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Frataxin interacts functionally with mitochondrial electron transport chain proteins

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Frataxin deficiency is the main cause of Friedreich ataxia, an autosomal recessive neurodegenerative disorder. Frataxin function in mitochondria has not been fully explained yet. In this work, we show that *Saccharomyces cerevisiae* frataxin orthologue Yfh1p interacts physically with succinate dehydrogenase complex subunits Sdh1p and Sdh2p of the yeast mitochondrial electron transport chain and also with electron transfer flavoprotein complex ETFα and ETFβ subunits from the electron transfer flavoprotein complex. Genetic synthetic interaction experiments confirmed a functional relationship between *YFH1* and succinate dehydrogenase genes *SDH1* and *SDH2*. We also demonstrate a physical interaction between human frataxin and human succinate dehydrogenase complex subunits, suggesting also a key role of frataxin in the mitochondrial electron transport chain in humans. Consequently, we suggest a direct participation of the respiratory chain in the pathogenesis of the Friedreich ataxia, which we propose to be considered as an OXPHOS disease.

INTRODUCTION

Friedreich ataxia is an autosomal recessive neurodegenerative disorder affecting sensory neurons of dorsal root ganglia and spinocerebellar tracts (1,2). The causing gene *FRDA* maps on chromosome 9q13 and encodes frataxin, a small protein of 210 amino acids (3), associated with the internal mitochondrial membrane (4–7). Frataxin is a compact and globular protein consisting of six-stranded antiparallel β-sheets flanked on one side by two α-helices. Twelve acidic residues from α1-helix and β1-sheet form a large, contiguous anionic patch on the protein surface, creating a significant charged dipole (8,9). The size and nature of this structure predicts potential interaction with other proteins or ligands. Both the global structure and the anionic patch are conserved from bacteria to eukaryotes, including animals, plants and yeast (10,11).

A number of physiological functions for frataxin in mitochondria have been proposed. First reports related frataxin with the homeostasis of mitochondrial iron and with the response to oxidative stress (5,12,13). Frataxin has also been proposed as an iron-storage protein maintaining iron in a non-toxic and bioavailable form (14–16). In addition, frataxin seems to be involved in the maturation of several biological processes that utilizes iron. These include biogenesis of iron–sulfur clusters (ISC) (17–19), maturation of hemo-containing proteins (15,20,21) and, more recently, the interaction with aconitase, converting the inactive $[3Fe–4S]^{1+}$ enzyme to the active $[3Fe–4S]^{2+}$ form of the enzyme (22). Finally, some data coming from an *in vivo* study of ATP production by phosphorus magnetic resonance spectroscopy in patients (23) and from a cellular model overexpressing frataxin (24) suggest that frataxin may have a role on the mitochondrial energy conversion and oxidative phosphorylation.

Saccharomyces cerevisiae has been a powerful tool to investigate the function of frataxin. In fact, human frataxin may rescue the phenotype of yeast *yfh1*Δ mutants but complementation disappears when mutant frataxin alleles associated with the disease are introduced into the *yfh1*Δ mutants (12,25). To investigate the biological function of frataxin, we decided to search for putative interacting proteins in *S. cerevisiae*. In this study, we show that Yfh1p interacts physically with yeast mitochondrial electron transport chain

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succinate dehydrogenase complex subunits Sdh1p and Sdh2p and also with yeast putative electron transfer flavoprotein complex ETFα and ETFβ subunits from the electron transfer flavoprotein complex. We also observed that this interaction is conserved in humans because we were able to detect a physical interaction between the human forms of frataxin and the succinate dehydrogenase proteins. These results suggest a direct role of frataxin in the regulation of the mitochondrial respiratory chain, which could be of major relevance in the pathogenesis of Friedreich ataxia.

RESULTS

Identification of proteins that interact with Yfh1p

To identify proteins that could interact with the *S. cerevisiae* frataxin orthologue Yfh1p, we used it as bait in a two-hybrid screening of a *S. cerevisiae* cDNA library. From a total of 100 000 transformants, we recovered four positive clones. Plasmids containing the cDNA fragments of the putative frataxin interacting partners were extracted and sequenced. cDNA sequences were characterized by BLAST analysis (26). One of the cDNAs encoded for the C-terminal domain (from aminoacids 106–399) of yeast Yhb1p, a flavohemoglobin involved in the response to oxidative stress (27,28). By co-immunoprecipitation analyses using full-length proteins, we confirmed that Yhb1p interacted with yeast frataxin (Fig. 1). Yeast Yhb1p is located in both cytosol and mitochondria (29). Whereas its localization within the mitochondria may suggest a role in relation with Yfh1p physiology, Yhb1p is not a good candidate for the pathogenesis of FRDA because it is not conserved in multicellular organisms. However, it is noteworthy that Yhb1p has two domains, a heme domain located at the N-terminus that binds oxygen and a flavo domain at the C-terminus that binds flavin adenine dinucleotide (FAD); thus, we reasoned that the observed interaction may reflect, in fact, a binding between Yfh1p and a domain of Yhb1p. Then, we postulated that proteins containing either flavo or heme domain may be good candidates to interact physiologically with Yfh1p.

