic mechanism of ferric siderophore reduction has also been found in cytosolic extracts of *Azotobacter vinelandii*, which produces catecholate siderophores

like protochelin or azotochelin.[107] There, the partially hydrolyzed catecholate-type ferric siderophores as well as ferric citrate were reduced with much higher specific activities than the non-hydrolyzed intact catecholate iron complexes. Similarly, *E. coli* YqjH was found to reduce the hydrolyzed complex of ferric enterobactin (ferric [2,3-dihydroxybenzoylserine]$_3$; $E^{\circ\prime} \sim -0.35$ V)[108] with more than ten-fold higher rates than the uncleaved complex.[102] Thus, the strong redox potential increase (about +0.4 V) of the hydrolyzed compared to the intact ferric complex was of clear advantage for the reaction. Hence, it can be speculated if complex hydrolysis might be a key factor of catalytic efficiency during reduction of high-affinity species such as ferric triscatecholates. Indeed, the *in vitro* and *in vivo* observations of YqjH-mediated ferric siderophore reduction in *E. coli* led to the conclusion that iron release from ferric enterobactin preferentially takes place in two steps: the first one being catalyzed by the Fes esterase, which can already lead in part to a facilitated removal of ferric iron from the ligand, while the second one depends on the YqjH reductase, which in turn generates ferrous iron for further metabolic assimilation (Fig. 2). It is of interest in this respect that *E. coli* utilizes the catecholate precursors of enterobactin as well as its hydrolytic cleavage products for iron acquisition *via* distinct transport routes,[2] thereby circumventing the need for Fes-dependent complex hydrolysis during frequent cycles of iron uptake and reductive release.

Additional possibilities for a redox potential increase of ferric triscatecholates may include symmetry changes in their iron coordination modes as well as the involvement of softer ligand donor atoms, especially in the cases of non-hydrolyzable triscatecholate scaffolds. Interestingly, several of these siderophores contain one or two oxazoline rings adjacent to their peripheral aryl-caps (Fig. 3A). In the case of ferric vibriobactin in complex with the binding protein ViuP, an iron coordination was observed in which the iron was liganded by five of the arylic hydroxo groups and by one nitrogen of the second oxazoline ring.[109] It is not clear if this binding mode is primarily adopted in particular protein binding sites or if it is also preferred in free solution;[110,111] however, such oxazoline-dependent coordination modes in aryl-capped siderophores might play an interesting role during reductive iron release, especially from those siderophores that are equipped with oxazoline rings adjacent to salicylate caps (Fig. 3A). Further, pH-dependent switches between the catecholate and salicylate coordination modes of the iron-binding arylamide groups could contribute to a facilitated reduction, especially in the case of triscatecholates like protochelin possessing neither a hydrolyzable trilactone backbone nor any oxazoline rings. Thus, the mechanistic details of reductive iron release within this group of ferric siderophore reductases have to be studied further, not least since several of their siderophore substrates are important factors for virulence development in a variety of pathogens. A deeper understanding of their iron release mechanisms could lead to the definition of novel target-specific inhibition strategies.

5 Intracellular pathways for iron trafficking and assimilation

The relevance of an efficient cellular iron homeostasis is best reflected by the number of enzymes and processes that require iron as an essential cofactor. Several hundreds of different proteins in a regular cell may require iron binding in various forms, including single iron sites, diiron-oxo centers, heme and siroheme groups, Fe/S clusters or mixed metal sites such as in [NiFe]-hydrogenases or purple acid phosphatases, in which iron can adopt a broad range of oxidation states or may operate as redox factor or activating Lewis acid.[112–114] The main intracellular iron channeling routes include trafficking to storage and cofactor assembly systems as well as the directed transfer of the iron cofactors to their target apo-proteins. It is important to note that ferrous iron is relevant for most of these processes, in particular for transfer into ferritins, for *de novo* heme and iron–sulfur cluster biogenesis,[115–118] as well as for intracellular iron sensing and associated regulatory processes.[7,119,120]

