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Iron-Sulfur Cluster Synthesis, Iron Homeostasis and Oxidative Stress in Friedreich Ataxia

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Abstract

Friedreich ataxia (FRDA) is an autosomal recessive, multi-systemic degenerative disease that results from reduced synthesis of the mitochondrial protein frataxin. Frataxin has been intensely studied since its deficiency was linked to FRDA in 1996. The defining properties of frataxin—(i) the ability to bind iron, (ii) the ability to interact with, and donate iron to, other iron-binding proteins, and (iii) the ability to oligomerize, store iron and control iron redox chemistry—have been extensively characterized with different frataxin orthologues and their interacting protein partners. This very large body of biochemical and structural data [reviewed in (Bencze et al., 2006)] supports equally extensive biological evidence that frataxin is critical for mitochondrial iron metabolism and overall cellular iron homeostasis and antioxidant protection [reviewed in (Wilson, 2006)]. However, the precise biological role of frataxin remains a matter of debate. Here, we review seminal and recent data that strongly link frataxin to the synthesis of iron-sulfur cluster cofactors (ISC), as well as controversial data that nevertheless link frataxin to additional iron-related processes. Finally, we discuss how defects in ISC synthesis could be a major (although likely not unique) contributor to the pathophysiology of FRDA via (i) loss of ISC-dependent enzymes, (ii) mitochondrial and cellular iron dysregulation, and (iii) enhanced iron-mediated oxidative stress.

Keywords

Friedreich ataxia; frataxin; mitochondria; iron-sulfur clusters; heme; oxidative stress; anti-oxidants; iron-chelators

FRIEDREICH ATAXIA

Friedreich ataxia (FRDA; OMIM number 229300) is the most common genetically inherited ataxia with an estimated incidence of 1:40,000 [for recent reviews of FRDA clinical and pathological aspects see (Koeppen, 2011; Pandolfo, 2009)]. Patients are largely

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CONFLICT OF INTEREST

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asymptomatic during the first 5–10 years of life until they develop a progressive loss of movement coordination and other deficits, including cardiac disease, muscle weakness, skeletal deformities, vision and hearing impairment, and diabetes. Often as young as teenagers, patients become wheelchair-bound and unable to perform daily activities independently, with cardiac failure representing a frequent cause of death as early as the second decade of life. The majority of patients carry intronic GAA triplet repeat expansions that cause transcriptional silencing of the FRDA gene via different mechanisms [reviewed in (Gottesfeld, 2007; Wells, 2008)], ultimately leading to reduced levels of frataxin, a mitochondrial protein encoded by the FRDA gene (Campuzano et al., 1996). Although frataxin is ubiquitously expressed (Campuzano et al., 1997), frataxin deficiency affects primarily certain regions of the central and peripheral nervous systems as well as the heart, skeleton, and endocrine pancreas, which accounts for the major clinical and pathological aspects of FRDA (Koeppen, 2011). The underlying factors responsible for this tissue-specific susceptibility remain undefined. Currently, there is no cure for FRDA but several therapeutics are in preclinical or clinical development [reviewed in (Wilson, 2012)].

DEFICIENT ISC SYNTHESIS AND THE PATHOPHYSIOLOGY OF FRDA

ISC are essential and highly adaptable enzyme co-factors that can participate in a wide range of cellular reactions and processes [reviewed in (Johnson et al., 2005)]. In eukaryotic cells, ISC-containing enzymes have been identified in the mitochondria (*e.g.* Krebs's cycle and electron transport chain), the cytoplasm (*e.g.* ribosome biogenesis), and the nucleus (*e.g.* DNA synthesis and repair mechanisms) [reviewed in (Lill et al., 2012; Ye and Rouault, 2010)]. Yeast and animal cells synthesize ISC primarily in the mitochondrial matrix (Lill et al., 2012; Muhlenhoff et al., 2002; Schilke et al., 1999), while ISC synthesis in other cellular compartments depends on as yet undefined factors or signals that are available only when ISC synthesis is functional in the mitochondria (Gerber et al., 2004; Kispal et al., 1999; Lill et al., 2012; Martelli et al., 2007; Ponderre et al., 2006; Ye and Rouault, 2010). In addition, it is well established that defects in mitochondrial ISC synthesis are associated with a rapid increase in cellular iron uptake and a redistribution of iron within the cell, leading to mitochondrial iron accumulation and cytoplasmic iron depletion (Babcock et al., 1997; Chen et al., 2004; Knight et al., 1998; Li et al., 1999; Whitnall et al., 2008) [reviewed in (Rouault and Tong, 2005)]. Thus, mitochondrial ISC synthesis is key to the maintenance of numerous vital enzymatic activities as well as the maintenance of cellular iron homeostasis. Not surprisingly, a complete loss of mitochondrial ISC synthesis is incompatible with life (Cossee et al., 2000; Kispal et al., 2005; Lill and Kispal, 2000). Moreover, even partial defects in mitochondrial ISC synthesis can lead to severe phenotypes typically dominated by mitochondrial abnormalities, including impaired energy metabolism, oxidative damage and loss of mitochondrial DNA integrity (Karthikeyan et al., 2003; Knight et al., 1998; Li et al., 1999). Concomitant extra-mitochondrial abnormalities include multiple ISC-containing enzyme deficiencies that lead to nuclear genome instability (Veatch et al., 2009), impaired ribosome biogenesis (Kispal et al., 2005), impaired amino acid metabolism (Kispal et al., 1999), and other effects [reviewed in (Ye and Rouault, 2010)].

The pathophysiology of FRDA was linked to defects in ISC synthesis early on, when Rustin and Colleagues first reported a deficient activity of the ISC-containing subunits of mitochondrial respiratory complexes I, II and III in the endomyocardial biopsy of two FRDA patients (Rotig et al., 1997). Mitochondrial aconitase, a [4Fe-4S] enzyme in the Krebs's Cycle, was also found deficient. Similar multiple ISC-dependent enzyme deficiencies were observed upon deletion of the *YFH1* gene (encoding the yeast frataxin homologue, Yfh1) in *S. cerevisiae* (Foury, 1999; Rotig et al., 1997). Subsequent studies showed that the lack of human or mouse frataxin caused early defects in ISC-dependent enzymes, which preceded other mitochondrial alterations such as mitochondrial iron