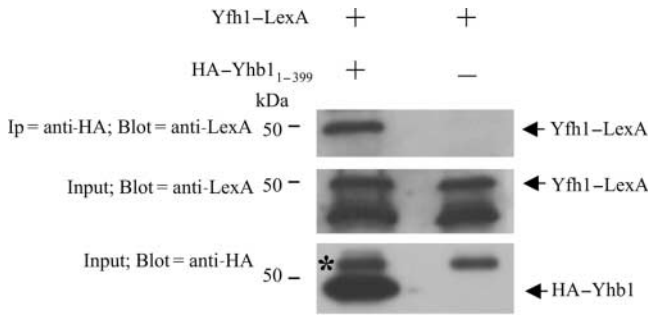

Figure 1. Yfh1p interacts physically with flavohemoglobin Yhb1p. Crude extracts (500 μg) were prepared from FY250 yeast glucose growing cells expressing Yfh1–LexA (pRS424–ADH1p–Yfh1–LexA) and HA–Yhb1p (pWS93–Yhb1) or containing the corresponding empty vectors. HA–Yhb1p fusion proteins were immunoprecipitated with anti-HA monoclonal antibodies. Pelleted proteins were analyzed by SDS–PAGE and immunodetected with anti-LexA polyclonal antibodies (upper panel). Proteins in the input crude extracts (2.5 μg) were also immunodetected with either anti-LexA (middle panel) antibodies or anti-HA antibodies (lower panel). The position of a protein in the crude extracts that cross-reacted non-specifically with anti-HA is denoted with an asterisk. Molecular weight markers are indicated in kilo-Daltons. The faster migrating band in the middle panel may correspond to the mature form of the frataxin fusion protein or to an N-terminal defined proteolytic product, independent of the pre-prosequence of frataxin.

Yfh1p interacts with components of mitochondrial complex II

It has been described that a yeast frataxin mutant (*yfh1*Δ) shows a deficient respiratory growth and has reduced mitochondrial succinate dehydrogenase and aconitase activities (30,31). Moreover, reduced activities of mitochondrial complexes I, II and III and aconitase have also been observed in cardiomyocytes from Friedreich ataxia patients (32). On the other hand, it is also known that overexpression of human frataxin in human adipocytes increases the activity of the electron transport chain, the mitochondrial membrane potential and ATP production (24).

All these data induced us to study whether FAD-containing flavoproteins of the mitochondrial electron transport chain could interact with frataxin. We started our study with yeast succinate dehydrogenase, a component of mitochondrial complex II, well conserved throughout the phylogenetic tree. Complex II, or succinate dehydrogenase-ubiquinone oxidoreductase, is composed of four subunits: a flavoprotein (Sdh1p) and an ISC-containing protein (Sdh2p), which make up the catalytic core and are oriented to the mitochondrial matrix, and proteins Sdh3p and Sdh4p that anchor the catalytic core to the inner mitochondrial membrane (33).

Sdh1p has an N-terminal FAD-binding domain and a C-terminal domain also present in other flavoproteins. We decided to investigate first the putative interaction of Sdh1p with full-length Yfh1p by co-immunoprecipitation analysis. With this aim, we generated four constructs: pWS93–Sdh1$_{32-640}$, which corresponded to the full-length Sdh1 protein without the mitochondrial signal peptide; pWS93–Sdh1$_{153-640}$, with an extended N-terminal deletion; pWS93–Sdh1$_{153-485}$, which contained only the FAD binding domain; pWS9–Sdh1$_{506-640}$, expressing the C-terminal domain (Fig. 2A). Using these constructs, we observed a positive interaction between Yfh1p and all the forms containing the FAD binding domain but not with the C-terminus of the Sdh1 protein (Fig. 2B). These results suggested that Sdh1p might be a target for Yfh1p and also suggested that the interaction occurred within the FAD domain of Sdh1p. Because Sdh1p and the ISC-containing Sdh2p subunit interact with each other at the matrix side of the mitochondrial complex II, we decided to perform similar co-immunoprecipitation experiments with Sdh2p and, as shown in Figure 2C, we observed a specific interaction between Yfh1p and Sdh2p. As a control of the specificity of the interactions as described earlier, we used malate dehydrogenase, Mdh1p (a protein located in the mitochondrial matrix), and observed no physical interaction between Yfh1p and Mdh1p (Fig. 2C).

