Cells Lacking Pfh1, a Fission Yeast Homolog of Mammalian Frataxin Protein, Display Constitutive Activation of the Iron Starvation Response*1,2,3

Received for publication, September 21, 2012, and in revised form, October 27, 2012 Published, JBC Papers in Press, October 31, 2012 DOI 10.1074/jbc.M112.421735

Natalia Gabrielli, José Ayté1, and Elena Hidalgo1,2

From the Oxidative Stress and Cell Cycle Group, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, C/Dr. Aiguader 88, 08003 Barcelona, Spain

Background: Defects in the protein frataxin give rise to Friedreich ataxia.

Results: A new Friedreich ataxia model using fission yeast has been generated and its phenotype and proteome characterized.

Conclusion: Frataxin absence triggers a complete iron starvation program, sufficient to generate all the associated respiratory defects.

Significance: Our new model system may contribute to decipher the role of frataxin.

Friedreich ataxia is a genetic disease caused by deficiencies in frataxin. This protein has homologs not only in higher eukaryotes but also in bacteria, fungi, and plants. The function of this protein is still controversial. We have identified a frataxin homolog in fission yeast, and we have analyzed whether its depletion leads to any of the phenotypes observed in other organisms. Cells deleted in pfh1 are sensitive to growth under aerobic conditions, display increased levels of total iron, hallmarks of oxidative stress such as protein carbonylation, decreased aconitate activity, and lower levels of oxygen consumption compared with wild-type cells. This mitochondrial protein seems to be important for iron and/or reactive oxygen species homeostasis. We have analyzed the proteome of cells devoid of Pfh1, and we determined that gene products up- and down-regulated upon iron depletion in wild-type cells are constitutively misregulated in this mutant. Because of the particular signaling pathway components governing the iron starvation response in fission yeast, our experiments suggest that cells lacking Pfh1 display a decrease of cytosolic available iron that triggers activation of Grx4, the common regulator of the iron starvation gene expression program. Our Schizosaccharomyces pombe Δpfh1 strain constitutes a new and useful model system to study Friedreich ataxia.

Friedreich ataxia is an inherited autosomal recessive disease causing degeneration in the central and peripheral nervous system, cardiomyopathy, skeletal abnormalities, and increased risk of diabetes mellitus (1–4). A decreased expression of a highly conserved nucleus-encoded mitochondrial protein, known as frataxin, causes Friedreich ataxia. In 98% of the cases, an unstable hyper-expansion of a GAA triplet repeat in the first intron of the gene is the most common genetic mutation (2, 5). The expanded GAA repeat impairs frataxin transcription by adopting an abnormal triple helical structure.

This disease is thought to be the consequence of a mitochondrial defect related to iron metabolism. Thus, the cardiac tissues from patients with Friedreich ataxia contain iron deposits, are deficient in respiratory complexes I–III and aconitase activities, and have reduced mitochondrial DNA (6, 7). The finding of iron accumulation in the hearts of both patients and mouse models (6, 8, 9) suggests that excess free iron is involved in the production of reactive oxygen species and is responsible for the oxidative damage to iron-sulfur clusters (ISCs)3 and loss of mitochondrial DNA (10).

Iron is a vital metal for most biological organisms and participates in an astonishing array of biological reactions such as DNA synthesis, cell cycle progression, and respiration (11). Nevertheless, this redox-active transition metal presents a dilemma to cells, because iron can also catalyze the deleterious oxidation of biomolecules via Haber-Weiss/Fenton chemistry when combined with reactive oxygen species (ROS) (12). Accordingly, the concentration of iron in cells is tightly regulated by control of its uptake and intracellular storage (13). Frataxin is a mitochondrial iron-binding protein, and its primary function remains controversial, because iron homeostasis, intracellular fluctuations of ROS, oxidation and damage of ISCs, and mitochondrial dysfunctions are all intimately linked, and it is not trivial to establish the sequential order of events leading to the disease. Homologs to human frataxin have been found in bacteria, fungi, and plants (14). In particular, unicellular model systems are being exploited to determine the origin of the pleiotropic phenotypes displayed by patients. In particular, Saccharomyces cerevisiae cells deficient in the frataxin homolog YFH1 are unable to carry out oxidative phosphorylation, lose mitochondrial DNA (15), display impaired iron efflux out of

* This work was supported in part by Spanish Ministry of Science and Innovation Grants BFU2009-06933 and BFU2012-32045, PLAN E, FEDER, by Spanish Program Consolider-Ingenio 2010 Grant CSD 2007-0020, and by Generalitat de Catalunya (Spain) Grant SGR2009-196 (to E. H.).