accumulation (Puccio et al., 2001; Stehling et al., 2004). Since these initial studies, eukaryotic frataxin orthologues have been universally shown to stimulate ISC synthesis *in vitro* (Cook et al., 2010; Gakh et al., 2010; Li et al., 2009; Tsai and Barondeau; Yoon and Cowan, 2003) [prokaryotic frataxin was recently shown to inhibit ISC synthesis under certain conditions (Adinolfi et al., 2009) that will be discussed later]. Thus, a large body of data supports early and recent proposals that the complex phenotypes associated with frataxin deficiency in humans and other eukaryotes reflect at least in part an impaired ability to synthesize ISC (Foury, 1999; Pandolfo, 2006; Rotig et al., 1997; Wilson, 2006). Indeed, frataxin depletion is consistently associated with multiple ISC enzyme deficiencies in mitochondria and throughout the cell (Foury, 1999; Martelli et al., 2007). These deficiencies are accompanied by global dysregulation of cellular iron homeostasis that leads to mitochondrial iron accumulation and cytosolic iron depletion (Babcock et al., 1997; Foury and Cazzalini, 1997; Huang et al., 2009; Whitnall et al., 2008). Although mitochondrial iron accumulation is inconsistently observed in FRDA cell lines (Delatycki et al., 1999; Wong et al., 1999), the state of mitochondrial iron in these cells is clearly altered independent of a net increase in mitochondrial iron content (Wong et al., 1999). Similarly, total iron content was not significantly higher in the left ventricle of FRDA patients compared to controls (Michael et al., 2006), however, iron deposits were observed inside FRDA cardiomyocytes (Koeppen, 2011; Lamarche et al., 1980; Michael et al., 2006) and electron-dense inclusions inside the mitochondria of those cardiomyocytes, which appeared to result from accumulation of iron-loaded mitochondrial ferritin (Koeppen, 2011). Furthermore, total iron content was not significantly increased in FRDA dorsal root ganglia, however, the presence of increased ferritin immunoreactivity in satellite cells of dorsal root ganglia neurons suggested regional iron accumulation and the possibility that both satellite cells and dorsal root ganglia neurons are affected by iron dysregulation in FRDA (Koeppen et al., 2009). Magnetic resonance imaging suggested iron accumulation in the nucleus dentatus of the cerebellum of young patients, indicating that iron dysregulation occurs early in FRDA in select regions of the nervous system (Boddaert et al., 2007; Velasco-Sanchez et al., 2011).

The mechanisms leading to mitochondrial iron dysregulation and regional iron accumulations in FRDA are complex. It is clear that the processes of mitochondrial iron accumulation and cytoplasmic iron depletion are linked to each other and are transcriptionally regulated (Chen et al., 2004; Foury and Talibi, 2001; Huang et al., 2009). Although the underlying signals are not completely understood, deficient ISC synthesis is a probable initiating event. In *S. cerevisiae*, mitochondrial iron accumulation is a hallmark of defects in ISC synthesis (Knight et al., 1998; Li et al., 1999). Similarly, the lack of human or mouse frataxin caused early defects in ISC enzymes prior to detectable mitochondrial iron accumulation (Puccio et al., 2001; Stehling et al., 2004). Moreover, frataxin depletion in mouse heart was associated with (i) global down-regulation of molecules involved not only in mitochondrial ISC synthesis but also iron storage and heme synthesis, and (ii) changes in the expression of molecules involved in cellular and mitochondrial iron uptake; these effects could synergistically contribute to mitochondrial iron accumulation in mammalian cells (Huang et al., 2009). While the accumulated iron is progressively converted to an oxidized and insoluble or ferritin-bound form (Knight et al., 1998; Koeppen, 2011; Seguin et al., 2010), studies in yeast and human cells indicate that iron dysregulation is an early effect of frataxin depletion, which increases the fraction of labile redox-active iron inside mitochondria (Karthikeyan et al., 2003; Wong et al., 1999). The combination of deficits in aconitase and respiratory chain activities with mitochondrial iron dysregulation is believed to result in progressive impairment of energy metabolism and accumulation of oxidative damage (Pandolfo, 2006; Wilson, 2006) (discussed extensively later). Importantly, in multicellular organisms these mitochondrial alterations in turn lead to changes in various cellular pathways involved in antioxidant, metabolic, and inflammatory responses, which most likely amplify the pathophysiology of FRDA and promote disease progression in

susceptible tissues (Coppola et al., 2009; Lu et al., 2009; Paupe et al., 2009; Pianese et al., 2002; Sparaco et al., 2009; Wagner et al., 2012) [reviewed in (Pandolfo, 2012)].

THE ROLE OF FRATAXIN IN IRON-SULFUR CLUSTER SYNTHESIS

Mitochondrial ISC Synthesis: From Individual Components to Macromolecular Machineries

The mitochondrial proteins known to be involved in ISC synthesis are highly conserved from yeast to humans, and many were actually inherited from bacteria (Fontecave and Ollagnier-de-Choudens, 2008). They include early components that provide the building blocks to assemble new [2Fe-2S] and [4Fe-4S] clusters (elemental iron and sulfur, and reducing equivalents), and late components that transfer these clusters to mitochondrial apo-proteins [reviewed in (Lill et al., 2012; Ye and Rouault, 2010)]. Additional components are thought to transport ISC precursors to the cytoplasm for the biogenesis of cytoplasmic and nuclear ISC-containing enzymes [reviewed in (Ye and Rouault, 2010)]. The general roles played by individual components have been assessed through genetic and biochemical studies in prokaryotes and *S. cerevisiae* and confirmed for the corresponding human components [reviewed in (Fontecave and Ollagnier-de-Choudens, 2008; Lill, 2009; Ye and Rouault, 2010)]. According to current models, synthesis of [2Fe-2S] clusters in the mitochondrial matrix is initiated on a scaffold protein (yeast Isu1/mammalian ISCU) and involves (i) a cysteine desulfurase (yeast Nfs1/mammalian NFS1, stabilized by a small binding partner, Isd11/ISD11) that serves as the sulfur donor; (ii) frataxin (yeast Yfh1/mammalian FXN) that serves as the iron donor; and (iii) the electron donor chain formed by ferredoxin reductase and ferredoxin (yeast Arh1-Yah1/mammalian FDXR-FDX2). Subsequently, release of ISC from Isu1/ISCU and their transfer to apo-enzymes are assisted by various chaperone and co-chaperones, proteins needed to reduce cysteine residues on apo-enzymes, and ISC carrier proteins (Lill et al., 2012; Ye and Rouault, 2010). In these models, the ISC scaffold protein, Isu1/ISCU, is thought to cycle between the early and the late components. However, recent studies indicate that ISC synthesis occurs on stable complexes built-up of at least four components: Nfs1, Isd11, Isu1 and Yfh1 in yeast; NFS1, ISD11, ISCU and FXN in humans. These complexes have been reconstituted *in vitro* with purified proteins (Gakh et al., 2010; Li et al., 2009; Schmucker et al., 2011; Tsai and Barondeau, 2010) and have also been reconstituted with native proteins from yeast or human cells (Gakh et al., 2010; Gerber et al., 2003; Li et al., 2009; Schmucker et al., 2011). In addition, multiple molecular chaperones are associated with human frataxin in cells (Shan and Cortopassi, 2011; Shan et al., 2007). These findings together point to the existence of complex macromolecular machineries that eukaryotic cells have developed to perform ISC synthesis. The vital functions controlled by these machineries (mitochondrial energy metabolism, cellular iron homeostasis and nuclear genome stability, among others) justify the substantial effort that many different laboratories are investing toward understanding their structures and mechanisms.