As described above, most of the iron is released from siderophore complexes by reduction and is hence available in the ferrous state. Further important iron sources like heme scaffolds can be degraded in the cytosol by heme oxygenases which is accompanied by ferrous iron release,[121,122] or could be used directly as cofactors for incorporation into target proteins.[123] Iron that is released or imported in the ferric state may be subjected to a number of yet unknown reductive events before entering the main cytoplasmic trafficking routes. It can be assumed that cytosolic reduction of ferric iron also occurs in fungi, either after its cellular import *via* the Fet3p–Ftr1p system or after its transfer from vacuolar storage sites into the cytosol by homologous transporters like Fet5p–Fth1p in *Saccharomyces*.[124] Another process of intracellular iron reduction could be associated with the release from cytosolic storage compounds such as hyphal ferricrocin or conidial hydroxyferricrocin in *Aspergillus*.[81,82,125]

Prior to a directed cytosolic trafficking, imported or released iron may become part of the ''labile iron pool'' that putatively comprises low-affinity interactions with diverse protein ligands, metabolic intermediates or even phosphorylated sugar compounds.[11,126] A direct route from this transient pool can lead to the long-term storage of intracellular iron in the form of a rather inert mineral core in ferritins. Active Fe(II) uptake by bacterial ferritins is generally assumed, while the mechanism of mineral core formation depends on the ferritin subfamily type and can further be coupled with intracellular ROS detoxification.[127,128] While a ligand-mediated Fe(II) delivery to bacterial ferritins is not known, the delivery of Fe(II) to mammalian ferritins depends on iron chaperones like PCBP1 and PCBP2, which are also involved in a direct metallation of metabolic target enzymes such as Fe(II)-/2-oxoglutarate-dependent dioxygenases.[129,130] The release process of iron from the ferritin mineral core is generally reductive, and may require external reductases. In heme-containing bacterioferritins, the electron transfer processes for both iron core formation and for core reduction appear to be strictly dependent on the intrinsically bound heme cofactors.[131,132]