Genetic synthetic interaction between *YFH1* and *SDH* genes

The results described earlier suggested a functional relationship between Yfh1p and Sdh proteins. To demonstrate this point, we

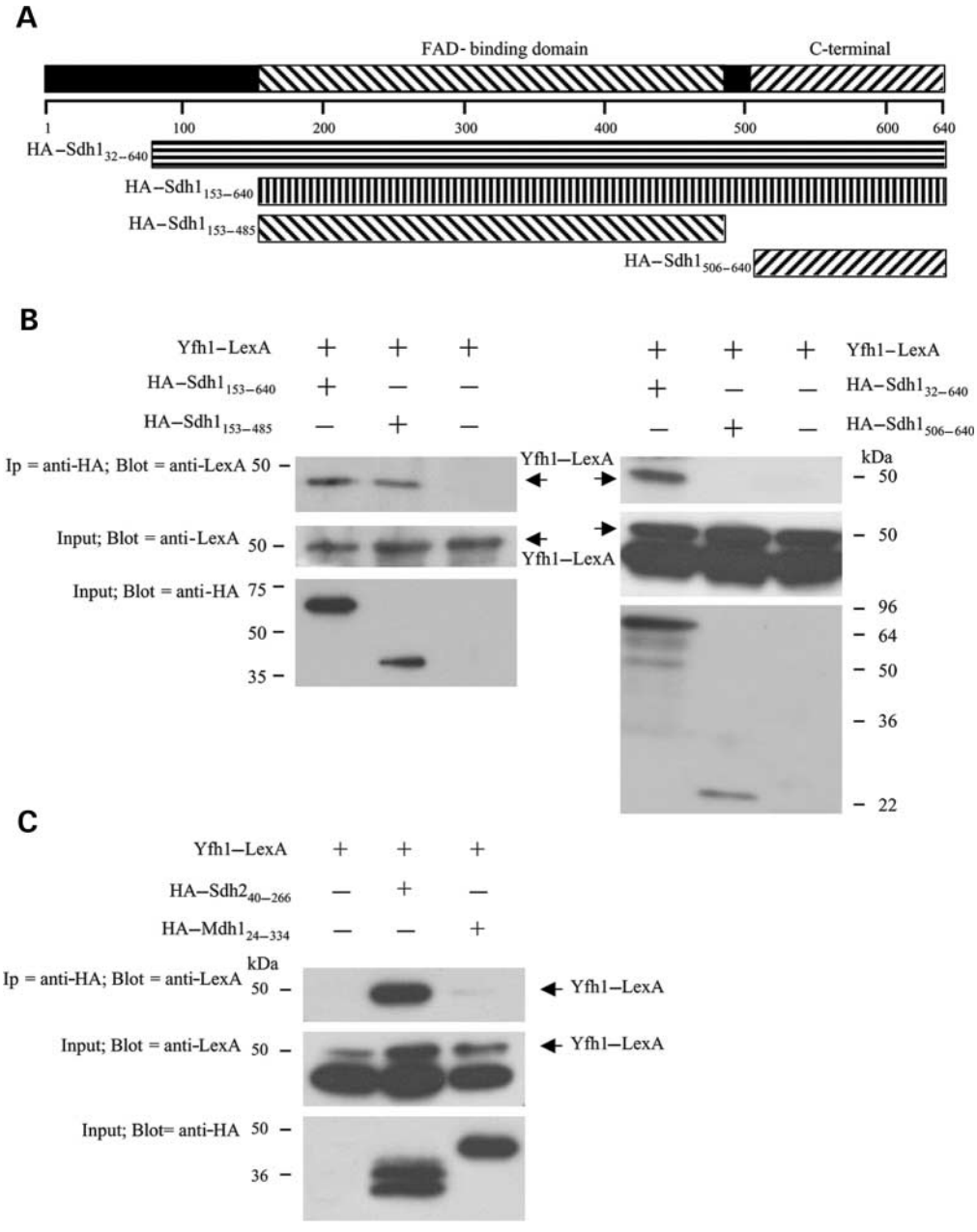

Figure 2. Yfh1p interacts physically with succinate dehydrogenase. **(A)** Diagram of Sdh1p and the constructs used in the co-immunoprecipitation assays. (**B, C**) Physical interaction between Yfh1p and Sdh1p and Sdh2p. Crude extracts (500 μg) were prepared from FY250 yeast glucose growing cells expressing Yfh1–LexA (pRS424–ADH1p–Yfh1–LexA), HA–Sdh1p (pWS93–$Sdh1_{153-640}$; pWS93–$Sdh1_{32-640}$; pWS93–$Sdh1_{153-485}$; pWS93–$Sdh1_{506-640}$) (B) or HA–Sdh2p (pWS93–$Sdh2_{40-266}$) and HA–Mdhp (pWS93–$Mdh_{124-334}$) (C) or containing the corresponding empty vectors. HA-fusion proteins were immunoprecipitated with anti-HA monoclonal antibodies. Pelleted proteins were analyzed by SDS–PAGE and immunodetected with anti-LexA polyclonal antibodies (upper panel). Proteins in the input crude extracts (2.5 μg) were also immunodetected with either anti-LexA antibodies (middle panel) or anti-HA (lower panel) antibodies. Molecular weight markers are indicated in kiloDaltons.

measured mitochondrial succinate dehydrogenase activity in a *yfh1*Δ mutant strain and found a significant reduction (Fig. 3) in agreement with previously reported results (30,31,34). As an alternative way to confirm the functional relationship between Yfh1p and Sdh proteins, we studied the possible genetic synthetic interaction