The Yeast Frataxin Homologue (Yfh1) and ISC Synthesis in *S. cerevisiae*

Early studies in yeast begun to shed light on the interactions among eukaryotic ISC synthesis components. Supplementation of mitochondrial extract with Fe^{2+} allowed the co-isolation, by use of pull-down or co-immunoprecipitation assays, of Yfh1 and Isu1 together with Nfs1 and Isd11 (Foury et al., 2007; Gerber et al., 2003; Wang and Craig, 2008). Furthermore, pre-incubation of purified Yfh1 monomer with Fe^{2+} enabled Isu1 to be pulled-down with Yfh1 in the absence of other proteins (Wang and Craig, 2008). These studies led to the view that iron delivery for yeast ISC synthesis involved direct contacts between iron-loaded monomeric Yfh1 and Isu1 (Foury et al., 2007; Gerber et al., 2003; Wang and Craig, 2008). A subsequent study with purified proteins *in vitro* demonstrated that the Yfh1-Isu1 interaction is Fe^{2+} -dependent and involves the iron-binding surface of Yfh1, suggesting that

Yfh1-Isu1 contacts allow the transfer of iron to Isu1 (Cook et al., 2010). This large body of work focused primarily on the interaction between monomeric Yfh1 and Isu1. Because Yfh1 monomer was known to oligomerize in the presence of Fe^{2+} (Adamec et al., 2000; Wang and Craig, 2008) (Fig. 1), we explored the possibility that Isu1 might interact with oligomeric Yfh1. Using purified proteins and biochemical approaches, we showed that oligomeric Yfh1 can drive the assembly of the core machinery responsible for the initial step of mitochondrial ISC synthesis (Li et al., 2009). Specifically, our work showed that (i) Yfh1 and Nfs1-Isd11 directly bind to each other; (ii) this interaction is mediated at least in part by direct Yfh1-Isd11 contacts; (iii) Yfh1 and Nfs1-Isd11 can independently or simultaneously bind to Isu1; (iv) binding of Yfh1 to Nfs1-Isd11 or Isu1 requires oligomerization of Yfh1 and can occur in an iron-independent manner; (v) stronger contacts are formed when Yfh1 oligomerization is coupled with Fe^{2+} binding and oxidation; (vi) the ability to store Fe^{3+} enables oligomeric Yfh1 to release iron only when both Isu1 and elemental sulfur are available (Li et al., 2009). We also showed that in *S. cerevisiae* interactions between native Yfh1 and Isu1 or Nfs1-Isd11 are enhanced by conditions known to stimulate Yfh1 oligomerization *in vivo*, and inhibited by mutations in residues required for the iron-dependent oligomerization of Yfh1 (Li et al., 2009). We proposed that the iron-dependent oligomerization of Yfh1 promotes the formation of stable complexes in which Yfh1 and Nfs1-Isd11 can interact with each other and with Isu1 *simultaneously*, which leads to the productive release of iron and sulfur for ISC assembly on Isu1. A similar mechanism had already been proposed for prokaryotic ISC assembly on IscU (Layer et al., 2006). These two proposals are attractive because stable multi-component complexes would serve two important purposes: (i) to enable the protected delivery of potentially toxic iron and sulfur to ISC scaffold proteins, and (ii) to enable the protected transfer of oxygen-labile [2Fe-2S] and [4Fe-4S] clusters from scaffold proteins to apo-enzymes.

In contrast to the studies summarized above, one study showed that under anaerobic conditions, which prevent Fe^{2+} oxidation, the Fe^{2+} -loaded CyaY monomer inhibited ISC synthesis (Adinolfi et al., 2009). It was later shown that structural differences between the bacterial and mammalian cysteine desulfurases allow both CyaY or human frataxin to function as a repressor or a catalyst of ISC synthesis (Bridwell-Rabb et al., 2012). In addition, whereas Fe^{2+} -loaded monomeric CyaY inhibited ISC synthesis (Adinolfi et al., 2009), Fe^{3+} -loaded oligomeric CyaY stimulated the process (Layer et al., 2006). Recent structural studies have demonstrated complexes in which a dimer of *E. coli* NFS1 binds one ISCU monomer at each end (Shi et al., 2010) and CyaY monomer binds in a pocket between the two proteins (Prischi et al., 2010). It will be interesting to see how the protein composition and stoichiometry, protein-protein interaction surfaces and overall architecture of these complexes compare to those of the much larger complexes formed by oligomeric CyaY or Yfh1, which remain to be elucidated. Why would *E. coli* and *S. cerevisiae* cells need different complexes involving either monomeric or oligomeric frataxin? Aerobic conditions lead to iron-dependent oligomerization of both Yfh1 and CyaY (Adamec et al., 2000; Layer et al., 2006; Wang and Craig, 2008), and anaerobic conditions are required to stabilize the Fe^{2+} -loaded Yfh1 or CyaY monomer (Adinolfi et al., 2009; Cook et al., 2006; Cook et al., 2010). Furthermore, Fe^{3+} -loaded oligomeric Yfh1 or CyaY stimulate ISC synthesis, whereas Fe^{2+} -loaded monomeric Yfh1 stimulates ISC synthesis only minimally and Fe^{2+} -loaded monomeric CyaY inhibits it completely (Adinolfi et al., 2009; Layer et al., 2006; Li et al., 2009). Thus, the facultative anaerobes *E. coli* and *S. cerevisiae* may use monomeric frataxin to limit ISC synthesis during anaerobic growth, and may switch to oligomeric frataxin to increase the rate of ISC synthesis during aerobic growth, when oxidative damage greatly accelerates the turnover of ISC-containing enzymes (Fridovitch, 1995). As we will discuss below, human frataxin has moved away from iron- and oxygen-dependent oligomerization and has instead evolved a new mechanism involving the protein's N-terminal region.

Oligomeric Yfh1: Iron-Donor or Iron-Storage? (Or Both?)

While the biochemical data reviewed above demonstrate that Yfh1 can donate iron for ISC synthesis *in vitro*, this point remains to be conclusively demonstrated *in vivo*. The task is not simple and will require new technology to measure ISC assembly on Isu1 in living cells. Meanwhile, it is intriguing to consider that of the four core proteins required for the initial step of yeast ISC assembly (Nfs1, Isd11, Isu1 and Yfh1), only Yfh1 is not essential (Duby et al., 2002). Each of the other three proteins (Isu1 in conjunction with its orthologue Isu2) is essential for ISC synthesis, hence, for yeast viability (Lill et al., 2012). The fact that some degree of ISC synthesis can occur in the absence of Yfh1 (Duby et al., 2002) might be explained by the existence of additional iron donors, which would not be surprising given that at least two different iron donors participate in bacterial ISC synthesis (Ding et al., 2007). In a recent report, a single amino acid substitution in Isu1 was sufficient to rescue many deficient functions in Yfh1-depleted yeast (Yoon et al., 2011). In the presence of the mutant Isu1, the efficiency of ISC synthesis was markedly increased compared to Yfh1-depleted cells. Interestingly, the amino acid substitution in the mutant Isu1 was found to be normally present in bacterial orthologues. The Authors therefore suggested that unique features of the bacterial ISC assembly machinery might enable the mutant Isu1 to bypass the need for Yfh1 (Yoon et al., 2011), which might perhaps include the ability of the mutant Isu1 to interact with an alternative as yet unknown iron-donor.

