Recent advances in the molecular pathogenesis of Friedreich ataxia

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Friedreich ataxia, the most frequent cause of recessive ataxia, is due in most cases to a homozygous intronic expansion resulting in the loss of function of frataxin. Frataxin is a mitochondrial protein conserved through evolution. Yeast knock-out models and histological data from patient heart autopsies have shown that frataxin defect causes mitochondrial iron accumulation. Biochemical data from patient heart biopsies or autopsies have revealed a specific deficiency in the activities of aconitases and of mitochondrial iron-sulfur proteins. These results suggest that frataxin may play a role either in mitochondrial iron transport or in iron-sulfur cluster assembly or transport. Iron abnormalities suggest a pathogenic mechanism involving free radical production and oxidative stress, a process that might be sensitive to antioxidant therapies.

INTRODUCTION

Friedreich ataxia (FRDA) is the most frequent hereditary ataxia, with an estimated incidence in Caucasians of 1 in 30 000 (1,2). FRDA is an autosomal recessive neurodegenerative disease characterized by progressive gait and limb ataxia, dysarthria, lower limbs areflexia, decreased vibration sense, muscle weakness of the legs and positive extensor plantar response (3,4). Non-neurological signs include hypertrophic cardiomyopathy (5,6) and increased incidence of diabetes mellitus (7). The onset of symptoms usually occurs before the age of 25 years, and typically around puberty. The first pathological changes occur in the dorsal root ganglia (DRGs), with the loss of large sensory neurons, followed by neuron degeneration in Clarke’s and posterior columns, and pyramidal and spinocerebellar tracts of the spinal cord (8). Mild degenerative changes are also observed in the medulla, cerebellum and pons.

The present review describes how the discovery of the Friedreich ataxia gene and of its major mutation, a large GAA trinucleotide expansion, led to the understanding of the FRDA pathology as a disturbance of mitochondrial iron homeostasis and to new therapeutic prospects.

THE FRDA GENE

The human FRDA gene, X25, positionally cloned in 1996, is localized on chromosome 9q13 and is composed of seven exons spanning 80 kb of genomic DNA (9). Northern blot analysis in human and mouse, RNase protection assays and cDNA cloning indicate that the 1.3 kb major transcript is made from five exons (exons 1–5a). This transcript encodes a protein of 210 amino acids called frataxin. A very minor alternative transcript contains exon 5b instead of 5a, followed by the non-coding exon 6. The functional significance of this transcript, if any, is still uncertain.

The FRDA gene demonstrates tissue-specific and developmentally controlled expression by northern blot (in human and mouse tissues) and in situ hybridization (in mouse tissues only) (9–11). The expression partially correlates with the main sites of pathology of the disease. DRGs, where the sensory cell bodies are located, are the major sites of expression in the nervous system, from embryonic day 12 until adult life. Deep sensory neuropathy and degeneration of the posterior columns therefore appear as a direct consequence of reduced frataxin levels in these structures. Expression in the spinal cord is comparatively much lower, suggesting that degeneration of the spinocerebellar tracts and of the Clarke’s columns (containing, respectively, the axons and the cell bodies of secondary neurons projecting to the cerebellum) might be secondary to degeneration of the DRG neurons. Significant frataxin expression is also observed in the granular layer of the cerebellum. Degeneration of the motor corticospinal (pyramidal) tracts might correlate with frataxin expression in mature cells of the developing forebrain (10), though it was not detected in mouse adult cerebral cortex (11). Expression in mouse brain is restricted mostly to the periventricular zone in embryos and to the corresponding ependymal layer in adults (11).

The frataxin gene is also expressed in non-neuronal tissues, such as heart and pancreas, which may account for hypertrophic cardiomyopathy and the increased incidence of diabetes observed in FRDA patients (9,11). Frataxin is also expressed prominently in tissues apparently not affected by the disease, such as liver, muscle, thymus and brown fat. All tissues highly expressing frataxin are rich in mitochondria, with brown fat, present in newborns, being particularly rich (11). The difference between non-affected and affected tissues may lie in the non-dividing nature of the latter (neurons,
FRDA MUTATIONS

FRDA is caused most commonly by a large GAA triplet repeat expansion within the first intron of the gene encoding frataxin (9). Ninety-six percent of patients are homozygous for GAA trinucleotide repeat expansions, whereas the remaining 4% of cases are compound heterozygotes for a GAA expansion and a point mutation within the coding region of the gene (9,12). Truncating and missense mutations are equally represented, although missense mutations have only been found in the second half of the protein, suggesting that it is an important functional domain. In most cases, point mutations are found in clinically typical FRDA patients with few exceptions. The G130V missense mutation always appears associated with an atypical presentation (retracted knee reflexes, often brisk, absence of dysarthria, moderate ataxia, spastic gait and slow progression) (12–15). The G130V mutation is one of the most frequent frataxin point mutations and arose from a common founder event (15). Other missense mutations, each represented by a single case [L106S (16), D122Y (12), R165C and L182F (14)], also seem to be associated with mild presentation.

