Apn1 AP-endonuclease is essential for the repair of oxidatively damaged DNA bases in yeast frataxin-deficient cells

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Frataxin deficiency results in mitochondrial dysfunction and oxidative stress and it is the cause of the hereditary neurodegenerative disease Friedreich ataxia (FA). Here, we present evidence that one of the pleiotropic effects of oxidative stress in frataxin-deficient yeast cells (Δyfh1 mutant) is damage to nuclear DNA and that repair requires the Apn1 AP-endonuclease of the base excision repair pathway. Major phenotypes of Δyfh1 cells are respiratory deficit, disturbed iron homeostasis and sensitivity to oxidants. These phenotypes are weak or absent under anaerobiosis. We show here that exposure of anaerobically grown Δyfh1 cells to oxygen leads to down-regulation of antioxidant defenses, increase in reactive oxygen species, delay in G1- and S-phases of the cell cycle and damage to mitochondrial and nuclear DNA. Nuclear DNA lesions in Δyfh1 cells are primarily caused by oxidized bases and single-strand breaks that can be detected 15–30 min after oxygen exposition. The Apn1 enzyme is essential for the repair of the DNA lesions in Δyfh1 cells. Compared with Δyfh1, the double Δyfh1Δapn1 mutant shows growth impairment, increased mutagenesis and extreme sensitivity to H2O2. On the contrary, overexpression of the APN1 gene in Δyfh1 cells decreases spontaneous and induced mutagenesis. Our results show that frataxin deficiency in yeast cells leads to increased DNA base oxidation and requirement of Apn1 for repair, suggesting that DNA damage and repair could be important features in FA disease progression.

INTRODUCTION

Friedreich ataxia (FA) is the most common autosomal recessive inherited ataxia. This slowly progressive disease is characterized by neuron degeneration in the central and peripheral nervous system, cardiomyopathy and diabetes. FA is caused by a dynamic mutation in the FXN gene. The majority of patients are homozygous for a GAA trinucleotide repeat expansion in the first intron of the FXN gene that causes transcriptional silencing and low level of the encoded protein, frataxin (1–3). Frataxin is a mitochondrial protein implicated in iron–sulfur cluster biogenesis, iron homeostasis and resistance to oxidants (4–6). Convincing evidence show now that the primary function of frataxin is in Fe–S cluster biogenesis (7–9); however, oxidative stress seems to be the major pathological feature of the disease and the driving force that results in mitochondrial dysfunction and neuron degeneration (6,10).

One of the consequences of mitochondrial dysfunction and increase in reactive oxygen species (ROS) is the formation of nuclear and mitochondrial genome lesions (11). DNA damage
is implicated in the common aging process, but deficiencies in the DNA repair pathways can result in cancer and neurodegeneration (12). Mitochondrial and nuclear DNA damage have been reported in human, mouse and yeast (Δyfh1 mutant) frataxin-deficient cells (13–16). Loss of mitochondrial DNA was one of the first phenotypes reported for the Δyfh1 yeast cells (17–19) and for FA patient cells (20). Thirty years ago, it was found that skin fibroblasts from FA patients were sensitive to ionizing radiation and that the newly synthesized DNA was abnormal, which were suggestive of defects in DNA repair. Recently, transcriptome profiling of total blood from 28 children with FA revealed the molecular signature of cell response to DNA damage (16). Using quantitative PCR, the same authors showed increased number of mitochondrial and nuclear DNA lesions in blood cells from FA patients compared with controls. A link between frataxin expression, DNA repair and tumor initiation was observed in murine liver (13). Analysis of diploid frataxin-deficient yeast also showed nuclear DNA damage, which was evidenced by higher levels of illegitimate mating, higher rate of spontaneous mutation and increased sensitivity to the DNA-alkylating methyl methanesulfonate (MMS) and to the replication inhibitor hydroxyurea than controls (14). Furthermore, deletion of the glutathione peroxidase encoding gene GPX1 in frataxin-deficient cells resulted in a marked increase in the nuclear mutation rate. These results led the authors to suggest that the increased spontaneous nuclear damage in Δyfh1 cells was caused by H2O2 generated in the mitochondria (14). In line with this hypothesis, Δyfh1 cells are extremely sensitive to H2O2 treatment (21). In addition, different studies in yeast, Drosophila and patient fibroblasts showed that H2O2 levels increase in frataxin-deficient cells (22–24).

ROS can induce several types of lesions in DNA, such as oxidized bases, apurinic/apyrimidinic (AP) sites, base deamination products, oxidized sugar fragments and DNA-strand breaks. The four DNA bases can be directly oxidized by ROS. The major products of oxidation of purines are 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 2, 6-diamino-4-hydroxy-5-formamidopyrimidine (FapydG) for guanine and 8-oxoA and FapydA for adenine. Oxidation of cytidine results mainly in the 5-hydroxy-2′-deoxycytidine (OH5dC) and oxidation of thymidine results in thymine glycol. These and other damaged bases, if not repaired, may have miscoding properties and lead to mutation upon replication or block progression of the replication fork (11,25). The vast majority of damaged and mispaired DNA bases, resulting not only from oxidation but also from alklylation or X-ray exposure, are repaired by the base excision repair (BER) pathway in yeast and mammalian cells (12,26). The BER pathway is initiated by the recognition and removal of the damaged or inappropriate bases by bifunctional N-glycosylases/AP-lyases (three enzymes in yeast, Ogg1, Ntg1 and Ntg2), creating an AP site. This abasic site is then repaired by the AP endonucleases, Apn1 and Apn2, or by AP lyases (25,26). In addition, the nucleotide excision repair (NER) pathway can be used to repair AP sites when the BER pathway is deficient (26). Ntg1 and Ntg2 are closely related and, as opposed to Ogg1, exhibit a broad substrate specificity catalyzing the removal of oxidized pyrimidines and purines (26,27). Ntg1 is localized to the nucleus or to the mitochondria in response to oxidative DNA damage, whereas Ntg2 is exclusively nuclear and has an iron–sulfur cluster (27–29). Single deletion of the NTG1 and NTG2 genes causes increased spontaneous and H2O2-induced mutagenesis compared with the wild-type (27). Apn1 is the major AP endonuclease in the cell accounting for >97% of the AP-endonuclease/3′-dioesterase activity in wild-type cells (30). It is localized in the nucleus and in the mitochondria through physical interaction with Pir1 (31). Apn1-deficient cells show increased spontaneous nuclear and mitochondrial mutation frequencies (32).

Oxygen exposition causes the emergence of the two main deleterious phenotypes observed in the yeast Δyfh1 mutant: iron–sulfur cluster deficiency and oxidative stress. Transition from anaerobiosis to aerobicism leads to degradation of the aconitase iron–sulfur cluster and a decrease of its activity, accumulation of cytosolic and mitochondrial carbonylated proteins and inhibition of the proteasome activity in Δyfh1 cells (33). Here, we report that transition from anaerobiosis to aerobicism of Δyfh1 cells causes down-regulation of antioxidant defenses and that the increase in the amounts of ROS induces mitochondrial and nuclear DNA damage. In addition, we show that the Apn1 AP-endonuclease is essential for the repair of oxidatively modified DNA bases in Δyfh1 cells.