An early study reported that the iron-induced oligomerization of Yfh1 was dispensable in *S. cerevisiae* based on the observation that an oligomerization-deficient form of Yfh1, unable to store iron *in vitro*, was phenotypically silent *in vivo* (Aloria et al., 2004). It was later shown that mutations that inhibit Yfh1 oligomerization are asymptomatic only in unstressed cells, and otherwise enhance the sensitivity of yeast cells to oxidative stress, shortening chronological life span and causing lethality when combined with lack of copper-zinc superoxide dismutase (Gakh et al., 2006). Nevertheless, we still have a limited understanding of how the monomeric and oligomeric forms of Yfh1 are regulated in *S. cerevisiae*. Lesuisse and Colleagues were able to carefully manipulate Yfh1 levels in *S. cerevisiae* and showed that while Yfh1 trimer was the predominant species present in wild-type cells, larger oligomers formed in Yfh1-overproducing cells (Seguin et al., 2009). Similarly, our laboratory found that Yfh1 oligomerization *in vivo* was induced by overexpression of wild type Yfh1 monomer and also by mitochondrial iron uptake, mutations that stabilize Yfh1 trimer, or heat stress (Gakh et al., 2008). In our experiments, only monomeric Yfh1 could be detected in unstressed yeast cells when mitochondrial iron uptake was kept at a steady, low nanomolar level. However, a rapid increase in mitochondrial iron uptake induced stepwise assembly of Yfh1 into trimer and higher-order oligomers, and Yfh1 oligomerization correlated with reduced oxidative damage and higher levels of aconitase activity, suggesting that oligomeric Yfh1 was able to simultaneously promote ISC synthesis and oxidative stress tolerance (Gakh et al., 2008). Lesuisse and Colleagues observed that overexpression of *YFHI* resulted in an increased level of Yfh1 oligomerization; however, the shift in Yfh1 distribution analyzed by gel filtration was not accompanied by a shift in iron distribution, suggesting that iron was not stored in the Yfh1 oligomers (Seguin et al., 2009). Under these conditions, they too observed increased oxidative stress tolerance; however, ISC synthesis was significantly reduced while heme synthesis was enhanced. They further analyzed the iron accumulated in the mitochondria of ISC synthesis mutants in the presence of Yfh1, and concluded that Yfh1 did not store iron *in vivo* at least under their experimental conditions (Seguin et al., 2010). On the other hand, we found that under growth conditions that resulted in a rapid >100 fold increase in mitochondrial iron uptake, Yfh1 formed homopolymers larger than 600 kDa that were peripherally associated with mitochondrial membranes and were barely detected in the soluble mitochondrial fraction (Gakh et al., 2008). These polymers co-migrated with iron on

native PAGE; however, attempts to directly analyze their composition failed because of their limited solubility (Gakh et al., 2008). These data together indicate that additional work is needed to define if, and under what conditions, Yfh1 functions as an iron-storage molecule *in vivo*. Alternatively, Lesuisse and Colleagues have proposed a model in which oligomeric Yfh1 associates only transiently with iron to catalyze biological processes such as ISC or heme synthesis (Seguin et al., 2010).

Human Frataxin (FXN) Is a Multifunctional Component of ISC Synthesis

Human frataxin (FXN) is initially synthesized in the cytoplasm as a precursor polypeptide (FXN^{1–210}) (Campuzano et al., 1996) that is imported to the mitochondrial matrix and processed step-wise to shorter isoforms, FXN^{42–210}, FXN^{56–210}, FXN^{81–210}, and FXN^{78–210} (Cavadini et al., 2000; Condo et al., 2007; Schmucker et al., 2008). FXN^{42–210} and FXN^{81–210} are the most abundant isoforms both in normal individuals as well as FRDA carriers and patients (Condo et al., 2007; Gakh et al., 2010; Schmucker et al., 2008), and interestingly, they have strikingly different biochemical properties that have been characterized by independent groups (Table 1): (i) FXN^{42–210} forms stable oligomers, while FXN^{81–210} is a stable monomer; (ii) oligomeric FXN^{42–210} forms stable complexes with NFS1-ISC11 in the absence or presence of ISCU, while monomeric FXN^{81–210} only binds to a pre-formed NFS1-ISC11-ISCU complex, and importantly, all of these interactions are iron-independent; (iii) in the presence of NFS1-ISC11 and ISCU, both isoforms stimulate the cysteine desulfurase activity of NFS1 and catalyze ISC synthesis; (iv) however, FXN^{81–210} can stimulate NFS1 activity and support ISC synthesis only under non-oxidizing conditions, whereas oligomeric FXN^{42–210} is equally able to activate NFS1 and to support ISC synthesis under non-oxidizing or oxidizing conditions; (v) formation of stable Fe²⁺-loaded FXN^{81–210} monomer requires anaerobic conditions (i.e. conditions that prevent iron oxidation) whereas oligomeric FXN^{42–210} is able to convert Fe²⁺ to a stable protein-bound mineral in the presence of oxygen (Table 1). It should be noted that the contacts formed by ISC11 within these complexes are still poorly defined due to the inability thus far to express the human protein separately from NFS1; however, it has been shown that *in vivo* human frataxin forms direct contacts with ISC11 (Shan et al., 2007), consistent with the observation that oligomeric Yfh1 forms direct contacts with isolated Isd11 *in vitro* (Li et al., 2009).

A working model to reconcile the functional differences between FXN^{81–210} and oligomeric FXN^{42–210} is discussed below and is illustrated in Fig. 2. FXN^{81–210} cannot store iron *in vitro* (Gakh et al., 2010) and therefore it is not expected to be able to store or transport iron in the mitochondrial matrix. Moreover, FXN^{81–210} forms stable contacts only with the complete NFS1-ISC11-ISCU complex *in vitro* (Gakh et al., 2010; Schmucker et al., 2011; Tsai and Barondeau, 2010), and it is recovered mostly as a free monomer in cell extracts (Gakh et al., 2010). Thus, in our model, FXN^{81–210} binds Fe²⁺ only transiently and supports basal levels of ISC synthesis through dynamic interactions with the NFS1-ISC11-ISCU complex (Fig. 2A). On the other hand, oligomeric FXN^{42–210} stores iron tightly *in vitro* (Gakh et al., 2010) and is expected to be able to store iron *in vivo*. In addition, oligomeric FXN^{42–210} forms stable complexes with NFS1-ISC11 both *in vitro* and in human cells (Gakh et al., 2010). Thus, in our model, stable complexes of oligomeric FXN^{42–210} and NFS1-ISC11 provide a mechanism to (i) bind and store iron when mitochondrial iron uptake exceeds the iron-binding capacity of FXN^{81–210}; and (ii) increase the rate of ISC synthesis above the rate allowed by the FXN^{81–210}-NFS1-ISC11-ISCU complex (Fig. 2B). This model raises interesting biological questions and implications for the pathophysiology of FRDA:

- I. It is well established that the FXN^{42–210} isoform is an obligate processing intermediate for the production of FXN^{81–210} (Cavadini et al., 2000; Condo et al., 2007; Schmucker et al., 2008). Interestingly, FXN^{81–210} was consistently more

abundant than FXN^{42–210} in normal heart or cerebellum, however, both forms were almost equally abundant in actively dividing human lymphoblasts or yeast cells (Gakh et al., 2010). Moreover, monomeric FXN^{42–210} interacted with the preformed NFS1-ISP11-ISCU complex just like monomeric FXN^{81–210} *in vitro* (Gakh et al., 2010; Schmucker et al., 2011); and actively dividing human lymphoblasts or yeast cells contained distributions of monomeric and oligomeric FXN^{42–210} (Gakh et al., 2010). These data indicate that in non-dividing tissues at steady state most FXN^{42–210} is normally cleaved to FXN^{81–210}, whereas during cell growth a significant proportion of FXN^{42–210} is not proteolytically processed. Inhibition of MPP by increasing levels of FXN^{81–210} (*i.e.* product inhibition) could be a simple mechanism to accumulate FXN^{42–210} during cell growth, and to enhance ISC synthesis by use of both monomeric and oligomeric FXN^{42–210}.