This article was selected as a Paper of the Week.

This article contains supplemental Fig. S1, Tables S1–S5, and additional references.

1 Recipients of ICREA Academia Awards (Generalitat de Catalunya).
2 To whom correspondence should be addressed: Universitat Pompeu Fabra, C/Dr. Aiguader 88, 08003 Barcelona, Spain. Tel.: 34-93-316-0848; Fax: 34-93-316-0901; E-mail: elena.hidalgo@upf.edu.

3 The abbreviations used are: ISC, iron-sulfur cluster; ROS, reactive oxygen species; DIP, dipyridyl.
mitochondria with a consequent accumulation of iron in this compartment (16), suffer iron depletion in the cytosol, show elevated expression of high affinity iron uptake, exhibit heme deficiency (17), and have an increased sensitivity to oxidative stress. Additional properties of these ΔYFH1 mutant cells include defects in the synthesis of ISCs with a consequent deficiency of ISC-containing proteins and loss of respiratory competence. Furthermore, in vitro studies indicate that YFH1 can bind iron and deliver it to Isu1 (ISC assembly scaffold protein) (18, 19). The phenotypes caused by decreased levels of frataxin point to the notion that it plays a role in ISC synthesis and mitochondrial and cellular iron misregulation with a consequent oxidative stress. However, studies on mice and yeast also show that the loss of ISCs precedes iron accumulation (9, 20), so that the anomalies may not be a direct result of iron-induced oxidative damage. Furthermore, studies using an S. cerevisiae model of conditional expression of frataxin demonstrated that the primary consequence of frataxin depletion is to trigger up-regulation of the iron transport system before affecting iron-sulfur enzyme activities (21).

The success of previous simple models of Friedreich ataxia prompted us to investigate whether we could isolate a Schizosaccharomyces pombe strain mimicking the phenotypes observed in other organisms. We have found an open reading frame (ORF), pfh1, coding for the fission yeast homolog of human frataxin. We have constructed a strain carrying a deletion of the gene, and we found phenotypes resembling those of other Friedreich ataxia model systems. Despite the great amount of data obtained with previous model systems, the analysis of the proteome of our frataxin-deficient strain has provided us with new data and has prompted us to discard previous hypotheses, such as the possible role of frataxin on ISC biogenesis.

**EXPERIMENTAL PROCEDURES**

Alignment of Frataxin Sequence—The alignment of the frataxin protein sequence of S. pombe, S. cerevisiae, and human isofrom 1 was performed by multiple sequence alignment with hierarchical clustering software (22).

Growth Conditions and Yeast Strains—Cells were grown in rich medium (YE5S) or synthetic minimal medium as described previously (23). Yeast cells were grown in anaerobic liquid cultures (50 ml). Cells were harvested by centrifugation at 4 °C. Supernatants were collected, and 10^7 cells/ml. Standard curves were prepared from 10 to 40 nmol of FeCl3 dissolved in 3% nitric acid. All chemicals (except ferrozine from Fluka) were purchased from Sigma and resuspended in ultrapure water obtained from a Millipore Milli-Q Advantage. Data were obtained from three independent experiments and are expressed as mean ± S.E.

Protein Carbonylation—The detection method was performed as described (33).

Enzymatic Activity Assay for Aconitase—To perform aconitase enzymatic activity assay for wild-type and Δpfh1 strains, S. pombe cells were grown in YE5S in anaerobic conditions to an A600 of 0.5. Aconitase activity was assayed as described (34) following method 2. Aconitase activity can be measured spectrophotometrically at 340 nm using citrate as the substrate of aconitase, and the isocitrate formed was then converted to α-ketoglutarate by NADP⁺-dependent isocitrate dehydrogenase. Yeast cells were washed once with PBS buffer, pH 7.4. Total protein extracts were prepared by homogenization with glass beads in Tris buffer (50 mM Tris-HCl, pH 7.6, 1 mM cysteine, 1 mM citrate, 0.5 mM MnCl2). Insoluble material was removed by centrifugation at 10 min at 16,000 × g at 4 °C. Supernatants were collected, and 10 μl of the total extract were mixed with 90 μl of reaction buffer (50 mM Tris-HCl, pH 7.4, 30 mM sodium citrate, 0.5 mM MnCl2, 0.2 mM NADP⁺, isocitrate dehydrogenase (2 units/ml, Fluka)). Absorbance at 340 nm (ε340 = 6.22 mM⁻¹ cm⁻¹) was recorded by UV-visible spectrophotometer (UV-1700 Pharma Spec Shimadzu).
from three independent experiments and are expressed as mean ± S.E.

