**INTRODUCTION**

FRDA (Friedreich’s ataxia) is an autosomal recessive neuro- and cardio-degenerative disorder that is caused by the deficient expression of the nuclear-encoded mitochondrial protein frataxin [1,2]. It is characterized by gait and limb ataxia, dysarthria, absent muscle stretch reflexes in the lower limbs, sensory loss and pyramidal signs [2,3]. In addition, patients generally suffer from skeletal deformities, diabetes and cardiomyopathy [2]. The disorder has an estimated prevalence of 1 in 50,000 in European populations, which makes it the most common hereditary ataxia in this population grouping. In contrast, the disease is far rarer among Asians, sub-Saharan Africans and American-Indians [2,4–6].

Frataxin deficiency is primarily due to a homozygous GAA triplet repeat expansion in the first intron of the frataxin gene FXN [3,7], which is located on the proximal long arm of chromosome 9 [8] at position 9q21.11 [9]. However, a minority of FRDA patients (<4%) are compound heterozygotes possessing point or small deletion mutations in the non-expanded allele and a GAA triplet repeat expansion in the other [10,11]. Although a normal FXN allele contains 6–36 triplets, an expanded FXN allele contains 66–1700 triplets, although in the majority of cases there are 600–900 triplets [3,7,12]. Expansions in the gene cause an abnormal conformation in the DNA, resulting in decreased transcription of the FXN gene and a subsequent reduced expression of the frataxin protein [13]. Importantly, the size of the GAA repeat has been found to positively correlate with the severity of the disease and negatively correlate with the age of onset [3].

There are a number of atypical variants of FRDA [2]: (i) late-onset FRDA, where disease progression is slower and onset occurs after 25 years of age; (ii) acadian-type FRDA, in which symptoms are less severe and cardiomyopathy rarely occurs; and (iii) FRDA with retained reflexes, in which tendon reflexes in the lower limbs are preserved and clinical features are less pronounced. These atypical variants with less severe symptoms such as late-onset FRDA typically have shorter GAA expansions [14]. The pathogenesis of the typical form of FRDA has a mean age of onset at 10.5 ± 7.4 years and death at 37.5 ± 14.4 years of age [15].

The association of FRDA with mitochondrial dysfunction is supported by a large body of evidence. The mitochondrion is not only a vital organelle for energy transduction and the regulation of cell death [16], but it is also important in iron metabolism, as it is the primary site for haem and ISC (iron–sulfur cluster) synthesis [17–20]. In fact, iron is incorporated into haem and ISCs for utilization in the cytosol for incorporation into a variety of proteins. Both the haem and ISC biosynthetic pathways are significantly affected in FRDA [17,18,21,22]. Hence, as a result of the disruption of normal mitochondrial function, some of the notable features that are observed in the disorder include mitochondrial iron accumulation in the heart and ISC deficiency [21,23–27]. In addition, FRDA primarily affects mitochondria-rich tissues, particularly those containing post-mitotic cells and those that are
largely dependent on oxidative energy metabolism. This results in the selective loss of dorsal root ganglia neurons, cardiomyocytes and pancreatic \( \beta \)-cells in neuronal, cardiac and pancreatic tissue respectively [9,28]. However, it is not known why certain neuronal types, such as spinal cord and brainstem motor neurons, are exempted in the pathogenesis of the disorder [9].

Although there is no known cure for FRDA, several treatment modalities are available. These include antioxidant therapy (which only targets cardiomyopathy), physical therapy and standard treatment for patients suffering from diabetes, scoliosis and arrhythmias [6,29,30]. It is anticipated that the elucidation of frataxin’s role in iron metabolism will provide insight into its function and into disease pathogenesis, as well as further therapeutic opportunities for FRDA.

**FRATAXIN**

Frataxin is initially synthesized as a 210-amino-acid nuclear-encoded protein that is primarily targeted to the mitochondrial matrix, where it is proteolytically processed by a mitochondrial processing peptidase to up to four N-terminally truncated forms [31–34]. Once within the matrix, frataxin is found associated with the inner mitochondrial membrane and crests and is also found as a soluble protein [1,35].

Although frataxin is ubiquitously expressed, its highest levels of expression are in the heart and spinal cord, which are two of the main sites affected by the disease [9]. Although it is known that frataxin is linked to neuro- and cardio-degeneration, the proximal biochemical function of the protein remains unclear. However, mounting evidence indicates that frataxin is likely to play a role in regulating mitochondrial iron metabolism [17,18,36,37]. As such, it has been proposed to interact with, and functionally modulate, proteins such as ferrochelatase and the ISCUs (ISC scaffold proteins), which are proteins involved in the mitochondrial iron utilization pathways of haem and ISC synthesis respectively [38,39]. Further support for a role for frataxin in modulating iron metabolism comes from a recent study in the fission yeast *Schizosaccharomyces pombe*, in which deletion of the *pfh1* gene encoding the frataxin orthologue PfH1 causes mitochondrial iron overload while simultaneously activating the ‘iron starvation’ gene expression programme that is regulated in *S. pombe* by glaturaredoxin [40]. Similarly, lymphoblasts and fibroblasts taken from FRDA patients display probable cytosolic iron deficiency as indicated by increased levels and/or IRE (iron-responsive element) binding activity of the IRPs (iron-regulatory proteins), IRP2 and IRP1, respectively [41].

Analogously, studies in other mammalian systems indicate that frataxin deletion in the heart triggers a state of cytosolic iron deficiency, characterized by an up-regulation of IRP2, TIR1 (transferrin receptor 1; a protein responsible for transferrin-dependent iron uptake), and down-regulation of ferritin (a cytosolic iron storage protein) and ferroportin (a cellular iron exporter in mammals) [21,25]. In the latter model, this cytosolic ‘iron deficiency’ is concomitant with mitochondrial iron overload and a derangement of mitochondrial iron metabolism pathways [21,25]. Interestingly, membrane-permeant iron chelators, which mediate cellular iron depletion, do not markedly affect the steady-state levels of frataxin in Friend cells that have been induced to undergo haemoglobinization by dimethyl sulfoxide [42], although frataxin expression has been reported to be modulated by iron chelation in other cell lines and types [41].

Several important hypotheses have been proposed [9,17,18,36,37,43] regarding frataxin’s role in mitochondrial iron homeostasis and these are briefly discussed below.

**PROPOSED ROLES OF FRATAXIN**

**Frataxin as an iron-binding protein**

*In vitro* studies have revealed that the yeast (i.e. *Saccharomyces cerevisiae*) and bacterial (i.e. *Escherichia coli*) orthologues of frataxin, Yfh1 and CyaY respectively, homo-oligomerize in the presence of increasing concentrations of ferrous iron, and that these aggregates can both store iron in a mineralized ferric state and possess ferroxidase activity [44,45] (Figure 1a). Similarly, the overexpression of human frataxin in *E. coli* results in the assembly of a homo-oligomer, which is able to bind approximately ten iron atoms per frataxin molecule [46]. This activity of frataxin may function to keep iron in its inert state, which would prevent it from redox cycling and would limit iron-dependent oxidative stress [28,36]. Of relevance, the mineralization and storage of iron via protein oligomerization occurs in the iron storage protein ferritin, which suggests that frataxin may play a similar role [28]. Consistent with this proposal, the structures of three frataxin orthologues were resolved, and it was found that there is a highly conserved N-terminal region consisting of metal-binding aspartate and glutamate residues [36].