- II. What are the specific roles of each of the four known FXN isoforms under different metabolic conditions? In particular, what is the significance of FXN^{56–210}? Similar to FXN^{42–210}, this isoform can serve as an alternative processing intermediate for the generation of FXN^{81–210} (Schmucker et al., 2008) and can also oligomerize (O'Neill et al., 2005). Moreover, what is the significance of FXN^{78–210} and other ~14 kDa FXN products? These products result from cleavages in the region between serine 56 and serine 81 (Adinolfi et al., 2002; Babady et al., 2007; Schmucker et al., 2008; Yoon et al., 2007). *In vitro*, these cleavages were catalyzed by the proteolytic activity of dihydrolipoamide dehydrogenase (Babady et al., 2007) and were also induced by iron-mediated chemical cleavage (Yoon et al., 2007). Formation of FXN^{78–210} was also observed in human cells (Schmucker et al., 2008) and was shown to result from cleavage of either FXN^{42–210} or FXN^{56–210} in isolated mitochondria (Vaubel et al., 2011). However, the mechanism(s) responsible for formation of ~14 kDa FXN products *in vivo* are not yet defined. These mechanisms should be further studied in the context of FRDA cells where cleavage to ~14 kDa products could aggravate depletion of the longest FXN isoforms and contribute to FXN^{81–210} depletion.

ADDITIONAL FUNCTIONS OF FRATAXIN

In this paragraph we will review long proposed additional biological roles of frataxin that remain controversial.

Role of Frataxin in Repairing Oxidatively Inactivated [3Fe-4S] Aconitase

Mitochondrial aconitase requires a [4Fe-4S]²⁺ cluster for activity. However, the cluster is readily inactivated via superoxide-induced release of the solvent-exposed Fe- α yielding a [3Fe-4S]¹⁺ cluster (Vasquez-Vivar et al., 2000), such that the loss of mitochondrial aconitase activity is an intracellular indicator of oxidative damage (Fridovitch, 1995). Szweda and Colleagues initially found that mitochondrial aconitase was rapidly inactivated and reactivated when isolated rat cardiac mitochondria were treated with H₂O₂ (Bulteau et al., 2003). The presence of the aconitase substrate, citrate, diminished enzyme inactivation and was required for reactivation via an unknown mechanism (Bulteau et al., 2003). At that time both yeast and human frataxin had been shown to be able to donate iron to other iron-binding proteins and to also be able to detoxify Fe²⁺ *in vitro* [reviewed in (Bencze et al., 2006)]. Moreover, Kennedy and Colleagues had shown that Fe²⁺ ions are released from superoxide-induced oxidation of the aconitase [4Fe-4S]²⁺ cluster (Vasquez-Vivar et al., 2000). Thus, in a subsequent study, the Szweda, Kennedy and Isaya laboratories asked if frataxin might be the long sought-after molecule responsible for the redox-dependent modulation of aconitase activity. They showed that upon H₂O₂ treatment of intact mitochondria from rat heart or *S. cerevisiae*, frataxin was associated with endogenous

aconitase in a citrate-dependent manner, as determined by co-immunoprecipitation of the two proteins from solubilized mitochondria (Bulteau et al., 2004). In mitochondria from yeast cells expressing different levels of human frataxin, aconitase activity, measured before and after H₂O₂ treatment, was directly proportional to the levels of human frataxin. In the same study, oligomeric FXN^{56–210} was able to convert inactive [3Fe-4S]¹⁺ aconitase to the active [4Fe-4S]²⁺ enzyme *in vitro*. These findings led the Authors to propose that frataxin plays a critical role in protecting aconitase from pro-oxidant-induced inactivation and in facilitating aconitase reactivation (Bulteau et al., 2004). In a recent study, co-immunoprecipitation of frataxin with mitochondrial aconitase could not be reproduced (Schmucker et al., 2011). The Authors performed immunoprecipitation from HeLa cell mitochondrial extracts expressing a recombinant human frataxin with a C-terminal flag epitope as well as GST pull-down experiments from HeLa mitochondrial extracts using FXN^{81–210} fused to an N-terminal GST protein tag. Whereas ISCU, NFS1, ISD11 and MPP were co-immunoprecipitated or pulled-down with frataxin, mitochondrial aconitase was not, even when experiments were carried out under the conditions described in Bulteau et al. 2004. We think this discrepancy reflects our still limited understanding of the frataxin-aconitase interaction, which is probably much more complex than other readily observed frataxin interactions. In particular, evidence indicates that the presence of citrate within the mitochondrial matrix is required for the frataxin-aconitase interaction to occur and for inactivation of aconitase by H₂O₂ to be reversible (Bulteau et al., 2003; Bulteau et al., 2004). Nevertheless, the content and regulation of the mitochondrial citrate carrier and, therefore, concentration of citrate within the matrix differs between tissues and is profoundly affected by the nutritional state of the organism and the metabolic status of specific cells (Gnoni et al., 2009). Thus, while it is clear that frataxin is required for anti-oxidant protection of aconitase activity both in yeast and heart (Bulteau et al., 2004; Foury, 1999; Gakh et al., 2008; Rotig et al., 1997), the frataxin-aconitase interaction needs to be further characterized in different cellular and metabolic settings.

Role of Frataxin in Delivering Iron to Ferrochelatase for Heme Synthesis

A large body of evidence links frataxin to ferrochelatase and heme synthesis. *In vitro*, both yeast and human frataxin were found to be able to deliver Fe²⁺ to ferrochelatase, the enzyme that catalyzes the insertion of one Fe²⁺ atom into protoporphyrin IX in the final step of heme synthesis (Park et al., 2003; Yoon and Cowan, 2004). In Park et al., oligomeric Yfh1 enabled heme synthesis to proceed efficiently in the presence of atmospheric O₂ at neutral pH and without reducing agents, conditions that in the absence of Yfh1 promoted conversion of Fe²⁺ to Fe³⁺, which cannot be utilized by ferrochelatase [reviewed in (Al-Karadaghi et al., 2006)]. The transfer of Fe²⁺ from Yfh1 to ferrochelatase occurred in the presence of an excess of citrate, a physiological iron chelator, implying a protein-protein interaction (Park et al., 2003). Shortly thereafter, the ferrochelatase-binding surfaces of monomeric yeast and human frataxin were characterized by nuclear magnetic resonance spectroscopy; in both cases, ferrochelatase appeared to bind to a region of frataxin that includes its iron-binding surface (Bencze et al., 2007; He et al., 2004). These studies were corroborated by observations in *S. cerevisiae*, where Yfh1 was shown to protect bioavailable iron within mitochondria and to facilitate its use for heme synthesis (Zhang et al., 2005). Moreover, Yfh1-deficient yeast formed Zn²⁺-protoporphyrin, not heme, strongly suggesting that Yfh1 was required for iron utilization by ferrochelatase (Lesuisse et al., 2003) and/or that frataxin controls the type of metal ion that is delivered to ferrochelatase (Al-Karadaghi et al., 2006). On the other hand, Lill and Colleagues questioned a direct role of Yfh1 in heme synthesis; they showed that the heme deficit present in Yfh1-depleted yeast was due to reversible inhibition of ferrochelatase, although the nature of the inhibition could not be defined (Lange et al., 2004). Lesuisse and Colleagues later showed that yeast cells overexpressing Yfh1 displayed ISC synthesis deficits while their endogenous mitochondrial

iron was more available to ferrochelatase, which resulted in higher levels of heme synthesis compared to wild-type cells (Seguin et al., 2009). The fact that they could observe robust heme synthesis in spite of defective ISC synthesis led these Authors to conclude that frataxin has independent roles in both processes, and that the optimal conditions for these independent roles are different (Seguin et al., 2009). These results underscore the need to further elucidate the Yfh1-ferrochelatase interaction under appropriate metabolic conditions.