of the corresponding genes. We generated simple *sdh1*Δ and *sdh2*Δ mutants, double *yfh1*Δ *sdh1*Δ, *yfh1*Δ *sdh2*Δ and *sdh1*Δ *sdh2*Δ mutants and the triple *yfh1*Δ *sdh1*Δ *sdh2*Δ mutant (see Materials and Methods). We verified that all the strains were rho+ by genetic crossing with a rho0 tester strain (see Materials and Methods). Every simple mutant showed a regular growth in rich medium (YPD) (Fig. 4A, left panel). However, the double *yfh1*Δ *sdh1*Δ and *yfh1*Δ *sdh2*Δ mutants showed a poor growth on YPD, which was even poorer in the triple *yfh1*Δ *sdh1*Δ *sdh2*Δ mutant (Fig. 4A, middle panel), suggesting a synthetic interaction

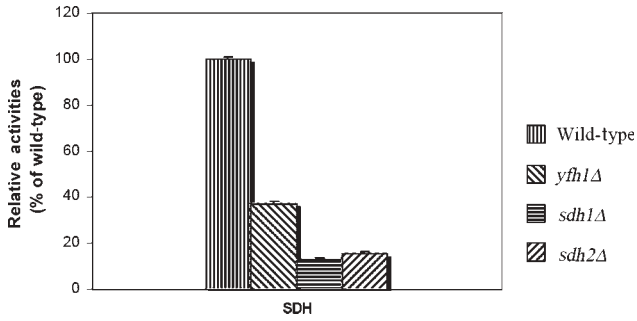

Figure 3. Yfh1p depletion affects the activity of succinate dehydrogenase. Wild-type, *yfh1*Δ *sdh1*Δ and *sdh2*Δ cells were grown in glucose-containing media under aerobic conditions until exhaustion of the carbon source. Mitochondria were isolated and used to determine the succinate dehydrogenase activity by following the reduction of 2,6-dichlorophenolindophenol (DCPIP) at 600 nm. Error bars indicate the standard deviation of at least three independent measurements.

between *yfh1*Δ and either *sdh1*Δ or *sdh2*Δ mutant. Growth of the different strains in minimal SCD medium followed the same trend as in rich YPD medium but aggravating the growth defect of mutants having the frataxin gene deleted (*yfh1*Δ) (Fig. 4B, left and middle panels). Reduced growth was observed in any mutant strain in glycerol/ethanol medium (data not shown).