The clinical equivalence between the GAA intronic expansion and the truncating mutations suggests that the expansion acts by loss of function on frataxin. Indeed, RT–PCR and RNase protection experiments revealed that frataxin mRNA levels are markedly decreased in comparison with those of controls or unrelated ataxias (9,17,18). RNase protection and in vitro transcription experiments suggest that the expansion acts at the transcriptional level, rather than by interfering with the splicing of intron 1 (18,19). Experiments using in vitro and in vivo expression systems revealed that the GAA repeat interferes with transcription in an orientation- and length-dependent manner (18,19). The molecular basis for these results was proposed to be the formation of a non-B DNA conformation, probably a triple helical structure. Indeed, Sakamoto et al. (20) recently described a novel DNA structure, “sticky DNA”, for long tracts of GAA from the frataxin gene. Sticky DNA is formed by the association of two purine–purine–pyrimidine (R–R–Y) triple helical structures under the influence of negative supercoiling. Therefore, the formation of sticky DNA in an FRDA patient would be the mechanism by which the expansion suppresses gene expression, resulting in reduced levels of frataxin.

The direct involvement of the GAA expansion as the cause of FRDA is demonstrated by the very significant inverse correlation between the size of the smaller of the two expansions and the age of onset, the severity of the disease and the risk of occurrence of optional signs such as cardiomyopathy, scoliosis and diabetes (21–26). These observations suggest that some frataxin is produced from alleles carrying smaller expansions, in a length-dependent manner. This was confirmed using a monoclonal antibody directed against frataxin (27) and is in agreement with the transcript analyses. The smallest pathological expansions are in the range of 90–110 repeats, and are found in patients with late onset and atypical presentation of the disease (22) [one exception described (1) is most likely due to the presence of an unstable pre-mutation in one of the parents]. As a consequence, the molecular definition of FRDA based on the presence of the GAA expansion mutation is broader than the previous clinically based definition. Patients with small expansions (<400 repeats) often show onset of the symptoms after age 25 years or have retained tendon reflexes (21–25), features previously considered as exclusion criteria (4). Detection of the expansion mutation thus provides a most useful diagnostic test. The length of expansion, however, has little value for individual prognosis, given the large scattering of points along the correlation curve.

FRATAXIN MITOCHONDRIAL LOCALIZATION

Frataxin is a 210 amino acid protein showing no similarity to protein domains of known function. Therefore, the function of frataxin cannot be inferred from its amino acid sequence. The protein, however, shows a striking degree of evolutionary conservation among eukaryotes, in particular in a stretch of 27 amino acids encoded by exons 4 and 5a.

A first suggestion that frataxin could be a mitochondrial protein came from phylogenetic studies. Sequence comparisons showed the presence of more distant homologues in Gram-negative (γ purple), but not in Gram-positive, bacteria (28). This suggests that the frataxin gene might be derived from the bacterial precursor of the mitochondrial genome which shares phylogenetic ancestry with Gram-negative bacteria, and then underwent transfer to the nuclear genome. Furthermore, computer analysis predicted a mitochondrial targeting signal at the N-terminus of yeast and mouse frataxin, a domain that is absent in the bacterial homologues (11). Human and yeast frataxin were demonstrated directly to be mitochondrial proteins by epitope tagging experiments and colocalization with well-established mitochondrial markers (11,29–31). The mitochondrial localization of endogenous frataxin was demonstrated using specific monoclonal antibodies. Immunoelectron microscopy results indicate that frataxin, which has no hydrophobic transmembrane segment, is nevertheless associated with mitochondrial membranes and crests (27).

The N-terminal mitochondrial targeting sequence of both mammalian and yeast frataxin is removed proteolytically in a two-step cleavage resulting in an 18 kDa mature protein (27,32,33). Both cleavage steps involve the mitochondrial processing peptidase (MPP), a dimeric protease whose β-subunit binds to the N-terminus of frataxin (32,33). A conflicting report suggesting that maturation of mammalian frataxin is a single-step processing event by MPP (34) failed to demonstrate that the single-step product is indeed mature frataxin and might well correspond to the intermediate product, since the second step cleavage is very inefficient in vitro (33).