Measurement of Oxygen Consumption—Oxygen consumption of ~10^7 cells in 1 ml, collected from anaerobic cultures at an A_{600} of 0.5, was measured as described before (35).

Fluorescence Microscopy—Cells expressing PfH1-GFP were grown in YE5S until an A_{600} of 0.5. They were then incubated with 0.1 μg/ml MitoTracker Red CMXRos (Invitrogen) during 30 min. Cells were pelleted and resuspended in YE5S. Fluorescence microscopy and image capture was performed as described previously (36).

RNA Analysis—Total RNA from exponentially growing S. pombe cells in YE5S was extracted, processed, and transferred to a membrane as previously reported (37). Membranes were hybridized with [α-^32P]dCTP-labeled pfh1, pap1, tpr1, trxl, tpx1, ctt1, zwf1, caf5, obr1, SPCC663.08c, fio1, str3, pcl1, and isa1 probes. Ribosomal RNAs and act1 were used as loading controls.

Cell Extracts and Immunoblot Analysis of Pap1—Preparation of S. pombe trichloroacetic acid (TCA) protein extracts to measure Pap1 concentration was performed as described before (38). Samples were separated by 8% SDS-PAGE. Gels were transferred to membranes, and they were probed with polyclonal anti-Pap1 antiserum and anti-Sty1 antiserum (as loading control) (36)

Growth Curves—Yeast cells were grown in YE5S, and the cultures of NG60, NG147, and NG148 strains were grown anaerobically. The initial A_{600} of the growth curves was 0.1, and recording of the growth curves was performed as described (30).

Quantification of Proteins by Dimethyl Labeling—50 ml of wild-type and Δpfh1 cells were grown to exponential phase (A_{600} 1.0) anaerobically. Cells were passed to aerobic conditions with shaking for 3 h, which did not significantly affect viability of the cultures (supplemental Fig. S1). Cells were collected after shaking for 3 h, which did not significantly affect viability of the cultures (supplemental Fig. S1).

RESULTS

Identification and Deletion of the Gene pfh1, Which Codes for the Fission Yeast Frataxin Homolog—We searched the S. pombe genome for genes with homology to the human frataxin gene, and found an ORF (SPCC1183.03c) coding for a 158-amino acid-long polypeptide sharing 42 and 44% of protein identity with human and S. cerevisiae frataxin, respectively (Fig. 1A). We named the protein PfH1 (pombe frataxin homolog 1), following the nomenclature used for the S. cerevisiae frataxin (yeast frataxin homolog, YFH1). YFH1 diverged from the human gene earlier in evolution than S. pombe pfh1 (Fig. 1A), which further motivated us to analyze the effect of pfh1 deletion on the cell's physiology. We generated a strain lacking the whole ORF by genetic recombination, as shown by Northern blot analysis (Fig. 1B), and we had to perform the selection of the deleted clones under semi-anaerobic conditions. The substitution of the whole pfh1 ORF by an antibiotic resistance cassette was checked by PCR (data not shown). When anaerobic cultures of strain Δpfh1 were spotted on agar plates, we observed severe growth defects in the presence of oxygen (Fig. 1C). In fact, even the viability of liquid cultures of strain Δpfh1 was severely compromised when grown under aerobic conditions, as shown in Fig. 1D. We also determined that cells lacking PfH1 display sensitivity to exogenous oxidative stress (Fig. 1E). These results are consistent with an essential role of PfH1 in cellular fitness, because it is required for respiratory growth and for survival in front of intrinsic (Fig. 1, C and D) or extrinsic (Fig. 1E) accumulation of ROS.