RESULTS

Antioxidant defenses in Δyfh1 cells are down-regulated in aerobicism but not in anaerobiosis

Previous data showed that the anaerobiosis to aerobicism transition is an inducer of oxidative stress in Δyfh1 cells (33). In addition, transcription of several genes encoding critical antioxidant enzymes (superoxide dismutase SOD2; catalases CTT1 and CTA1; thiol peroxidase HYR1; glutaredoxins GRX4 and GRX5; thioredoxin TRX1 and thioredoxin reductase TRR1) were significantly repressed in Δyfh1 compared with wild-type cells in aerobicism (21). Real-time quantitative PCR (RT-qPCR) was therefore used to investigate the expression of 22 genes implicated in the oxidative stress response in cells cultivated under strict anaerobiosis (2 ppm O2) and aerobicism (Table 1). In anaerobiosis, the expression of most genes in Δyfh1 was similar to the wild-type or induced (AHPI, GJT2 and TRX3). However, expression of the GRX4, GRX5 and TRR1 genes, which were repressed in aerobicism, was also moderately repressed (1.2–1.5-fold) under anaerobiosis in Δyfh1 cells. In aerobicism, only one gene was induced in Δyfh1 cells (GJT2), while nine genes were repressed. The expression profile of the 11 genes that were either induced or repressed in anaerobiosis or aerobicism was determined during transition from anaerobiosis to aerobicism (Table 1 and Fig. 1A). All genes were repressed 30–60 min after oxygen exposition in Δyfh1 cells. For SOD2, CTT1, CTA1, AHPI, GRX4, GRX5 and TRR1 genes repression occurred 15 or 30 min after transition, which persisted under aerobicism. The expression of GJT3 and TRX2 increased 15 min after transition to aerobicism but declined after 30 min. These findings show that frataxin deficiency in yeast cells leads to deregulation of the transcription of several genes that encode enzymes central to protection against oxidative damage in aerobic conditions.

Glutathione is a main protective antioxidant in yeast cells. We have shown that the glutathione levels of the Δyfh1
Table 1. Expression of genes implicated in the antioxidant defense and in the BER pathway in Δyfh1 cells

| Gene                              | Δyfh1/WT ratio (mean ± SE) | Anaerobiosis | 15 min | 30 min | 60 min | Aerobiosis |
|-----------------------------------|----------------------------|--------------|--------|--------|--------|------------|
| SOD1, Cu,Zn superoxide dismutase  | 1.10 ± 0.03                | 0.79 ± 0.02  |        |        |        |            |
| SOD2, Mn superoxide dismutase     | 1.32 ± 0.08                | 0.44 ± 0.02  |        |        |        |            |
| CTT1, catalase T                  | 1.11 ± 0.07                | 0.20 ± 0.02  |        |        |        |            |
| CTA1, catalase A                  | 1.70 ± 0.09                | 0.50 ± 0.03  |        |        |        |            |
| AHP1, peroxiredoxin              | 2.72 ± 0.21                | 0.64 ± 0.02  |        |        |        |            |
| GSH1, gamma-glutamylcysteine synthetase | 1.24 ± 0.03           | 0.90 ± 0.04  |        |        |        |            |
| GPX1, glutathione peroxidase      | 1.89 ± 0.06                | 0.96 ± 0.09  |        |        |        |            |
| GRX2, glutathione peroxidase      | 1.21 ± 0.03                | 1.17 ± 0.07  |        |        |        |            |
| GRX1, thiol peroxidase            | 0.97 ± 0.03                | 0.56 ± 0.02  |        |        |        |            |
| GRX3, thiol transferase           | 1.20 ± 0.09                | 1.40 ± 0.12  |        |        |        |            |
| GTT2, glutathione transferase     | 2.08 ± 0.24                | 3.09 ± 0.84  |        |        |        |            |
| GTT3, glutathione transferase     | 1.20 ± 0.08                | 2.16 ± 0.01  |        |        |        |            |
| GRX4, glutaredoxin                | 1.62 ± 0.06                | 1.76 ± 0.13  |        |        |        |            |
| GRX5, glutaredoxin                | 1.02 ± 0.04                | 0.73 ± 0.04  |        |        |        |            |
| TRX1, thioredoxin                 | 1.29 ± 0.02                | 0.85 ± 0.04  |        |        |        |            |
| TRX2, thioredoxin                 | 0.65 ± 0.01                | 0.16 ± 0.01  |        |        |        |            |
| TRX3, thioredoxin                 | 0.82 ± 0.03                | 0.33 ± 0.02  |        |        |        |            |
| TRR1, thioredoxin reductase       | 0.94 ± 0.05                | 0.36 ± 0.02  |        |        |        |            |
| TRR2, thioredoxin reductase       | 1.08 ± 0.03                | 0.36 ± 0.02  |        |        |        |            |
| TRX1, thioredoxin                 | 2.11 ± 0.06                | 0.36 ± 0.02  |        |        |        |            |
| TRX3, thioredoxin                 | 0.77 ± 0.02                | 0.51 ± 0.02  |        |        |        |            |
| TRR1, thioredoxin reductase       | 0.10 ± 0.13                | 0.74 ± 0.02  |        |        |        |            |
| APN1, AP-endonuclease             | 1.48 ± 0.15                | 1.02 ± 0.18  |        |        |        |            |
| NAG1, N-glycosyltransferase/AP-lyase | 0.91 ± 0.09             | 0.82 ± 0.15  |        |        |        |            |
| NAG2, N-glycosyltransferase/AP-lyase | 1.26 ± 0.12             | 1.28 ± 0.08  |        |        |        |            |
| FET3, ferroxidase                 | 17.55 ± 2.99               | 50.25 ± 4.39 |        |        |        |            |

The FET3 gene was included as a control.

mutant are disturbed compared with the wild-type in aerobic conditions (21,34). Interestingly, under anaerobiosis, the total glutathione levels in Δyfh1 and wild-type cells were significantly different but in the same range (1.2-fold difference, P < 0.01; 78.9 ± 2.1 nmoles glutathione/mg protein in Δyfh1 cells compared with 93.1 ± 3.8 nmoles glutathione/mg protein in wild-type cells; Fig. 1B). As observed previously, in aerobic conditions the total glutathione specific content was 2-fold decreased in Δyfh1 compared with wild-type cells (115.0 ± 8.6 nmoles glutathione/mg protein in Δyfh1 cells compared with 206.7 ± 23.5 nmoles glutathione/mg protein in wild-type cells). Time course experiments showed that the total glutathione levels of wild-type cells increased progressively from 10 to 30 min after transition to aerobicis to attain values 2-fold higher than those observed in aerobically cultivated cells (Fig. 1C). Compared with wild-type cells, the response of Δyfh1 cells is more rapid but transient. A 3-fold increase in total glutathione content is observed 10 min after transition to aerobiosis and a return to basal level after 15 min (Fig. 1C). This shows that in wild-type cells, glutathione levels increase with the transition from anaerobiosis to aerobicis, but in Δyfh1 cells this response to oxygen does not occur and could result in less protection against oxidative damage.