The oligomerization of human frataxin does not occur spontaneously *in vitro*, and, in some cases, there was no clear interaction between human frataxin and iron [45]. In addition, one study showed that a mutant form of Yfh1, which was unable to oligomerize and store iron, was phenotypically silent *in vivo*, suggesting that iron storage is not a primary function of frataxin [47]. Finally, the discovery of Pmtt (mitochondrial ferritin), which functions as a mitochondrial iron storage protein, in higher organisms suggests that the proposed role of frataxin as an iron storage protein in the mitochondrion may be redundant in humans and other higher organisms [48–51].

**Frataxin as an iron chaperone**

On the basis of isothermal titration calorimetry and fluorescence quenching experiments, it was found that holo-frataxin (i.e. the iron-bound form of frataxin) has a high binding affinity for ferrochelatase [39], the enzyme responsible for the insertion of ferrous iron into PPIX (protoporphyrin IX) during the terminal step of heme biosynthesis. This was subsequently confirmed by the isolation of a frataxin-based chaperone activity that mediates the transfer of iron from a soluble iron-bound protein to ferrochelatase [40].

Frataxin’s role as an iron chaperone is further supported by its ability to interact with and functionally modulate the ISCUs (ISC scaffold proteins) [38,39]. In this context, frataxin has been shown to interact with ferrochelatase in a yeast two-hybrid assay, and to inhibit the activity of the ISCUs in a variety of cell lines and species [41].

**Figure 1** The proposed roles of frataxin in iron metabolism in mammals

<table>
<thead>
<tr>
<th>a</th>
<th>Iron storage</th>
<th>b</th>
<th>Iron chaperone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frataxin</td>
<td>Iron</td>
<td>Fech</td>
<td>Frataxin</td>
</tr>
<tr>
<td>ISC</td>
<td>Frataxin</td>
<td>ISC synthesis</td>
<td>ISC regulator</td>
</tr>
<tr>
<td>At high [I(_{\text{ER}})]</td>
<td>Iron sensor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron regulator</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Frataxin has been suggested to be involved in a number of iron-related processes such as (a) iron storage, (b) acting as an iron chaperone, (c) iron sensing and (d) iron regulation.
of haem synthesis [52]. Therefore frataxin may function as an iron chaperone in haem synthesis that delivers iron atoms directly to ferrochelatase, and consequently may act to limit the toxicity of free iron within the cell [36] (Figure 1b).

There is also evidence suggesting that frataxin serves as an iron chaperone in the assembly of ISCs [38, 53, 54]. ISCs are essential for a wide range of biological processes, such as the electron transport chain and other enzymatic and regulatory processes [55]. Biosynthesis begins with the formation of a disulfide bridge between NFS1 (cysteine desulfurase) and mitochondrial cysteine residues, leading to the formation of [2Fe–2S] or [4Fe–4S] clusters on scaffold proteins such as ISCU [55].

Furthermore, it was found that holo-frataxin, but not apo-frataxin, forms a complex with ISCU with sub-micromolar binding affinities [38]. In the presence of a source of sulfide, this bound frataxin donates iron to nucleation sites on ISCU to form the [2Fe–2S]-containing holo-ISCU [38]. Indeed, when free iron was used, negligible ISCU-dependent cluster formation was observed in the absence of frataxin [38]. However, as ISCs could still be assembled in vitro in the absence of accessory proteins, albeit at a lower rate, proteins such as frataxin may act as iron carriers rather than as catalysts in the formation of ISCs. Hence human frataxin is likely to act as an iron donor for ISC biogenesis through the interaction of holo-frataxin with apo-ISCU [38]. A similar interaction between the ISC assembly complex and the yeast orthologue of frataxin Yfh1 has been reported, as holo-Yfh1 has been shown to form a complex with the yeast scaffold protein Isu1 and the yeast Nfs1 [53].

**Frataxin as a metabolic regulator**

Contrary to frataxin’s proposed role as an iron chaperone, it was found that the bacterial orthologue of frataxin, CyaY, may function as a negative regulator of ISC biogenesis that specifically inhibits [2Fe–2S] cluster synthesis under conditions of high iron availability and low ISC apo-acceptor concentrations [54] (Figure 1c). Effectively, this model of frataxin function suggests that the protein can exert ‘kinetic control’ over the rate of ISC biogenesis, which is governed by the relative availabilities of iron and ISC apo-acceptors [17]. This ‘iron sensor’ hypothesis is consistent with the moderate iron-binding affinity (i.e. low micromolar range) of bacterial, yeast and mammalian frataxin orthologues [43]. Additionally, although yet to be tested in higher organisms, this model may help explain the observed ability of frataxin to protect against oxidative stress [43]. That is to say, a frataxin-dependent down-regulation of ISC biogenesis under conditions of surplus iron relative to ISC apo-acceptors would be expected to diminish the precipitation and oxidative degradation of unbound ISCs [45].

Another role that frataxin may play in the regulation of iron metabolism is that of a metabolic ‘switch’, which may allow for an increased input of iron into the haem synthesis pathway via ferrochelatase at the expense of a decrease in ISC biogenesis in cells that are undergoing haemoglobinization [17, 18, 37, 42] (Figure 1d). Although yet to be thoroughly tested, this hypothesis is supported by the observed down-regulation of frataxin expression that occurs during erythroid differentiation and in response to exogenous PIX, the iron-devour haem precursor [42]. Consistent with this hypothesis, it was found that the rate of haem synthesis was inversely related to the ratio of the levels of frataxin and ferrochelatase [39]. That is to say, higher levels of frataxin relative to ferrochelatase were found to lead to decreased rates of haem synthesis, which might enable the trafficking of iron towards ISC biogenesis.

**CELLULAR EFFECTS OF FRATAXIN-DEFICIENCY**

**Iron accumulation**

The importance of frataxin in cellular iron metabolism is further supported by the observations that mitochondrial iron accumulates in frataxin-deficient yeast and cardiomyocytes [21, 23, 25, 27, 56, 57], and that this accumulation occurs as putatively redox-active biominal iron aggregates, which are distinct from isolated holo-ferritin molecules [27]. Iron accumulation has been detected in the heart, liver and spleen, but not in the dorsal root ganglion, spinal cord, skeletal muscle, cerebellum, peripheral nerves or pancreas of FRDA patients [58]. A similar observation was made in 10-week-old MCK (muscle creatine kinase) conditional frataxin-KO (knockout) mice, in which significant iron deposition was detected in frataxin-deficient cardiomyocytes [57]. In those mice, the targeted deletion of exon 4 in the *Fxn* gene is under the control of the MCK promoter and, consequently, the mutated gene is restricted to striated muscle [57]. Herein, these mice will be referred to as ‘MCK frataxin-KO mice’ and the related ‘NSE (neurone-specific enolase) conditional frataxin-KO mice’, in which the defective *Fxn* gene is expressed under the control of the NSE promoter, and occurs predominantly in neuronal tissue, will be referred to as ‘NSE frataxin-KO mice’, as described previously [21, 25, 57].