Phenotypic Characterization of Strain Δpfh1—Because our Δpfh1 strain seemed to share the sensitivity to aerobic growth of other Friedreich ataxia model systems, we tested whether it also recapitulated some of the characteristic features related to iron homeostasis and mitochondrial metabolism. We detected over 3-fold increases in total iron in extracts from strain Δpfh1 compared with wild-type cells (Fig. 2A). The strain, either before or after a transient shift to aerobic conditions, displayed hallmarks of oxidative stress, such as increased levels of reversibly oxidized thiols (Fig. 2B) or protein carbonylation (Fig. 2C). The activity of the ISC-containing protein aconitase was also significantly reduced in the mutant (Fig. 2D), and respiratory competence was also severely affected as determined by measuring oxygen consumption (Fig. 2E). All these features have also been reported for previous models of Friedreich ataxia and confirmed the relevance of our newly developed system. Consistently, protein PfH1 displayed mitochondrial localization, as...
reported previously for human and *S. cerevisiae* frataxin proteins (Fig. 2F).

**Characterization of the Proteome of Δpfh1 Cells**—To decipher the role of Pfhl in the cell’s physiology, we analyzed the protein composition of total cell extracts of cells lacking Pfhl and of wild-type cells, when anaerobic cultures were shifted for 3 h to aerobic conditions (supplemental Fig. S1). Tryptic peptides derived from cell extracts were labeled with either normal (wild type) or deuterium-labeled (Δpfh1) formaldehyde. Peptides were then mixed and analyzed by LC-MS/MS, as reported previously for human and *S. cerevisiae* frataxin homologs with human isoform1 frataxin sequence. Red letters represent the consensus sequence of amino acids that are conserved in the three organisms, and blue letters indicate the amino acid sequence that is present just in two of the organisms. The phylogenetic tree relative to the frataxin gene for these organisms is represented in the right panel. B, Northern blot analysis of pfh1 mRNA. Total RNA from strains 972 (WT) and NG60 (Δpfh1) was obtained from cultures growing anaerobically. The RNA was analyzed by Northern blot using a pfh1 probe. Total ribosomal RNA was used as a loading control. C, cells lacking Pfhl displays severely compromised aerobic growth. Strains 972 (WT) and NG60 (Δpfh1) were grown anaerobically in YE5S to a final A600 of 0.5, and serial dilutions from 10^5 to 10^2 cells were spotted in duplicate into YE5S plates and incubated at 30 °C for 2–3 days in aerobic or anaerobic conditions. D, viability of wild-type (WT) and NG60 (Δpfh1) cultures in response to aerobic growth. Strains 972 (WT) and NG60 (Δpfh1) were grown overnight (ON) in YE5S in aerobic (+ ON + O2) or anaerobic (+ ON − O2) conditions, and then serial dilutions of log phase cells were spotted as described in C plates were grown under anaerobic conditions (− O2 plate). E, strain Δpfh1 is sensitive to H2O2 stress. Survival of wild-type (WT), AV18 (Δsty1), and NG60 (Δpfh1) strains in response to the indicated concentration of H2O2 in plates under anaerobic conditions is shown.

We first checked by Northern blot analysis whether the expression of the genes coding for some of the Pap1-dependent proteins overexpressed in Δpfh1 extracts are also up-regulated under basal conditions. As shown in Fig. 3B, the levels of Pap1-dependent drug resistance mRNAs are up-regulated by aerobic growth even prior to the addition of H2O2 in cells lacking Pfhl, but not the antioxidant ones such as trr1. Because the presence of Pap1 at the nucleus can also be accomplished by overexpression of the transcription factor Pap1 (Fig. 3C), we tested whether the mRNA for *pap1* in strain Δpfh1 was elevated. Surprisingly, both *pap1* mRNA (Fig. 3C) and Pap1 protein levels (Fig. 3D) are 3–10-fold higher in extracts from cells lacking Pfhl (supplemental Table S4). In response to H2O2, the transcription factor Pap1 up-regulates transcription of genes required for adaptation to oxidative stress and for tolerance to toxic drugs. H2O2 induces oxidation of Pap1, its nuclear accumulation, and expression of more than 50 Pap1-dependent genes, some of which are antioxidant genes and others drug resistance genes (Fig. 3A) (36, 42). We have recently reported that the ability of Pap1 to bind and activate the second subset of promoters, those of drug tolerance genes, is independent of Pap1 oxidation. Thus, nuclear localization of nonoxidized Pap1 or overexpression of the protein is sufficient to trigger activation of drug resistance genes, but Pap1 has to be not only nuclear but also oxidized to bind to another transcription factor, Ppr1, and activate antioxidant genes (43).
shown), which discards enhanced H2O2 levels triggering its transcription factor is not altered in this mutant (data not of

