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REVIEW

# Fixing frataxin: 'ironing out' the metabolic defect in Friedreich's ataxia

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The metabolically active and redox-active mitochondrion appears to play a major role in the cellular metabolism of the transition metal, iron. Frataxin, a mitochondrial matrix protein, has been identified as playing a key role in the iron metabolism of this organelle due to its iron-binding properties and is known to be essential for iron–sulphur cluster formation. However, the precise function of frataxin remains elusive. The decrease in frataxin expression, as seen in the inherited disorder Friedreich's ataxia, markedly alters cellular and mitochondrial iron metabolism in both the mitochondrion and the cell. The resulting dysregulation of iron trafficking damages affects tissues leading to neuro- and cardiodegeneration. This disease underscores the importance of iron homeostasis in the redox-active environment of the mitochondrion and the molecular players involved. Unravelling the mechanisms of altered iron metabolism in Friedreich's ataxia will help elucidate a biochemical function for frataxin. Consequently, this will enable the development of more effective and rationally designed treatments. This review will focus on the emerging function of frataxin in relation to the observed alterations in mitochondrial iron metabolism in Friedreich's ataxia. Tissue-specific alterations due to frataxin loss will also be discussed, as well as current and emerging therapeutic strategies.

**LINKED ARTICLES**
This article is part of a themed issue on Mitochondrial Pharmacology: Energy, Injury & Beyond. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2014.171.issue-8

**Abbreviations**
ALAS, aminolevulinate synthase; CyaY, bacterial frataxin; DFP, deferiprone; eIF2α, eukaryotic translation initiation factor 2α; Fech, ferrochelatase; Fpn, ferroportin; FRDA, Friedreich's ataxia; Ftmt, mitochondrial ferritin; HJV, haemojuvelin; IRE, iron-responsive elements; IRP, iron regulatory protein; ISC, iron-sulphur clusters; LIP, labile iron pool; Mfrn, mitoferrin; MPP, mitochondrial processing peptidase; PCBPs, poly(rC)-binding proteins; RARS, ring sideroblasts; Tf, transferrin; TfR1, transferrin receptor 1; UTR, untranslated regions; XLSA, X-linked sideroblastic anaemia; Yfh1, frataxin in yeast

## Introduction

The mitochondrion is a vital cellular organelle due to its central roles in energy transduction, apoptosis and the synthesis of crucial metabolites (Holley *et al.*, 2011; Kubli and Gustafsson, 2012). As the sole site of haem synthesis, and a major generator of iron–sulphur clusters (ISC) and ISC precursors, the mitochondrion is integral to cellular iron metabolism (Napier *et al.*, 2005; Richardson *et al.*, 2010). Iron is an essential transition metal for cells and the mitochondrion depends upon it as a substrate for these biosynthetic processes. The ability of iron to redox cycle between the ferrous [Fe(II)] and ferric [Fe(III)] oxidation states endows it with the versatility to be used in vital processes including DNA synthesis (Wu and Brosh, 2012) and cellular respiration (Munoz *et al.*, 2011). However, redox-labile iron has the propensity to react with oxygen and catalyse the production of toxic reactive oxygen species (ROS) that can readily oxidize

proteins, lipids and nucleic acids (Eaton and Qian, 2002). Iron in the redox-active mitochondrion can promote the generation of ROS, and therefore, the regulation of mitochondrial iron must be highly coordinated to avoid damage to this key organelle.

Frataxin is a mitochondrial iron-binding protein (Foury *et al.*, 2007), encoded by the *FXN* gene on chromosome 9 in mammals, and is emerging as a key player in mitochondrial iron metabolism (Foury and Roganti, 2002; Bencze *et al.*, 2007; Pandolfo and Pastore, 2009). Although its exact function is unknown, the current major hypotheses include its roles as an 'iron sensor', 'metabolic switch' and/or iron chaperone for ISC and haem biosynthesis (Becker *et al.*, 2002; Yoon and Cowan, 2004; Lane and Richardson, 2010; Richardson *et al.*, 2010; Colin *et al.*, 2013). Current knowledge of this protein's function has been gleaned from studying the inherited disorder Friedreich's ataxia (FRDA), which is a neuro- and cardiodegenerative disease caused by a deficiency in frataxin expression (Napoli *et al.*, 2006; Martelli *et al.*, 2012).

Decreased frataxin expression within the heart and skeletal muscle, such as that occurring in the muscle creatine kinase (MCK) conditional-*Fxn* null mice [herein referred to as MCK-*Fxn*-knockout (KO) mice], results in the dysregulation of cellular and systemic iron metabolism. The hearts of these mice are characterized by mitochondrial iron loading and, somewhat paradoxically, cytosolic iron depletion (Whitnall *et al.*, 2008; Huang *et al.*, 2009). Altered iron metabolism, and the resultant pathology, of this model accurately reproduce the observed cardiac iron deposits in tissue samples from FRDA patients (Santos *et al.*, 2010). The observed alterations in cardiac iron trafficking may cause an increase in toxic free radical production that ultimately leads to cell death (Gomez-Sarosi *et al.*, 2010; Kell, 2010) and pathological cardiac remodelling (Lane *et al.*, 2013b). Furthermore, studies based on FRDA patients, as well as mice, yeast and other cellular models of this disease have revealed a deficiency in ISC proteins, highlighting the importance of frataxin in mitochondrial iron handling (Huang *et al.*, 2009; Pandolfo and Pastore, 2009; Santambrogio *et al.*, 2011; Martelli *et al.*, 2012).

Intriguingly, the consequences of frataxin deficiency appear to be tissue specific. Unlike the MCK *Fxn*-KO mouse, no detectable iron deposits were observed in the complete *Fxn*-KO mouse or in the CNS of the 'neuron-specific' *Fxn*-KO mouse models (see the following section for further discussion; Santos *et al.*, 2010). These observations add to the complexity in unravelling the biological function of frataxin.

This review will focus on frataxin and its key role in mitochondrial iron metabolism, as well as the pathological effects of iron dysregulation in the absence of frataxin. Additionally, it will discuss tissue-specific alterations in iron trafficking and highlight why the mitochondrion is an ideal therapeutic target in diseases where iron homeostasis is adversely affected, as in FRDA.

## Journey of iron through the cell

### *Cellular iron uptake*

Iron is transported in the circulation bound to the glycoprotein transferrin (Tf), which contains two high-affinity iron(III)-binding sites (Aisen and Brown, 1977; Morgan, 1981). Mono- and di-ferric Tf bind to the transferrin receptor 1 (TfR1) on the cell surface, and are internalized via receptor-mediated endocytosis (Wyllie, 1977; Figure 1). A vacuolar-type proton-pumping ATPase localized in the endosomal membrane leads to acidification of the vesicle, which liberates iron from Tf (Morgan, 1981; Morgan, 1983; Yamashiro and Maxfield, 1984). Once released from Tf, iron(III) is reduced to iron(II), either by a ferrireductase, such as the six transmembrane epithelial antigen of the prostate 3 (Ohgami *et al.*, 2005; 2006), or possibly by small MW reductants such as ascorbate (Nunez *et al.*, 1990; Lane *et al.*, 2013a). The resulting iron(II) can then be exported to the cytosol via ferrous-selective iron transporters such as the divalent metal transporter-1 (DMT1; Fleming *et al.*, 1997; Andrews, 1999). Iron-free Tf (apo-Tf) is recycled back to the cell surface where it dissociates from TfR1, re-enters the circulation and is free to engage in further cycles of iron binding, uptake and release (Ciechanover *et al.*, 1983; Dautry-Varsat *et al.*, 1983).