Before 10 weeks of age, levels of mitochondrial iron in the 7-week-old MCK frataxin-KO mice appeared to be either normal or only slightly increased, whereas ISC enzyme activities were already diminished at this time point [57]. In addition, although iron deposits were apparently absent in the NSE frataxin-KO mice, both models showed signs of cardiac hypertrophy and deficits in ISC enzymes [57]. Importantly, analysis of gene and protein expression in the MCK frataxin-KO mice identified a mechanism of iron redistribution characterized by an up-regulation of genes involved in cellular and mitochondrial iron uptake (i.e. *TIR1* and *mitoferrin 2* respectively), and a down-regulation of genes encoding proteins involved in cytosolic iron storage (i.e. ferritin-H and -L chains), cellular iron efflux (i.e. ferroportin 1) and mitochondrial iron-processing, namely, proteins involved in haem synthesis (i.e. 5-aminolevulinate dehydratase, uroporphyrinogen III synthase and ferrochelatase) and ISC synthesis (*Iscl1* and *Nfs1*) [21] (Figure 2). In addition, it was shown in vivo in MCK frataxin-KO mice that 59Fe uptake from radiolabelled holo-transferrin was markedly increased in frataxin-deficient hearts relative to that of wild-type mice, and furthermore, that redistribution of 59Fe from the cytosol to the stromal mitochondrial membrane fraction occurred [25]. These data strongly suggest that the increase in transferrin-dependent iron uptake occurred via the marked elevation in *TIR1* expression, and that the increased accumulation of iron by mitochondria may result from the decrease in cytosolic ferritin and ferroportin, coupled with the increase in both haem oxygenase 1 and the mitochondrial iron importer, mitoferrin 2 [21]. Under these conditions, the mitochondrial may have an increased ability to import iron from the cytosolic labile iron pool [59]. Therefore mitochondrial iron accumulation results from an up-regulation of cellular iron uptake, decreased cytosolic iron storage and increased iron uptake by mitochondria, coupled with defective incorporation into haem and ISCs [21, 25]. Although there is marked iron uptake into the frataxin-deficient mitochondria, it is not effectively incorporated into haem or ISCs, which, under physiological conditions, are then shuttled out of this organelle for cytosolic use [17, 18, 22]. Hence the increased iron uptake and decreased iron release by the mitochondrion leads to iron loading of this organelle. In support of this model, genome-wide analyses of gene expression in *S. cerevisiae* have demonstrated that when
Figure 2 Alterations in cardiomyocyte iron metabolism in FRDA

Yfh1 is knocked out, the resulting Yfh1-KO yeast displays an up-regulation of all known genes of the so-called ‘iron regulon’ [60]. The iron regulon contains genes that encode proteins involved in cellular iron uptake, such as the Fte (ferric reductase) 1–3, the high affinity iron uptake system (Fet3/Ftr1), siderophore transporters (Arn1–4) and the vacuolar iron transporter (Fet5/Fth1) [60–64].

Importantly, the expression of iron regulon genes is controlled in an iron-dependent manner by the transcription factors Aft1p/Aft2p, which are inhibited from binding to their target genes by iron, but are active under iron deficiency [60,64–66]. Thus, in S. cerevisiae, low levels of cytosolic iron [65,66], as well as defective mitochondrial ISC biogenesis [67], induce the Aft1p/Aft2p regulatory system, which helps to explain the increase in cellular iron uptake and the redistribution of cytosolic iron to mitochondria in response to frataxin deficiency.

It is worth pointing out that Aft1p/Aft2p-dependent activation of the iron regulon in response to frataxin deficiency in S. cerevisiae is analogous to the recently described activation of the iron regulon in response to frataxin deficiency in S. pombe [40] (discussed above), as well as activation of the cytosolic iron deficiency response in mammalian cells (e.g. up-regulation of Tfr1, down-regulation of ferritin and ferroportin 1 [21,25] (discussed above). Therefore the development of cytosolic iron deficiency concomitant with mitochondrial iron overload appears to be a common feature of frataxin deficiency in eukaryotes.

More recent studies have assessed the biochemical form of cardiomyocyte iron deposits in FRDA by examining the heart of the MCK frataxin-KO mouse at 10 weeks of age [27]. In these animals, it was shown by a variety of techniques, including native fast protein liquid chromatography, alternating current magnetic susceptibility, Mössbauer spectroscopy, transmission electron microscopy and energy-dispersive X-ray analysis, that the accumulation of mitochondrial iron was consistent with a non-ferritin mineral of high-spin iron(III) that contained both phosphorus and sulfur [27]. Importantly, the mitochondrial iron accumulation in the heart of the MCK frataxin-KO mice was distinct from the iron found in the liver of these animals, which occurred as distinct iron-containing spheres that were characteristic of ferritin [27]. Indeed, the mitochondrial iron accumulation occurred as very small inter-connected particles of ~1.9 nm in diameter, whereas the iron in ferritin appeared as larger discrete spheres of ~4.6 nm in diameter [27]. The presence of these non-ferritin biomineral iron aggregates in mitochondria could be redox-active and deleterious by promoting the formation of cytotoxic free radicals [27].

Intriguingly, the selective loss of frataxin in the heart and skeletal muscle in the MCK frataxin-KO mouse leads not only to iron loading in the heart, but also to a significant increase in liver, spleen and kidney iron levels [21,27]. These alterations in iron metabolism in non-striated muscle tissues that express frataxin at normal levels presumably occur by modifications in systemic iron homeostasis, which are probably mediated by changes in the expression of key molecules involved in the regulation of systemic iron metabolism that affect the expression of the hepcidin in the liver [27], as well as the increased expression of haemojuvelin and interleukin-6 [21], were all significantly misregulated in MCK frataxin-KO mice. Although the increase in hepatic hepcidin is a possible consequence of the up-regulation of interleukin-6, the latter of which is a myokine that triggers hepcidin expression [19], the series of molecular events leading to these alterations in key systemic iron metabolism molecules is not yet clear. However, it is clear that the deletion of frataxin in a restricted set of tissue types (e.g. striated muscle) can lead to disruptions in systemic iron levels and iron metabolism, including serum iron levels. These changes may reflect compensatory systemic alterations in response to the misregulation of cardiac iron metabolism occurring in MCK frataxin-KO mice [27].

Of relevance to the cardiomyopathy and systemic alterations in iron metabolism occurring in MCK frataxin-KO mice [27], it is notable that previous studies examining a cardiac-specific Ctr1 (copper transporter 1)-KO mouse model demonstrated the development of severe cardiomyopathy, systemic alterations in copper metabolism and cardiac copper deficiency [69]. Additionally, these mice demonstrate increased serum copper and elevated expression of proteins involved in intestinal and liver copper uptake and mobilization respectively. Thus alterations in the systemic regulation of copper trafficking were identified in the context of a cardiac-restricted Ctr1-KO mouse model that initially deregulates cardiac copper homeostasis followed by the systemic signalling of this misregulated copper status to copper storage organs [69]. Those findings mirror our data in MCK frataxin-KO mice, where up-regulation of iron acquisition mechanisms in the duodenum and liver were observed, together with increased serum iron, which may act as a response to correct the cardiac iron deficiency [27]. Hence inter-organ communication may occur.
between the heart and other organs important for iron and copper metabolism to aid homeostasis [27].

Oxidative stress

The accumulation of ineffectively sequestered iron within a highly redox-active environment such as the mitochondrion may potentiate production of highly damaging and reactive hydroxyl radicals through the Fenton reaction [70,71] (eqn 1):

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}
\]

Therefore the accumulation of mitochondrial iron observed in FRDA patients may result in oxidative stress and free-radical-induced cytotoxicity. In support of this hypothesis, it was shown that, in the absence of Yfh1, mitochondrial damage in yeast cells was proportional to the concentration and duration of exposure to extracellular iron, suggesting that iron accumulation is indeed responsible for the cellular damage observed in FRDA [72]. Moreover, mitochondrial iron accumulation in the heart of MCK frataxin-KO mice appears to occur as a non-protein aggregate [27]. Interestingly, this latter form of iron is unlike holo-ferritin and appears to lack a protein shell, which may facilitate its redox activity [27].