Transition from anaerobiosis to aerobicis leads to increased ROS-mediated DNA-strand breaks in Δyfh1

Since the cellular antioxidant defenses appear to be deregulated in Δyfh1 cells, increase in ROS accumulation was tested during the transition from anaerobiosis to aerobicis using the fluorescent dye dihydroethidium (DHE). After exposure to oxygen, Δyfh1 cells showed significantly more ROS-driven DHE to ethidium conversion than wild-type cells at all time points investigated, as estimated by flow cytometry analysis (Fig. 2A). ROS accumulation in Δyfh1 cells peaked at 30 min (median DHE fluorescence at 30 min was 13.1 ± 0.8 in Δyfh1 cells and 7.9 ± 0.9 in wild-type cells, P < 0.001) and then decreased at 60 min. This decline in the ROS level is certainly transitory because in aerobic conditions, Δyfh1 mutant cells show higher ROS levels than wild-type cells (Fig. 2A).

We tested if cells accumulating ROS failed to adapt to oxygen conditions and died. ROS production is a key event in the induction of apoptosis in yeast cells. In addition, treatment of wild-type and Δyfh1 cells with low doses of H2O2 induces apoptosis (21,35). To test this possibility, cell death and apoptosis were monitored using the clonogenic test and TUNEL staining after transition from anaerobiosis to aerobicis. No decrease in viability was observed in wild-type and Δyfh1 cells (data not shown), suggesting that cell death was not induced in these conditions. However, when formaldehyde fixed cells were tested for DNA-strand breaks by TUNEL assay, a significantly high level of positive cells was observed in Δyfh1 cells in aerobicis. In anaerobiosis, <10% of the cells were positively stained for TUNEL in both wild-type and Δyfh1 strains (Fig. 2B). Significantly (P < 0.01), fewer Δyfh1 cells were stained after 60 min than 15 min of oxygen exposition. However, a high proportion of Δyfh1 cells from aerobic cultures were positively stained for TUNEL (60.2 ± 2.0% for Δyfh1 compared with 15.5 ± 0.2% for wild-type), consistent with the findings for ROS staining.
The effect of the spin trap phenyl N-tert-butyl-nitrone (PBN) was tested. Treatment with 5 mM PBN reduced TUNEL staining of Δyfh1 cells close to wild-type levels during transition from anaerobiosis to aerobiosis and in aerobicosis (Fig. 2B), indicating that oxygen exposition caused an increase in ROS production and in DNA-strand breaks.
**Δyfh1 cells show increased oxygen-dependent nuclear and mitochondrial DNA damage**

The preceding results suggested that increased nuclear DNA lesions occurred when Δyfh1 cells were exposed to oxygen. Since DNA damage was previously reported in diploid Δyfh1/Δyfh1 cells in aerobiosis (14), the spontaneous mutation rate at the *CAN1* locus was determined by canavanine resistance in anaerobiosis and aerobiosis. The results showed that haploid Δyfh1 cells have a 2-fold increased CanR mutation frequency compared with the wild-type in aerobiosis and in anaerobiosis (Fig. 3A and B). In addition, both wild-type and Δyfh1 strains showed increased mutation frequencies in aerobiosis compared with anaerobiosis. Transition from anaerobiosis to aerobiosis did not cause a significant change in the CanR mutation frequency in wild-type cells (Fig. 3A), in agreement with the increase in ROS levels and DNA-strand breaks observed. Moreover, addition of PBN to aerobic cultures led to a reduction in the mutation frequency in Δyfh1 cells to values similar to that obtained in wild-type cells (with PBN, 21.4 ± 7.6 CanR mutants/10^7 viable cells in wild-type and 26.3 ± 4.9 CanR mutants/10^7 viable cells in Δyfh1 cells; without PBN, 18.3 ± 1.7 CanR mutants/10^7 viable cells in wild-type and 40.0 ± 6.9 CanR mutants/10^7 viable cells in Δyfh1 cells) (Fig. 3B). Together, these data show that oxygen exposure and ROS accumulation cause oxidative nuclear DNA lesions in Δyfh1 cells.

Other indicators of nuclear DNA damage were tested. Double-strand breaks in DNA arise mainly during replication and require homologous recombination for repair (36). Illegitimate mating can be used as an indirect measure of recombination and double-strand break repair. The frequency of mating of *Matα* wild-type and Δyfh1 cells with a *Matα* tester strain was identical and close to 0 in anaerobiosis; however, it was moderately increased in aerobiosis in Δyfh1 compared with wild-type cells (2.6 ± 0.8 colonies/10^8 Δyfh1 cells and 0 colonies with wild-type cells, *n* = 16; Fig. 3C). In addition, the Δyfh1 cells were not sensitive to hydroxyurea (up to 100 mM; data not shown), which inhibits replication and causes double-strand breaks in the DNA. Sensitivity to the base-alkylating MMS was also determined. Although the mutant was more sensitive than the wild-type, the difference between the survival of Δyfh1 and wild-type cells was only statistically significant when treated with 40 mM MMS (Fig. 3D). These data suggest that double-strand

![Figure 3](https://example.com/f3.png)
breaks are a minor type of lesion in the genomic DNA of 
Dyfh1 cells.

Loss of mitochondrial DNA (rho− and rho+ cells) is a characteristic phenotype of frataxin deficiency in yeast cells (17–19). To detect complete or partial loss of mitochondrial DNA, colonies resulting from crosses with the Mata ρ0 tester strain were performed in anaerobiosis and aerobiosis and diploid colonies were tested for growth in the medium containing glycerol as the only carbon source. In anaerobiosis, no loss of mitochondrial DNA was observed (400 colonies analyzed) compared with only 1.2% of cells that retain mitochondrial DNA in aerobiosis (900 colonies analyzed). Therefore, mitochondrial DNA loss is oxygen exposition dependent. Loss of mitochondrial DNA mediates mutagenesis of the nuclear genome (37); however, it cannot explain the increased mutation frequency in 
Dyfh1 cells because 6–8 h (two generations) after transition from anaerobiosis to aerobiosis, no glycerol-negative colonies were observed (400 colonies analyzed).