In non-erythroid cells newly assimilated cytosolic iron, released from the endosome, enters a poorly characterized compartment known as the 'labile iron pool' (LIP; Jacobs, 1977; Kakhlon and Cabantchik, 2002; Shvartsman and Ioav Cabantchik, 2012; Lawen and Lane, 2013). Although the precise nature of the LIP remains unclear, the iron is typically thought to be bound to low MW ligands such as citrate, ATP, amino acids, etc. (Jacobs, 1977; Shvartsman and Ioav Cabantchik, 2012). Attempts to characterize the LIP in erythroid cells strongly suggest that no significant low MW cytosolic intermediate exists (Richardson *et al.*, 1996) and has prompted alternate hypotheses in order to explain intracellular iron transport in erythroid cells. For instance, the 'kiss-and-run' hypothesis postulates a scenario in which iron, still enveloped by the endosome, is transported directly to the mitochondrion (Figure 2). According to this model, once in close proximity, the endosome transiently docks with the mitochondrion in order to transfer the iron directly through protein-based conduits; and thereby bypassing the cytosol (Sheftel *et al.*, 2007). The recent identification of the putative chaperone proteins human poly(rC)-binding proteins 1–4 (PCBPs; Shi *et al.*, 2008; Leidgens *et al.*, 2013) has elucidated another modality of intracellular iron transport. These proteins bind cytosolic iron and facilitate iron loading into cytosolic ferritin and the iron-dependent prolyl hydroxylases, as well as an asparaginyl hydroxylase, that are responsible for hydroxylating the hypoxia-inducible factor (HIF)1α prior to its proteasomal degradation (Shi *et al.*, 2008; Nandal *et al.*, 2011; Leidgens *et al.*, 2013). The discovery of the PCBPs as cytosolic iron chaperones suggests that the LIP may involve cytosolic chaperone proteins with moderate (e.g. micromolar) iron-binding affinities. There are likely to be other cytosolic chaperone proteins with a function similar to PCBPs that are yet to be identified. If so, the existence of a low MW LIP may be superfluous.

Iron that is not further metabolized or stored by ferritin can be exported from the cell by the trans-plasma-membrane protein, ferroportin (Fpn1; Donovan *et al.*, 2000). The expression of Fpn1 is post-translationally regulated by secretion of the liver-derived hormone, hepcidin (Nemeth *et al.*, 2004). When hepcidin binds to Fpn1, the receptor is internalized and degraded by the lysosome, which decreases iron export

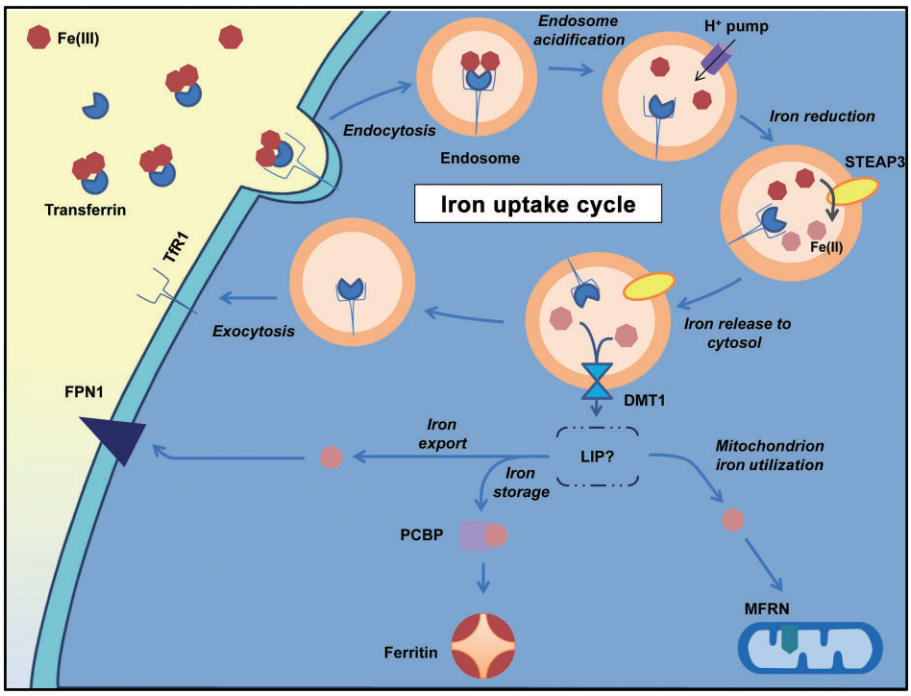



**Figure 1**

Scheme ofcellular iron uptake and utilization. Transferrin-bound iron(III) binds to TfR1 and enters the cell via endocytosis. Acidification of the endosome by a proton pump releases iron from transferrin. Iron(III) is reduced to iron(II) by the ferrireductase, six-transmembrane epithelial antigen of the prostate family member 3 (STEAP3) and then transported through the endosomal membrane by DMT1. Transferrin and TfR1 are recycled back to the cell surface to become available for further iron uptake cycles. Once in the cytosol, iron(II) potentially enters a labile intracellular iron pool (LIP) where it can either (i) be trafficked to the mitochondrion for biosynthesis of essential metabolites; (ii) is potentially chaperoned by PCBPs to cytosolic ferritin for storage; or (iii) exported from the cell via the export pump, Fpn1.

(Nemeth *et al.*, 2004). This hepcidin-mediated regulation of cellular iron export is particularly important in systemic iron metabolism in which dietary iron absorption can be regulated by modulating duodenal Fpn1 that releases iron into the circulation (Nemeth *et al.*, 2004; Lawen and Lane, 2013). In fact, intracellular iron levels regulate the expression of crucial proteins involved in cellular iron metabolism. The precise regulation of these proteins ensures that iron is utilized efficiently in order to minimize toxicity.

## Regulation of cellular iron metabolism

Two RNA-binding proteins, iron regulatory proteins (IRPs) 1 and 2, are largely responsible for moderating cellular iron levels (Muckenthaler *et al.*, 2008). These proteins bind to iron-responsive elements (IRE) in the 5′ and 3′ untranslated regions (UTR) in mRNAs encoding proteins crucial for iron homeostasis (i.e. TfR1, Fpn1, DMT1, ferritin, etc.; Figure 3). The specific IRE-binding activity of IRP1 and the rate of degradation of IRP2 are decreased by the level of iron in the cell (Rouault, 2006). When cellular iron levels are low, IRPs bind with high affinity to the 5′ IREs in *ferritin* and *Fpn1* mRNAs, among others, which sterically suppresses their translation, while IRP binding to 3′ IREs in *TfR1* and *DMT1* mRNAs stabilize these molecules against nuclease-dependent degradation (Hentze and Kuhn, 1996; Chen *et al.*, 1997; Siddappa *et al.*, 2003). Conversely, in iron-replete cells, the level of IRE binding by the IRPs is greatly diminished due to ISC assimilation in IRP1 and increased iron-dependent degradation of IRP2. This leads to increased ferritin translation and decreased TfR1 translation (Casey *et al.*, 1988; Henderson and Kuhn, 1995; Popovic and Templeton, 2004). In this way, the IRPs ensure iron homeostasis is maintained with minimal cytotoxicity (Anderson *et al.*, 2012). The concerted action of IRP–IRE binding and its effects on cellular iron metabolism have been extensively studied and thoroughly reviewed elsewhere (Hentze and Kuhn, 1996; Rouault, 2006; Muckenthaler *et al.*, 2008; Recalcati *et al.*, 2010).

## Mitochondrial iron handling

### *Crossing the mitochondrial membranes*

Precisely how iron is transported into the mitochondria is unclear. However, in order to gain entry to the mitochondrial matrix, the iron must cross both the inner and outer membranes. Identification of the inner mitochondrial membrane solute carriers, mitoferrins 1 and 2 (Mfrn1/2), has provided 'one piece of the puzzle.' First identified in *Saccharomyces cerevisiae* (MRS3/MRS4; Foury and Roganti, 2002), the Mfrns are currently the only known transport proteins in eukaryotes to import iron across the inner membrane (Shaw *et al.*, 2006).

