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REVIEW

Frataxin: a protein in search for a function

Annalisa Pastore* and Helene Puccio†,‡,§,¶,**

*MRC National Institute for Medical Research, London, UK

†Institut de Genetique et de Biologie Moleculaire et Cellulaire, 1 rue Laurent Fries, BP10142, Illkirch, France

‡Inserm, U596, Illkirch, France

§3CNRS, UMR7104, Illkirch, France

¶Universite' Louis Pasteur, Strasbourg, France

**College de France, Chaire de genetique humaine, Illkirch, France

Abstract

Reduced levels of the protein frataxin cause the neurodegenerative disease Friedreich's ataxia. Pathology is associated with disruption of iron–sulfur cluster biosynthesis, mitochondrial iron overload, and oxidative stress. Frataxin is a highly conserved iron-binding protein present in most organisms. Despite the intense interest generated since the determination of its pathology, identification of the cellular function of frataxin has so far remained elusive. In this review, we revisit the most significant milestones that have led us to our current understanding of frataxin and its functions. The picture that emerges is that frataxin is a crucial element of one of the most essential cellular machines specialized in iron-sulfur cluster biogenesis. Future developments, therefore, can be expected from further advancements in our comprehension of this machine.

Keywords: Friedreich's ataxia, iron metabolism, mitochondria, oxidative stress.

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Frataxin is a small protein which owes its 'notoriety' to its link to the neurodegenerative disease Friedreich's ataxia (FRDA) (Campuzano *et al.* 1996). This pathology is caused by an abnormal expansion of a non-coding GAA triplet repeat in the first intron of the *FRDA* gene, leading to lower expression levels of frataxin through heterochromatization of the locus (Campuzano *et al.* 1997). In healthy individuals, the number of repeats range from 6–36, whereas in FRDA patients, the number of repeats is in the 70–1700 range, most commonly 600–900 (Pandolfo 2009). The severity of the disease and the age at onset inversely correlate with the number of repeats. Nothing besides the primary sequence was known about frataxin when the *FRDA* gene was first linked to FRDA (Campuzano *et al.* 1996). The pace of advancement in the field of FRDA has been rapid. Sixteen years later, we have accumulated a fair amount of knowledge about the protein localization, its cellular forms and its interactome, even though we seem to be still far from a complete understanding of the frataxin cellular function. In this review, we shall highlight the most important steps that have led us to our current knowledge and discuss possible future developments. Here, we have underlined some of the main contributions to the investigation of frataxin's function. We apologize in advance to the colleagues who we have not cited. Other recent reviews may complement the information provided here (Ye and Rouault 2010; Martelli *et al.* 2012; Vaubel and Isaya 2012).

Structural and biophysical studies on frataxin

The frataxin sequence and its cellular localization

Frataxin is a small acidic protein (with isoelectric point on average around 4.9) highly conserved in most organisms from bacteria to mammalians (Gibson *et al.* 1996; Adinolfi *et al.* 2002) (Fig. 1a). A frataxin homolog was also identified in the human parasite *Trichomonas vaginalis* (Dolezal *et al.* 2007). This finding is interesting since these unicellular eukaryote organisms do not have mitochondria

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Address correspondence and reprint requests to Annalisa Pastore, MRC National Institute for Medical Research, The Ridgeway, London NW71AA. UK. E mail: apastor@nimr.mrc.ac.uk

Abbreviations used: FRDA, Friedreich's ataxia; MMP, mitochondrial processing peptidase; RMSD, root mean square deviations; XAS, X-ray absorption spectroscopy.

but hydrogenosomes which share common ancestry with mitochondria. Sequence alignment of the frataxin family shows two distinct regions. An N-terminal block of 70–90 residues is completely absent in prokaryotes and poorly conserved also among eukaryotes (Huynen *et al.* 2001). Its sequence has the features typical of intrinsically unfolded proteins. The C-terminus of the protein comprises a highly conserved block of ca. 100–120 amino acids that is conserved in most organisms. The sequence identity of this region is as high as ~ 25%, whereas the similarity is 40–70%, indicating that this is the functionally important part of the molecule. Among the most conserved residues are three tryptophans that are residues with a relatively low occurrence in proteins. Their conservation suggests that they could have an important structural and/or functional role. Semi-conserved is a stretch of negatively charged residues.