Apn1 is necessary for repair of modified DNA bases in 
Dyfh1 cells

Given that Dyfh1 cells accumulate oxidative DNA lesions, we investigated whether the BER pathway was defective in these cells. To this end, the function of Ntg1, Ntg2 and Apn1 was investigated. Although the expression of these genes in anaerobiosis and aerobiosis was not altered in 
Dyfh1 compared with wild-type cells, they were repressed after 30 min of oxygen exposition (Table 1). We examined the phenotypes of the double deletions 
Dyfh1Δntg1, 
Dyfh1Δntg2 and 
Dyfh1–Δapn1 and overexpression of the NTG1 and APN1 genes in wild-type and 
Dyfh1 cells. The 
Dyfh1Δapn1 strain, but not the 
Dyfh1Δntg1 and 
Dyfh1Δntg2 strains, presented a growth defect in liquid and solid media compared with the 
Dyfh1 cells (data not shown; see below). Deletion of APN1 in 
Dyfh1 cells resulted in an important increase in the spontaneous CanR mutagenesis (Fig. 4A, Table 1). On the contrary, deletion of the NTG1 and NTG2 genes in 
Dyfh1 cells caused only a small, non-significant, increase in the spontaneous mutation frequencies (Fig. 4A). In agreement with this, 
Dyfh1 cells overexpressing the APN1 gene showed a 2-fold decrease in mutation frequencies compared with cells harboring the vector (Fig. 4B). As for deletion, overexpression of the NTG1 gene did not cause any change in 
Dyfh1 cells (data not shown). Since the 
Dyfh1 strain is highly sensitive to 
H2O2, all mutants were treated 30 min with 0.5 to 2 mM 
H2O2 and the surviving fraction of the population determined. The 
Dyfh1Δapn1 mutant was extremely sensitive to 
H2O2 (Fig. 4C), but not the 
Dyfh1Δntg1 and 
Dyfh1Δntg2 double mutants (data not shown). In contrast, overexpression of APN1 in 
Dyfh1 cells did not lead to an increase in resistance to 
H2O2 (Fig. 4C). Taken together, these data suggest that Apn1 AP-endonuclease is a central enzyme in the repair of damaged bases in 
Dyfh1 cells.

Figure 4. Apn1, but not Ntg1 or Ntg2, is necessary for repair of damaged bases in 
Dyfh1 cells. (A) CanR spontaneous mutant frequencies determined in aerobic stationary cultures. Data represent means ± SE for 5 ≤ n ≤ 10 cultures; ***P < 0.01. (B) CanR spontaneous mutant frequencies in aerobic stationary cultures of wild-type and 
Dyfh1 cells carrying the vector p426GPD1 or a construct that overexpresses APN1. Data represent means ± SE for n = 5 cultures; *P = 0.07. (C and D) The survival ratio of exponentially growing cells in SD medium to 30 min 
H2O2 treatment in aerobiosis. Data represent ratio of means ± SE for n = 3 cultures; ***P < 0.001.
Mutagenesis in Δyfh1 cells exposed to oxidizing and alkylating chemicals

Exposure of yeast cells to H2O2 and MMS causes oxidation and alkylation of DNA bases, respectively, resulting in increased mutagenesis. The data presented here show oxidative damage to DNA in Δyfh1 cells but no noticeable sensitivity to MMS. Therefore, we investigated the effect of H2O2 and MMS on the mutation frequency in frataxin-deficient mutants. Exposure of Δyfh1 cells to rising H2O2 concentrations led to a marked increase in CanR mutation frequencies, while in wild-type cells the increase was small between 0.5 and 2 mM H2O2 (Fig. 5A). The double Δyfh1Δapn1 mutant showed higher mutation frequency to 0.5 mM H2O2 (higher concentrations were difficult to test because the survival of Δyfh1Δapn1 cells treated with H2O2 was very low) than Δyfh1 and Δapn1 cells (Fig. 5B). Accordingly, overexpression of the APN1 gene in Δyfh1 reduced the mutation frequency after H2O2 treatment (Fig. 5C). In addition, the fact that mutation frequencies in Δapn1 are higher than those observed in Δyfh1 cells argues in favor of the essential role of Apn1 in the repair of oxidized bases in Δyfh1.

The results obtained after treatment of Δyfh1 cells with MMS were unexpected. Although exposure of Δyfh1 cells to MMS caused an increase in CanR mutation frequencies compared with untreated conditions, the levels were 1.6 to 3.7 times lower than those observed for wild-type cells (Fig. 5A). The Δyfh1Δapn1 mutation frequencies were...
intermediate between Δyfh1 and Δapn1, again pointing to a resistance of frataxin-deficient cells to MMS-induced mutagenesis (Fig. 5B). However, the mutant Δyfh1Δapn1 was very sensitive to MMS with <0.5% survival with a 20 mM treatment for 30 min (Fig. 5D). Overexpression of APN1 in Δyfh1 cells increased cell survival and reduced by more than 6-fold the mutation frequency in response to MMS treatment (Fig. 5C and E). In contrast, when Δyfh1 cells were exposed to H2O2, the overexpression of APN1 did not change cell survival (Fig. 4D) and only reduced mutagenesis by a factor of 2.5 (Fig. 5C).

Transition from anaerobiosis to aerobiosis leads to cell cycle arrest in Δyfh1 cells

Cells respond to DNA damage and defects in chromosome replication by delaying cell cycle that allows time for DNA repair to occur. Checkpoints are signaling pathways that identify damage and arrest cell cycle. In S. cerevisiae, DNA damage checkpoints delay the G1/S transition, slow replication (S-phase) and block G2/M transition of the cell cycle (38). Therefore, we tested the impact of oxidative DNA damage on the cell cycle in Δyfh1 cells shifted from anaerobiosis to aerobiosis. To follow cell cycle progression in an asynchronous cell population, we chose to use multispectral imaging flow cytometry because it allows the analysis of DNA content and morphological markers of cell cycle progression in a large number of cells (39,40). The analysis of the data is based on the following criteria: cells in G1 have 1n DNA content, single round nuclei and present no buds; the DNA content of cells in S-phase progressively increase from 1n to 2n, cells have emergent buds and undivided nuclei; G2/M is characterized by 2n DNA content and divided nuclei. The DNA content is determined by the intensity of the SYTOX Green stain and the nuclear morphology by the aspect ratio (ratio of width to length) of the DNA stain. In a scatter plot showing these two features, the three populations of cells can be clearly identified as shown in Figure 6A.

In aerobiosis, no significant difference in the cell cycle was observed between wild-type and exponentially growing Δyfh1 cells, although the number of cells in G1 was slightly higher in the mutant (Fig. 6B). No difference was observed between the two strains in anaerobiosis either. The percentage of cells in G1-phase was increased in anaerobiosis compared with aerobiosis (Fig. 6B), in agreement with the slower growth rate of the wild-type and Δyfh1 in anaerobiosis (data not shown). Transition from anaerobiosis to aerobiosis induced a temporary cell cycle arrest in Δyfh1 but not in wild-type cells. After 30 min of oxygen exposition, a significant (P < 0.01) increase in the number of cells in G1 and a decrease in the number of cells in G2/M were visible (Fig. 6B). Two hours later cell cycle resumed. These data show that exposition of Δyfh1 cells to oxygen causes cell cycle arrest at G1 and delays replication. The single Δapn1 mutant showed no perturbation in cell cycle when the cells were exposed to oxygen (Fig. 7A). The growth of the double Δyfh1Δapn1 mutant was defective in anaerobiosis and aerobiosis, which was perceptible in the distribution of cells in G1 (almost 50%) and G2/M (less than 20%) populations (Fig. 7A). Exposition of these cells to oxygen block cells in G1- and S-phases (1n DNA content). On the contrary, the overexpression of APN1 rescues the cell cycle arrest phenotype in Δyfh1 cells (Fig. 7B). These data further establish the role of Apn1 in the repair of oxidized bases in Δyfh1 cells.
Oxidative genome damage has been implicated in the etiology of several neurological disorders. Here, we present evidence that one of the pleiotropic effects of increased ROS and oxidative stress in frataxin-deficient yeast cells is oxidation of DNA bases and requirement of Apn1 of the BER pathway for repair and cell survival.