The situation is even more complex in mammalian cells. An early study reported that the levels of free erythrocyte protoporphyrin were elevated in FRDA patients, indicative of a heme synthesis defect (Morgan et al., 1979); more recently, a one-year prospective study of eight patients with FRDA revealed that six patients had elevated baseline levels of erythrocyte protoporphyrin IX, which were reduced by idebenone treatment in five patients (Buyse et al., 2003). Consistent with these reports, FRDA lymphoblasts were resistant to aminolevulinate-dependent toxicity, as expected if the heme pathway were inhibited (Schoenfeld et al., 2005). In addition, FRDA cells showed increased cellular protoporphyrin IX levels, reduced mitochondrial heme a and heme c levels, and reduced activity of cytochrome *c* oxidase, suggesting a biosynthetic block between protoporphyrin IX and heme a (Schoenfeld et al., 2005). In this study, ferrochelatase activities were similar in FRDA and normal cells, while Zn-chelatase activities were slightly elevated in FRDA cells (Schoenfeld et al., 2005), supporting once again the idea that frataxin deficiency alters the metal-specificity of ferrochelatase (Al-Karadaghi et al., 2006). The Authors suggested that frataxin deficiency causes defects late in the heme pathway in human cells. Whether this effect is direct or indirect is difficult to define because both ferrochelatase and adrenodoxin, another protein involved in heme synthesis, contain an ISC cofactor (Napoli et al., 2007), and ferrochelatase is unstable without sufficient ISC synthesis (Crooks et al., 2009). In addition, in cardiac tissue of conditional frataxin knockout mice, frataxin deficiency was found to cause down-regulation of several enzymes involved in heme synthesis including ferrochelatase (Huang et al., 2009). Moreover, a direct interaction between human frataxin and ferrochelatase could not be detected in HeLa cells (Schmucker et al., 2011), while the mitochondrial iron importer mitoferrin-1 was shown to form a complex with ferrochelatase in erythroid cells (Chen et al., 2010). Thus, it remains unclear how the frataxin-ferrochelatase interactions observed *in vitro* (Bencze et al., 2007; He et al., 2004; Park et al., 2003; Yoon and Cowan, 2004) correlate with the situation *in vivo*. On the other hand, it is clear that frataxin deficiency affects heme synthesis probably via multiple mechanisms. Cortopassi and Colleagues noted that ataxic symptoms occur in other diseases of heme deficiency, and therefore the heme defect observed in frataxin-deficient cells could be important to the pathophysiological process of FRDA and should be further investigated (Schoenfeld et al., 2005).

Role of Frataxin as a Mitochondrial Iron-Storage Molecule

The ability of oligomeric frataxin to detoxify and store iron has been extensively demonstrated with biochemical approaches ([reviewed in (Bencze et al., 2006)], and recently also with structural studies (Karlberg et al., 2006; Schagerlf, 2008) (Fig. 1). On the other hand, an iron-storage role for frataxin *in vivo* remains to be demonstrated as studies that have directly addressed this issue have yielded indirect (Gakh et al., 2008) or negative results (Seguin et al., 2010) as discussed earlier above. How can this dichotomy be reconciled? Although oligomeric yeast and human frataxin can be readily loaded with iron in the absence of other proteins *in vitro* (Adamec et al., 2000; Cavadini et al., 2002; Wang and Craig, 2008), it is unlikely that oligomeric frataxin would work like an iron chelator and scavenge iron indiscriminately *in vivo*. In fact, independent studies have shown that oligomeric yeast or human frataxin expressed in *E. coli* are unable to scavenge the accessible iron in those cells (Adamec et al., 2000; Cavadini et al., 2002; Karlberg et al.,

2006; Lu et al.). *In vivo* it is more likely that oligomeric frataxin acquires iron from specific iron transporters (Zhang et al., 2005; Zhang et al., 2006), and releases iron in the context of ISC synthesis and possibly other iron-dependent processes (Seguin et al., 2010). Thus, being able to demonstrate iron storage inside oligomeric frataxin *in vivo* will require a better understanding of how these pathways are physiologically regulated.

OXIDATIVE STRESS IN FRDA

Whether oxidative stress plays a role in the pathophysiology of FRDA is probably the most controversial topic in the scientific literature related to this disease. A number of studies suggest that an increase in oxidative stress is a direct and early consequence of frataxin deficiency, but several studies do not. We will review these studies below and try to reconcile their differences.

Studies that support a role for oxidative stress in FRDA

Several studies have reported the presence of markers of oxidative damage in association with partial or complete loss of frataxin, most notably, studies in humans (Emond et al., 2000; Schultz, 2000), mice with conditional frataxin knock-out in pancreatic β cells or hepatocytes (Ristow et al., 2003; Thierbach et al., 2005), mice with the GAA repeat expansion mutation (Al-Mahdawi et al., 2006) as well as *C. elegans* (Vazquez-Manrique et al., 2006) and yeast models (Karthikeyan et al., 2003). Increased ROS production of superoxide and hydrogen peroxide was demonstrated directly in FRDA lymphoblasts by use of fluorescent probes (Napoli et al., 2006), and DNA damage was detected in peripheral blood cells from FRDA patients (Haugen et al., 2010). In yeast, frataxin deficiency had little effect when cells were grown anaerobically, but a shift to aerobic growth resulted in loss of aconitase activity and oxidative protein damage (Bulteau et al., 2007).

Several studies have also consistently demonstrated that the complete or partial loss of frataxin enhances sensitivity to oxidative stress in a wide variety of model systems including yeast (Santos et al., 2010), *C. elegans* (Vazquez-Manrique et al., 2006), *Drosophila* (Runko et al., 2008), mouse (Al-Mahdawi et al., 2006), and FRDA patient cells (Wong et al., 1999). In these studies, frataxin deficiency enhanced the sensitivity to a variety of pro-oxidants, including H_2O_2 and other peroxides as well as iron. The mechanism underlying this enhanced sensitivity is complex. Superoxide dismutases are not induced in frataxin-deficient cells exposed to iron or oligomycin (Chantrel-Groussard et al., 2001; Jiralerspong et al., 2001). Similarly, superoxide dismutases are not upregulated in Yfh1-depleted yeast or in conditional frataxin knock-out mice (Chantrel-Groussard et al., 2001; Foury and Talibi, 2001; Seznec et al., 2005). As a decrease in total superoxide dismutase activity was also observed in mice lacking H-ferritin (Isler et al., 2002; Thompson et al., 2003), it is possible that iron dysregulation is responsible for the lack of superoxide dismutase induction in organisms lacking frataxin. Given that the superoxide dismutase reaction produces H_2O_2 , limiting superoxide dismutase induction perhaps helps in limiting iron-catalyzed Fenton chemistry in FRDA cells. This view is consistent with several observations that H_2O_2 detoxification (achieved via supplementation with selenium, reduced glutathione or glutathione peroxidase mimetics, or up-regulation of catalase) protects FRDA cells and Yfh1-depleted yeast from oxidant mediated damages (Jauslin et al., 2003; Jauslin et al., 2002; Kucej and Foury, 2003; Pastore et al., 2003). Similarly, in a *Drosophila* FRDA model, expression of enzymes that scavenge H_2O_2 suppressed the phenotype while expression of enzymes that scavenge superoxide had no effect (Anderson et al., 2008). Interestingly, in FRDA fibroblasts the cellular redox equilibrium was shifted toward more protein-bound glutathione, and oxidative stress resulted in the glutathionylation of actin and the impairment of cytoskeletal functions (Pastore et al., 2003). This in turn led to impaired activation of the Nrf2/KEAP1 signaling pathway, which regulates cellular response to oxidative stress (Paupe

