Manganese superoxide dismutase induction by iron is impaired in Friedreich ataxia cells

Sarn Jiralerspong[a], Bing Ge[b], Thomas J. Hudson[b,c], Massimo Pandolfo[a,d,*]

[a] *Department of Medicine, Centre Hospitalier de l'Université de Montréal, Hôpital Notre-Dame – Y5608, 1560 rue Sherbrooke est, Montreal, QC, Canada H2L 4M1*
[b] *Montréal Genome Centre, McGill University Health Centre Research Institute, Montreal, QC, Canada H3G 1A4*
[c] *Departments of Human Genetics and Medicine, McGill University, Montreal, QC, Canada H3G 1A4*
[d] *Service de Neurologie, Hôpital Erasme, 1070 Brussels, Belgium*

Received 28 August 2001; revised 26 October 2001; accepted 31 October 2001

First published online 13 November 2001

Edited by Vladimir Skulachev

Abstract Iron-mediated oxidative stress has been implicated in the pathology of the neurodegenerative disease Friedreich ataxia (FRDA). Here, we show that normal upregulation of the stress defense protein manganese superoxide dismutase (MnSOD) fails to occur in FRDA fibroblasts exposed to iron. This impaired induction was observed at iron levels in which increased activation of the redox-sensitive factor NF-κB was absent. Furthermore, MnSOD induction could only be partially suppressed by antioxidants. We conclude that an NF-κB-independent pathway that may not require free radical signaling is responsible for the reduction of MnSOD induction. This impairment could constitute both a novel defense mechanism against iron-mediated oxidative stress in cells with mitochondrial iron overload and conversely, an alternative source of free radicals that could contribute to the disease pathology. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Friedreich ataxia; Frataxin; Manganese superoxide dismutase; Iron; Nuclear factor-κB; Triplet-repeat disease

1. Introduction

Friedreich ataxia (FRDA), the most common hereditary ataxia, is a recessive neurodegenerative disease caused by a partial loss of the mitochondrial matrix protein frataxin [1,2]. FRDA patients exhibit cardiomyopathy [3] and have a phenotype similar to that produced by severe deficiency of the antioxidant vitamin E [4], consistent with the idea that, as in other neurodegenerative diseases such as amyotrophic lateral sclerosis, a significant component of the underlying pathology of FRDA is cellular oxidative stress [2,5,6]. Removal of the yeast frataxin homolog (YFH) produces gross mitochondrial accumulation of unchelated iron, hypersensitivity to oxidative stress-inducing agents such as hydrogen peroxide, and inhibition of mitochondrial iron-sulfur (Fe-S) proteins, most of which can be alleviated by limiting exogenous iron availability [5,7]. FRDA tissues and cells, which still contain residual levels of frataxin, show similar changes of a milder nature and also exhibit a cytotoxic response to externally added iron [6,8–10]. In these cells, Fe-S protein impairment does not respond to therapy with iron chelators, but can be partially corrected by treatment with antioxidants [11]. Taken together, these results suggest a model by which excess free iron in the mitochondria of FRDA cells participates in Fenton reactions with the reactive oxygen species (ROS) hydrogen peroxide to generate highly reactive hydroxyl radicals which can damage cell membranes, proteins, and DNA structures [12,13]. The subsequent harm to the respiratory complexes could lead to increased leakage of the ROS superoxide that would account for the inactivation of Fe-S proteins and possibly induce even greater free iron accumulation [14]. The validity of this model is called into question by the observations that restoration of normal mitochondrial iron levels in yeast null strains does not restore activity of the mitochondrial Fe-S protein aconitase [7] and that in mouse-deficient strains, inhibition of Fe-S proteins appears to precede iron buildup [15], suggesting that these abnormalities do not result from iron-induced hydroxyl radical damage.