Previous observations showed that exposition of yeast frataxin-deficient cells to oxygen for 16 h caused oxidative stress and loss of activity of aconitase, an iron–sulfur cluster enzyme (33). Although in anaerobiosis no defect is observed in antioxidant defense, in aerobiosis several genes encoding important antioxidant enzymes are repressed, glutathione levels are low and ROS levels are high (21, 34). The effect of oxygen in anaerobically grown Δyfh1 cells is very rapid. The first response to oxygen exposition is the increase in total glutathione content after 10 min. At 15 min, glutathione levels decrease, several antioxidant genes are repressed and DNA-strand breaks can be detected by the TUNEL assay. At 30 min, cell cycle is arrested at G1/S and mutation frequency is increased. Two hours later, cell cycle is reinitiated despite the oxidative stress state and high spontaneous mutation frequency in Δyfh1 cells. Therefore, exposition of Δyfh1 cells to oxygen causes an oxidative burst in the first 15 min due to inefficient antioxidant defense and adaptation to aerobiosis after 60–120 min. Mitochondrial iron–sulfur cluster biosynthesis and integrity of mitochondrial DNA are important factors for the maintenance of nuclear genome stability (37, 41, 42). Loss of mitochondrial DNA in yeast leads to cell cycle arrest at G1 and progressive loss of viability. However, the cells reentering the cell cycle exhibit increased genome instability and selection for nuclear mutations that improve growth in the absence of mitochondrial DNA (41). This process is mediated by the reduction in the inner mitochondrial membrane electrochemical potential, increase in

**DISCUSSION**

Oxidative genome damage has been implicated in the etiology of several neurological disorders. Here, we present evidence that one of the pleiotropic effects of increased ROS and oxidative stress in frataxin-deficient yeast cells is oxidation of DNA bases and requirement of Apn1 of the BER pathway for repair and cell survival.

Previous observations showed that exposition of yeast frataxin-deficient cells to oxygen for 16 h caused oxidative stress and loss of activity of aconitase, an iron–sulfur cluster enzyme (33). Although in anaerobiosis no defect is observed in antioxidant defense, in aerobiosis several genes encoding important antioxidant enzymes are repressed, glutathione levels are low and ROS levels are high (21, 34). The effect of oxygen in anaerobically grown Δyfh1 cells is very rapid. The first response to oxygen exposition is the increase in total glutathione content after 10 min. At 15 min, glutathione levels decrease, several antioxidant genes are repressed and DNA-strand breaks can be detected by the TUNEL assay. At 30 min, cell cycle is arrested at G1/S and mutation frequency is increased. Two hours later, cell cycle is reinitiated despite the oxidative stress state and high spontaneous mutation frequency in Δyfh1 cells. Therefore, exposition of Δyfh1 cells to oxygen causes an oxidative burst in the first 15 min due to inefficient antioxidant defense and adaptation to aerobiosis after 60–120 min. Mitochondrial iron–sulfur cluster biosynthesis and integrity of mitochondrial DNA are important factors for the maintenance of nuclear genome stability (37, 41, 42). Loss of mitochondrial DNA in yeast leads to cell cycle arrest at G1 and progressive loss of viability. However, the cells reentering the cell cycle exhibit increased genome instability and selection for nuclear mutations that improve growth in the absence of mitochondrial DNA (41). This process is mediated by the reduction in the inner mitochondrial membrane electrochemical potential, increase in
oxidative damage and impaired mitochondrial and nuclear/cytoplasmic Fe–S cluster assembly (41). It is possible that a similar process of adaptation occurs when Δyfh1 cells are exposed to oxygen for the first time, although the chronology of the events is not the same. Increase in nuclear genome instability takes place before mitochondrial DNA loss, and this could be due to the low iron–sulfur cluster biosynthesis in Δyfh1 cells. Upon oxygen exposition, oxidative damage to DNA causes cell cycle delay and selection for mutations that improve survival of Δyfh1 cells. In agreement with this hypothesis, accumulation of spontaneous suppressor mutations at a high frequency has been described in Δyfh1 (43). Independent analysis of several spontaneous mutants identified a single amino acid substitution in the Isu1 scaffold protein that bypasses the need of frataxin for iron–sulfur cluster assembly (44).

In yeast, frataxin deficiency does not induce a general sensitivity to DNA-damaging agents (14). Here, we show that lesions in the DNA in Δyfh1 cells are mainly due to oxidative modifications of DNA bases and single-strand breaks. Accordingly, H2O2 treatment causes a considerable increase in mutation frequency. However, the reason why Δyfh1 cells are more resistant than wild-type cells to MMS-induced mutagenesis is not clear. Treatment of wild-type yeast cells growing on a glucose-rich medium with MMS triggers inhibition of respiration and glycolysis, enhanced ROS production and increased glucose flow through the pentose-phosphate pathway (45). The metabolism in Δyfh1 cells is considerably different from that observed in wild-type cells; in particular, the pentose-phosphate pathway is strongly stimulated (34), which could account for the resistance of Δyfh1 cells to MMS treatment.

Oxidative DNA damage is largely repaired by the BER pathway. The AP endonucleases and AP lyases act at the same step in the BER pathway but have different enzymatic activities (26). AP-endonucleases cleave DNA at the 5′-side of an AP site or of a modified base yielding single-strand breaks with a 5′-deoxyribosephosphate. AP lyases excise oxidized bases by cleavage of the phosphodiester backbone on the 3′-side yielding single-strand breaks with a 3′-α,β-unsaturated-daldehydic end. Apn1 is essential for the repair of oxidative lesions in the nuclear DNA of Δyfh1 cells. In the absence of Apn1, AP sites are mostly incised by Ntg1 and Ntg2, but Ogg1 and the NER pathway can serve as backup activities (26,27). It is possible that these N-glycosylases/AP lyases and the NER pathway are less active in Δyfh1 cells. Ntg2 and the helicase Rad3, a subunit of the RNA polymerase II initiation complex TFIIH, from the NER pathway are iron-cluster enzymes and expected to have decreased activities in Δyfh1 cells (26,46). However, Δyfh1Δntg1 and Δyfh1Δntg2 mutants do not show increased spontaneous mutagenesis. The double Δyfh1Δapn1 mutant presents growth defects, high spontaneous and induced mutagenesis and is extremely sensitive to oxygen treatment. These phenotypes ascertain the deleterious effect of oxidative DNA damage and the importance of Apn1 for repair in frataxin-deficient yeast cells.