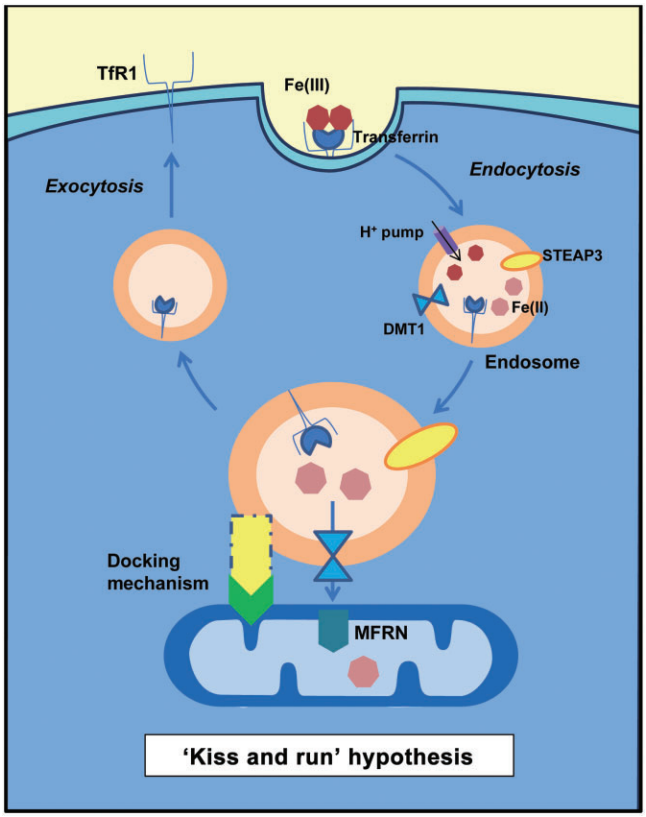



**Figure 2**

Scheme ofthe 'kiss and run' hypothesis of mitochondrial iron uptake from transferrin. This mechanism proposes a route for iron trafficking between the cytosol and the mitochondrion. Iron still bound to transferrin in the endosome is transported by endocytosis to the mitochondrion where it docks by an unknown mechanism and enters the mitochondrion directly. Further studies are required to definitively prove its existence.

To date, the conduit(s) by which iron traverses the outer mitochondrial membrane are unknown. This has influenced hypotheses concerning how iron is transported into the mitochondrion from the cytosol. The previously described 'kiss-and-run' hypothesis is one such proposed mechanism for mitochondrial iron import (Sheftel *et al.*, 2007), although the issue of how iron traverses the mitochondrial membranes is not directly addressed by this model. Alternatively, it has been suggested that iron can be acquired directly from the cytosol without an intermediate chaperone protein to facilitate the transfer. However, this model also fails to address how iron crosses the outer mitochondrial membrane. An iron uptake study in mitochondria isolated from yeast demonstrated that iron(II) is transported across the inner mitochondrial membrane in a manner dependent on the membrane potential rather than an ATPase transporter (Lange *et al.*, 1999).

## Made in the mitochondrion: essential biological metabolites requiring iron

Once in the mitochondrion, iron is utilized by the three major metabolic pathways: (i) ISC biogenesis, (ii) haem biosynthesis or (iii) mitochondrial iron storage, all of which are vital to the actively metabolizing cell.

### *Iron–sulphur cluster synthesis*

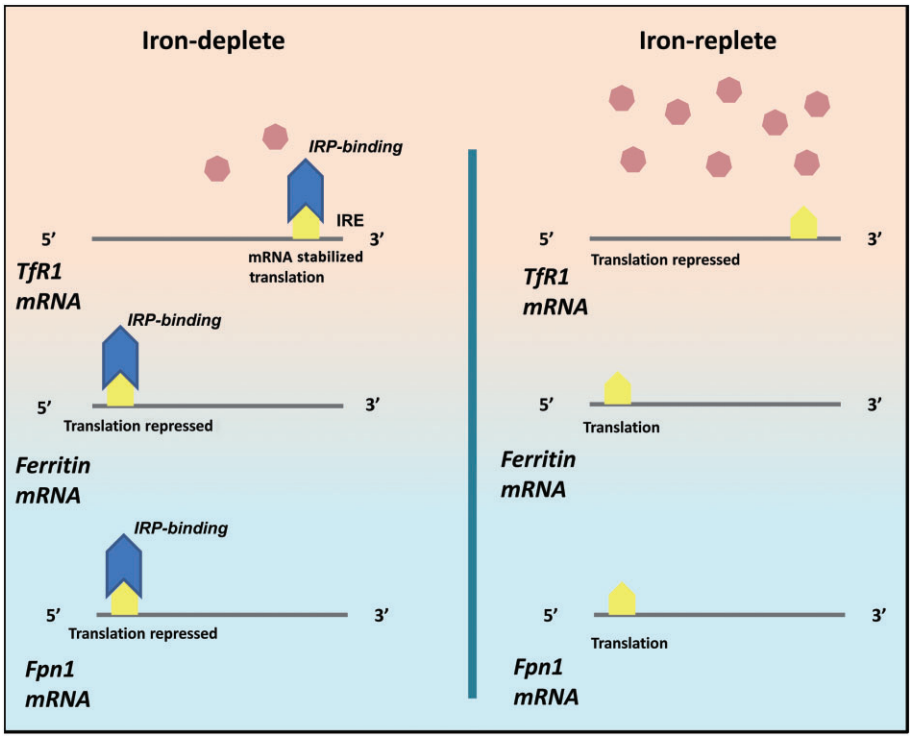
ISCs consist of iron and sulphide ($S^{2-}$) atoms that assemble to form [2Fe-2S] or [4Fe-4S] clusters (Lill, 2009). They are important cofactors in proteins that are vital for electron transport, redox reactions and metabolic catalysis (Tong and Rouault, 2000). In humans, *de novo* ISC synthesis is facilitated by the cysteine desulphurase, NFS1, that provides sulphur (Land and Rouault, 1998; Tong *et al.*, 2003), and the assembly of the cluster occurs on the scaffold protein, ISCU (Tong and Rouault, 2000; Frazzon and Dean, 2003). Mitochondrial ISC assembly and export systems serve as key regulators of iron homeostasis in eukaryotes, their status has a critical influence on the cellular uptake of iron and its intracellular distribution and utilization (Schalinske *et al.*, 1997; Galy *et al.*, 2010). For example, IRP1 is central to the post-transcriptional regulation of cellular iron homeostasis. In iron-replete cells, incorporation of the ISC in IRP1 converts this protein to a cytosolic aconitase, thereby abolishing its IRE mRNA-binding activity. (Hentze and Kuhn, 1996; Theil and Eisenstein, 2000; Lill *et al.*, 2012). Conversely, in iron-deficient cells, IRP1 without an ISC binds IREs with high affinity to promote cellular iron uptake (Rouault and Tong, 2008).

In fact, several diseases have been linked to mutations in genes that encode proteins involved in ISC biogenesis and are associated with a substantial accumulation of iron within the mitochondria (Allikmets *et al.*, 1999; Wingert *et al.*, 2005; Rouault and Tong, 2008). In the case of FRDA, the reduced expression of frataxin results in the iron loading of the mitochondria (Babcock *et al.*, 1997). Frataxin is thought to be an integral part of ISC biogenesis as a dearth of ISC cluster proteins and enzymes are observed in FRDA patients, KO mice, yeast and other cellular models of frataxin deficiency (Becker *et al.*, 2002; Martelli *et al.*, 2007).

Like FRDA, sideroblastic anaemia is characterized by iron loading in the mitochondria (Wingert *et al.*, 2005). A mutation in the gene *glutaredoxin 5* (*GLRX5*) results in the presence of ringed sideroblasts representing erythroid precursor cells with iron-loaded mitochondria that form 'rings' in the perinuclear region. In yeast and zebrafish, *Glrx5* homologues have been shown to be required for ISC biogenesis, although their precise functions in this process are unknown (Ye and Rouault, 2010).

### *Haem synthesis*

The mitochondrion also uses iron for the synthesis of haem (Sano *et al.*, 1959). Haem prosthetic groups are essential components of many critical proteins, including haemoglobin and myoglobin (Ponka, 1997). The formation of these prosthetic groups involves sequential catalysis by eight enzymes with intermediate steps in both the cytosol and mitochondrion (Ponka, 1997). The first enzyme in the pathway, aminolevulinate synthase (ALAS), exists in two isoforms: the ubiquitous ALAS1 and the erythroid-specific ALAS2. ALAS2 is the only variant with an IRE in its 5′ UTR and is thus subject to regulation via the IRP system. This unique characteristic enables the rate of haem synthesis to be governed by the availability of cytosolic iron in erythroid cells (Ponka, 1997).




## Figure 3

Scheme ofthe IRP/IRE mechanism of regulating cellular iron homeostasis. Cellular iron metabolism is regulated by RNA-binding proteins known as IRP1 and 2. The IRPs bind to IREs in the 5′ and 3′ UTRs of mRNAs of molecules responsible for iron homeostasis, for example, TfR1, ferritin, Fpn1, DMT1, etc. The IRE-binding activity of IRP1 and IRP2 are dictated by cellular iron levels by different molecular mechanisms. Under iron-deplete conditions, IRPs bind IREs in the 5′ UTR of *ferritin* and *Fpn1* mRNA suppressing their translation, while IRP binding to the 3′ UTR of *TfR1* and *DMT1* mRNA stabilize them against degradation. Conversely, under iron-replete conditions, IRP-IRE binding is greatly diminished leading to translation of *ferritin* and *Fpn1* mRNA and the degradation of *TfR1*.