Yfh1p interacts with components of the electron transfer flavoprotein complex

In our experiments, we observed that the double *sdh1*Δ *sdh2*Δ mutant exhibited a regular growth on both YPD and SCD media (Fig. 4, middle panels). To explain this finding, we reasoned that ubiquinone (Q) should be correctly reduced in the absence of Sdh proteins, possibly by means of electrons arriving to this molecule from alternative pathways. In mammalian cells, it is known that ETF, a system composed of ETF-dehydrogenase (ETF-QO) and a heterodimer composed of two subunits (ETFα and ETFβ), delivers electrons coming from β-oxidation of fatty acids and amino acid catabolism to ubiquinone (35). In *S. cerevisiae*, β-oxidation of fatty acids occurs in peroxisomes and not in mitochondria; however, homologous genes for mammalian ETF complex genes have been reported in yeast (http://www.yeastgenome.org). To test whether the yeast Etf complex might be used as an alternative pathway for ubiquinone reduction in double *sdh1*Δ *sdh2*Δ mutants, we constructed different combinations of mutants lacking the yeast *ETF*α homologue, *YPR004c.* As observed in Figure 4 (right panels), although the double *sdh1*Δ *ypr004c*Δ mutant showed regular growth in YPD medium, it displayed a poor growth in SCD medium, indicating a synthetic interaction of both genes under the latter conditions and suggesting that yeast may use both Sdh and Etf complexes to introduce electrons to ubiquinone. Ypr004cp contains an FAD binding domain, so we tested the putative physical interaction between Ypr004cp and Yfh1p. As shown in Figure 5, co-immunoprecipitation analyses confirmed the interaction between Yfh1p and Ypr004cp. However, *YPR004c* did not show a synthetic interaction with *YFH1* (Fig. 4A, right panels). We extended our studies to the yeast homologue of the mammalian ETFβ subunit (*YGR207c*) and also observed co-immunoprecipitation with Yfh1p (Fig. 5). Finally, we checked the interaction of Yfh1p with yeast ETF-dehydrogenase homologue (*YOR356w*), also an FAD-containing protein, and observed no interaction of Yfh1p with this protein (data not shown), suggesting that not all FAD-containing proteins interact with yeast frataxin.

Interaction of frataxin with succinate dehydrogenase subunits is conserved in humans

To relate these findings with the human disease, we expressed in yeast human frataxin and the human complex II proteins SDHA and SDHB and performed co-immunoprecipitation analyses. As shown in Figure 6, human frataxin was able to interact physically with the FAD binding domain of SDHA ($SDHA_{33-493}$) but not with the C-terminal domain of this protein ($SDHA_{512-664}$). Human frataxin was also able to interact physically with $SDHB_{47-280}$ protein. These data suggested that the interaction of frataxin with succinate dehydrogenease complex subunits was well conserved from yeast to humans.

DISCUSSION

In this work, we present evidence that yeast frataxin is able to interact physically with complexes providing electrons to ubiquinone (Sdh and putative Etf homologues). We also present evidence that yeast frataxin gene displays a genetic synthetic interaction with *SDH1* and *SDH2* and also that the activity of the succinate dehydrogenase complex is severely impaired in yeast mutants lacking frataxin. All these data indicate a functional relationship between yeast frataxin and succinate dehydrogenase complex and suggest a direct role of frataxin in regulating the entry of electrons towards the electron transport chain, at least via complex II. Alternatively, frataxin could also affect the stability or the assembly of the succinate dehydrogenase complex, regulating in this way its activity.

In previous reports, the reduction in succinate dehydrogenase enzymatic activity in yeast *yfh1*Δ mutants was explained as a secondary effect. The authors suggested that the primary defect of frataxin deficiency was an impaired biogenesis of ISC, which directly affected appropriate synthesis of ISC-containing proteins, such as the subunit 2 of the succinate dehydrogenase (17,18). However, the fact that a yeast lacking frataxin (*yfh1*Δ) shows a regular growth in rich medium, which is only impaired when, in addition, either the *SDH1* gene or the *SDH2* gene is deleted, indicates a direct effect of frataxin on the activity of succinate dehydrogenase complex. Our results also indicate a physical interaction of frataxin with succinate dehydrogenase subunits 1 and 2, and therefore, propose frataxin as an essential component of the electronic transport chain. All these results agree with the fact that abnormal respiration has been described in all frataxin deficient model organisms studied so far (5,36–38).

How does frataxin exert its effects in the electron transport chain? If we assume that frataxin may be involved in the entrance of electrons into the electronic transport chain, the lack of frataxin would impede the correct use of electrons by complex II. If electrons were not incorporated properly to