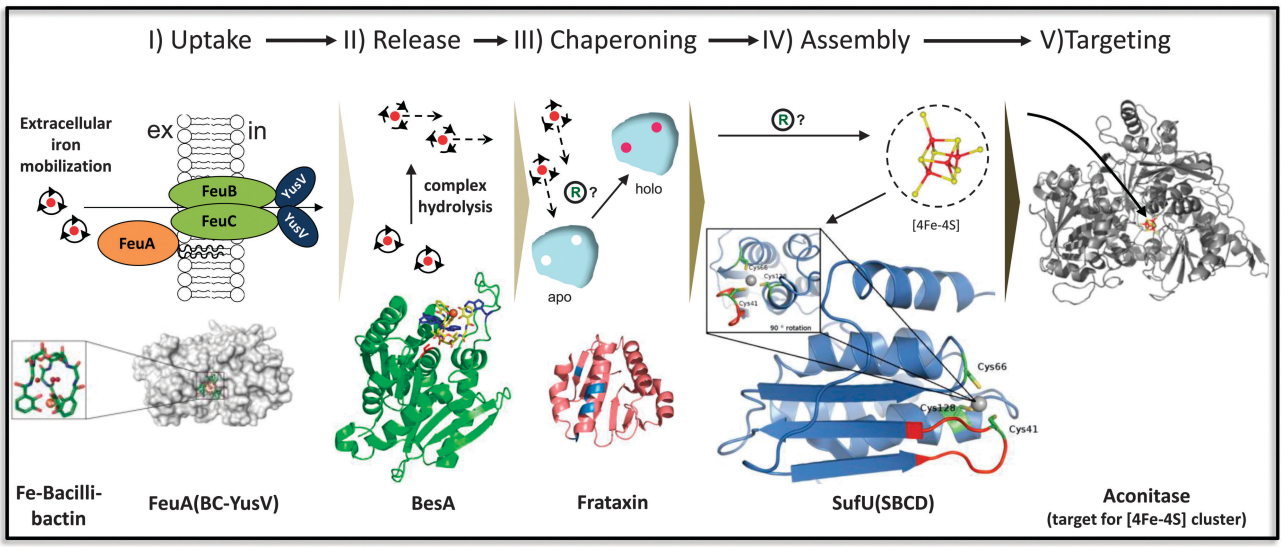

Fig. 4 A proposed iron channeling pathway in *Bacillus subtilis* leading to aconitase maturation. The pathway starts with extracellular iron mobilization by the high-affinity ferric iron chelator bacillibactin (Fe-BB; log K_f = 47.6),[172] and proceeds *via* high-affinity uptake of the ferric bacillibactin complex by the FeuABC-YusV transporter (K_D of the FeuA/Fe-BB complex which is 27 nM;[173] Fe-BB and FeuA/Fe-BB structures are from PDB entry 2WHY[105]). Cytosolic hydrolysis of the ferric siderophore complex is catalyzed by the BesA esterase ($K_{m(obs)}$ for Fe-BB is ~0.5 μM;[72] the *B. cereus* BesA structure from PDB entry 2QM0 [green] is shown in a model with the Fe-BB ligand [yellow] attached to the three conserved tryptophan residues [blue] in the lid domain adjacent to the catalytically active serine [red]). Enzymatic hydrolysis leads to the formation of a ferric (2,3-dihydroxybenzoate–glycine–threonine)$_3$ complex, from which iron release is facilitated by ligand exchange with the ''labile iron pool'' or, alternatively, by interaction with the iron binding sites of apo-frataxin containing high-affinity and lower affinity sites with K_D's of 0.1 μM and 2.4 μM for ferrous iron, respectively (*B. subtilis* frataxin [Fra; formerly YdhG] from PDB entry 2OC6 is shown [salmon] with highlighted conserved acidic surface regions possibly involved in metal binding [blue]).[141] A possible step of ferric iron reduction (''®?'') might occur prior to iron-charging of frataxin or during the next stage, in which holo-frataxin delivers the bound metal ions to the SUF system for Fe/S biogenesis that includes the cysteine desulfurase SufS and the scaffold protein SufU (SufU from PDB entry 2AZH is depicted [blue] with its three conserved cysteines [green; –SH in yellow] that coordinate a Zn^{2+} ion *in vitro* [grey] and form the putative Fe/S binding site *in vivo*; a flexible loop region that contains the critical Cys_{41} is possibly involved in interaction with the catalytic center of SufS during sulfide transfer [red]).[151,153] During Fe/S cluster formation on SufU, SufS activity is enhanced both by SufU and frataxin (the SufU-dependent $K_{m(app)}$ of SufS is ~2.6 μM; the specific activity of SufS is ~20-fold enhanced in the presence of 20-fold molar excess of SufU,[152] and still several fold further in the additional presence of Fra [personal communication, A. Albrecht]). During the transfer of the assembled cluster to the apo-aconitase target enzyme (shown is a structure of a homologous holo-aconitase from PDB entry 1FGH [grey] with bound 4Fe–4S cluster [red-yellow]), the *in vitro* targeting efficiency in the presence of a tripartite holo-Fra/SufSU reconstitution system is about 80% higher after 20 min of transfer than in the presence of the same amounts of free iron and sulfide.[140]