In the final step of haem synthesis, the enzyme ferrochelatase (Fech) is responsible for inserting iron into protoporphyrin IX to produce haem. Mutations in the *FECH* gene, as seen in the inherited disorder, erythropoietic protoporphyria, causes a deficiency in Fech enzyme activity manifesting in skin photosensitivity due to protoporphyrin accumulation (Ye and Rouault, 2010). Furthermore, since mammalian Fech requires an ISC as a cofactor to govern its catalytic activity (Ferreira *et al.*, 1995), it is possible that any disruption in ISC synthesis could also adversely affect the rate of haem synthesis (Richardson *et al.*, 2010; Huang *et al.*, 2011).

### *Mitochondrial iron storage: mitochondrial ferritin (Ftmt)*

Iron not used for haem or ISC synthesis is sequestered by Ftmt (Levi *et al.*, 2001). Since the mitochondrion is a major source of cytotoxic ROS, it is essential that intracellular iron is stored in an inert form to prevent adventitious redox-cycling reactions (Richardson *et al.*, 2010). Like cytoplasmic ferritin, Ftmt has ferroxidase activity and both binds and sequesters iron (Levi *et al.*, 2001; Bou-Abdallah *et al.*, 2005). Notably, Ftmt is preferentially expressed in cells characterized by high energy consumption (i.e. brain, heart, kidney, thymus and smooth muscle), but absent from tissues with a known iron storage function (i.e. liver and spleen; Santambrogio *et al.*, 2007). Unlike the mRNAs encoding cytosolic ferritins, the *FtMt* mRNA lacks an IRE that would allow IRP-dependent regulation by iron (Levi *et al.*, 2001). Interestingly, Ftmt expression can strongly influence cellular iron homeostasis (Corsi *et al.*, 2002; Nie *et al.*, 2005). In tissues expressing Ftmt, cellular iron gets redistributed from the cytosol to the mitochondria (Corsi *et al.*, 2002; Nie *et al.*, 2005). Furthermore, Campanella and colleagues have demonstrated that Ftmt can protect cells from oxidative damage, while maintaining the balance between mitochondrial iron availability and ROS formation (Campanella *et al.*, 2009). In these studies, HeLa cells were transfected with *Ftmt* and treated with either $H_2O_2$ or antimycin A in order to generate ROS in the mitochondria (Campanella *et al.*, 2009). Ftmt-expressing cells demonstrated reduced ROS and increased ATP levels in response to oxidative stress and increased activity of mitochondrial ISC enzymes (Campanella *et al.*, 2009). Moreover, Ftmt has demonstrated a protective role against cellular defects caused by frataxin deficiency in FRDA models in yeasts and human cells, as well as in fibroblasts of FRDA patients (Campanella *et al.*, 2004; 2009; Zanella *et al.*, 2008).

Although Ftmt expression can be beneficial to the cell, the overexpression of Ftmt in cells can have pathological effects. Indeed, the overexpression of Ftmt *in vitro* increases Ftmt iron loading, IRP-binding activity and TfR1 expression, and decreases cytosolic and mitochondrial aconitase activity (indicating decreased ISC synthesis), cytoplasmic ferritin

levels and haem synthesis (Nie *et al.*, 2005). Expression of Ftmt that is usually absent from erythroid cells, was observed in X-linked sideroblastic anaemia (XLSA) and also in patients with refractory anaemia with ring sideroblasts (RARS; Cazzola *et al.*, 2003). Indeed, Ftmt is thought to be the source of iron identified in the characteristic ring sideroblasts (Cazzola *et al.*, 2003). Following a study demonstrating that the STAT5/JAK2 pathway was important for erythropoiesis (Grebien *et al.*, 2008), *IRP2* and *TfR1* were established as transcriptional targets of STAT5 in erythroid cells of *STAT5*-null mice (Kerenyi *et al.*, 2008). Subsequent studies in erythroleukaemic cells demonstrated that Ftmt overexpression increased apoptosis and limited haem synthesis via decreased STAT5 phosphorylation and decreased transcript levels of the anti-apoptotic protein, Bcl-xL (Santambrogio *et al.*, 2011). Indeed, gene profiling of erythroblasts from RARS patients confirmed the suppression of *STAT5* gene expression (Camaschella, 2009). Hence, the deregulation of the STAT5 pathway by Ftmt overexpression may represent one mechanism promoting mitochondrial iron loading in XLSA and RARS. These studies underscore how hyperexpression of Ftmt can disrupt iron homeostasis with pathological consequences.

Iron is essential for the formation and function of the proteins and enzymes involved in mitochondrial metabolism (Richardson *et al.*, 2010; Huang *et al.*, 2011). Therefore, it is clear how inadequate regulation of mitochondrial iron levels could create a 'domino effect' resulting in dysregulation of cellular iron metabolism. An important protein thought to be essential for mitochondrial iron metabolism is frataxin, which has postulated roles in all three mitochondrial iron metabolic pathways and whose deficiency results in marked iron accumulation in the mitochondrion (Babcock *et al.*, 1997; Alper and Narayanan, 2003).

## Frataxin

Frataxin is a small (210 amino acids) evolutionarily conserved mitochondrial protein encoded in the nucleus and targeted to the mitochondria, where a two-step proteolysis removes the targeting sequence to produce the mature protein (Li *et al.*, 2013). Mitochondrial processing peptidase (MPP) cleaves frataxin twice converting the pro-peptide into a 19 kDa intermediate and then into the 17 kDa mature protein (Pandolfo and Pastore, 2009; Li *et al.*, 2013). While frataxin was initially found to be associated with the inner mitochondrial membrane (Campuzano *et al.*, 1997), there is no canonical structural motif present in the sequence that would anchor it there. Therefore, it is possible that this association is derived from complex formation between frataxin and mitochondrial membrane proteins, such as Fech (Yoon and Cowan, 2004).

The basic structure of frataxin consists of *N*- and *C*-terminal α-helices, flanked by a 5–7 strand antiparallel β-sheet in a 'sandwich' motif (Musco *et al.*, 2000). The iron-binding regions were mapped to a semi-conserved acidic ridge on the first helix and β-strand using NMR (Dhe-Paganon *et al.*, 2000) and were subsequently validated by mutating frataxin in yeast (Yfh1; Foury *et al.*, 2007). Substitution of the acidic residues with lysine or alanine increased the pI of this region with concomitant loss of iron-binding efficacy (Foury *et al.*, 2007) and, depending on the degree of loss, also impaired ISC biosynthesis and increased oxidative stress compared with wild-type (WT) yeast (Foury *et al.*, 2007). Over the years, subsequent studies of frataxin deficiency in various mouse and cell models have generally recapitulated what has been observed in yeast (Rotig *et al.*, 1997; Puccio *et al.*, 2001). Taken together, these studies confirm the role of frataxin in orchestrating ISC formation, its most widely accepted function.

Frataxin's role in haem synthesis is more obscure and is based on attenuated haem production (Lesuisse *et al.*, 2003; Huang *et al.*, 2009), as well as alterations in enzymes involved in haem synthesis when frataxin expression is reduced (Yoon and Cowan, 2004; Boddaert *et al.*, 2007). It is unclear whether frataxin participates directly in haem synthesis since the paucity of haem does not manifest as anaemia in FRDA patients as might have been expected. Instead, the role of frataxin in haem synthesis may manifest as a by-product of its role in ISC formation, as Fech is an ISC-containing protein (Ferreira *et al.*, 1995). Curiously, decreased expression of Yhf1 also disrupts haem synthesis, even though the yeast orthologue of Fech does not contain an ISC (Lesuisse *et al.*, 2003). This supports the hypothesis that the involvement of frataxins in haem synthesis goes beyond its interaction with Fech. The notion that frataxin can contribute directly to haem synthesis is bolstered by the observations that (i) human frataxin binds Fech with high affinity and significantly stimulates its activity (Yoon and Cowan, 2004; Bencze *et al.*, 2007) and (ii) haemoglobinization and addition of the haem precursor, protoporphyrin IX, down-regulates frataxin expression in murine erythroleukaemic Friend cells (Becker *et al.*, 2002).