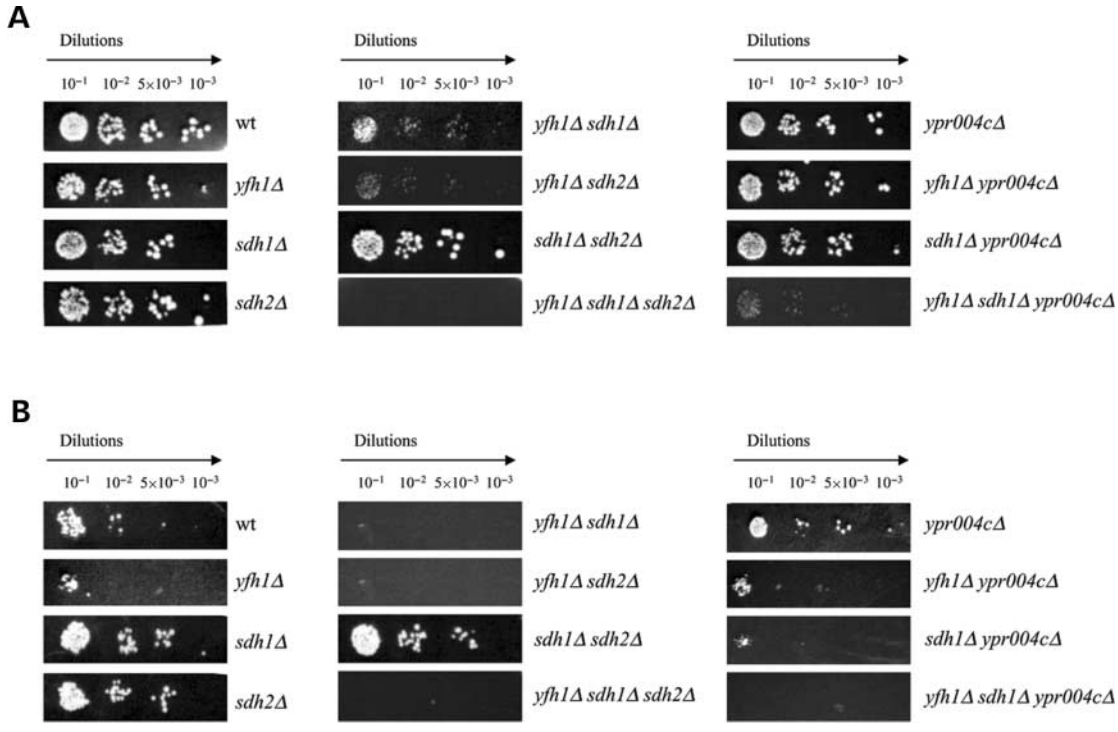

Figure 4. Genetic interaction between *YFH1* and electron transport chain protein coding genes. Serial dilutions of cell suspensions of the different strains were spotted on the indicated media and incubated at 30°C for 48 h (Materials and Methods). **(A)** Cells grown on rich YPD plates. **(B)** Cells grown on SCD medium plates.

respiratory chain, ubiquinone (Q) would not be completely reduced to ubiquinol (QH_2) and there would be an excess of the intermediate semiquinone ($Q\cdot^-$) form. The generation of this radical semiquinone has been associated with a pro-oxidizer effect by its interaction with molecular oxygen, producing superoxide radicals and oxidative stress in the mitochondria (39,40). In this way, frataxin could prevent the accumulation of semiquinone ($Q\cdot^-$) form and play a positive role in response to oxidative stress.

We also suggest that this function of frataxin may be conserved from yeast to humans, because human frataxin was also able to interact physically with the human succinate dehydrogenase complex subunits SDHA and SDHB. These findings may explain some of the observed phenotypes in frataxin-deficient cells and patients, such as decreased ATP production (23), abnormal function of the respiratory chain and production of free radical oxygen species, resulting in increased oxidative stress (32,41). In this way, Friedreich ataxia could be considered an OXPHOS disorder caused by mutations in a nuclear gene.

Frataxin interacts also with other proteins in mitochondria. A direct interaction between the yeast Yfh1p and the central ISC-assembly complex Isu1p/Nfs1p has been established; Yfh1p would participate in the *de novo* synthesis of ISC on the Isu1p scaffold (17). It has also been suggested that frataxin acts as an iron chaperone that modulates the mitochondrial aconitase activity, a protein of tricarboxylic acid cycle (22). All these results together with our data on the functional interaction of frataxin with complex II proteins suggest that frataxin has not just a unique function but may have a dynamic and wide role on the mitochondrial physiology.