Koutnikova et al. (32) suggested that frataxin binding and cleavage by MPP appear partially affected by disease-causing missense mutations in its C-terminal moiety (G130V and I154F), and that maturation of the I154F mutant was reduced compared with wild-type frataxin in an in vivo overexpression system, whereas Gordon et al. (34) clearly found no evidence...
of reduced first step cleavage for the I154F mutant. Further investigations are needed to determine whether the disease-causing missense mutations affect the maturation and, if so, which step and whether this relates to the pathological process in FRDA patients.

MITOCHONDRIAL IRON ACCUMULATION

Yeast as a model organism proved to be an invaluable system for unravelling frataxin function within the mitochondria. Three independent groups observed that deletion of the yeast frataxin gene, YFH1 (yeast frataxin homologue 1), results in impaired growth on glycerol, a non-fermentable source of carbon, accumulation of mitochondrion-deficient rho– clones (deletion of mitochondrial DNA) and reduced respiration (11,29,30,35). Moreover, the mutant yeast showed a higher sensitivity to oxidative agents such as hydrogen peroxide, iron and copper than did the wild-type strains (29,35). The yeast frataxin gene was isolated independently as a multicopy suppressor able to rescue a yeast mutant strain unable to grow on iron-limited medium (29). Measurements of iron content revealed that mitochondrial iron is 10-fold higher in the ∆YFH1 mutants than in wild-type yeast, whereas total cellular iron concentration is doubled (29,35). The fact that the high affinity iron import system is constitutively turned on in the ∆YFH1 mutants suggests that cytosolic iron is low (29). Iron is a well known catalyst of free radicals, and excess iron in mitochondria most probably explains the increased sensitivity to hydrogen peroxide and the mitochondrial dysfunction through irreversible oxidative damages (Fig. 1).

If the function of human frataxin is similar to that of the yeast protein, this would suggest that iron accumulates in mitochondria of FRDA patients, and could result in hypersensitivity to oxidative stress, as a consequence of the Fenton reaction (Fe²⁺-catalysed production of hydroxyl radicals). Indeed, iron deposits are observed consistently on autopsy in some heart myofibrils of FRDA patients and sometimes are also observed in liver and spleen (36,37). Cardiomyopathy in FRDA patients could thus be a result of iron overload (as in thalassaemia or in haemochromatosis) or might reflect a selective sensitivity of heart mitochondria to frataxin deficiency. Recent magnetic resonance imaging data indicate that iron also accumulates in the dentate nucleus, an affected cerebellar structure (38). Total iron content of FRDA fibroblasts, an unaffected tissue in this disease, is within the normal range, with a minimal mitochondrial iron increase just at the limit of significance (39).

IRON–SULFUR CLUSTER PROTEIN DEFICIENCY

Rötig et al. (40) found selective deficiencies of the respiratory chain complexes I, II and III and of both mitochondrial and cytosolic aconitase activities in the heart biopsy of two patients. Other respiratory chain complexes and Krebs cycle
activities were normal. Aconitase activity deficiency was profound (~10-fold reduction), whereas deficiencies in the activities of complexes I, II and III were significant only when their ratios to other mitochondrial activities, such as complex IV, were calculated. No deficiency was found in the muscle, fibroblasts or lymphocytes of the same patients. All the deficient enzymes and complexes contain iron–sulfur (Fe–S) clusters in their active sites. Fe–S proteins are remarkably sensitive to free radicals, and their inactivation further suggests oxidative stress in FRDA-affected tissues. Deficiency restricted to the Fe–S proteins has not been found in 60 biopsies of patients with cardiomyopathy, several of whom had mitochondrial DNA mutation (40), indicating that this inactivation is associated specifically with the depletion of frataxin. These results have been confirmed recently on autopsy material of nine patients, although the deficiencies of complexes I, II and III were more pronounced (36), possibly due to a longer disease duration. Fe–S protein deficiency was not found in autopsies of two patients analysed, presumably because the large sensory neurons, the primary target cells in FRDA, are entirely replaced by gliosis at the time of death.