Cells Lacking Pfh1 Display Decreased Levels of Proteins Normally Down-regulated upon Iron Starvation—As shown in supplemental Table S5, 32 proteins down-represented in extracts from strain Δpfh1 are also down-regulated at their gene levels upon iron starvation, most of them in a Php4-dependent man-

this strain background than in wild-type cells, which could explain the constitutive activation of some drug resistance genes (Fig. 3E). Further work will be required to understand how up-regulation of pap1 levels is occurring upon deletion of pfh1; this is the first reported genetic mutation that affects Pap1 activity at the level of transcription. Oxidation of the transcription factor is not altered in this mutant (data not shown), which discards enhanced H2O2 levels triggering its activation.
FIGURE 3. **Cells lacking pfh1 overexpress a subset of Pap1-dependent genes.**

A, scheme of the activation of Pap1 pathway in *S. pombe* wild-type cells. Pap1 activates two subsets of genes, the antioxidant and the drug resistance genes. In wild-type cells, oxidation of Pap1 upon H$_2$O$_2$ stress induces its nuclear (NUC) accumulation, and a heterodimer with Prr1 is formed, which is able to activate both sets of promoters, the antioxidant (trr1, srx1, and ctt1) and the drug resistance (obr1, caf5, and C663.08c) genes. CYT, cytosol.

B, Northern blot analysis of Pap1-dependent genes. Total RNA from strains 972 (WT) and NG60 (Δpfh1) was obtained from cultures growing anaerobically or shifted to aerobic conditions for the times indicated and treated or not with H$_2$O$_2$. The RNA was analyzed by Northern blot using probes for the Pap1-dependent genes zwf1, trx1, tpx1, obr1, caf5, C663.08c, and trr1. Total ribosomal RNA was used as a loading control.

C, Northern blot analysis of *pap1* gene. Total RNA from strains 972 (WT) and NG60 (Δpfh1) strains was obtained from cultures growing anaerobically or aerobically. The *pap1* ORF was used as a probe.

D, amount of Pap1 protein is higher in Δpfh1 than in wild-type cells. Total TCA protein extracts were analyzed by Western blot with anti-Pap1 antibodies; anti-Sty1 antibodies were used as a loading control.

E, hypothetical scheme of Pap1 activation in Δpfh1 cells. There is an accumulation of reduced Pap1 that is able to enter in the nucleus and activate the drug resistance genes.

FIGURE 4. **Cells lacking Pfh1 show increased expression of proteins normally up-regulated in response to iron starvation.**

A, scheme of the iron starvation response in *S. pombe* cells. Grx4 is a glutaredoxin whose role is to sense the iron levels in cells and transmit the iron status to two repressor factors, Fep1 and Php4. Fep1 is the repressor of the iron uptake genes, and Php4 is a protein that represses a set of genes that code for iron-containing proteins and iron storage. B, Northern blot analysis of Fep1-dependent genes. Total RNA from strains 972 (WT), NG60 (Δpfh1), NG1 (Δfep1), and NG147 (Δpfh1 Δfep1) was obtained from cultures growing in YE5S aerobically treated or not with 250 μM of the iron chelator DIP for 90 min. C, aerobic growth defects of strain Δpfh1 are not due to the increment in the iron uptake system. Strains 972 (WT), NG60 (Δpfh1), NG1 (Δfep1), and NG147 (Δpfh1 Δfep1) were grown in YE plates under aerobic or anaerobic conditions, as described in Fig. 1C. D, growth curves of 972 (WT), NG60 (Δpfh1), and NG1 (Δfep1) strains were done in YE5S liquid medium, and $A_{600}$ was recorded at the indicated times for each culture during 35 h.
Proteome of Frataxin Homolog in Fission Yeast