Further evidence supporting a role of oxidative stress in FRDA was obtained in a study in which the administration of the antioxidant idebenone (a coenzyme Q, mimetic that is an approved treatment for FRDA in Canada) led to a decrease in myocardial hypertrophy and improved myocardial energy efficiency [73]. Other studies showed increased concentrations of markers of oxidative DNA damage (e.g. 8-hydroxy-2′-deoxyguanosine) and of lipid peroxidation (e.g. serum malondialdehyde) in patients [46,74,75]. Finally, it has been shown that there is a higher mitochondrial DNA mutation rate in genes encoding certain subunits of complex I in FRDA patients than in controls, indicating the occurrence of mitochondria-localized ROS (reactive oxygen species) production [9]. It is likely that these mutations contribute to mitochondrial instability.

There have also been contradicting studies suggesting that frataxin deficiency is not linked to oxidative stress. For example, Seznec et al. [76] showed that frataxin deficiency did not induce oxidative stress in cardiac and neuronal tissues, and that it also led to the eventual down-regulation of the mitochondrial antioxidant enzyme MnSOD (manganese superoxide dismutase). Consequently, it was proposed that the reduced levels of MnSOD were indicative of the lack of endogenous free radical production, instead of as a causative factor in the oxidative damage observed in FRDA patients [76].

Regulation by hypoxia-inducible factors

Expression of the mouse frataxin gene *Fxn* is regulated by HIF (hypoxia-inducible factor)-2α, an iron metabolism-regulating transcription factor subunit that is up-regulated by iron deficiency and down-regulated by iron repletion [77]. As levels of frataxin were reduced in response to a deletion of the HIF-2α-encoding gene *Epas1* [78]. Similarly, it was observed in certain human tumour cell lines that frataxin is up-regulated in response to hypoxia. That is to say, frataxin was observed to modulate expression of the p53-encoding tumour suppressor gene *TP53* (tumour protein p53) in an HIF-dependent manner [79]. Although it is unclear how HIFs are activated by frataxin deficiency, a likely possibility is that HIF expression is increased in response to a decrease in iron-dependent prolyl hydroxylases, which are enzymes that are responsible for the iron- and dioxegen-dependent regulation of HIF protein turnover by hydroxylation and subsequent degradation by the proteasome [77]. A decrease in the activity of these enzymes would be expected to result from the cytosolic iron deficiency that occurs in FRDA [17,18,21]. Further studies are required to identify the pathways that are responsible for the tissue-specific pathology observed FRDA patients.

**CARDIOMYOPATHY IN FRDA**

**Symptoms and treatment**

Cardiomyopathy is the most common cause of death in FRDA and it affects two-thirds of patients [3,9,26]. Patients are often found with left ventricular hypertrophy, which can progress to the more fatal dilated cardiomyopathy. Adequate systolic function is usually maintained until shortly before death [26,80,81]. Both ventricles are affected, and arrhythmias of atrial origin are common and important in determining the prognosis of the patient as they are indicative of left ventricular dysfunction [9,82]. At a histological level, it has been observed that cardiomyocyte hypertrophy, focal necrosis and diffuse fibrosis occur in the left ventricle [26].

Moreover, a significant decrease in myocardial energy generation was shown to correlate with the degree of hypertrophy [26]. Accordingly, short-term studies have shown that the use of idebenone has been successful in improving myocardial energy output and decreasing hypertrophy [73]. However, longer-term studies have failed to show a decrease in arrhythmias or progression of heart failure [83]. Another therapeutic option for the cardiomyopathy in FRDA is heart transplantation. In one case in which this procedure was performed the patient’s ataxia improved considerably, possibly due to the reperfusion of blood to the limbs [84].

In the experimental MCK frataxin-KO model, cardiac hypertrophy was determined by heart/body weight ratio [57]. The mutants did not display any significant differences in this ratio compared with wild-type mice until late onset, after which the mass of the MCK frataxin-KO hearts increased significantly until death [57]. The cardiac hypertrophy was observed to develop into a dilated cardiomyopathy, which recapitulates progression of the human disease [57,85]. The aforementioned ISC deficiency was not apparent until late onset, which suggests that this process occurs in parallel with the pathogenesis of cardiomyopathy in FRDA [57]. In addition, the application of antioxidant therapy using idebenone had a similar effect on the mouse models as it also slowed the progression of cardiomyopathy [86].
animal models of FRDA strongly suggest the involvement of autophagy and apoptosis [94–96].

In vitro studies have demonstrated that an increase in apoptosis occurs in cultured cells from FRDA patients in response to oxidative damage [95,97]. However, these observations have yet to be confirmed in vivo. The observation of apoptotic cells in hearts of FRDA patients has not been reported, which may be a consequence of the fact that apoptotic cells are readily phagocytosed by neighbouring cells and are consequently difficult to detect [98]. An accumulation of lipofuscin, which is indicative of membrane lipid damage and oxidation, was also found in both the dorsal root ganglia and the cardiomyocytes of FRDA patients [99,100]. Lipofuscin is a lipoprotein aggregate that is formed after the degradation of iron-containing proteins by the lysosome, and it is known to be an indicator of the insufficient or defective autophagy of mitochondria that occurs progressively in aging post-mitotic cells [101,102] (e.g. neurones and cardiomyocytes; see below). This raises the possibility that pathologic autophagy occurs in the hearts of FRDA patients, and it might therefore be a major contributor to cardiomyopathy in FRDA.

Interestingly, autophagic cardiomyocytes are observed at a significantly higher frequency in failing hearts than apoptotic cardiomyocytes [103]. Although it has been observed that there is an increase in autophagic markers in affected neuronal tissue in FRDA patients [96], it is not known whether a similar up-regulation of autophagy occurs in cardiac tissue. These findings are important because autophagy and apoptosis can play a vital role in the development of cardiac failure in FRDA, and they might also act synergistically to result in heart failure [104]. Hence these processes and the pathways that trigger them need to be further considered in the pathogenesis of the cardiomyopathy of FRDA.

AN EMERGING ROLE FOR THE ISR IN FRDA

The ISR [87,89] is a highly conserved adaptive stress response initiated by specific kinases that results in the phosphorylation of eIF2α (eukaryotic initiation factor)-2α at Ser51 [87], which is a protein that is necessary for the initiation of translation in eukaryotes. This event is central to the advent of an adaptive cellular stress response and results in a reduction of general translation while a specific subset of ‘stress-response’ transcripts is translated. Four known kinases upstream of eIF2α are phosphorylated in response to cellular stressors: (i) Hri (haem-regulated inhibitor) [eIF2AK (eIF2α kinase) 1], which autophosphorylates in response to haem deficiency; (ii) Pkr (protein kinase R) (eIF2AK2), which is activated after viral infection; (iii) PERK [PKR (double-stranded-RNA-dependent protein kinase)-like ER (endoplasmic reticulum kinase)] (eIF2AK3), which is up-regulated following ER stress; and (iv) Gcn2 (general control non-derepressing 2) (eIF2AK4), which is activated in response to amino acid deficiency [105–109] (Figure 3).