It is not yet clear whether the trafficking of iron to cofactor assembly systems principally requires a mediating ligand transfer; however, several mobile scaffolding compounds are known that bind iron with moderate affinity and contribute to its directed trafficking. In eukaryotes, such a ligand-based transport takes place through the bridging [2Fe–2S] centers of monothiol glutaredoxins like Grx3/4, which form independently of an Fe/S biogenesis machinery and may instead deliver iron to components of the cytosolic Fe/S protein assembly (CIA) system.[133] In contrast, a targeted iron delivery to mitochondrial Fe/S and heme biosynthesis systems is still speculative. The role of the mitochondrial protein frataxin, which might act as a general iron chaperone as well as a possible iron donor for directed cofactor assembly, is under discussion.[115] Frataxin-like proteins are conserved from human to bacteria and possess iron-binding acidic surface regions with K_D's in the lower micromolar range.[134] While frataxin-mediated iron delivery to assembly components such as the Fe/S scaffold IscU or porphyrin-metallating ferrochelatase has been demonstrated *in vitro*, its *in vivo* function for iron trafficking has to be further elucidated.[135,136] The role of frataxins to act as allosteric effectors of cysteine desulfurase activities in eukaryotes and in several bacteria including *E. coli* seems to be established especially in association with ISC-type Fe/S cluster assembly systems.[137–139] However, further structural frataxin homologs such as *B. subtilis* Fra, which can bind both Fe(II) and Fe(III) in similar stoichiometry, seem to be more generally involved in cellular iron homeostasis.[140,141] Indeed, *B. subtilis* Fra appears to be the missing link for iron delivery in a multi-step channeling pathway that includes components of the SUF-type Fe/S assembly machinery (Fig. 4). Nevertheless, several molecular details of the directed iron transfer processes regarding ligand- and component-specific interactions as well as binding site occupations due to possible affinity gradients between different ligand environments remain to be shown.

The Fe/S assembly on ISC-scaffolds in bacteria and mitochondria is further dependent on ferredoxin, which might be involved in several reductive events such as sulfide generation from cysteine desulfurase-delivered sulfur, reduction of ferric iron sources such as Fe(III)-charged frataxin, and may also permit a reductive $[2Fe–2S]^{2+}$ to $[4Fe–4S]^{2+}$ coupling during the later stages of cluster maturation.[142,143] The cluster transfer to acceptor proteins in mitochondria is mediated by the monothiol glutaredoxin Grx5 that takes the [2Fe–2S] cluster possibly

in complex with glutathione from the Isu assembly scaffold, a process which is enhanced by an ATP-hydrolyzing chaperone system.[144,145] The generation of [4Fe–4S] clusters takes place in association with late-acting ISC targeting factors, which also allow the insertion of the cluster into specific target proteins.[115] Similarly, monothiol glutaredoxins like GrxS14/S16 in chloroplasts take Fe/S clusters from Nfu-type or Suf-type assembly scaffolds and transfer them to target proteins in this cellular compartment.[146]

In contrast, bacterial Fe/S cluster transfer is thought to occur *via* a transient complex formation between a scaffold protein carrying the surface exposed cluster and a target apo-protein by cysteine–thiol ligand exchanges. The transfer reaction and possibly also the subsequent complex dissociation can be enhanced by the ATP-dependent chaperone/co-chaperone system HscA/HscB in ISC-type systems.[116,147] In the case of bacterial SUF biogenesis systems with A-type scaffolds,[148] primary cluster building is thought to take place on the SufBCD complex which appears to use $FADH_2$ instead of ferredoxin for the reductive assembly.[149] The generated clusters are then transferred either directly from SufBCD or through interacting carrier proteins like SufA to their targets.[150] SUF systems with U-type scaffolds are mainly present in Gram-positive clades such as the Firmicutes.[151] Here, cluster assembly and transfer can be mediated by the SufU scaffold protein *in vitro*, but it is not clear which function(s) SufU fulfills *in vivo*, since it has functional relations to both the sulfur carrier SufE and the Fe/S carrier SufA of the distinct Gram-negative SUF systems.[152,153]