FIGURE 5. Cells lacking Pfh1 down-regulate genes normally down-regulated under iron starvation. A, genes coding for iron-containing proteins (such as isa1) or involved in iron storage (such as pcl1) are down-regulated in a Δpfh1 strain. Total RNA from strains 972 (WT), NG60 (Δpfh1), NG40 (Δphp4), and NG148 (Δpfh1 Δphp4) was obtained from cultures growing in YESS aerobically treated or not with 250 μM of DIP for 90 min. Northern blot analysis of Php4-dependent genes was performed. B: deletion of the php4 gene partially suppresses the aerobic defects of strain Δpfh1. Survival spots were performed for 972 (WT), NG60 (Δpfh1), NG40 (Δphp4), and NG148 (Δpfh1 Δphp4) strains in different agents under aerobic and anaerobic conditions. Those strains were grown in YESS under anaerobic conditions to a final A500 of 0.5 and then spotted in solid plates of low iron conditions (Dx) or high iron concentrations (Fe), at the indicated concentrations. C, growth curves of 972 (WT), NG60 (Δpfh1), NG40 (Δphp4), and NG148 (Δpfh1 Δphp4) strains were done in YESS liquid medium, and A500 was recorded at the indicated times for each culture during 35 h.

Thus, upon low iron, Php4 accumulates at the nucleus and represses transcription of genes coding for iron storage proteins or iron-consuming proteins (Fig. 4A) (46). First of all, we confirmed by Northern blot that not only protein levels but also mRNA levels for some Php4-dependent genes, such as pcl1 and isa1, are constitutively low in Δpfh1 cells (Fig. 5A). It is important to point out that many of the down-regulated by Php4 upon iron starvation are ISC- or iron-containing proteins, and many of them are essential (supplemental Table S5). Because deletion of the php4 gene, coding for a repressor, leads to the lack of iron starvation-dependent gene repression (Fig. 5A) (41), we tested whether the oxygen-sensitive phenotype of cells lacking Pfh1 could be alleviated by further deletion of the php4 gene. As shown in Fig. 5A, expression of pcl1 and isa1 was constitutively up-regulated in the double knock-out strain, as expected. Furthermore, the sensitivity to grow under aerobic conditions was partially suppressed in the double mutant as shown both on solid plates (Fig. 5B) and in liquid cultures (Fig. 5C). Excess iron or addition or iron chelators did not significantly improve or impair the growth of Δpfh1 cells (Fig. 5B). Therefore, the constitutive down-regulation observed in strain Δpfh1 of some Php4-dependent genes, many of which are essential, is partially, but not totally, contributing to the severe phenotype of our Friedreich ataxia model. It is also worth mentioning that genes coding for the ISC-containing proteins acotinase are also Php4-dependent and are therefore down-regulated in our Δpfh1 strain; this fact would be sufficient to justify the low activity levels of this and other ISC-containing proteins in this genetic background (Fig. 2D).

Transcriptome of Δpfh1 Cells Resembles That of Δgrx4 Δfep1 Cells—As explained above, both the transcriptome (as observed by Northern blots) and the proteome of cells lacking Pfh1 are very similar to that of wild-type cells under iron limitation. Thus, iron import is exacerbated, and iron storage and iron-containing proteins are down-regulated. Because the glutaredoxin Grx4 is the common link between up-regulation (Fep1-dependent) and down-regulation (Php4-dependent) of transcription under iron deprivation (Fig. 4A), our frataxin homolog could be participating in signaling by regulating Grx4. However, deletion of the gene coding for this glutaredoxin does not lead to the same transcriptome changes as deletion of pfh1 (Fig. 6A). Indeed, the only strain that misregulates the iron regulon in the same directions as strain Δpfh1 is the double deletion Δgrx4 Δfep1 (Fig. 6A). We interpret these results by hypothesizing that the absence of Pfh1 does not directly participate in the signaling cascade leading to the complete iron starvation gene program; instead, it may really trigger an iron starvation situation, which should consequently activate Grx4. It is worth mentioning that Δgrx4 Δfep1 cells also display severe growth phenotypes in the presence of oxygen (Fig. 6B). That indicates that many of the phenotypes of our Friedreich ataxia model system, strain Δpfh1, are caused by the misregulation of the iron regulon itself, and in particular by the constitutive Php4-dependent repression of many essential ISC-containing proteins.