These divergent stress signals converge at the level of eIF2α hyperphosphorylation, which inhibits the guanine nucleotide exchange activity of eIF2B thereby causing a blockade of translation initiation [110]. Additionally, eIF2α phosphorylation triggers the formation of ‘stress granules’, which are transient dynamic aggregates of stalled pre-initiation complexes [111]. These stress-induced RNA–protein granules appear to function as triage centres that determine whether a given mRNA is translated, stored or decayed [112]. This process participates in the suppression of general translation during stress. Importantly, and somewhat paradoxically, the phosphorylation of eIF2α triggers the up-regulation of translation of a specific subset of stress-response transcripts that are required for the affected cell to mount an adequate homeostatic response [110,112]. One such transcript includes ATF4 (activating transcription factor 4) [87,108,113] (Figure 3). The encoded transcription factor ATF4 is responsible for the activation of a wide range of adaptive cellular processes by directly up-regulating the expression of stress-inducible genes involved in the response to oxidative stress, amino acid synthesis, differentiation, metastasis and angiogenesis [108,113]. However, if this adaptive response fails to alleviate the original stress, prolonged activation of the ISR can lead to the induction of autophagy and eventually to apoptosis [88,89,108] (Figure 3).

Autophagy is also induced in response to a number of stress signals (e.g. nutrient stress, ER stress, hypoxia, anoxia and oxidative stress), and the activation of Gcn2 and Pkr can up-regulate autophagy in response to nutrient deprivation [89,114]. It has also been found that cells expressing a point mutant of eIF2α in which the normally phosphorylatable Ser51 has been replaced...
Figure 4  Autophagic pathway

The Atg12–Atg5–Atg16L (autophagy related 16-like) complex interacts with the isolation membrane and localizes asymmetrically as it elongates in response to stress signals sent from damaged organelles [119]. The pre-autophagosome engulfs these organelles along with a bulk of cytoplasm, and the Atg complex dissociates from the membrane following autophagosome formation [119]. Thereafter, the autophagosome fuses with a lysosome. The resultant autolysosome is thus responsible for the degradation of its contents. Reprinted from Trends Mol. Med., 13(11), Martinet W, Knaapen MW, Kockx MM, De Meyer GR, Autophagy in cardiovascular disease, 482–491, © (2007), with permission from Elsevier.

with an alanine residue, which cannot be phosphorylated, are unable to up-regulate autophagy in response to starvation [115] and viral infection [114]. However, it is still unclear how the ISR up-regulates autophagy [89,115].

We have demonstrated recently that in the MCK frataxin-KO mouse model there is an up-regulation of ATF4-inducible genes at a relatively early stage of disease progression [115a]. These genes include Asns (asparagine synthetase), Mthfd2 [methylene-tetrahydrofolate dehydrogenase (NADP⁺-dependent) 2] and Psat1 (phosphoserine aminotransferase 1), all of which are associated with the response to amino acid deficiency; Chop [C/EBP (CCAAT/enhancer-binding protein)-homologous protein] or DDIT (DNA-damage-inducible transcript 3), which has been implicated in different forms of cardiomyopathy leading to heart failure and is up-regulated in apoptotic cardiomyocytes [88]; and Trib3 [Tribbles homologue 3; Skip3]. The protein encoded by the last gene, at least in human cells, regulates the ISR via a negative feedback mechanism involving Trib3-dependent down-regulation of both ATF4 expression [116] and Chop activity [88,117]. Moreover, it is noteworthy that Chop and Trib3 are important for ISR-dependent cardiomyocyte apoptosis [88,117,118].

Although it is not currently known which particular stress signals cause the activation of the ISR, or indeed precisely which eIF2α kinases are crucial in FRDA, we have observed recently that there is an increase in the phosphorylated form of Hri at 3–5 weeks of age in the MCK frataxin-KO mouse [115a]. As frataxin is essential for ferrochelatase activity [39] and ferrochelatase expression is significantly diminished in the MCK frataxin-KO mouse [21], the activation of Hri is a plausible trigger for early-onset eIF2α phosphorylation and consequent ISR activation in FRDA hearts. Additionally, prolonged ER stress leading to Chop activation is significant in the progression of cardiomyopathy during cardiac hypertrophy and ensuing heart failure caused by transverse aortic constriction [88]. Given these observations, it is now of paramount importance to investigate further the significance of the ISR in FRDA cardiomyopathy and heart failure. As activation of the ISR can activate autophagy [89,115], and an up-regulation of key autophagy genes occurs in the MCK frataxin-KO mouse model [115a], we now turn to the latter process and its potential relevance to FRDA pathology.

**IS THERE A ROLE FOR AUTOPHAGY IN FRDA CARDIOMYOPATHY?**

Autophagy is a cell-autonomous and catabolic process of bulk recycling of protein aggregates that is vital to cellular homoeostasis in the face of extracellular and/or intracellular stress [119,120]. Whether autophagy is adaptive or maladaptive may be context-dependent, although a large body of evidence suggests that autophagy is an essential cytoprotective response to cellular stress that contributes indispensably to cellular homoeostasis [103,120]. However, it is also apparent that excessive autophagy may precipitate cellular demise, particularly if the autophagic catabolism is not counterbalanced by appropriate biosynthetic processes [103,121,122]. An increase in autophagy has been documented in a number of human neurodegenerative disorders such as Alzheimer’s, Parkinson’s and Huntington’s diseases (reviewed in [119]), as well as in heart injury and failure [123] (see below for further discussion).

Of the three major types of autophagy (i.e. macroautophagy, microautophagy and chaperone-mediated autophagy), which differ primarily in the mode of cargo delivery to the lysosome, macroautophagy (hereinafter referred to as ‘autophagy’) is the best described [119,120], particularly with reference to cardiac tissue [121,124,125]. During macroautophagy, an isolation membrane (also known as a phagophore), which is derived from the ER, outer mitochondrial membrane or plasma membrane, extends into a double-membrane structure (termed an autophagosome) that encapsulates bulk cytosolic components and organelles such as mitochondria [89,119]. The autophagosome is then targeted to the lysosome, which then fuses with the
autophagosome forming an autolysosome that then degrades its intravesicular contents [119,120] (Figure 4).

The regulation of autophagy is highly complex and a detailed analysis of this area is outside the scope of the present review. For an excellent review on the core machinery and regulation of the mammalian autophagy pathway, see Mizushima et al. [126].

Mitophagy, cardiac homeostasis and FRDA

Although autophagy was once regarded as a largely non-selective process in which portions of the cytoplasm were randomly sequestered into an autophagosome [119], it is now appreciated that autophagy is highly selective [122] and is in some instances responsible for caspase-independent programmed cell death [127] (see below for further discussion). For example, mitochondrial autophagy, or mitophagy, is a targeted form of autophagy in which damaged and/or effete mitochondria are selectively earmarked for autophagic degradation [128–130] (Figure 5). This form of autophagy occurs in a range of different physiological processes such as erythroid differentiation, the metamorphosis of silk moth muscles and in the development of T-lymphocytes [131–133]. Importantly, it is also of crucial relevance to cardiac homeostasis and dysfunction [122,125].

Notably, although mitochondria are essential for energy transduction and cell signalling, damaged and dysfunctional mitochondria can be detrimental to cellular health by promoting oxidative stress and cell death through the release of pro-death factors such as cytochrome c [134] (see below for further discussion). Hence the selective sequestration and autophagic degradation of defective mitochondria by mitophagy may be a stress-response mechanism to prevent further cellular damage and eventual cell death. For example, this particular function of mitophagy has been proposed to occur in models of ischaemia/reperfusion in cardiac tissue [125]. Indeed, mitophagy is probably critical to cardiomyocyte homeostasis, as these cells are post-mitotic and mitochondria make up ~30% of cardiac volume [122,135].