During the biogenesis of heme, a controlled cofactor trafficking is of similar importance for subsequent side-chain modifications and protein targeting. The Fe(II) redox state of ferrochelatase-derived heme *b* seems to be strictly required for farnesylation reactions that lead to heme *o* or heme *a*, as well as for thioether bond formation during cytochrome *c* maturation.[154,155] For the latter process, heme *b* has to leave the bacterial cytosol or the mitochondrial matrix in eukaryotes. It possibly maintains its ferrous state after crossing the inner mitochondrial membrane, since it requires only the cytochrome *c* heme lyase (CCHL) for apocytochrome attachment in the intermembrane space.[156,157] In contrast, the control of the redox state is more complicated in bacteria. One prominent bacterial heme trafficking pathway employs the CcsAB heme channel, which translocates heme across the cytoplasmic membrane and keeps the ferrous state at the extracellular site by attachment to conserved histidines ligand. Another heme trafficking pathway that is mainly found in α- and γ-Proteobacteria and Archaea utilizes the multilayered Ccm system, in which the membrane-associated CcmABCD complex translocates ferrous heme and delivers it to the periplasmic heme chaperone CcmE.[158,159] The membrane-attached chaperone forms a covalent heme adduct which results in cofactor oxidation, and transfers the ferric heme to the cytochrome *c* synthetase complex CcmF/H, which re-reduces the heme prior to its ligation to the target protein.[158,160] In addition to periplasmic heme chaperoning, an ankyrin-containing protein in *Campylobacter* is proposed to act as a heme chaperone for cytosolic heme trafficking and targeting during intracellular catalase maturation.[161]

Relatively little is known about directed insertion mechanisms of iron into mononuclear sites such as in non-heme iron dioxygenases or into binuclear sites like diiron-oxo centers associated with ferroxidase activity. In humans, the PCBP chaperones fulfill a role in the maturation of proteins containing those kinds of binding sites.[129] In contrast, the situation in microbes is still unclear, and it will be exciting to explore if specific components or rather unspecific cytosolic ligands are involved, and further if gradients of increasing binding affinities are necessary to acquire iron from ligands or chaperones with lower affinities, a mechanism that has been described as a main driving force of directed copper trafficking.[162] On the other hand, iron sites containing unusual ligands such as CN^- and CO in hydrogenases require highly complex batteries of auxiliary proteins for ligand synthesis, delivery and metal coordination such as the Hyp machinery for [NiFe]-hydrogenase maturation.[163–166] Interestingly, the *E. coli* YqjH ferric siderophore reductase is regulated in response to iron and nickel, which indicates that reductive iron assimilation interacts already at an early stage with nickel homeostasis with yet unknown implications for mixed metal site maturation.[106] Altogether, various iron trafficking routes and cofactor assembly systems have been defined today, but the knowledge of cellular iron channeling processes in general is still rather fragmentary. Components which may act as intermediate ligands for trafficking between different protein units and cellular compartments are yet to be identified or further characterized, as well as factors and mechanisms that determine the sequences and directions of the complex trafficking pathways. Further investigations may also comprise the mechanisms of communication between intracellular iron pools as well as different iron cofactors and the various sensor proteins that bind either ionic iron, Fe/S clusters or heme in order to regulate the expression of genes associated with cellular iron homeostasis.

6 Conclusions

Understanding the molecular mechanisms of microbial iron assimilation remains a primary task in light of the vast complexity of iron-driven redox and sensing processes in cellular systems. Bacterial and fungal model organisms, and among them a number of important pathogens, have been employed to establish the basic principles of diverse iron homeostasis strategies. The next layer of studies in this field should entail the various interconnections between the involved system elements, including a steady advancement of the still elementary knowledge of cellular iron trafficking processes. Further in-depth investigations of already known and yet unknown iron acquisition and assimilation components and their functions at both the molecular and the global cellular level are expected to bring about a positive impact on the exploitation of novel iron-dependent targets and the development of novel therapeutic strategies for the purpose of a pathway-specific pathogen defense.

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