In mammalian cells, Apn1 is the functional homologous protein of yeast Apn1. Apn1 is a multifunctional protein; in addition to its AP endonuclease activity in the BER pathway, it is a redox-dependent activator of several stress-induced transcription factors, such as AP-1, NF-kB, HIFα and p53 (47). Although the AP endonuclease activity is conserved from bacteria to humans, the redox function is specific to mammals. The redox activity of Apn1 depends on several cysteine residues that are reduced by thioredoxin and maintain transcription factors in a reduced (active) state. Therefore, Apn1 is a master regulator of oxidative stress response in mammalian cells through regulation of gene transcription and repair of DNA, but also by its control of the intracellular redox state by inhibiting ROS production (48). Decrease in the Apn1 levels in primary rat hippocampal and sensory neuron cultures leads to oxidative DNA damage, increase in apoptosis and sensitivity to H2O2 (49). Although some reports exist on this aspect, DNA damage has been disregarded in the pathophysiology of FA. However, oxidation of DNA bases and repair could be important factors in the progression rate of the disease.

MATERIALS AND METHODS

Yeast strains and growth conditions

The Saccharomyces cerevisiae strains used in this study were derived from cycloheximide-resistant YPH499 (MATa ura3-52 lys2-801(amber) ade2-101(ochre) trp1-Δ63 his3-Δ200 leu2-Δ1 cyh2) and YPH500 (MATa ura3-52 lys2-801(amber) ade2-101(ochre) trp1-Δ63 his3-Δ200 leu2-Δ1 cyh2) strains, referred to here as ‘wild-type’, and the yfh1 shuffle strain described previously (50). In the shuffle strain, the yfh1 mutation is covered by pRS318-YFH1, a plasmid containing the CEN, CYH2 and the YFH1 HindIII genomic fragment. To avoid the appearance of suppressor mutations and loss of mitochondrial DNA, the plasmid was removed regularly under anaerobic conditions by counter selection on medium containing 10 μg/ml cycloheximide, which is toxic in the presence of the CYH2 allele. The resulting mutant was named Δyfh1 (MATa ura3-52 lys2-801(amber) ade2-101(ochre) trp1-Δ63 his3-Δ200 leu2-Δ1 cyh2 Δyfh1::TRP1).

The YPH500 (MATa) wild-type strain was used to construct strains deleted for the NTG1, NTG2 and APN1 genes and strains overexpressing the NTG1 and APN1 genes. For the construction of the Δntg1::HIS3MX6, Δntg2::HIS3MX6 and Δapn1::HIS3MX6 strains, the coding regions were replaced by the HIS3MX6 gene, which was amplified from plasmid pFA6a-HIS3MX6 by PCR using the primer sets: 5′-GCCACA CATTGGACCCCTCGATCCTTGTCACTCCGGCCTCGG ATCCCGGGGTAAAATTTTA-3′ and 5′-CTGTTTCTCTCCG TGTGTAATACATTGATTTCCGCTCACGTAATCTCGAG CTCGTTTTAAC-3′ for the NTG1 gene; 5′-CGATCAAGG ACTTGTGATGATCAGGAACACTGGGAGGACGATCC CCGGGTTAATTTAA-3′ and 5′-CGATCAAGG ACTTGTGATGATCAGGAACACTGGGAGGACGATCC CCGGGTTAATTTAA-3′ for the NTG2 gene and 5′-ATGCTCTGACAC CTAGCTTTGTAGATCTGCTGTCGAGCTTATCGGGGGG GTAAATTAA-3′ and 5′-ATGCTCTGACAC CTAGCTTTGTAGATCTGCTGTCGAGCTTATCGGGGG GGTAAATTAA-3′ for the APN1 gene as described previously (51). Successful integration was verified using external primers to the genomic region deleted using the primer sets: 5′-AGACTGAAACTG GACCGAGAC-3′ and 5′-ACATATTAAACACTAGGCCCTC C-3′ for NTG1; 5′-TTGACTGGAATCAGGAC-3′ and
p426GPD was used as a vector for the overexpression of the NTG1 and APN1 genes (52). The multicopy plasmid p426GPD vector was amplified by PCR using 5’-CGGGATCCATGCAAAGATCAG TAAAATAC-3’ and 5’-CGGAATTCTTAGTCCTCTACTTTA ACAGA-3’ primers for the NTG1 and 5’-CGGGATCCATGCG CTTCGACACCTAGCTT-3’ and 5’-CGGAATTCTTATTCTT TTCTAGTCTTCTCTC-3’ primers for the APN1, which carry 5’ BamHI and EcoRI restriction sites, respectively. The amplified fragment was digested with BamHI and EcoRI and inserted into the p426GPD plasmid under the control of the constitutive glyceraldehyde-3-phosphate dehydrogenase promoter (52). Overexpression of these genes was monitored by RT-qPCR and a 20–50-fold increase in mRNA was detected in wild-type and Δyfh1 strains carrying APN1/NTG1 overexpressing plasmids compared with strains carrying the p426GPD vector.

Unless otherwise stated, liquid cultures were grown at 30°C in synthetic defined media (SD; yeast nitrogen base, ammonium sulfate, 2% D-glucose and 200 mg/l adenine). For growth under anaerobic conditions, the medium was supplemented with 30 mg/l of ergosterol and 200 mg/l arginine. For growth under aerobic conditions, potassium phosphate monobasic, pH 7.4) for 1–4 h and the cell wall was digested with 20 μl β-glucuronidase/arylsulfatase (Roche Diagnostics) and 5 U lyticase (Sigma) for 1.5 h at 30°C. Spheroplasts were permeabilized with 0.1% Triton X-100 and labeled following the manufacturer’s instructions for the kit. For staining in aerobic conditions, exponential cells (OD600nm ≈ 0.4) were cultivated 2 h in the dark, centrifuged, resuspended in PBS buffer and analyzed. Data were analyzed as quantified as previously reported (21).

**Illegitimate mating and loss of mitochondrial DNA**

The same haploid strains were used as mating-type and p+ tester strains (Mata pΔ thr1 and Mata pΔ thr1). For illegitimate mating strains, 107 cells of the tester strain were spread onto dropout base media (DOB; yeast nitrogen base, ammonium sulfate, 2% D-glucose) plates. After drying, 50 μl drops containing 108 cells of wild-type and Δyfh1 mutant were added and plates were incubated for 2–3 days at 30°C. To evaluate mitochondrial DNA integrity, haploid tester strain and wild-type or Δyfh1 strains were streaked together in the same DOB plate and incubated 3 days at 30°C. Isolated diploid colonies were streaked in YP-2% glycerol plates and replica-plated onto YPD plates. After 3 days at 30°C, the number of colonies capable of growing on glycerol media was counted.

**Spontaneous CanR mutation frequencies**

Spontaneous mutagenesis was monitored by forward mutation in the CAN1 gene that leads to inactivation of the encoded protein, arginine permease and canavanine resistance. For each strain, one isolated colony was inoculated into 5 ml SDTe and grown for 3 days in anaerobiosis or aerobicism. Cell density was measured by plating appropriate dilutions into YPD or SD-uracil plates and counting the colonies after 3–5 days at 30°C. The quantification of canavanine-resistant mutants (CanR) was assayed by plating the cell culture in SD without arginine plates containing 60 mg/l of L-canavanine (Sigma) and counting the colonies after 5–8 days at 30°C. All experiments were repeated at least three times. Mutation frequency was calculated as the number of CanR mutants per 107 surviving cells (data presented in Figs 3B and 4A).