The mitochondrial enzyme manganese superoxide dismutase (MnSOD) is one of the primary proteins involved in cellular defense against oxidative stress and catalyzes the step immediately upstream of the Fenton reaction, namely the conversion of superoxide into the stabler hydrogen peroxide [16,17]. Overexpression of the protein has well-documented anti-apoptotic effects [18–20] and its deletion in mice leads to neurodegeneration and cardiomyopathy [21,22]. MnSOD exhibits complex regulation by a variety of factors that generate oxidative stress, including proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) which serve as strong inducers of expression [23–26]. For some stimuli, the early response factor nuclear factor-κB (NF-κB) is the primary upstream mediator of induction, although ATF-1/CREB-1, AP-2, and other factors may also modulate expression [27–29]. Free radical species, especially hydroxyl radical, serve as key signalling molecules in NF-κB activation and probably account for at least part of the redox sensitivity in MnSOD expression [30,31].

In this study, we sought to address the inconsistencies in the proposed model of FRDA pathology by using MnSOD ex-

*Corresponding author. Fax: (1)-514-412 7661.
E-mail address: massimo.pandolfo@umontreal.ca (M. Pandolfo).

Abbreviations: FRDA, Friedreich ataxia; YFH, yeast frataxin homolog; Fe-S, iron-sulfur; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; MnSOD, manganese superoxide dismutase; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-κB; FAC, ferric ammonium citrate; EMSA, electromobility shift assay

0014-5793/01/$20.00 © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(01)03140-4

pression and NF-κB induction as molecular markers to confirm increases in Fenton-based oxidative stress in FRDA fibroblasts exposed to high levels of external iron. Furthermore, we hoped to delineate the specific roles of NF-κB and ROS in the regulation of MnSOD by exogenous iron and to determine if differences in MnSOD expression might account for some of the biochemical defects in FRDA.

2. Materials and methods

2.1. Cell culture

Primary fibroblast lines were established from patients diagnosed with classical FRDA and normal, unaffected controls. PCR analysis was used to confirm and size the presence of alleles containing GAA hyperexpansions in FRDA cells [1]. All experiments were conducted on lines between passages 7 and 12 maintained under 5% CO_2 in modified Eagle's medium containing 10% fetal bovine serum and antibiotics.

2.2. RNA analysis

Fibroblasts were incubated 18 h with 200 μg/ml ferric ammonium citrate (FAC), followed by direct Trizol extraction of RNA (Gibco). Determination of expression levels by microarray analysis was done essentially as described elsewhere [32]. Briefly, total RNA was used for cDNA synthesis followed by in vitro transcription with a T7 promoter primer having a poly-T tail. The resulting product was hybridized and processed with the GeneChip system (Affymetrix) to a HuGeneFL DNA microarray containing oligonucleotides specific for approximately 7000 human transcripts. Data analysis was performed with Microarray Suite Software v3.3. For Northern analysis, total RNA (8 μg) was run on a 1% formaldehyde/MOPS gel system and probed with ^{32}P-labeled probes for a 276-bp mouse β-actin fragment (Ambion) and a 772-bp human MnSOD fragment amplified with the primers 5′-CGGGGTACCCGGCGGCATCAGCGGTACG-3′ and 5′-CGGGGTACCCTGCAGTACTCTATACCACTAC-3′. Transcript levels were estimated by densitometric scanning of the autoradiographs.

2.3. Nuclear extract preparation

Extracts were prepared as described previously [33]. Briefly, 5×10^5 cells (10-cm dishes) were incubated with or without 200–800 μg/ml FAC for 12 or 18 h, respectively, then trypsinized, washed once with ice-cold Tris-buffered saline (100 mM Tris, pH 7.5, 150 mM NaCl), and resuspended in swelling buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF). After 15 min on ice, the cells were lysed with 0.6% Nonidet P-40 detergent, vigorously vortexed for 10 s, then centrifuged at $15\,000\times g$ for 30 s to recover the nuclear pellet. The pellet was resuspended in extract buffer (20 mM HEPES, pH 7.9, 10% glycerol, 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF) and agitated for 30 min before centrifugation at $15\,000\times g$ for 5 min. Nuclear protein concentrations in the supernatant were determined by Bradford assay (Bio-Rad).