It is clear that the iron-binding activity of frataxin makes it an important player in the creation of mitochondrial metabolites, though its exact role remains elusive. The association of frataxin with multiple aspects of iron metabolism, from ISC and haem synthesis to regulation of oxidative stress, has prompted suggestions for its roles as an iron chaperone, iron sensor, a scavenger of ROS, an iron storage protein and as a metabolic switch (Lane and Richardson, 2010; Richardson *et al.*, 2010). The details of each possible role will be discussed in the following section.

### *Frataxin: the iron chaperone*

Frataxin's involvement in both ISC assembly and haem synthesis makes it difficult to reconcile its primary function. The iron-binding status and connection to ISCs and haem synthesis has elicited the hypothesis that frataxin may be an iron chaperone. The formation of [2Fe-2S] clusters on the scaffold protein, ISCU, requires contribution of sulphur, by NFS1, and iron, by an unknown donor. Physical interactions between frataxin and the core ISC proteins (NFS1, ISCU and ISD11) have been demonstrated and frataxin proffered as the iron donor in this system (Wang and Craig, 2008; Schmucker *et al.*, 2011; Rouault, 2012).

Further evidence for the iron-chaperone function of frataxin comes from bioinformatics analyses in yeast and bacteria. The chaperone proteins hscA and hscB, required for ISC formation, co-evolved with and exhibit identical distribution patterns to bacterial frataxin (CyaY; Huynen *et al.*, 2001). The iron-sensitive association of HSC20, the mamma-

lian homologue of hscA/B, to frataxin was recently demonstrated (Shan and Cortopassi, 2012) and loss of HSC20 attenuated cellular iron pools, expression enzymes and proteins containing ISCs (Shan and Cortopassi, 2012). The similarities between frataxin and a known iron chaperone further support frataxin's status as a chaperone for ISC formation.

In a similar regard, frataxin is thought to be the iron donor in the Fech-catalysed haem synthesis through the delivery of iron(II) for insertion into protoporphyrin IX. This is supported by the finding that (i) iron-laden human frataxin binds Fech with nanomolar affinity and markedly stimulates Fech activity (Yoon and Cowan, 2004), and (ii) frataxin is required for correct metallation of protoporphyrin in yeast, as zinc protoporphyrin is formed in place of haem in the absence of frataxin (Lesuisse *et al.*, 2003).

## *Frataxin as an iron sensor and metabolic switch*

Another possibility is that frataxin acts as an iron sensor that detects perturbations in cellular iron levels. This hypothesis comes from an observation that CyaY negatively regulates ISC synthesis when the amount of iron exceeded the availability of ISC apo-acceptor proteins (Adinolfi *et al.*, 2009). If frataxin can indeed exert kinetic control over ISC formation, it would follow that a decrease in frataxin would create an excess of ISCs. Contrary to this notion, ISC formation is decreased when frataxin expression is deficient (Chen *et al.*, 2002; Huang *et al.*, 2009). Therefore, it is unlikely that frataxin functions as an iron sensor, at least in higher organisms. However, this does not exclude the possibility that frataxin has a regulatory function. A rise of protoporphyrin IX levels in murine erythroleukaemic cells decreases frataxin expression as a failsafe to ensure haem generation at the expense of other mitochondrial iron products, such as ISCs (Becker *et al.*, 2002). In fact, haem synthesis is determined by the molar ratio of frataxin to Fech with an increase in this ratio diminishing haem synthesis (Yoon and Cowan, 2004). Therefore, based on its expression relative to its binding partners, it is plausible that frataxin acts as a metabolic switch (Becker *et al.*, 2002).

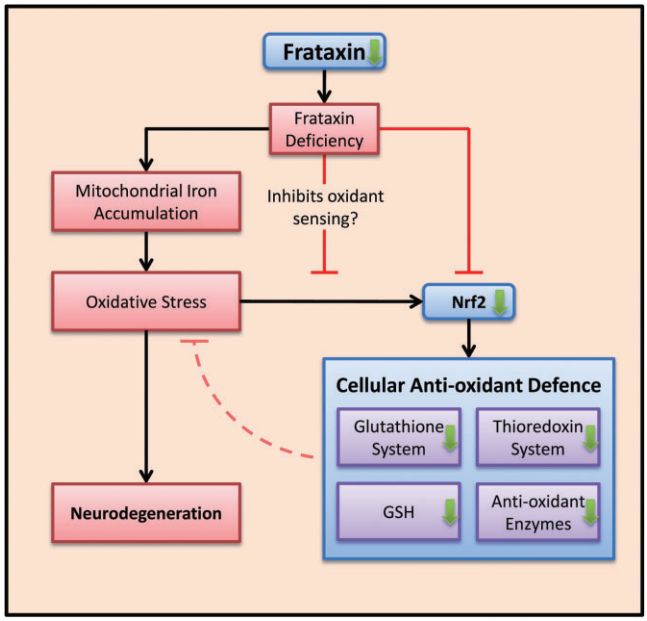
## *Frataxin and iron storage*

The hypothesized role of frataxin as an iron storage protein, similar to ferritin, was fostered by the oligomerization of Yhf1 and CyaY in iron-replete conditions (Park *et al.*, 2003). These large aggregates showed greater iron-binding capacity, sequestering up to 50 atoms of iron per monomer (Adamec *et al.*, 2000), and retained iron in a redox-inactive state readily mobilized by physiological chelators, such as citrate, for use in haem synthesis (Park *et al.*, 2003). However, this is unlikely to be a physiological mechanism, as the calcium/magnesium levels normally present in the mitochondrion would stabilize Yhf1 and CyaY in the iron-bound monomeric state preventing oligomerization (Adamec *et al.*, 2000; Adinolfi *et al.*, 2002). This proposed role becomes even less likely considering that (i) human frataxin does not self-assemble, even under iron-loaded conditions (Cavadini *et al.*, 2002) and (ii) the mitochondrial iron storage protein, Ftmt, was discovered in human tissues (Drysdale *et al.*, 2002).

## *Frataxin as a regulator of oxidative stress*

Impaired activity of the respiratory chain complexes in the absence of frataxin is well documented and the ensuing leakage of electrons resulting from decreased activity of the respiratory chain may be a source of ROS (Napoli *et al.*, 2006; Armstrong *et al.*, 2010) Indeed, increased markers of oxidative stress were reported in FRDA patients' blood and urine samples (Pandolfo, 2008; Schmucker and Puccio, 2010) and lymphoblasts from FRDA patients contain markedly increased levels of $H_2O_2$ and oxidized glutathione (Napoli *et al.*, 2006; Santos *et al.*, 2010). The increase in oxidative stress markers, together with the observed hypersensitivity of FRDA patient fibroblasts and lymphoblasts to oxidative stress, (Yang *et al.*, 1999) has culminated in the hypothesis that frataxin plays a role in ROS formation. Despite mounting evidence detailing the role of frataxin in the detoxification of ROS (Armstrong *et al.*, 2010), there is significant debate over whether frataxin deficiency leads to an increase in cellular ROS (Seznec *et al.*, 2005). This is primarily due to a lack of direct evidence linking frataxin expression and ROS generation. Thus, whether the oxidative damage associated with frataxin deficiency manifests from loss of antioxidant protection, thus rendering the cell hypersensitive to oxidative stress or an actual increase in ROS formation is not yet known. Gakh and colleagues elegantly demonstrated that these functions are not mutually exclusive using mutant Yhf1 with impaired frataxin ferroxidase activity (Gakh *et al.*, 2006). The ferroxidase-devoid Yhf1 protein could not mineralize and detoxify iron, producing hypersensitive to oxidative stress, yet chaperone function remained intact (Gakh *et al.*, 2006). These results suggest that yeast frataxin directly participates in iron detoxification in addition to its role as an iron chaperone. However, the correlation of loss of frataxin and increased oxidative stress is not so conclusive in mammals. The detoxification of iron by Yhf1 depends on both ferroxidase activity and self-assembly into oligomers (Park *et al.*, 2003). The inability to form oligomers may preclude human frataxin from manifesting ferroxidase activity. Indeed, the ferroxidase activity observed with forced expression of recombinant human frataxin in *Escherichia coli* (O'Neill *et al.*, 2005) is yet to be validated with endogenous expression from mammalian systems.