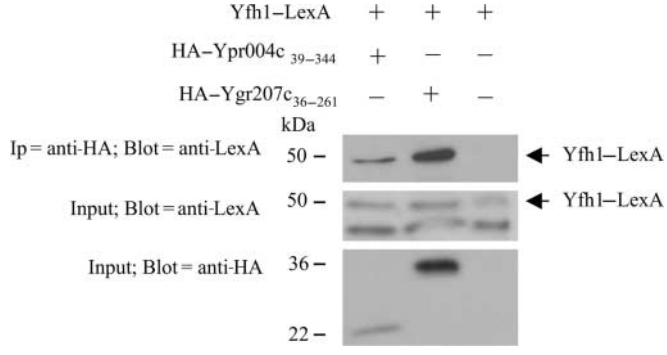

Figure 5. Yfh1p interacts physically with Etf proteins. Crude extracts (500 μg) were prepared from FY250 yeast glucose growing cells expressing Yfh1–LexA (pRS424–ADH1p–Yfh1–LexA), HA–Ypr004c (pWS93–Ypr004c$_{39-344}$) and HA–Ygr207c (pWS93–Ygr207c$_{36-261}$) or containing the corresponding empty vectors. HA-fusion proteins were immunoprecipitated with anti-HA monoclonal antibodies. Pelleted proteins were analyzed by SDS–PAGE and immunodetected with anti-LexA polyclonal antibodies (upper panel). Proteins in the input crude extracts (2.5 μg) were also immunodetected with either anti-LexA antibodies (middle panel) or anti-HA (lower panel) antibodies. Molecular weight markers are indicated in kiloDaltons.

MATERIALS AND METHODS

Strains

Strains used in this study are listed in Supplementary Material, Table S1. Deletion of *SDH1, SDH2* and *YPR004C* was performed by homologous recombination (42) in a W303-1A wild-type yeast haploid strain using PCR products as follows: the corresponding reporter gene (*LEU2, TRP1* and

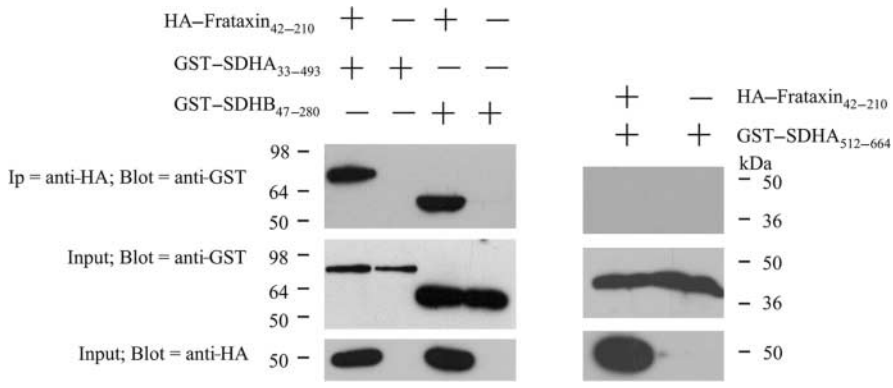

Figure 6. Human frataxin interacts physically with human succinate dehydrogenase complex subunits. Crude extracts (500 μg) were prepared from FY250 yeast glucose growing cells expressing GST–SDHA (pGST–$SDHA_{33-493}$; pGST–$SDHA_{511-664}$) or GST–SDHB (pGST–$SDHB_{47-280}$) and HA–frataxin (pWS93–$frataxin_{42-210}$) or containing the corresponding empty vectors. HA-fusion proteins were immunoprecipitated with anti-HA monoclonal antibodies. Pelleted proteins were analyzed by SDS–PAGE and immunodetected with anti-GST polyclonal antibodies (upper panel). Proteins in the input crude extracts (2.5 μg) were also immunodetected with wither anti-GST antibodies (middle panel) or anti-HA (lower panel) antibodies. Molecular weight markers are indicated in kiloDaltons.

URA3) was amplified by PCR using primers with 3′ sequences specific for reporter gene sequence and 5′ tails homologous to upstream/downstream sequences of the *SDH1, SDH2* and *YPR004C* open reading frames, respectively. To delete *SDH1*, PCR amplifications containing *LEU2* were made using primers DisSDH1.dir/DisSDH1.rev (Supplementary Material, Table S2) and YDp-L plasmid (42) as template. To delete *SDH2*, PCR amplifications containing *TRP1* were made using primers DisSDH2.dir/DisSDH2.rev (Supplementary Material, Table S2) and YDp-W plasmid (43) as template. To delete *YPR004C*, PCR amplifications containing *URA3* were made using primers DisETFα.dir/DisETFα.rev (Supplementary Material, Table S2) and YDp-U plasmid (43) as template. Haploid mutants were confirmed by PCR using primers YFH1dis.dir/YFH1dis.rev., SDH1-1.dir/SDH1-1.rev, SDH2-1.dir/SDH2-1.rev and ETFα-1.dir/ETFα-2.rev for deletion of *YFH1, SDH1, SDH2* and *YPR004c*, respectively. Haploid double and triple mutants were generated by crossing the corresponding single and double mutants, subsequent sporulation of the respective diploids and tetrad dissection. The presence of deletions in the spore progeny was confirmed by PCR. Mutants were crossed with rho0 tester strains to confirm that all were rho+ (all the diploids were able to grow on glycerol–ethanol culture medium).