Clues on the cellular localization of frataxin were provided already from the sequence alignment of the family (Gibson *et al.* 1996). The distribution of the FRDA gene in different genomes, and some clinical similarities with another ataxia linked to vitamin E deficiency and with neuropathies with mitochondrial DNA instability caused by mutations in nuclear genes, suggested that frataxin could be a protein imported to mitochondria. It was argued that the absence of the non-conserved N-terminus in most prokaryotes and the presence of homologs in purple bacterial genomes, but not in other bacteria, suggest a mitochondrial localization. This hypothesis was confirmed by tagging experiments which showed that human frataxin co-localizes with a mitochondrial protein (Koutnikova *et al.* 1997). It is now well accepted that frataxin is nuclearly encoded, expressed in the cytoplasm and imported in the mitochondrion through an import signal contained in the N-terminus. This explains the poorer conservation of this region.

Frataxin maturation in mitochondria

The mature form of the yeast frataxin ortholog, Yfh1, was established early on to start at residue 52 of the transcript (Branda *et al.* 1999). On the other hand, the exact nature of the human mature frataxin has been the subject of a long-standing controversy. Human frataxin is synthesized as a precursor of 210 amino acids imported to the mitochondrion, and undergoes maturation by the mitochondrial processing peptidase (MMP) through a two-step process that leads to the successive generation of an intermediate form of 19 kDa cleaved between G41 and L42 (residues 42–210) and a mature form of 14 kDa (residues 81–210) (Koutnikova *et al.* 1998; Condò *et al.* 2007; Schmucker *et al.* 2008). Another form starting at S56 was also reported (Cavadini *et al.* 2000), but it is now widely accepted that the 81–210 form is the most abundant species both in normal individuals and in FRDA patients (Condò *et al.* 2007; Schmucker *et al.* 2008; Gakh *et al.* 2010). The frataxin 81–210 mature form is fully functional for cell survival and is thus the most suitable species to investigate in further functional studies.

The 3D structure of frataxin

Recombinant frataxins from *E. coli*, *S. cerevisiae* and *H. sapiens* have been intensively studied structurally: there are 19 entries responding to the keyword frataxin in the PDB Database (Fig. 1b). The structure of the conserved C-terminal domain of the human protein was the first to be characterized both by NMR and crystallography, together

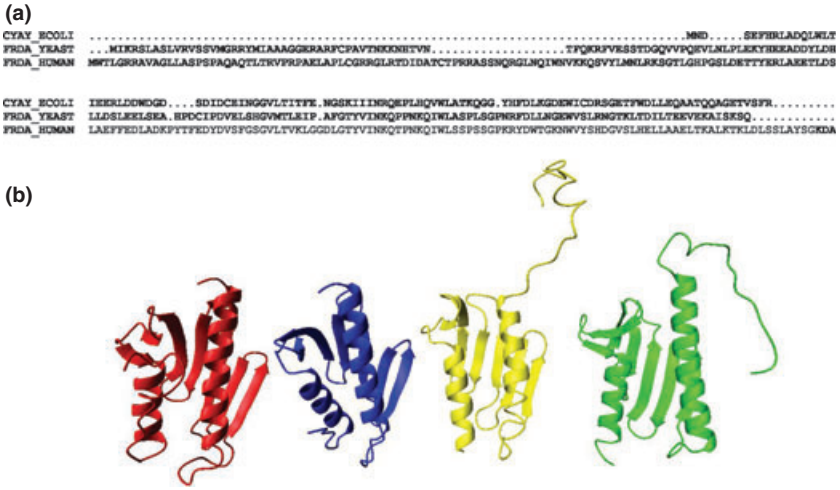

Fig. 1 Frataxin sequence and structure. (a) Sequence alignment of bacterial, yeast, and human frataxin. (b) Four representative structures taken from the 19 entries present in PDB. Red: X-ray structure of E. coli CyaY (1ew4), blue: X-ray structure of human frataxin (1ekg), yellow: NMR structure of yeast Yfh1 (2ga5), green: X-ray structure of Yfh1 (3oeq). The differences between the NMR and X-ray structures of Yfh1 are almost certainly because of the appreciable lower accuracy of the NMR structure.