DISCUSSION

Deficiencies in frataxin give origin to Friedreich ataxia. We have here developed a new model system to study the molecular events leading to the disease; fission yeast cells lacking Pfh1...
display all hallmarks of other previously reported model systems. Importantly enough, the function of frataxin (and therefore the molecular events leading to the disease in the absence of this protein) is still controversial, and our new model system may shed light onto the essential function of this protein under aerobic conditions.

S. cerevisiae YFH1, the budding yeast frataxin homolog, was earlier reported to directly participate in ISC biogenesis, because aconitate activity was reduced in cells lacking the protein, and this inactivation seemed to precede iron accumulation (9, 47). In particular, yeast frataxin has been suggested to participate in ISC maturation as an iron donor, based on its reported interaction with Isu1 (19, 48). However, it has also been demonstrated that restricting oxidative damage, either by decreasing ROS production (49) or by diminishing available iron (50), prevents aconitate inactivation. Furthermore, a conditional knockdown of YFH1 expression has allowed establishing the order of sequential events occurring upon frataxin depletion, indicating that induction of iron import is a primary event leading to the late inactivation of ISC-containing proteins (21). Our results suggest that the low levels of aconitate activity in cells lacking Pfh1 are a consequence of the earlier activation of the Php4 repressor, which in wild-type cells lowers expression of most ISC-containing proteins in an iron starvation-dependent manner. Therefore, our work does not support the idea that frataxin is required for ISC biogenesis, but rather directly or indirectly participates in iron sensing and signaling.

As explained above, the fission yeast gene expression program upon iron starvation is governed by Grx4, which ultimately is responsible of both up- and down-regulation of several genes. Downstream of Grx4, the inactivation of Fep1 and activation of Php4 transcriptional repressors mediate the cellular response to iron deficiency (44). Briefly, when iron is experimentally depleted by the use of chelators, Fep1 is released from promoters of genes involved in iron uptake (45), whereas Php4 accumulates at the nucleus and represses transcription of genes coding for iron storage or iron-consuming proteins (46). Importantly enough, Grx4 is the real sensor of iron deprivation, probably through its ISC. However, the apoprotein does not mimic an iron starvation response, indicating that the loss of the ISC is not the mechanism by which wild-type Grx4 becomes active. Similarly, cells devoid of Grx4 only mimic the iron starvation response with regard to gene down-regulation by Php4 but cannot trigger activation of Fep1-dependent genes (Fig. 6A). As we have shown here, cells devoid of Pfh1 display all hallmarks of an iron deprivation situation, which can hardly be accomplished by genetic modulation of iron sensing/signaling components. In fact, only cells carrying double deletion of grx4 and fep1 display a similar transcriptome and phenotype as Δpfh1 cells (Fig. 6, A and B). Pfh1 could modulate Grx4 activity by, for instance, stabilizing the inactive, iron-rich conformation via chaperone or scaffold properties, its deficiency leading to the basal accumulation of the iron starvation-induced conformation. However, it is difficult to reconcile this putative chaperone role of Pfh1 on Grx4 activity when the first protein has mitochondrial localization (Fig. 2F) and Grx4 displays cytoplasmic and nuclear localization. Our results unambiguously indicate that the absence of S. pombe frataxin causes a real iron starvation situation able to trigger the complex up- and down-regulation of the gene expression program. Indeed, activation of a complete iron starvation response, including up-regulation of the high affinity iron transport system Fet3-Ftr1 and increased rate of iron uptake, has been described before in the yeast model of Friedreich ataxia (10). Furthermore, both increased iron uptake and a decrease in the major pathways of mitochondrial iron utilization have been described in a mouse model of Friedreich ataxia (51). A possible role for frataxin, which nicely fits with these results, is its participation in the regulation of cellular iron homeostasis from the mitochondria. Maybe Pfh1 depletion triggers accumulation of the metal in this compartment, with the concomitant decrease of available cytosolic iron and Grx4 activation. Further experiments to confirm this hypothesis are of course required.

Our study also strongly suggests that constitutive repression of many essential ISC-containing proteins in strain Δpfh1 contributes to the severe phenotypes observed, because they can be partially suppressed by deletion of php4 (Fig. 5, B and C). Studies on our new S. pombe model system on Friedreich ataxia will hopefully contribute to understanding the function of frataxin and to easily test different therapeutic interventions, which may prevent the onset of the disease.