However, the studies described above, do not preclude frataxin from a direct role in antioxidant protection. There is evidence that the decrease in frataxin, and subsequent increase in ROS generation, results from impaired frataxin-dependent antioxidant responses. In fact, cultured fibroblasts from FRDA patients are hypersensitive to oxidative insults due to faulty signalling of the Nrf2 pathway impairing the response of antioxidant enzymes (Paupe *et al.*, 2009; Figure 4). This association is supported by microarray data obtained from the YG8R neuronal mouse model of FRDA (Shan *et al.*, 2013), which showed down-regulation of Nrf2-regulated antioxidant enzymes from both the GSH and thioredoxin redox systems (Shan *et al.*, 2013). In addition, Nrf2 itself was down-regulated and demonstrated to be frataxin dependent in several cell lines (Shan *et al.*, 2013). Indeed, decreased *Nfr2* mRNA was observed in motor neurons




## Figure 4

Scheme offrataxin's effect on cellular redox state. Frataxin-deficient cells exhibit increased sensitivity to oxidative stress. Iron accumulation in the mitochondrion is a potential source of ROS formation in the cell resulting in oxidative stress. This may be due to the proposed role of frataxin in regulating ROS formation by activating cellular antioxidant defences. Nrf2 is down-regulated and Nrf2-regulated antioxidant enzymes from both the glutathione (GSH) and thioredoxin redox systems fail to be activated in frataxin-deficient cells. The combination of increased ROS formation and failure of the cell to neutralize ROS by antioxidant mechanisms may lead to apoptosis and neurodegeneration.

silenced for frataxin (D'Oria *et al.*, 2013) and that activation of the residual Nfr2 was defective even after treatment with pro-oxidants (D'Oria *et al.*, 2013). Given the importance of Nrf2 in protecting neurons from oxidative stress (Shih *et al.*, 2003), it is easy to reconcile this frataxin-dependent mechanism with the resulting neurodegeneration seen in FRDA.

No matter how the specific molecular mechanisms by which the iron-binding properties of frataxin are utilized, it is obvious that cellular and mitochondrial iron metabolism is dependent on appropriate expression and function of frataxin. The autosomal recessive disorder, FRDA, is a perfect example of the deleterious consequences of iron dysregulation.

## Friedreich's ataxia

FRDA is a rare neuro- and cardiodegenerative disease affecting approximately 1:50 000 individuals of European ancestry (Koeppen, 2011). Onset of symptoms usually occurs around puberty and are characterized by progressive gait and limb ataxia, decreased perception of vibration, muscular weakness and positive extensor plantar response, which ultimately results in wheelchair confinement. These neurological symptoms are a consequence of degeneration of the large sensory neurons of the dorsal root ganglia (DRG) and spino-cerebellar tracts (Pandolfo, 2009). Additional manifestations of the disease include hypertrophic cardiomyopathy, the most frequent cause of death and an increased incidence of diabetes (Koeppen, 2011). Life expectancy for FRDA patients is approximately 40 years of age (Koeppen, 2011).

The pathogenic mutation in FRDA is a homozygous guanine-adenine-adenine (GAA) trinucleotide repeat expansion on chromosome 9q13 that leads to transcriptional silencing of the *Fxn* gene (Koeppen, 2011). The decrease in frataxin expression perturbs iron homeostasis promoting neuro- and cardiodegeneration (Huang *et al.*, 2009; Koeppen, 2011). A more recent study has indicated that the GAA repeat expansion can lead to silencing of the gene, *PIP5K1B*, which encodes phosphatidylinositol 4-phosphate 5-kinase β type 1 (Bayot *et al.*, 2013). This enzyme is functionally linked to the actin cytoskeleton dynamics with silencing of the gene leading to abnormal remodelling of the actin cytoskeleton in fibroblasts (Bayot *et al.*, 2013). While these findings remain preliminary, they raise the question of whether *PIP5K1B* silencing plays a role in FRDA pathogenesis.

The mechanisms dysregulating iron and causing degeneration of frataxin-deficient tissues are still being unveiled. It has been suggested that the mitochondrial iron accumulation observed in FRDA patient tissue and mouse models is mostly a late event, and that the iron is largely found as inert precipitates in the mitochondria and does not contribute to the pathology (Sturm *et al.*, 2005; Seguin *et al.*, 2010; Bayot *et al.*, 2011) Notably, studies with specific iron chelators demonstrate that removal of mitochondrial iron in the MCK-*Fxn*-KO mouse indicates that while removal of the iron improves cardiac function it does not prevent the pathology (Whitnall *et al.*, 2008). These studies suggest that it is the function of frataxin in iron metabolism rather than the iron accumulation *per se* that is the major mediator of the pathology observed. Nonetheless, it has recently been demonstrated that the iron accumulation observed in the MCK-*Fxn*-KO mouse is not stored in Ftmt and is present as iron aggregates that could be redox active and contribute to the neurodegeneration in the disease (Whitnall *et al.*, 2012).

Further insights into FRDA pathology have been gleaned from various transgenic animals created to mimic FRDA pathology. These models were created using a number of strategies, such as conditional frataxin deletion using a lox/cre system or the introduction of expanded GAA repeats in frataxin-null mice (Puccio *et al.*, 2001; Martelli *et al.*, 2012; Shan *et al.*, 2013). Research implementing these animal models has uncovered tissue-specific aspects of frataxin-mediated iron metabolism (Figure 5). For instance, the MCK conditional frataxin KO strategy accurately reproduces the cardiomyopathy seen in FRDA patients (Puccio *et al.*, 2001). Targeted deletion of frataxin from striated muscle, using the MCK-Cre mice, caused up-regulation of TfR1 and IRP2 and decreased ferritin expression in the heart, relative to the WT mice (Whitnall *et al.*, 2008; 2012). Conversely, skeletal muscle from the same mouse showed the opposite response (Whitnall *et al.*, 2012). This observation implies that frataxin deficiency has a different effect on iron metabolism in the heart, relative to that in skeletal muscle, and may relate to the distinct functions and metabolic requirements of the these tissues.

| Alterations | Heart | Neurons | Skeletal muscle |
|---|---|---|---|
| Cytosolic iron deficiency | Yes Huang *et al.* 2009<br>Michael *et al.* 2006 | ? | No Puccio *et al.* 2001<br>Whitnall *et al.* 2012 |
| Mitochondrial iron loading | Yes Babcock *et al.* 1997<br>Puccio *et al.* 2001<br>Whitnall *et al.* 2008<br>Huang *et al.* 2009<br>Santos *et al.* 2010<br>Whitnall *et al.* 2012 | No Puccio *et al.* 2001<br>Koeppen *et al.* 2007<br>Santos *et al.* 2010 | No Puccio *et al.* 2001<br>Whitnall *et al.* 2012 |
| Perturbed ISC synthesis | Yes Becker *et al.* 2002<br>Pandolfo *et al.* 2009<br>Huang *et al.* 2009<br>Santambrogio *et al.* 2011<br>Martelli *et al.* 2012 | Yes Simon *et al.* 2004<br>Puccio *et al.* 2001 | ? |
| Autophagy/apoptosis | Yes Knaapen *et al.* 2001<br>Shimomura *et al.* 2001<br>Gustafsson *et al.* 2009<br>Huang *et al.* 2013 | Yes Santos *et al.* 2001<br>Simon *et al.* 2004<br>Ristow *et al.* 2003 | ? |
| Oxidative stress | ? | Yes Michalec *et al.* 2002<br>Chodaczek *et al.* 2007<br>Shan *et al.* 2013<br>D'oria *et al.* 2013 | ? |
| Inflammatory response | ? | Yes Chodaczek *et al.* 2007<br>Lu *et al.* 2009<br>Koeppen *et al.* 2009<br>Urrutia *et al.* 2013 | ? |

**Figure 5**
Tissue-specific FRDA pathology.

## Tissue-specific effects of frataxin deletion on iron metabolism

### *Mitochondrial iron accumulation and autophagy in the frataxin-deficient heart*

Analysis of cardiac tissues from MCK-*Fxn*-KO mice has revealed variations in iron metabolism genes leading to mitochondrial iron accumulation (Whitnall *et al.*, 2008; 2012; Huang *et al.*, 2009). Although the up-regulation of TfR1 and Mfrn2 greatly increased cellular and mitochondrial iron uptake relative to WT mice, cytosolic iron levels were found to be decreased (Huang *et al.*, 2009). Furthermore, ferritin and Fpn1 were down-regulated in MCK-*Fxn*-KO mice, suggesting that the frataxin-deficient heart is responding to cytosolic iron deprivation by increasing iron uptake (Huang *et al.*, 2009). In support of this hypothesis, IRP2-RNA binding activity was markedly increased in MCK-*Fxn*-KO relative to WT mice (Whitnall *et al.*, 2008). However, despite the greater influx of iron, the molecules involved in mitochondrial iron handling were significantly down-regulated, indicating iron was not properly utilized within the mitochondrion and resulted in iron accumulation (Huang *et al.*, 2009).