Plasmids

YFH1 was amplified from yeast FY250 (Supplementary Material, Table S1) genomic DNA. The final construct, pRS424–ADH1p–Yfh1–LexA, contained a recombinant yeast frataxin protein fused to LexA at its C-terminus, under the control of the *ADH1* promoter. We used this construct as bait in the yeast two-hybrid screening. To construct plasmids pWS93–Yhb1, pWS93–Sdh1, pWS93–Sdh2, pWS93–Ypr004c, pWS93–Ygr207c and pWS93–Mdh1, primers Yhb1L.dir/Yhb1L.rev ($Yhb1_{1-399}$), SDSdh1Blev.dir/SDterL.rev ($Sdh1_{32-640}$), SDFadL.dir/SDterL.rev ($Sdh1_{153-640}$), SDFadL.dir/SDFadL.rev ($Sdh1_{153-485}$), SdterL2.dir/SDterL.rev $Sdh1_{506-640}$), SDSdh2lev.dir/SDSdh2lev.rev ($Sdh2_{40-266}$), ETFα.dir/ETFα.rev ($Ypr004c_{39-344}$), ETFbeta2.dir/ETFbeta2.rev ($Ygr207c_{36-261}$) and malato.dir/malato.rev ($Mdh1_{24-334}$) (Supplementary Material, Table S2) were used to amplify the corresponding open reading frames from FY250 genomic DNA by PCR (in brackets, the resulting amplified protein). Amplified fragments were subcloned into pWS93 (44) to produce fusion proteins with three hemagglutinin tags at the N-teminus. Plasmid pWS93–$frataxin_{42-210}$, expressing human frataxin, was constructed in the same way: human *FRDA* gene was amplified by PCR using primers Frataxin.dir/Frataxin.rev (Supplementary Material, Table S2) and a cDNA pool, obtained from total RNA from human adipocytes using M-MLV reverse transcriptase and oligo $(dT)_{18}$, as template. The same cDNA pool was used to amplify the *SDHA* and *SDHB* human genes using primers SDFadH.dir/SDFadH.rev ($SDHA_{33-493}$), SDTerH.dir/SDTerH.rev ($SDHA_{511-664}$) and SDHBH.dir/SDHBH.rev ($SDHB_{47-280}$) by PCR (Supplementary Material, Table S2), respectively. The amplified fragment was subcloned in pEG–GST plasmid (45) to produce fusion proteins with a GST tag at the N-teminus. All amplified fragments were sequenced to rule out the presence of undesired mutations.

Yeast two-hybrid screening

A two-hybrid screening (46) for proteins that interacted with Yfh1–LexA (pRS424–ADH1p–Yfh1–LexA; as mentioned earlier) was carried out in the yeast TAT-7 strain (Supplementary Material Table S1), which contained two chromosomally located reporter genes, *lexAop-HIS3 y lexAop-lacZ* (47). The strain was transformed with a library of *S. cerevisiae* cDNAs fused to the activation domain of Gal4 (GAD; genorous gift of Dr S. Elledge, Baylor University) (48). Transformants were selected in SC + 2% glucose and were subsequently screened for β-galactosidase activity using a filter lift assay (49).

Co-immunoprecipitation assays

Preparation of yeast protein extracts and co-immunoprecipitation was essentially as described earlier (50). The extraction buffer was 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 0.1% (v/v) Triton X-100, 10% (v/v) glycerol, 1 mM dithiothreitol,

1 mM phenylmethylsulfonyl fluoride and complete protease inhibitor cocktail (Roche). Commercial monoclonal anti-HA (Sigma) (1 μl) was used in each co-immunoprecipitation. Precipitates were analyzed by western blotting using commercial polyclonal anti-LexA (Invitrogen) for yeasts proteins or polyclonal anti-GST (Amersham) for human proteins. Antibodies were detected by enhanced chemiluminescence (Roche).

Synthetic interaction and phenotypic analyses

Synthetic interaction of different mutants in combination with *yfh1*Δ deletion was assessed by growing the strains in rich (YPD) and minimal media (SCD). Previously, cells growing exponentially in YPD medium were harvested and adjusted to 0.1 U of absorbance at 600 nm. Serial dilutions were made with sterile water and 3 μl of each dilution was spotted on the different culture media. Plates were incubated at 30°C for 48 h.

Biochemical methods

Isolation of yeast mitochondria was as in Daum *et al.* (51). Succinate dehydrogenase activity was measured as in Maneiro *et al.* (52).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

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Conflict of Interest statement: None declared.

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