It is of significance to the discussion in the present review that electron microscopy of hearts from both the MCK and NSE frataxin-KO mouse models revealed the presence of myofibrils with abnormal alignment and disruption, as well as disorganized and swollen mitochondria, which are suggestive of mitochondrial dysfunction [57]. Similar observations of myocardial fibre disarray with hypertrophic cardiomyopathy and left ventricular wall thickening have been observed in necropsy studies of FRDA patients [136]. In further support of mitochondrial dysfunction in FRDA, mitochondrial myopathy associated with mitochondrial proliferation and structural abnormalities has been observed in skeletal muscle biopsies of a 13-year-old child with FRDA [137]. Considering that mitophagy is critical to normal cardiomyocyte function and protection against insult [122], it is probable that this process would be activated, or at least affected, in FRDA hearts and, additionally, may contribute to disease pathogenesis. In support of this notion, we have observed recently in hearts of the MCK frataxin-KO mouse a significant early up-regulation of: (i) LC3 (microtubule-associated protein 1 light chain 3)-I into LC3-II conversion, which is indicative of autophagy initiation (Figure 6); (ii) expression of Atg (autophagy-related) 3, which is an E2-like enzyme responsible for the conversion of LC3-I into LC3-II; and (iii) an increase in p62/Sqstm1, which is an essential autophagy substrate that binds to ubiquitinated proteins via its interaction with LC3-II before autophagic degradation [115a].

Importantly, the question of whether an increase in autophagy is significantly affected in authentic FRDA hearts has yet to be examined. However, given the role that early-onset autophagy appears to play in cardioprotection against various insults, it is at least plausible that autophagy in FRDA hearts may constitute an initial adaptive/cytoprotective response to the accumulation of damaged and/or dysfunctional mitochondria resulting from a deficiency of frataxin. It is worth noting that lipofuscin accumulation has been observed in dorsal root
because although autophagy is often associated with heart failure, it is also essential for the survival of cardiomyocytes through the production of free amino acids and fatty acids from the breakdown of proteins and organelles [144] and to the sequestration and degradation of unstable and potentially deleterious mitochondria [122,125]. It has been reported that autophagy might be beneficial in the progression of cardiomyopathy up to a point, but when it reaches a certain threshold autophagy could lead to heart failure [144].

Autophagy has been shown to protect against β-adrenergic stimulation, which enhances apoptosis, cardiac hypertrophy and heart failure [145–147]. This was observed in a study where autophagy-deficient mice displayed increased sensitivity to isoproterenol, a synthetic β-adrenergic agent, leading to left ventricular dilatation and cardiac dysfunction [147]. In addition, cardiac hypertrophy from thyroid hormone treatment was prevented following treatment with rapamycin, an activator of autophagy [148]. Rapamycin treatment was also able to reduce ischaemia/reperfusion injury on Langendorff-perfused rat hearts [149,150]. Furthermore, it was shown that glucose deprivation, which is important in ischaemia, resulted in the up-regulation of autophagy in isolated cardiac myocytes [124]. The inhibition of autophagy was correspondingly found to enhance glucose deprivation-mediated death [151].

Even though autophagy appears to play a protective role in ischaemic cardiac injury, in one study autophagy had the opposite effect on myocytes during reperfusion [151]. Matsui et al. [151] demonstrated that autophagy was regulated by AMPK (AMP-activated protein kinase) in ischaemia and by Beclin-1 in reperfusion. Although AMPK-activated autophagy appeared to be protective in ischaemia, the knockdown of Beclin-1 increased cell viability, suggesting that autophagy in the reperfusion phase was potentially detrimental [151]. Although this interpretation has been challenged [125], such results may be reflective of the ‘double-edged sword’ and/or context-dependent role for cardiomyocyte autophagy.

Support for the role of autophagy in cardiomyocyte death was found in human patients suffering from idiopathic dilated cardiomyopathy in which Beclin-1 was up-regulated during cardiac injury by ischaemia/reperfusion [152]. Consistent with these findings, Akazawa et al. [153] demonstrated that cardiomyocyte degeneration was induced in transgenic mice expressing the human diphtheria-toxin receptor in the heart, which led to heart failure and subsequent death in 80 % of the animals. Thereafter, it was found that there were signs of autophagic cell death, such as an accumulation of autophagosomes and an up-regulation of lysosomal markers, strongly suggesting a role of autophagy in cell death [153]. However, it remains unknown whether the presence of autophagic markers signifies a failed protective mechanism or a pathway for cell death. Regardless, it is clear that cardiomyocytes with a loss of organelles will be unable to produce functional contractile power, thus exacerbating the effect of heart failure [154].

In addition to the role that autophagy might play in cell death, under extreme conditions of stress, the loss of membrane potential across the mitochondrial inner membrane can result in ineffective ATP production and/or eventually mitochondrial permeability transition leading to necrotic cell death [16,121]. Alternatively, moderately damaged mitochondria can also release of pro-apoptotic factors that lead to apoptotic cell death [121]. Therefore multiple forms of cell death are often observed in the pathogenesis of heart failure, and these can be distinguished by a number of characteristic morphological features [141] (Table 1). However, it should also be noted that cross-talk exists between these cell death mechanisms, as autophagy and apoptosis have been shown to both counter each other or act synergistically,

Figure 6 Formation of LC3-II

The C-terminus of pro-LC3 is cleaved by a cysteine protease known as autophagin to form LC3-I [119]. LC3-I then undergoes a number of ubiquitination-like reactions to form LC3-II, which is tightly bound to the autophagosomal membrane [119].

ganglion neurones and cardiomyocytes from necropsy studies of FRDA patients [99,100,138]. Lipofuscin accumulation within the lysosomal compartment is suggestive of insufficient or defective autophagy, which is often observed in aging post-mitotic cells such as neurones and cardiomyocytes [139]. The accumulation of lipofuscin progressively down-regulates autophagic efficacy, which occurs due to the largely futile deployment of lysosomal hydrolases to lipofuscin-loaded lysosomes [139]. Thus the results of the human necropsy studies discussed above suggest that mitophagy and/or autophagy is defective and, consequently, a possible contributor to mitochondrial abnormalities and disease pathogenesis in FRDA.

As very little is known about the role of autophagy in FRDA, we now turn to a more general discussion of the role of autophagy in heart failure resulting from different cardiac diseases. An understanding of the role of autophagy in these disease states may shed light on, and suggest further experimental avenues for, subsequent FRDA research.

Autophagy in heart failure

Heart failure occurs after a process known as cardiac remodelling, during which the heart undergoes an alteration in structure and function in response to cardiac load and injury, which results in an increased rate of cardiomyocyte death [121,140]. Multiple forms of cell death mechanisms including apoptosis and necrosis have been observed in heart failure [141]. However, signs of autophagy from dead and dying cardiomyocytes have also been reported to occur in heart failure caused by dilated cardiomyopathy, hypertensive disease, chronic ischaemia and stunned myocardium, but have not been found in healthy human hearts [92,123,141–143]. In addition, it was found that there is a higher incidence of autophagic cardiomyocytes in heart failure than apoptotic cells [123,141]. Therefore autophagy is an important process to consider in the progression of heart failure. However, it is not known if autophagy plays a protective role or if it in fact contributes to the pathogenesis of heart failure. This is because although autophagy is often associated with heart failure,
Figure 7  The extrinsic pathway by the death receptors FasR and TNFR1 leading to activation of executioner caspases

The binding of the TNF ligand to TNFR1 results in the recruitment of FADD (Fas-associated death domain) and TRADD (TNFR1-associated death domain), and the eventual activation of an executioner caspases (cas), such as caspase 3. A similar process occurs in the binding of Fas to the FasR (Fas receptor). CAD, caspase-activated DNase; DISC, death-inducing signalling complex; ICAD, inhibitor of caspase-activated DNase.