2.4. Electrophoretic mobility shift assay

Nuclear extracts (5 μg) were incubated with 25 000 cpm of a 22-bp NF-κB consensus oligonucleotide (Promega) that had been previously end-labeled with [γ-^{32}P]ATP (3000 Ci/mmol, Amersham-Pharmacia Biotech) by T4 polynucleotide kinase. Incubations were done for 20 min at room temperature in binding buffer (10 mM Tris, pH 7.5, 9% glycerol, 1 mM $MgCl_2$, 1.5 mM EDTA, 1.5 mM DTT, 250 mM NaCl) containing 0.5 μg poly(dI•dC) as a non-specific competitor. For competition experiments, excess unlabeled NF-κB or SP1 oligonucleotides (Promega) were added to the binding buffer 10 min before addition of radiolabeled probe.

2.5. Pharmacological manipulation and Western blot analysis

Fibroblasts (5-cm dish) were grown to 70–80% confluence and exposed to either FAC (200–800 μg/ml; Fisher Scientific), TNF-α (1 ng/ml; Sigma), hydrogen peroxide (H_2O_2) (30 μM; Sigma), or paraquat (500 μM; Sigma) for up to 20 h to induce MnSOD expression. To

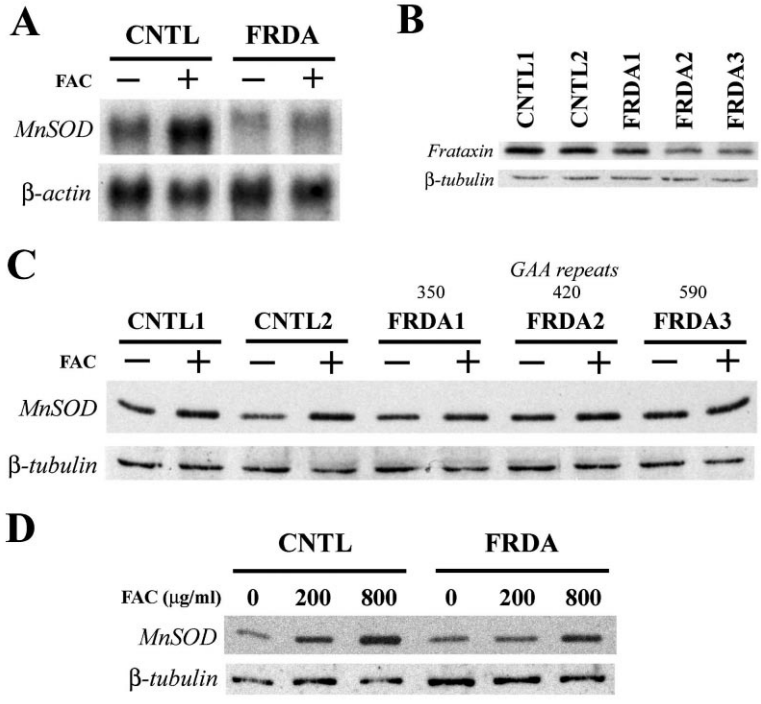

Fig. 1. Transcriptional induction of MnSOD by iron loading is impaired in FRDA fibroblasts. FRDA and control (CNTL) fibroblasts were cultured for 18 h in the absence (−) or presence (+) of FAC (200 μg/ml) to induce MnSOD expression. The 4.2-kb transcript of MnSOD was evaluated by Northern blot analysis of total RNA (8 μg/lane) as described in Section 2 using β-actin to control for RNA loading (A). FRDA cells expressing different levels of residual frataxin (B) were also exposed to the same iron stress, and protein levels of MnSOD were measured by Western blot (4 μg/lane) using β-tubulin as a loading control (C). The number of GAA repeats in the shorter disease allele of these cell lines was determined by PCR as an additional marker of residual frataxin levels. Dosage effect of iron loading was evaluated by culturing cells with the increasing amounts of FAC indicated (D) for 18 h. The results are representative of those obtained from two independent experiments.