et al., 2009). Such defect was rescued by a catalase mimetic, suggesting that H₂O₂ is responsible for impaired Nrf2 signaling in FRDA (Paupe et al., 2009). These studies together implicate an oxidative insult mediated by H₂O₂ via iron-catalyzed Fenton chemistry. Importantly, Fenton chemistry can be prevented not only via detoxification of H₂O₂, as in the studies described above, but also via sequestration of labile Fe²⁺. Indeed, overexpression of mitochondrial ferritin, which is an avid iron chelator (Corsi et al., 2002), restored respiratory function in frataxin-depleted yeast (Campanella et al., 2004), HeLa cells (Zanella et al., 2008) and FRDA fibroblasts (Campanella et al., 2009). Similarly, the iron chelator, deferoxamine, rescued FRDA fibroblasts from oxidant induced cell death (Wong et al., 1999); and the mitochondria-targeted chelator, deferiprone, improved mitochondrial energy metabolism in frataxin-deficient HEK-293 cells (Kakhlon et al., 2008).

In addition to the studies reviewed above, worth mentioning are early observations that FRDA is clinically similar to ataxia with vitamin E deficiency, an autosomal recessive disease resulting from defective transport of α -tocopherol, the most active form of vitamin E [reviewed in (Di Donato et al., 2010)]. Vitamin E is an important antioxidant, its primary and possibly only function being to prevent membrane lipid peroxidation (Traber and Atkinson, 2007). The common clinical features of FRDA, vitamin E deficiency and some mitochondrial pathologies led to an early proposal that a reduction in frataxin might result in oxidative damage (Campuzano et al., 1997). Indeed, treatment of FRDA patients with vitamin E and coenzyme Q improved mitochondrial energy metabolism and slowed disease progression (Calabrese et al., 2005).

The studies summarized above, together with the roles played by frataxin in ISC synthesis, heme synthesis, and aconitase activity suggest at least three, not mutually exclusive mechanisms that may lead to oxidative damage in FRDA: (1) a defect in ISC and heme synthesis leading to dysfunction of the electron transport chain and enhanced reactive oxygen species production (Rotig et al., 1997); (2) higher levels of mitochondrial labile iron resulting in Fenton chemistry (Isaya et al., 2004); (3) an impaired antioxidant response and inability of FRDA cells to cope with oxidative stress (Bayot et al., 2011). Consistent with each of these mechanisms, it has been recently shown that the anti-oxidant α -tocopheryl quinone (EPI-A0001) can potently prevent cell death in an FRDA cellular model upon sequential treatment with iron and an inhibitor of glutathione synthesis (Lynch et al., 2012). Moreover, a double-blind, randomized, placebo-controlled trial of 2 doses of EPI-A0001 in 31 adult FRDA patients demonstrated a dose-dependent improvement in neurologic function, as measured by the Friedreich Ataxia Rating Scale (Lynch et al., 2012). Promising results have also been obtained with the iron chelator deferiprone, believed to be a potent antioxidant because of its ability to reach extracellular and intracellular compartments (including mitochondria) in different tissues, and its ability to inhibit both iron- and copper-catalyzed free radical reactions [reviewed in (Kontoghiorghes, 2009)]. A six-month open-label single-arm study with deferiprone in conjunction with idebenone in nine adolescent FRDA patients showed selective iron chelation in the nucleus dentatus of the cerebellum as determined by magnetic resonance imaging, and apparently improved neuromotor function in the youngest patients (Boddaert et al., 2007). A 11-month open-labeled study in 20 patients further showed that combined therapy with idebenone and deferiprone improved significantly heart hypertrophy parameters and iron deposits in the dentate nucleus with a stabilizing effect in neurologic dysfunctions due to an improvement in the kinetic functions, although with a worsening of gait and posture scores (Velasco-Sanchez et al., 2011). A double-blind, randomized, placebo-controlled phase 2 trial of deferiprone for 80 FRDA patients was recently completed, and a preliminary report revealed no significant overall changes in Ataxia Scale scores, though improvements in posture, gait, and kinetic function were noted in some patients, and treatment was associated with a decrease in left ventricular mass [reviewed in (Wilson, 2012)].

Studies that do not support a role for oxidative stress in FRDA

Other reports have suggested that oxidative damage does not represent a relevant source of cell injury in FRDA. These reports are summarized below [along with any pertinent studies that suggest a more unified interpretation of the data]:

- i. In conditional knock-out mice with frataxin inactivation in cardiomyocytes or neurons, ISC-enzyme defects were detected prior to clinical symptoms, while oxidative stress markers were found only slightly increased or normal (Puccio et al., 2001; Seznec et al., 2004). [Oxidative damage was documented in mice with conditional frataxin knock-out in pancreatic β cells or hepatocytes (Ristow et al., 2003; Thierbach et al., 2005), and mice with the GAA repeat expansion mutation (Al-Mahdawi et al., 2006)].
- ii. A study reported that the iron accumulated in frataxin deficient yeast is largely in an oxidized and insoluble form and thus unable to participate in Fenton chemistry (Seguin et al., 2010). [Acute Yfh1 depletion led to a 6–10 fold increase in mitochondrial iron within five cell doublings (increasing 20-fold after a few more cell doublings) that was concomitant with considerable oxidative damage to mitochondrial proteins and preceded accumulation of mitochondrial DNA damage (Karthikeyan et al., 2003)].
- iii. Urinary 8-hydroxy-2'-deoxyguanosine, a marker of oxidative DNA damage, and urinary F(2)-isoprostanes, a marker of lipid peroxidation, were not increased in recent prospective studies of FRDA patients (Di Prospero et al., 2007; Myers et al., 2008; Schulz et al., 2009) [Nuclear and mitochondrial DNA damage was detected in peripheral blood cells from FRDA patients (Haugen et al., 2010)].
- iv. To date, no randomized clinical trial using idebenone has demonstrated significant improvement of neurological symptoms in FRDA patients (Kearney et al., 2012) [Idebenone has consistently shown a positive effect on left ventricular heart mass (Hausse et al., 2002) [reviewed in (Kearney et al., 2012; Tonon and Lodi, 2008)] and a dose-dependent effect on neurological functions was demonstrated in young patients (Tonon and Lodi, 2008); moreover, EPI-A0001, a more potent antioxidant, improved cellular and neurological functions (Lynch et al., 2012)].

Overview

A very large body of data, including pre-clinical and clinical data, support a role for oxidative damage in FRDA, regardless of whether this damage results from a net increase in the levels of pro-oxidants (i.e. labile Fe^{2+} and H_2O_2) or a reduction in the ability to tolerate normal levels of oxidative stress or both. On the other hand, (i) the inability to document oxidative damage in FRDA patients by use of urinary biomarkers, (ii) the inability to document oxidative damage in certain mouse models of FRDA, and (iii) the inconsistent and largely unsatisfactory efficacy of the anti-oxidant idebenone in clinical trials have called into question the role of oxidative stress in FRDA. When all of these data are considered together, it is clear that oxidative damage, although difficult to measure in humans and mice, is an important player in FRDA. Additional studies are warranted, especially studies to (i) understand the precise mechanism of action of idebenone and EPI-A0001, (ii) identify additional antioxidant molecules that specifically target the most relevant sites of FRDA pathology, and (iii) evaluate antioxidant and other therapies as early as possible in the disease course as strongly recommended by different Authors (Boddaert et al., 2007; Payne et al., 2011; Tonon and Lodi, 2008).