Table 1  Morphological features of selected major forms of cell death

Autophagy differs from apoptosis in that apoptosis is characterized by cell shrinkage, chromatin condensation and nuclear fragmentation. In addition, caspases are activated in apoptosis, whereas caspase activation is not needed in autophagy [103]. The distinction between apoptotic cell death and autophagy can be done through monitoring caspase activity, chromatin degradation and other apoptotic markers, as well as through the observation of the relationship between the specific inhibition of autophagy, and cell death [103,104]. Adapted from Martinet et al. [103], for a more complete list, see Galluzzi et al. [156].

<table>
<thead>
<tr>
<th>Cell death features</th>
<th>Apoptosis</th>
<th>Autophagy</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shrinkage</td>
<td>++</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Cellular swelling</td>
<td>−</td>
<td>−</td>
<td>+ +</td>
</tr>
<tr>
<td>Chromatin condensation</td>
<td>+ +</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Nuclear fragmentation</td>
<td>+ +</td>
<td>−</td>
<td>+ +</td>
</tr>
<tr>
<td>Loss of plasma membrane integrity</td>
<td>−</td>
<td>−</td>
<td>+ +</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>−</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Caspase activation</td>
<td>+ +</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Oligonucleosomal DNA fragmentation</td>
<td>+ +</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Random DNA degradation</td>
<td>+ +</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Processing of LC3</td>
<td>−</td>
<td>+ +</td>
<td>−</td>
</tr>
</tbody>
</table>

and they also share many of the same molecular regulators [104].

WHAT IS THE ROLE OF APOPTOSIS IN FRDA CARDIOMYOPATHY?

Apoptosis is a highly coordinated, typically energy-dependent and physiological mode of cell death (often also referred to as ‘programmed cell death’, although the terms are not synonymous [155,156]). It is essential for cell turnover and development, and is deregulated in a wide range of diseases (e.g. various neurodegenerative diseases and cancer) [155,157]. Apoptosis frequently occurs under physiological conditions as a means of maintaining cell populations in tissues, and it also functions as an organismal defence mechanism that occurs in response to cell damage and mutations [158]. Apoptosis is typically executed via one of two major pathways: the extrinsic pathway (Figure 7) and the intrinsic (or mitochondrial; Figure 8) pathway, although it is emerging that significant cross-talk exists between some of the specific pathways [157]. Both pathways can be subdivided further: the extrinsic pathway encompasses apoptosis induced by death receptors and by dependence receptors (see below), whereas the intrinsic pathway encompasses caspase-dependent and -independent mitochondria-mediated apoptosis [156]. It should be noted that there is also a third pathway to apoptosis, known as the immune pathway, which is mediated by the delivery of granzymes/perforins to cells by T-cells or natural killer cells [159], but this pathway is not known to be connected to heart failure or FRDA.

The extrinsic pathway is induced by death or dependence receptors. The former are transmembrane death receptors that engage with members of the TNF (tumour necrosis factor) family, and it is responsible for transmitting signals from the cell surface to intracellular signalling pathways by recruiting pro-caspase 8 [157,160,161]. The intrinsic caspase-dependent pathway, in contrast, acts through intracellular signals and involves non-receptor-mediated stimuli that lead to the activation of caspase 9 [157].
transduction pathways necessary to induce apoptosis [164]. It is also relevant to note that apoptosis occurs in several frataxin-deficient cell lines, raising the possibility that apoptosis has a role in the cardiomyopathy observed in FRDA [9]. The participation of apoptosis in heart failure has therefore been a subject of great interest. Signs of apoptosis in advanced heart failure have been found in explanted human hearts [91]. Apoptosis leading to heart failure has also been most commonly identified in idiopathic dilated cardiomyopathy and ischaemic cardiomyopathy, and it has also been proposed to contribute to the wall thinning observed in cardiac hypertrophy [165].

Studies have shown a low rate of apoptosis in heart failure [<1% TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling)-positive cells], but it must be noted that the detection of apoptosis could be dependent on the stage of the disease [166]. Most of the studies on human patients have been performed at an advanced stage of heart failure, and it is possible that signs of apoptosis were diminished at that point [166]. In addition, higher rates of apoptosis have been observed in rabbits following acute ischaemia and reperfusion [167]. Hence it appears that the observed occurrence of apoptosis is dependent on the models used, and on the conditions under which cardiomyopathy occurs.

Apoptosis in cardiomyocytes can be induced by stimuli such as hypoxia and ischaemia which are associated with the activation of Fas [165]. The Fas ligand as well as TNF-α were found to be up-regulated in patients with end-stage heart failure, and the extrinsic pathway also appears to be implicated in immune-mediated cardiomyopathy [166–168]. This suggests the importance of the death receptor-mediated pathway in apoptotic heart failure, although it appears to be more important in immune-mediated cardiomyopathy than in the more prevalent ischaemic or dilated cardiomyopathy [165]. It has also been observed that lowered serum glucose concentrations trigger the release of cytochrome c from the mitochondria (a pro-apoptotic event) in cardiomyocytes, suggesting that ischaemic injury might be mediated by the mitochondrial pathway in apoptosis [165,169]. It has indeed been shown that cytosolic cytochrome c and the subsequent activation of effector caspases occurs in different models of heart failure, and there has also been further evidence to suggest that cardiomyocytes utilize a mitochondrial-dependent apoptotic pathway [170–173]. Higher levels of the anti-apoptotic protein Bcl-2 have also been observed after acute coronary occlusion, but they are decreased after chronic heart failure by pressure overload [174–176]. In addition, the overexpression of Bcl-2 in the heart appears to reduce myocardial reperfusion injury by reducing the occurrence of myocyte apoptosis [177].

Interestingly, although oxidative stress in FRDA patient cells in vitro results in apoptotic cell death [95,97], clear evidence for apoptosis in vivo is limited [178]. Although increased apoptosis was detectable in the embryos of complete frataxin-KO mice [94], evidence of apoptotic cell death in the MCK and NSE conditional frataxin-KO models has not been forthcoming, with only cardiomyocyte necrosis and post-necrotic fibrosis being apparent [57,178]. Intriguingly, a more recent analysis of hearts from MCK frataxin-KO animal models has strongly suggested higher levels of apoptosis than age-matched wild-type mice, which is consistent with the cardiomyocyte loss observed in these animals [115a]. Additionally, β-islets from mice in which frataxin has been specifically knocked out in pancreatic β-cells suggest that increased apoptosis is responsible for loss of β-cell mass [179]. Furthermore, the use of the pan-caspase inhibitor, Z-VAD-FMK (benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone) can improve the viability of apoptosis-prone frataxin-deficient fibroblasts in vitro [97].