The yeast YFH1 mutants have a generalized mitochondrial dysfunction, including Fe–S protein deficiency as well as complex IV, and to a lesser extent complex V, deficiencies (35,40), presumably because iron accumulation is higher in yeast mutants than in patient hearts. Is the Fe–S protein deficiency a cause or a consequence of mitochondrial iron accumulation? Recent studies in the yeast suggest that frataxin mediates mitochondrial iron efflux (41), and demonstrate that the presence of an iron chelator in the culture medium restores normal intramitochondrial iron levels and normal oxidative respiration in ΔYFH1 yeast cells (42). However, in the latter experimental conditions, the activity of aconitase is not fully restored (42), suggesting that the reduction in the activity of the respiratory chain complexes is a consequence of mitochondrial iron accumulation, whereas the reduced aconitase activity is linked directly in part to frataxin deficiency. Mitochondrial aconitase deficiency therefore does not seem to be a mere consequence of mitochondrial dysfunction. In addition, the inactivation of cytosolic aconitase in FRDA patients (yeast do not have cytosolic aconitase) suggests that a general iron–sulfur defect is the underlying mechanism. The loss of cytosolic aconitase activity observed in FRDA might also reflect a decrease of cytosolic iron content, since cytosolic aconitase is converted to iron-responsive element-binding protein (IRE-BP1) in response to low iron concentration (43) (Fig. 1).

Two other yeast mutants that specifically accumulate iron in the mitochondria are worth a mention here. The ATM1 gene encodes a seven transmembrane ABC ATPase (a putative ABC transporter) that, when mutated, causes mitochondrial iron accumulation at 20-fold the normal level (44). ATM1 seems to be involved in export of Fe–S clusters, which are assembled in the mitochondria, since cytosolic Fe–S enzymes are inactivated before mitochondrial enzymes and before mitochondrial iron accumulation (45) (Fig. 1). Attempts to identify a direct functional relationship between the frataxin gene and ATM1 have failed so far. A conservative missense mutation in ABC7, the human homologue of the ATM1 gene, was found to cause a rare X-linked recessive disease, associating spinocerebellar ataxia and anaemia (46).

Mutants of the Ssq1 gene, encoding a low abundance mitochondrial heat shock 70 protein (mtHsp70), accumulate mitochondrial iron at a concentration up to 40-fold the normal level, depending on the extracellular iron concentration (47). Mtap70 proteins act as chaperones that pull imported peptides into the mitochondrial matrix, prior to their cleavage by MPP (48). Ssq1 mutants show partially altered frataxin second step maturation cleavage. The dramatic iron accumulation of Ssq1 mutants suggests that Ssq1 acts on the import of a cascade of proteins involved in mitochondrial iron homeostasis, a pathway that may also be altered in YFH1 mutants.

Studies of the biochemical defect in the yeast mutant should reveal the role of the frataxin homologue in iron homeostasis and Fe–S cluster biogenesis. Very recent in vitro biochemical studies suggest that yeast frataxin might instead act as a mitochondrial iron storage protein similar to cytoplasmic ferritin (49). Further biochemical analysis of appropriate target tissues may be difficult in man, and a mouse model of FRDA (by knock-out of the frataxin gene) should be extremely valuable to understand the pathological consequences of frataxin deficiency in mammalian cells and tissues.

THERAPEUTIC ADVANCES

Based on the recent discoveries on the potential function of frataxin, and more specifically on the consequences of frataxin reduction, therapeutic advances can be envisaged. Whether the mitochondrial iron accumulation is a primary or a secondary effect of frataxin deficiency, all the data suggest that intracellular iron imbalance leading to oxidative stress is involved in the pathogenesis of FRDA. This led to initial enthusiasm for iron chelators, such as desferrioxamine, as therapeutic reagents for treating the disease. However, desferrioxamine has a significant side effect profile and its effect on individuals who do not have a generalized iron overload is not well studied. FRDA patients have normal serum iron and ferritin levels (50). Rustin et al. (51) recently have shown, using an in vitro experimental system which mimics the abnormalities observed in heart biopsies from patients, that ferrous iron (Fe2+), but not ferric iron (Fe3+), causes peroxidation of lipids and inactivation of complex II without affecting aconitase activity. The addition of an iron chelator such as desferrioxamine in the extracts protects complex II and lipids from Fe2+ oxidation but causes a marked reduction in total aconitase activity (51), presumably by displacing the ferrous iron from membranes to the soluble fraction. These results suggest that care should be taken when using iron chelators as a therapeutic agent since they could displace rather than protect against Fe2+-mediated toxicity.