CONCLUSIONS

Multiple aspects of mitochondrial and cellular iron metabolism are affected by frataxin deficiency and the precise initiating events in FRDA pathophysiology are difficult to disentangle. We support a model in which deficient ISC synthesis is an early initiating event that, in turn, leads to increased levels of labile mitochondrial iron (enhanced by direct and indirect effects of frataxin deficiency on other iron-dependent processes) and oxidative stress. Thus, while ISC deficits may be an immediate consequence of frataxin deficiency, progressive accumulation of oxidative damage is a secondary event playing an important role in FRDA pathophysiology. This model is consistent with the natural history of FRDA, which is essentially silent for several years after birth until a threshold is reached and the disease becomes apparent, relentlessly progressive, and eventually fatal. An effective therapy for FRDA will ultimately require (i) combined treatments to increase frataxin expression and to limit oxidative damage and other biochemical consequences of frataxin deficiency, and (ii) strategies to diagnose and start treating patients as early as possible.

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ABBREVIATIONS

ISC	iron-sulfur clusters
FRDA	Friedreich ataxia
NFS1/Nfs1	human/yeast cysteine desulfurase
ISD11/Isd11	human/yeast NFS1-/Nfs1-binding protein
ISCU/Isu1	human/yeast scaffold protein
MPP	mitochondrial processing peptidase

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Figure 1. Structures of monomeric and oligomeric frataxin

The structure of yeast frataxin monomer (A) (PDB code 2GA5) (He et al., 2004) is compared to the structure of one subunit of yeast frataxin trimer (B) (PDB code 2FQL) (Karlberg et al., 2006). Note how both forms of the protein share the typical α/β sandwich fold of frataxin with two α helices packed against a five-strand β sheet. However, in the monomer helix $\alpha 2$ has seven turns, whereas in the trimer the first two turns of helix $\alpha 2$ unwind. This conformational change enables the interaction between the N-terminus of one subunit and the β sheet of another subunit to form trimer, as shown in (C). Helix $\alpha 2$ contains an extended acidic patch involved in iron binding (shown as green sticks). It is likely that iron binding to helix $\alpha 2$ disrupts the α -helical conformation, leading to partial unwinding and oligomerization. (D–E) Trimer is the building block of higher order oligomers; shown is the structure of yeast frataxin 24-mer in the apo (D) and iron-loaded (E) form (Schagerlöff, 2008; Soderberg et al., 2011). The structures of yeast frataxin trimer and 24-mer were

obtained with a mutant form of Yfh1, carrying an Y73A substitution; both *in vitro* and in yeast, the Y73A mutation promotes Yfh1 oligomerization without significantly altering other properties of Yfh1 (Gakh et al., 2008; Soderberg et al., 2011). The Y73A mutation most likely stabilizes a conformation required to initiate oligomerization, which in the wild-type protein is stabilized upon metal binding (Soderberg et al., 2011). Thus, the Yfh1-Y73A protein oligomerizes in an iron-independent manner (Gakh et al., 2008) enabling to uncouple oligomerization from iron binding and oxidation, which cannot be independently evaluated in wild-type Yfh1.

Figure 2. Proposed functions of human frataxin isoforms in ISC synthesis

The frataxin precursor is processed by MPP to FXN⁴²⁻²¹⁰, which can undergo further processing to FXN⁸¹⁻²¹⁰ or oligomerize. (A) Under basal conditions, most FXN⁴²⁻²¹⁰ is cleaved to FXN⁸¹⁻²¹⁰, which controls the labile Fe²⁺ pool and supports the bulk of [2Fe-2S] cluster assembly through dynamic interactions with the NFS1-ISC11/ISCU complex. Stable complexes of oligomeric frataxin and NFS1-ISC11 form in an iron-independent manner but may not significantly participate in ISC synthesis under these conditions. (B) Under conditions that require higher rates of ISC synthesis (e.g. rapidly dividing cells and/or increased mitochondrial biogenesis), a larger proportion of FXN⁴²⁻²¹⁰ is converted to oligomeric FXN⁴²⁻²¹⁰. Oligomeric FXN⁴²⁻²¹⁰ binds the iron that exceeds the iron-binding capacity of FXN⁸¹⁻²¹⁰, and stable complexes between oligomeric FXN⁴²⁻²¹⁰ and NFS1-ISC11 become an additional site of ISC assembly on ISCU. This augments the rate of ISC synthesis above the rate allowed by the FXN⁸¹⁻²¹⁰-NFS1-ISC11-ISCU complex. I, II, and

III: respiratory chain complex I, II and III; Aco; mitochondrial aconitase. Components involved in the transfer of [2Fe-2S] cluster from ISCU to Apo enzymes are not shown. This research was originally published in the Journal of Biological Chemistry. Gakh, O., et. al. Normal and Friedreich ataxia cells express different isoforms of frataxin with complementary roles in iron-sulfur cluster assembly. *J. Biol. Chem.* 2010; 285:38486-38501. © the American Society for Biochemistry and Molecular Biology.

Table 1Properties of human frataxin isoforms, FXN^{42–210} and FXN^{81–210}

	FXN ^{42–210}	FXN ^{81–210}
Biogenesis	Generated by cleavage of FXN ^{1–210} (precursor) by MPP (Cavadini et al., 2000; Schmucker et al., 2008).	Generated by cleavage of FXN ^{42–210} (or FXN ^{56–210}) by MPP (Condo et al., 2007; Schmucker et al., 2008).
Tissue prevalence <i>in vivo</i>	Abundant in heart, cerebellum and dividing lymphoblasts (Gakh et al., 2010).	Predominant isoform in fibroblasts (Gakh et al., 2010) and cells overexpressing FXN ^{1–210} (Condo et al., 2007; Schmucker et al., 2008).
Oligomerization state	Stable monomer or oligomer, both <i>in vitro</i> and <i>in vivo</i> (Gakh et al., 2010).	Stable monomer only, both <i>in vitro</i> and <i>in vivo</i> (Gakh et al., 2010; Tsai and Barondeau, 2010).
Iron binding properties	Oligomer binds Fe ²⁺ under aerobic or anaerobic conditions; monomer behaves like FXN ^{81–210} (Gakh et al., 2010).	Binds Fe ²⁺ under anaerobic conditions (Yoon and Cowan, 2003).
Interaction with ISCU and NFS1-ISD11	Oligomer forms stable complexes with NFS1-ISD11 in the absence or presence of ISCU; monomer behaves like FXN ^{81–210} (Gakh et al., 2010).	Binds to a pre-formed NFS1-ISD11-ISCU complex (Schmucker et al., 2011; Tsai and Barondeau, 2010).
Iron-dependence of interaction with ISCU and NFS1-ISD11	Iron-independent (Gakh et al., 2010).	Iron-independent (Gakh et al., 2010; Schmucker et al., 2011; Tsai and Barondeau, 2010).
Effect on NFS1 cysteine desulfurase activity	Activates cysteine desulfurase activity (Isaya Lab, unpublished results).	Activates cysteine desulfurase activity under non-oxidizing conditions (Tsai and Barondeau, 2010).