**Apoptosis in heart failure and FRDA**

It is generally accepted that heart failure is caused by the loss of cardiomyocytes. Although cells such as neurons and cardiomyocytes are generally considered post-mitotic in adult mammals, they still retain the capacity to activate signal

---

**Figure 8** The caspase-dependent intrinsic pathway of apoptosis

Apoptotic stimuli that can be either extra- or intra-cellular result in the stimulation of BAK (Bcl-2 homology domain 3)-only proteins, such as Bad (Bcl-2/Bcl-xL-antagonist, causing cell death) and BID (BHS-interacting domain death). BID is truncated by caspase 8 to form tBid, resulting in an amplification loop for the extrinsic pathway. Activation of BH3-only proteins results in Bax translocation to the mitochondrion and oligomerization of Bax and Bak (Bcl-2 homologous antagonist/killer) in the outer-mitochondrial membrane. This leads to the formation of a pore in the outer-mitochondrial membrane and release of cytochrome c (Cyt c), which binds together with pro-caspase 9 to apoptotic protease activating factor 1 (Apaf1) in the presence of dATP. This leads to the cleavage and activation of caspase 9, which in turn cleaves pro-caspase 3. Active caspase 3 is then responsible for execution of apoptosis. The release of Smac/DIABLO (direct IAP (inhibitor of apoptosis)-binding protein with low pi) inhibits the IAP, thus allowing apoptosis to proceed. Anti-apoptotic members of the Bcl-2 family are able to inhibit cytochrome c release. However, in the presence of pro-apoptotic BH3-only proteins, such as Bad, anti-apoptotic Bcl-2 family proteins, such as Bcl-2, are sequestered and the intrinsic pathway is allowed to progress.

These pathways of apoptosis are initiated by the activation of an upstream protease, and they have been found to converge at the cleavage of an effector or executioner caspases (caspases 3, 6 or 7) (Figures 7 and 8), and ultimately result in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors, and the uptake of phagocytic cells [157]. A comprehensive assessment of the various apoptosis signalling pathways is beyond the scope of the present review, although there are numerous excellent reviews in this area [16,98,156,161–163].
observation that sensitivity to oxidative stress and mitochondrial dysfunction are increased in the context of frataxin deficiency strongly suggests that activation of the intrinsic pathway, at least in vitro, is a likely cause of cardiomyocyte loss and eventual heart failure in this disease [180]. Unfortunately, there is also no evidence to date for the presence or absence of increased apoptosis in cardiac tissue from FRDA patients, which is probably a reflection of the paucity of available material for such studies. In conclusion, the available data from animal models of FRDA and in vitro studies of frataxin-deficient patient cells and cell lines indicate that the involvement of apoptosis in the pathogenesis of cardiomyopathy in FRDA should be thoroughly investigated.

**THERAPEUTIC IMPLICATIONS**

Considering the potential roles of autophagy and apoptosis in the progression of cardiomyopathy in FRDA, a number of therapeutic strategies could be employed to inhibit and/or manipulate these pathways. Several therapeutics have been found to be effective in experimental models of apoptosis. For example, the administration of caspase inhibitors in areas of risk resulted in the reduction of infarct sizes in rat hearts during ischaemia and reperfusion [181,182]. However, although it is possible to target apoptotic pathways, it still remains to be shown that inhibition of apoptosis will delay or prevent heart failure. It is possible that the inhibition of apoptosis will result in other forms of cell death, such as necrosis, which could be even more damaging [165]. In addition, it is not known what the long-term effects of inhibiting apoptosis will be, because apoptosis is beneficial in that it is needed for cell renewal and development. It should also be noted that excessive inhibition of apoptosis is known to be associated with autoimmune disorders and lymphoma [183].

Finally, as discussed above, the detection of apoptotic markers is dependent on the stage of the disease, as well as other factors. Hence the inhibition of apoptosis may not be effective for all forms of heart failure, and it may in fact not be beneficial in the treatment of FRDA.

Autophagic markers were found to be elevated during cardiac injury induced by ischaemia/reperfusion [151]. In addition, considering the correlation between cardiac hypertrophy and the prognosis in FRDA, it might be helpful to target players in the autophagic pathway [152]. However, as it is still not known if autophagy has a protective or deleterious effect in cardiac remodelling, the inhibition of autophagy as a therapeutic has to be approached with caution. Moreover, as in apoptosis, much is left to learn about the cross-talk between autophagy, apoptosis and necrosis, and inhibiting one pathway may lead to the stimulation of another.

**ACKNOWLEDGEMENTS**

We thank Dr Alfons Lawen (Monash University) for his comments on the paper before submission.

**FUNDING**

D.J.R.L. thanks the Cancer Institute NSW (CINSW) for an Early Career Fellowship (grant number 10/ECF/2-18) and the National Health and Medical Research Council of Australia (NHMRC) for an Early Career Postdoctoral Fellowship. D.R.R. acknowledges the NHMRC for a Senior Principal Research Fellowship and Project Grants and the Muscular Dystrophy Association U.S.A. for a Research Grant.

**REFERENCES**


© The Authors Journal compilation © 2013 Biochemical Society


157 Biochemistry of cardiomyopathy in Friedreich’s ataxia
misao, j., hayakawa, y., ohno, m., kato, s., fujiwara, t. and fujiwara, h. (1996) expression of bcl-2 protein, an inhibitor of apoptosis, and bax, an accelerator of apoptosis, in ventricular myocytes of human hearts with myocardial infarction. circulation 94, 1506–1512

liu, l., ather, g., gao, w., zhang, x. and wei, j. y. (1998) bcl-2 and bax expression in adult rat hearts after coronary occlusion: age-associated differences. am. j. physiol.: regul., integr. comp. physiol. 275, r315–r322

condorelli, g., morisco, c., stassi, g., notto, a., farina, f., sgaramella, g., de rienzo, a., roncarati, r., trimarco, b. and lembo, g. (1999) increased cardiomyocyte apoptosis and changes in proapoptotic and antiapoptotic genes bax and bcl-2 during left ventricular adaptations to chronic pressure overload in the rat. circulation 99, 3071–3078

brocheriou, v., hagihe, a. a., oubeniessa, a., lambert, m., mallet, v. o., duriez, m., wassef, m., kahn, a., menasché, p. and gilgenkrantz, h. (2000) cardiac functional improvement by a human bcl-2 transgene in a mouse model of ischemia/reperfusion injury. j. gene med. 2, 326–333

puccio, h. (2007) conditional mouse models for friedreich ataxia, a neurodegenerative disorder associated with cardiomyopathy. handb. exp. pharmacol. 178, 365–375

ristow, m., mulder, h., pomplun, d., schulz, t. j., muller-schmehl, k., krause, a., fex, m., puccio, h., multer, j. and isken, f. (2003) frataxin deficiency in pancreatic islets causes diabetes due to loss of β cell mass. j. clin. invest. 112, 527–534

bayot, a., santos, r., camadro, j. m. and rustin, p. (2011) friedreich’s ataxia: the vicious circle hypothesis revisited. bmc med. 9, 112

yaolta, h., ogawa, k., maeshara, k. and maruyama, y. (1998) attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. circulation 97, 276–281

holly, t. a., drincic, a., byun, y., nakamura, s., harris, k., klocke, f. j. and cryns, v. l. (1999) caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. j. mol. cell. cardiol. 31, 1709–1715


liu, l., feng, d., chen, g., chen, m., zheng, q., song, p., ma, o., zhu, c., wang, r., qi, w. et al. (2012) mitochondrial outer-membrane protein fundc1 mediates hypoxia-induced mitophagy in mammalian cells. nat. cell biol. 14, 177–185

received 14 january 2013/12 april 2013; accepted 15 may 2013
published on the internet 12 july 2013, doi:10.1042/bj20130079