Since it has been proposed that reducing the load of free radicals will slow the progression of the disease, antioxidants which are usually devoid of side effects can be considered as potential therapeutic reagents. Rustin et al. (51) have shown by in vitro experiments that ascorbic acid, a water-soluble anti-oxidant, is not protective since it reduces ferric iron to its ferrous toxic form. On the other hand, the antioxidant idebenone, a short chain analogue of coenzyme Q10, protects both membranes and respiratory chain enzymes against iron-induced injury without causing a reduction in aconitase activity (51,52). In addition, the short chain of idebenone allows it to cross membranes readily (including the blood–brain barrier). Preliminary results on the cardiomyopathy of
three FRDA patients are promising and warrant the establishment of a large clinical trial using idebenone.

An efficient method for the evaluation of potential treatment efficacy is a major prerequisite for such a clinical trial. Lodi et al. recently have reported in vivo evidence of impaired mitochondrial respiration in skeletal muscle of FRDA patients using phosphorus magnetic resonance spectroscopy (31P-MRS) (53). Moreover, the observed decreased oxidative activity shows some correlation with the size of the GAA expansion. These findings contribute to the growing body of evidence that treatments aimed at enhancing mitochondrial function and reducing toxic radical production are rational and may be a realistic hope for the patients and their families.

CONCLUSION

Friedreich ataxia is a remarkable disease. The major mutation, a large intronic GAA repeat expansion, is the most frequent trinucleotide repeat expansion presently known in the human population. It arises, probably recurrently, from alleles with a pure GAA repeat at the upper limit of the normal range, and has spread unselected in healthy carriers, up to a carrier frequency of 1 in 85 individuals in the Caucasian population. Initial studies of frataxin and of its yeast homologue have revealed that FRDA is a mitochondrial disease due to mutations in a gene from the nuclear genome. The fact that frataxin deficiency results in alteration of mitochondrial iron homeostasis strongly suggests that oxidative stress is underlying the pathology of the disease, since iron is a well established catalyst of free radical production from hydrogen peroxide, a by-product of the mitochondrial oxidative phosphorylation pathway. FRDA may, therefore, serve as a paradigm for the growing list of neurodegenerative diseases caused by free radical toxicity, such as ataxias due to vitamin E deficiency (54) and familial amyotrophic lateral sclerosis due to superoxide dismutase mutations (55). Many questions remain unsolved and in particular the cause for the relatively late onset and slow progression of the disease, as well as the specificity of neurodegeneration, while disruption of the corresponding gene in yeast results in a dramatic and rapid phenotype. We have found that a homozygous frameshift deletion in the frataxin gene in yeast results in a dramatic and rapid phenotype. We revealed that FRDA is a mitochondrial disease due to mutations in a gene from the nuclear genome. The fact that frataxin deficiency results in alteration of mitochondrial iron homeostasis strongly suggests that oxidative stress is underlying the pathology of the disease, since iron is a well established catalyst of free radical production from hydrogen peroxide, a by-product of the mitochondrial oxidative phosphorylation pathway. FRDA may, therefore, serve as a paradigm for the growing list of neurodegenerative diseases caused by free radical toxicity, such as ataxias due to vitamin E deficiency (54) and familial amyotrophic lateral sclerosis due to superoxide dismutase mutations (55). Many questions remain unsolved and in particular the cause for the relatively late onset and slow progression of the disease, as well as the specificity of neurodegeneration, while disruption of the corresponding gene in yeast results in a dramatic and rapid phenotype. We have found that a homozygous frameshift deletion in the mouse, which would be the equivalent of the yeast knock-outs, causes early embryonic lethality (M. Cossée et al., in preparation). This suggests that even the largest GAA expansions in FRDA patients do not extinguish frataxin expression totally, allowing the production of a residual amount of frataxin compatible with cellular survival. It is also possible that cell types requiring frataxin during embryonic development select precursors with a shorter expansion mutation, allowing for a higher residual frataxin level. Lastly, mammalian cells which cannot survive in the absence of oxidative phosphorylation, unlike yeast cells, may have developed numerous protective pathways against free radical toxicity, vitamin E being one of these, that can compensate in part for frataxin loss of function. The next challenge is to understand frataxin function and how its loss results in iron accumulation and Fe–S protein deficiency in mitochondria of some, but probably not all, cell types or tissues. This should shed light on a previously unsuspected regulatory process involved in the fine tuning of intracellular iron homeostasis, and should provide solid bases for designing eagerly awaited therapeutic approaches for FRDA.

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REFERENCES