**O2-, H2O2- and MMS-induced mutation frequencies**

Cells grown overnight in SD media were diluted to an OD600nm of 0.1, or 0.2 for Δyfh1 mutants, and allowed to grow for 5 h (~5 x 106 cells/ml) in anaerobiosis for transition to aerobicism experiments or in aerobicism for H2O2 or MMS treatments. Aliquots were transferred to aerobicism or challenged for 30 min with H2O2 and MMS at 30°C. Cell density was measured by plating appropriate dilutions into YPD or SD-uracil plates in aerobicism and counting the colonies after 3–5 days at 30°C. To screen CanR mutants, cells were plated onto SD without arginine plates containing 60 mg/l of L-canavanine in aerobicism and the colonies counted after 5–10 days at 30°C. Mutation frequency was calculated as the number of CanR mutants per 107 surviving cells.
Cell cycle analysis by multispectral imaging flow cytometry

Cell cycle progression was analyzed using the ImageStream™ imaging flow cytometer system (Amnis), which combines the advantage of analyzing large numbers of cells by flow cytometry while simultaneously acquiring image data from individual cells. The preparation of asynchronous populations of cells for SYTOX Green (Invitrogen) staining was performed as described (39). Briefly, overnight grown cultures in anaerobiosis or aerobiosis in SDTe were diluted to an OD<sub>600nm</sub> ~ 0.3 and allowed to reach OD<sub>600nm</sub> ~ 0.7 (3–6 h depending on the strain). At this point, anaerobic cultures were shifted to air and cells were collected at 30, 60 and 120 min. The 488 nm laser was used for excitation and fluorescence images using extended depth of field were collected on channel 3 (560–595 nm), and bright-field images were collected on channels 1 and 9. Between 10 000 and 50 000 cells were acquired for statistical analysis. The IDEAS software (Amnis) was used for image analysis. The bivariate plot of the nuclear intensity aspect ratio (ratio of minor axis intensity to major axis intensity) versus the SYTOX Green fluorescence intensity of single focused cells shows the segregation of the cell populations in G1-, S- and G2/M-phases based on intensity of the nuclear DNA content, and cell and nuclear morphology (39).

RNA isolation and RT-qPCR analysis

Total RNA for RT-qPCR analysis was extracted from cells, cultured in SD media in anaerobiosis to an OD<sub>600nm</sub> ~ 1.0, using the hot phenol method as described previously (34) and purified using QIAGEN columns (RNeasy kit). RT-qPCR analysis was performed exactly as described previously (21, 34). Primer pairs used were: 5′-TCGCCCTAAGAAGCAGTTAGACA-3′ and 5′-GCTCTGCACTGGCCTTCTC-3′ for the APNI gene; 5′-TAAAGCGGCGTTGGACAGAAA-3′ and 5′-ATCATCCGG TGCAAGGAA-3′ for the NTG1 gene and 5′-CCAGGGGTAAGAGTTGATT-3′ and 5′-TCCAATTTGATGAAATTTGGAGA-3′ for the NTG2 gene or were described previously for the other genes (21, 34). Relative expression was calculated using the equation described (53). The results reported were obtained from at least three biological replicates and PCR runs were repeated at least twice.

Determination of glutathione content

Total glutathione levels were determined using a modified version of the recycling enzymatic assay, as previously described (34).

Statistical analysis

All values represent means ± SE for at least biological triplicates (n ≥ 3). All experiments were repeated at least twice. Statistical analysis was performed using the paired t-test.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Campuzano, V., Montermini, L., Molto, M.D., Pianese, L., Cossée, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., Monticelli, A. et al. (1996) Friedreich’s ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science, 271, 1423–1427.
2. Wells, R.D. (2008) DNA triplexes and Friedreich ataxia. FASEB J., 22, 1625–1634.
3. De Blas, I., Chutake, Y.K., Rindler, P.M. and Bidichandani, S.I. (2009) Epigenetic silencing in Friedreich ataxia is associated with depletion of CTCF (CCCTC-binding factor) and antisense transcription. PLoS ONE, 4, e7914.
4. Stemmler, T.L., Lesuisse, E., Pain, D. and Dancis, A. (2010) Frataxin and mitochondrial FeS cluster biogenesis. J. Biol. Chem., 285, 26737–26743.
5. Santos, R., Lefevre, S., Sliwa, D., Seguin, A., Camadro, J.M. and Lesuisse, E. (2010) Friedreich ataxia: molecular mechanisms, redox considerations and therapeutic opportunities. Antioxid. Redox Signal., 13, 651–690.
6. 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.
7. Schmucker, S., Martelli, A., Colin, F., Page, A., Wattenhofer-Donzé, M., Reutenauer, L. and Puccio, H. (2011) Mammalian frataxin: an essential function for cellular viability through an interaction with a preformed ISC/NSF1/ISD11 iron-sulfur assembly complex. PLoS ONE, 6, e16199.
8. Gahk, O., Bedekovics, T., Duncan, S.F., Smith, D.Y., Berkholz, D.S. and Isaya, G. (2010) Normal and Friedreich ataxia cells express different isoforms of frataxin with complementary roles in iron-sulfur cluster assembly. J. Biol. Chem., 285, 38486–38501.
9. Bridwell-Rabb, J., Iannuzzi, C., Pastore, A. and Barondeau, D.P. (2012) Effector role reversal during evolution: the case of frataxin in Fe-S cluster biosynthesis. Biochemistry, 51, 2506–2514.
10. Bayot, A., Santos, R., Camadro, J.M. and Rustin, P. (2011) Friedreich ataxia: the vicious circle hypothesis revisited. BMC Med., 9, 112.
11. Svilar, D., Goellner, E.M., Almeida, K.H. and Sobol, R.W. (2011) Base excision repair and lesion-dependent subpathways for repair of oxidative DNA damage. Antioxid. Redox Signal., 14, 2491–2507.
12. Jeppesen, D.K., Bohr, V.A. and Stevnsner, T. (2011) DNA repair deficiency in neurodegeneration. Prog. Neurobiol., 94, 166–200.
13. Thierbach, R., Drewes, G., Fusser, M., Voigt, A., Kuhlow, D., Schulz, T.J., Reiche, C., Glatt, H., Epe, B. et al. (2010) The Friedreich’s ataxia protein frataxin modulates DNA base excision repair in prokaryotes and mammals. Biochem. J., 432, 165–172.
14. Karthikeyan, G., Lewis, L.K. and Resnick, M.A. (2002) The mitochondrial protein frataxin prevents nuclear damage. Hum. Mol. Genet., 11, 1351–1362.
15. Karthikeyan, G., Santos, J.H., Graziewicz, M.A., Copeland, W.C., Isaya, G., Van Houten, B. and Resnick, M.A. (2003) Reduction in frataxin causes progressive accumulation of mitochondrial damage. Hum. Mol. Genet., 12, 3331–3342.
16. Haugen, A.C., Di Prospero, N.A., Parker, J.S., Fannin, R.D., Chou, J., Meyer, J.N., Halweg, C., Collins, J.B., Dürr, A., Fischbeck, K. et al. (2010) Altered gene expression and DNA damage in peripheral blood cells from Friedreich’s ataxia patients: cellular model of pathology. PLoS Genet., 6, e1000812.
17. Wilson, R.B. and Roof, D.M. (1997) Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. Nat. Genet., 16, 352–357.
18. Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralserspong, S., Montermini, L., Pandolfo, M. and Kaplan, J. (1997) Regulation of mitochondrial iron accumulation by Yihlp, a putative homolog of frataxin. Science, 276, 1709–1712.