It is interesting to note that Ftmt is decreased in the KO mouse, despite mitochondrial iron loading, signifying that Ftmt is not the source of accumulated iron (Huang *et al.*, 2009; Whitnall *et al.*, 2012). In fact, examination of the heart by electron microscopy, chromatography, Mössbauer spectroscopy and magnetic susceptibility measurements revealed that cardiac iron accumulates as non-ferritin aggregates of iron, phosphorus and sulphur in the KO mouse (Whitnall *et al.*, 2012). Similar iron phosphate particles were observed in the mitochondria of *Yfh1*-null yeast (Lesuisse *et al.*, 2003). These observations are in contrast to the liver of MCK-*Fxn*-KO where frataxin expression is intact and iron is confined to cytosolic ferritin (Whitnall *et al.*, 2012). These findings are vital, as they reveal the nature of iron accumulating in the mitochondrion appears to be without the protection of the protein coat characteristic of ferritins (Whitnall *et al.*, 2012). Thus, the mitochondrial iron loading in this form is potentially an 'explosive' source of ROS.

More recent studies examining the MCK cardiac model of FRDA have identified the early and marked up-regulation of a gene cohort responsible for stress-induced amino acid biosynthesis (Huang *et al.*, 2013). This effect could be caused by activation of the integrated stress response, mediated by phosphorylation of the eukaryotic translation initiation factor 2α (p-eIF2α). In fact, p-eIF2α levels were up-regulated from 3 weeks of age in KO relative to WT mice, prior to any functional alterations in the heart (Huang *et al.*, 2013). Notably, the eIF2α-mediated integrated stress response has been previously implicated in heart failure via downstream processes such as autophagy and apoptosis (Knaapen *et al.*, 2001; Shimomura *et al.*, 2001; Gustafsson and Gottlieb, 2009). Considering this, a panel of autophagic and apoptotic markers were examined and found to be enhanced in MCK-*Fxn*-KO relative to WT mice, with autophagic activation preceding apoptosis (Huang *et al.*, 2013). Hence, the pathogenesis of the cardiomyopathy after frataxin deletion correlates with early and persistent eIF2α phosphorylation preceding activation of autophagy and apoptosis (Huang *et al.*, 2013).

The early induction of the stress response described by Huang and colleagues (Huang *et al.*, 2013) may be stimulated by the alterations in iron metabolism and in particular may be a response to induce autophagy that is required for the

release of iron from the iron storage protein, ferritin (Ollinger and Roberg, 1997; Asano *et al.*, 2011). This would explain the cytosolic iron deficiency and low levels of cytosolic ferritin in the heart of the MCK-*Fxn*-KO mouse (Whitnall *et al.*, 2008; 2012; Huang *et al.*, 2009). Moreover, autophagic activation in the frataxin-deficient cardiomyocytes may also be a survival response to recycle defective mitochondria, as indicated by an up-regulation of Fundc1, a marker of mitochondrial specific autophagy, 'mitophagy' (Huang *et al.*, 2013). In line with these observations, a recent study in *Yfh1*-null yeast indicated that the mitochondrion is the main site of ROS production, as treatment with rapamycin, an inducer of autophagy, resulted in decreased mitochondrial mass and ROS production (Marobbio *et al.*, 2012).

## *Frataxin deficiency in cardiac and skeletal muscle affects systemic iron homeostasis*

There is limited information on iron metabolism in the skeletal muscle of FRDA patients since it has not been thoroughly investigated. As mentioned earlier, profiling of iron metabolic molecules in skeletal muscle from the MCK-*Fxn*-KO, revealed that in clear contrast to the heart, TfR1 and IRP2 were down-regulated and ferritin expression was increased (Whitnall *et al.*, 2012). Considering that IRP2 expression inversely correlates with cytosolic iron levels (Hentze *et al.*, 2010), IRP2 down-regulation indicates that the skeletal muscle does not exhibit a cytosolic iron deficiency. Furthermore, the ISC protein deficiency accompanying decreased frataxin levels in the heart and brain, is not observed in the skeletal muscle, fibroblasts, lymphocytes or lymphoblasts of FRDA patients (Rotig *et al.*, 1997; Bayot *et al.*, 2011). This intimates that frataxin serves a different purpose in the skeletal muscle, compared to that in the heart.

It is interesting to note that the absence of frataxin in the heart and skeletal muscle of frataxin null mice leads to iron loading in the liver, spleen and kidney, demonstrating that frataxin absence in this very severe model of FRDA also affects systemic iron processing (Whitnall *et al.*, 2012). In fact, analysis of iron-related proteins in the livers of MCK-*Fxn*-KO mice revealed an increase in haemojuvelin (HJV), relative to WT mice (Whitnall *et al.*, 2012), which is known to induce expression of the hormone of iron metabolism, hepcidin (Huang *et al.*, 2005; 2009). This is consistent with the increase in hepcidin found in the blood of MCK-*Fxn*-KO mice (Whitnall *et al.*, 2012).

It has previously been proposed that HJV, shed from skeletal muscle and the heart, can affect systemic iron metabolism (Zhang *et al.*, 2007). HJV is thought to influence hepcidin expression through the bone morphogenetic protein pathway via a neogenin-dependent mechanism (Zhang *et al.*, 2007). Furthermore, HJV that is shed by skeletal muscle is believed to be soluble HJV, a negative regulator of hepcidin (Lin *et al.*, 2005), while the liver mainly expresses membrane-bound or GPI-linked HJV, which enhances hepcidin expression (Babitt *et al.*, 2007). It is the ratio of membrane GPI-linked HJV to soluble HJV that determines the concentration of hepcidin through this pathway (Babitt *et al.*, 2007). In fact, profiling of iron metabolism genes in human skeletal muscle and liver under physiological conditions revealed that HJV expression is higher in skeletal muscle relative to the liver (Polonifi *et al.*, 2010). This is of note, especially because of the high levels of HJV and hepcidin found in the liver of MCK-*Fxn*-KO (Whitnall *et al.*, 2012), pointing to a direct influence of frataxin on systemic iron metabolism.

## *Iron trafficking and neuroinflammation in frataxin-deficient neurons*

Iron metabolism in the brain and CNS remains poorly understood. What limited information there is, points to very different mechanisms of iron handling compared with other organs in the body (Moos and Morgan, 2004; Rouault, 2006; Crichton *et al.*, 2011). The dentate nucleus is one of several structures in the brain that are iron-rich under physiological conditions (Koeppen *et al.*, 2007). In FRDA, the selective degeneration of neurons was hypothesized to be the result of iron accumulation in this region as perceived in the heart (Koeppen *et al.*, 2007; Pandolfo and Pastore, 2009). Yet, collapse of this grey matter structure in FRDA, relative to normal tissues, does not result in a net increase or decrease of iron (Koeppen *et al.*, 2007). What is even more perplexing is that other iron-rich regions of the CNS are apparently unaffected in FRDA patients (Koeppen and Mazurkiewicz, 2013). In fact, X-ray fluorescence of the dentate nucleus not only showed comparable iron levels between FRDA and normal tissues, but also disclosed the presence of copper and zinc at a high concentration, overlapping the iron-rich regions (Koeppen *et al.*, 2012). However, it is unclear what role these metals play, if any, in the pathology of FRDA, as tissue staining for superoxide dismutase (SOD) and ATPase α-peptide provided inconclusive evidence that Cu and Zn excess contribute to damage of the dentate nucleus (Koeppen *et al.*, 2012).

Analysis of the dentate nucleus in FRDA patients exhibited differential expression of several iron-responsive proteins (Koeppen *et al.*, 2007). DMT1 (both $IRE^+/IRE^-$ isoforms) expression was not detected in the dentate nucleus, confirming neuronal loss, while cytosolic ferritin expression shifted from oligodendroglia to microglia and astrocytes in FRDA tissue (Koeppen *et al.*, 2007). Interestingly, Ftmt was not detected. Furthermore, Fpn1 was up-regulated in the cerebellar cortex and dentate nucleus and correlated with regions of grumose degeneration. This response is purportedly due to iron accumulation in or near the mitochondria (Koeppen *et al.*, 2007). Similar changes in expression patterns and levels of these proteins have been observed in the dorsal root ganglia (Koeppen *et al.*, 2009).