Acknowledgments—We thank members of the laboratories of Joaquim Ros and Jordi Tamarit for helpful discussions. We thank Mercé Carmona for technical assistance. We are grateful to the joint Centre for Genomic Regulation-Universitat Pompeu Fabra Proteomic Facility (Barcelona, Spain), where the mass spectrometry experiments were performed, and in particular to Henrik Molina and Guadalupe Espadas.

REFERENCES
1. Harding, A. E. (1981) Friedreich’s ataxia. A clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 104, 589–620
2. Campuzano, V., Montermini, L., Moltò, M. D., Panean, L., Cossée, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., Monticelli, A., Zara, F., Cañizares, J., Koutnikova, H., Bidichandani, S. I., Gellera, C., Brice, A., Trouillas, P., De Michele, G., Filla, A., De Frutos, R., Palau, F., Patel, P. L., Di Donato, S., Mandel, J. L., Cocozza, S., Koenig, M., and Pandolfo, M. (1996) Friedreich’s ataxia. Autosomal recessive disease caused by an intronic mutation of frataxin. Nat. Genet. 87, 346–349
3. Armstrong, J. S., Khdour, O., and Hecht, S. M. (2010) Does oxidative stress contribute to the pathology of Friedreich’s ataxia? A radical question. FASEB J. 24, 2152–2163
4. Bradley, J. L., Blake, J. C., Chamberlain, S., Thomas, P. K., Cooper, J. M., and Schapira, A. H. (2000) Clinical, biochemical, and molecular genetic correlations in Friedreich’s ataxia. Hum. Mol. Genet. 9, 275–282
5. Rötig, A., De Lonlay, P., Chretien, D., Foury, F., Koenig, M., S idi, D., Munich, A., and Rustin, P. (1997) Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. Nat. Genet. 17, 215–217
6. Lamarche, J. B., Côté, M., and Lemieux, B. (1980) The cardiomyopathy of 4. N. Gabrielli, M. Carmona, J. Ayté, and E. Hidalgo, unpublished results.

Proteome of Frataxin Homolog in Fission Yeast
Proteome of Frataxin Homolog in Fission Yeast

Friedrich's ataxia morphological observations in three cases. Can. J. Neurol. Sci. 7, 389–396

9. Puccio, H., Simon, D., Cossée, M., Criqui-Filipe, P., Tiziano, F., Melki, J., Hindelang, C., Matyas, R., Rustin, P., and Koenig, M. (2001) Mouse models for Friedrich ataxia exhibit cardiomyopathy, sensory nerve defect, and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. Nat. Genet. 27, 181–186

10. Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M., and Kaplan, J. (1997) Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. Science 276, 1709–1712

11. Kaplan, C. D., and Kaplan, J. (2009) Iron acquisition and transcriptional regulation. Chem. Rev. 109, 4536–4552

12. Halliwell, B., and Gutteridge, J. M. (1984) Oxygen toxicity, oxygen radicals, transition metals, and disease. Biochem. J. 219, 1–14

13. Corpet, F. (1988) Multiple sequence alignment with hierarchical cluster-
49. Bulteau, A. L., Dancis, A., Gareil, M., Montagne, J. J., Camadro, J. M., and Lesuisse, E. (2007) Oxidative stress and protease dysfunction in the yeast model of Friedreich ataxia. *Free Radic. Biol. Med.* **42**, 1561–1570

50. Chen, O. S., and Kaplan, J. (2000) CCC1 suppresses mitochondrial damage in the yeast model of Friedreich’s ataxia by limiting mitochondrial iron accumulation. *J. Biol. Chem.* **275**, 7626–7632

51. Huang, M. L., Becker, E. M., Whitnall, M., Suryo Rahmanto, Y., Ponka, P., and Richardson, D. R. (2009) Elucidation of the mechanism of mitochondrial iron loading in Friedreich’s ataxia by analysis of a mouse mutant. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16381–16386

52. Pelletier, B., Beaudoin, J., Mukai, Y., and Labbé, S. (2002) Fep1, an iron sensor regulating iron transporter gene expression in *Schizosaccharomyces pombe*. *J. Biol. Chem.* **277**, 22950–22958