19. Foury, F. and Cazzalini, O. (1997) Deletion of the yeast homologue associated with Friedreich’s ataxia elicits iron accumulation in mitochondria. FEBS Lett., 411, 373–377.

20. 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.

21. Lefèvre, S., Sliwa, D., Auchère, F., Brossas, C., Rukenstuhl, C., Boggetto, N., Lesuisse, E., Madeo, F., Camadro, J.M. and Santos, R. (2012) The yeast metacaspase is implicated in oxidative stress response in frataxin-deficient cells. FEBS Lett., 586, 143–148.

22. Paupe, V., Bayot, A., Dancis, A., Rogowska-Wrzesinska, A., Auche`re, F., Camadro, J.M., Bulteau, A.L., Gareil, M., Montagne, J.J., Camadro, J.M. and Santos, R. (2004) Abasic sites in DNA repair and biological consequences in Saccharomyces cerevisiae. DNA Repair (Amst.), 3, 1–12.

23. Seguin, A., Bayot, A., Dancis, A., Rogowska-Wrzesinska, A., Auche`re, F., Camadro, J.M., Bulteau, A.L. and Lesuisse, E. (2009) Overexpression of the yeast frataxin homolog (Yfh1): contrasting effects on iron-sulfur cluster assembly, heme synthesis and resistance to oxidative stress. Mitochondrion, 9, 130–138.

24. Anderson, P.R., Kirby, K., Orr, W.C., Hilliker, A.J. and Phillips, J.P. (2008) Hydrogen peroxide scavenging rescues frataxin deficiency in a Drosophila model of the ataxia. Proc. Natl Acad. Sci. USA, 105, 41–46.

25. Gros, L., Saparbaev, M.K. and Laval, J. (2002) Enzymology of the repair of free radicals-induced DNA damage. Oncogene, 21, 8905–8925.

26. Alseth, I., Eide, L., Pirovano, M., Rognes, T., Seeberg, E. and Bjøra˚s, M. (2004) Hydrogen peroxide scavenging rescues frataxin deficiency in a Drosophila model of Friedreich's ataxia. Proc. Natl Acad. Sci. USA, 101, 601–606.

27. Putnam, C.D., Jaehnig, E.J. and Rustin, P. (2009) Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia. Mol. Biol. Cell., 20, 1561–1570.

28. Foury, F. and Cazzalini, O. (1997) Deletion of the yeast homologue of the nuclear and mitochondrial dynamic localization in response to genotoxic DNA damage. Cytometry, 29, 13081–13087.

29. Gregor, I. and Tsugita, A. (1982) The amino acid sequence of cytochrome oxidase subunit VI from Saccharomyces cerevisiae. Biochem. J., 257, 13081–13087.

30. Brachat, A., Philippsen, P. and Pringle, J.R. (1998) Additional modules in providing iron for heme synthesis. Mol. Biol. Cell., 9, 2790–2802.

31. Putnam, C.D., Jaehnig, E.J. and Kolodner, R.D. (2009) Perspectives on the DNA damage and replication checkpoint responses in Saccharomyces cerevisiae. DNA Repair (Amst.), 8, 974–982.

32. Seguin, A., Bayot, A., Dancis, A., Rogowska-Wrzesinska, A., Auche`re, F., Camadro, J.M. and Santos, R. (2012) The yeast metacaspase is implicated in oxidative stress response in frataxin-deficient cells. FEBS Lett., 586, 143–148.

33. Bulteau, A.L., Dancis, A., Gareil, M., Montagne, J.J., Camadro, J.M. and Santos, R. (2004) Abasic sites in DNA repair and biological consequences in Saccharomyces cerevisiae. DNA Repair (Amst.), 3, 1–12.

34. Auche`re, F., Santos, R., Planamente, S., Lesuisse, E. and Camadro, J.M. (2007) Oxidative stress and protease dysfunction in the yeast model of Friedreich's ataxia. Free Radic. Biol. Med., 42, 1561–1570.

35. Madeo, F., Fröhlich, E., Ligr, M., Grey, M., Sigrist, S.J., Wolf, D.H. and Fröhlich, K.U. (1999) Oxygen stress: a regulator of apoptosis in yeast. J. Cell Biol., 145, 757–767.

36. Hicks, W.M., Yamaguchi, M. and Haber, J.E. (2011) Real-time analysis of double-strand DNA break repair by homologous recombination. Proc. Natl Acad. Sci. USA, 108, 3108–3115.

37. Putnam, C.D., Jaehnig, E.J. and Kolodner, R.D. (2009) Perspectives on the DNA damage and replication checkpoint responses in Saccharomyces cerevisiae. DNA Repair (Amst.), 8, 974–982.

38. Calvert, M.E. and Lannigan, J. (2010) Yeast cell cycle analysis: combining DNA staining with cell and nuclear morphology. Curr. Protoc. Cytom., Chapter 9, Unit 9.32.1–16.

39. Calvert, M.E., Lannigan, J.A. and Pemberton, L.F. (2008) Optimization of yeast cell cycle analysis and morphological characterization by multispectral imaging flow cytometry. Cytometry A, 73, 825–833.

40. Veatch, J.R., McMurray, M.A., Nelson, Z.W. and Gottschling, D.E. (2009) Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. Cell, 137, 1247–1258.

41. Diaz de la Loza, M.C., Gallardo, M., Garcia-Rubio, M.L., Izquierdo, A., Herrero, E., Aguilera, A. and Wellinger, R.E. (2011) Zim17/Tim15 links mitochondrial iron-sulfur-cluster biosynthesis to nuclear genome stability. Nucleic Acids Res., 39, 6002–6015.

42. Dı´az de la Loza, M.C., Gallardo, M., Garcı´a-Rubio, M.L., Izquierdo, A., Herrero, E., Aguilera, A. and Wellinger, R.E. (2011) Zim17/Tim15 links mitochondrial iron-sulfur-cluster biosynthesis to nuclear genome stability. Nucleic Acids Res., 39, 6002–6015.

43. Lesuisse, E., Santos, R., Matzanke, B.F., Knight, S.A., Camadro, J.M. and Dancis, A. (2003) Iron use for haeme synthesis is under control of the yeast frataxin homolog (Yfh1): contrasting effects on iron-sulfur cluster assembly, heme synthesis and resistance to oxidative stress. Mitochondrion, 9, 130–138.

44. Gregor, I. and Tsugita, A. (1982) The amino acid sequence of cytochrome oxidase subunit VI from Saccharomyces cerevisiae. J. Biol. Chem., 257, 13081–13087.

45. Popoff, S.C., Spira, A.I., Johnson, A.W. and Demple, J. (1997) Regulation of mitochondrial iron accumulation by Yihlp, a putative homolog of frataxin. Science, 276, 1709–1712.