There is mounting evidence that inflammatory responses in the CNS are associated with iron dysregulation and may contribute to neurodegeneration in FRDA (Koeppen *et al.*, 2009; Lu *et al.*, 2009; Urrutia *et al.*, 2013). Schwann cells and microglia in the CNS are partially immune competent and capable of secreting pro-inflammatory cytokines (Bergsteinsdottir *et al.*, 1991; Mathey *et al.*, 1999; Kaneko *et al.*, 2012). Interestingly, Schwann cells in the DRG of FRDA patients were the most affected by frataxin deficiency (Koeppen *et al.*, 2009). In fact, autopsy samples revealed that Schwann cells were fewer and smaller in FRDA patients relative to normal tissue, with inappropriate myelination of thin fibres (Koeppen *et al.*, 2009).

In a complimentary study by Lu and colleagues, the effects of frataxin depletion by siRNA on DRG neurons, Schwann cells and oligodendrocytes were investigated (Lu *et al.*, 2009). The comparison of neural cell types revealed that

frataxin deficiency inhibits proliferation in Schwann cells and oligodendrocytes, but had no effect on DRG neurons (Lu *et al.*, 2009). Furthermore, a significant decrease in viability of Schwann cells, but not oligodendrocytes, was found to result from strong activation of an inflammatory response leading to cell death (Lu *et al.*, 2009). In fact, treatment of frataxin-deficient Schwann cells with anti-inflammatory and anti-apoptotic drugs restored cell viability (Lu *et al.*, 2009). Thus, the observed inflammatory response and subsequent cell death of frataxin-deficient Schwann cells, which are in intimate contact with DRG neurons, could lead to demyelination and axon degeneration. It is notable that inflammation can be induced by iron accumulation and oxidative stress (Michalec *et al.*, 2002; Chodaczek *et al.*, 2007), which are consequences of frataxin deficiency. This represents one possible mechanism leading to neural degeneration (Urrutia *et al.*, 2013; Wang *et al.*, 2013). Interestingly, examination of heart samples from FRDA patients exhibited evidence of myocarditis with leukocyte infiltrations (Hejtmancik *et al.*, 1949; Michael *et al.*, 2006) and also presents a case for the role of inflammation in the progression of cardiomyopathy in FRDA.

Despite tissue-specific variations in frataxin-mediated iron handling, the dysregulation of iron metabolism due to frataxin deficiency remains the most prominent feature. Efforts to mitigate the iron loading and treat the underlying cause have employed a wide range of agents, from antioxidants to iron chelators, with varying success. To date, there is no effective treatment for FRDA. However, there are copious therapeutic strategies aimed at preventing the debilitating effects of frataxin loss and ameliorating the subsequent symptoms of FRDA. These are discussed in detail in the following section.

## Targeting the mitochondrion: potential therapeutics for FRDA

### *Iron chelation*

The 'free' iron that accumulates in the redox-active mitochondrion is a dangerous consequence of frataxin deficiency as it has the potential to form toxic free radicals that can result in oxidant-mediated cell death (Armstrong *et al.*, 2010; Whitnall *et al.*, 2012). Therefore, the use of iron chelators to eliminate the excess iron is a logical therapeutic option. *In vitro* examination of the therapeutic potential of iron chelators has shown that these compounds rescue cells from iron toxicity (Wong *et al.*, 1999) and restore aconitase activity as well as mitochondrial membrane and redox potentials (Kakhlon *et al.*, 2008). However, the generalized action of some of the chelators studied and the potentially detrimental effects thereof were seen as a shortcoming of this treatment. This culminated in the design and assessment of specifically targeted chelators directed to the mitochondrion (Richardson, 2003). Indeed, the concerted action of the iron chelator desferrioxamine combined with a mitochondrion-permeate ligand, pyridoxal isonicotinoyl hydrazone, *in vivo*, prevented cardiac iron loading and limited hypertrophy in the MCK-*Fxn*-KO without overt cardiac depletion of iron or toxicity (Whitnall *et al.*, 2008).

Deferiprone (DFP) is another small MW ligand that is able to cross both cellular and subcellular membranes (Kakhlon *et al.*, 2010) and is being tested in clinical trials (Boddaert *et al.*, 2007; Sohn *et al.*, 2008). One study, which assessed the utility of this compound in the brain, showed DFP successfully crossed the blood–brain barrier and chelated the iron accumulated in the dentate nucleus of FRDA patients (Boddaert *et al.*, 2007). However, concerns have been raised from studies in cell culture with DFP where chelation-impaired aconitase activity preceded a reduction in cellular proliferation (Goncalves *et al.*, 2008). Hence, treatment with these agents needs to be carefully monitored to ensure that excessive chelation does not lead to detrimental side effects.

### *Antioxidants*

The pathology of FRDA is strikingly similar to that of another metabolic disorder, namely, ataxia with vitamin E deficiency, which is treated with vitamin E supplements (Palau and Espinos, 2006). Taken together with the evidence of oxidative stress in FRDA pathology (Armstrong *et al.*, 2010), it is not surprising that antioxidants have been considered as a therapy. A wide range of antioxidants have been tested in clinical trials, including vitamin E, with varying efficacy (Kearney *et al.*, 2012; Lynch *et al.*, 2012; Mariotti *et al.*, 2012). Currently, the ubiquinone analogue, idebenone, is being tested in clinical trials because it is reported to act as a free radical scavenger and can potentially restore mitochondrial ATP production (Erb *et al.*, 2012). While studies have suggested that idebenone alleviates cardiac hypertrophy (Rustin *et al.*, 1999), the most frequent heart abnormality in FRDA, results from clinical trials have been mixed (Artuch *et al.*, 2002; Rustin *et al.*, 2002; Lagedrost *et al.*, 2011).

As an alternative to antioxidant supplementation, the pharmacological stimulation of antioxidant defences has been explored. The PPAR-γ agonist, pioglitazone, induces the expression of enzymes involved in mitochondrial metabolism, including SOD (Zou *et al.*, 2013). Testing in a 'knockin-knockout' mouse model of FRDA and patient fibroblasts showed pioglitazone up-regulated SOD2 specifically in the cerebellum and spinal cord (Marmolino *et al.*, 2010). The therapeutic use of pioglitazone is currently being evaluated in clinical trials.

### *Epigenetic therapy*

The transcriptional silencing of the *Fxn* gene by GAA repeat expansion is accompanied by histone hypoacetylation, consistent with a heterochromatin-mediated repression mechanism (Herman *et al.*, 2006). In fact, Herman and colleagues were the first to demonstrate the use of an epigenetic therapy to treat FRDA. This lab synthesized a class of histone deacetylase inhibitors that reversed frataxin silencing in primary lymphocytes from FRDA patients (Herman *et al.*, 2006). This novel treatment has since been tested in a mouse model of FRDA where it not only raised frataxin protein expression in the brain, but also increased mitochondrial aconitase activity and improved neuronal pathology in the DRG (Sandi *et al.*, 2011). These promising compounds are currently being evaluated in clinical trials.

Other epigenetic changes have since been proposed as mechanisms of gene silencing and have led to innovative new treatments, such as DNA-demethylating agents and antigene-RNA-based therapy, termed 'epigentic therapy'. An

in-depth description of the proposed epigenetic changes in FRDA was beyond the scope of the current article and is described in detail in a recent review on the subject (Sandi *et al.*, 2013).

## Summary

Frataxin is a crucial mitochondrial protein and its iron-binding capabilities appear essential to many biological processes. Given that tissues in the body have different metabolic requirements, the observed differences in iron metabolism in frataxin-deficient tissues are not surprising. It is now known that altered iron metabolism in the frataxin-deficient heart leads to mitochondrial iron accumulation and autophagy, while the pathological involvement of iron in neurodegeneration remains controversial. Currently, evidence of iron-mediated neurodegeneration is limited to oxidative stress and neuroinflammation leading to apoptosis. The investigation of these tissue-specific differences can further our understanding of frataxin's function in mitochondrial and cellular metabolism, and it is imperative to the development of more effective treatments.

## Conflict of interest

None.

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