examine the effects of antioxidants, cells were pretreated for 1 h with *N*-acetyl-L-cysteine (NAC; 30 mM; Sigma), glutathione ethyl ester (GSH, reduced form, 25 mM; Sigma), sodium formate (50 mM; Sigma), or Bayer 11-7082 (20 μM; Biomol Research Labs) prior to coincubation with one of the inducing agents. At the end of treatment, cells were washed twice with phosphate-buffered saline, immediately harvested by scraping with hot SDS buffer (125 mM Tris, pH 6.8, 4% SDS, 10% glycerol, 100 mM DTT), and sonicated briefly. Protein concentrations were determined by a modified Lowry assay (Sigma). Immunoprobing was done with MnSOD (1:10 000; Research Diagnostics) or IκB-α (1:1000, New England Biolabs) rabbit polyclonal antibodies, or β-tubulin (1:100; Calbiochem) or frataxin (1:10 000; Chemicon) mouse monoclonal antibodies. Horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch) were detected by chemiluminescent reaction (Dupont NEN). Protein levels were estimated by densitometric scanning of the autoradiographs.

3. Results

An initial study using cDNA hybridization to DNA microarrays to monitor changes in expression patterns induced by overnight incubation of human fibroblasts with iron revealed a 2-fold induction of MnSOD expression in unaffected control cells that was surprisingly absent in FRDA fibroblasts, a finding that was confirmed by Northern blot analysis (Fig. 1A). As a stimulus, the iron salt FAC was used under conditions known to produce cellular and mitochondrial iron loading. This lack of induction was further confirmed at the protein level by immunodetection and shown to vary inversely to the amount of residual frataxin in the cell line (Fig. 1B, C). There was no evidence of increased basal expression of MnSOD in FRDA cells that would account for the reduced induction. Moreover, strong MnSOD induction could be observed in the FRDA cells but only at higher levels of iron exposure (Fig. 1D), confirming that the reduced induction resulted from a dosage effect rather than the complete inactivation of the pathways responsible for upregulating MnSOD expression.

To examine if ROS might account for the iron-mediated MnSOD induction, control cells were also incubated with the intracellular superoxide generating agent paraquat and with hydrogen peroxide. Sublethal levels were employed to allow long term monitoring of slower translational effects

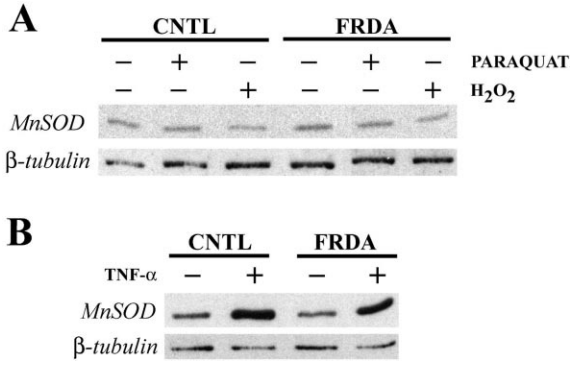

Fig. 2. Response of MnSOD expression to a variety of oxidative stresses. FRDA and control (CNTL) fibroblasts were incubated with (+) or without (−) H_2O_2 (30 μM) or paraquat (500 μM), a superoxide generating agent, for 20 h prior to analysis of MnSOD protein levels by Western blot (4 μg/lane), using β-tubulin as a loading control (A). Exposure to the ROS generating cytokine TNF-α (10 ng/ml) for 10 h was also evaluated (B). The results are representative of those obtained in two independent experiments.

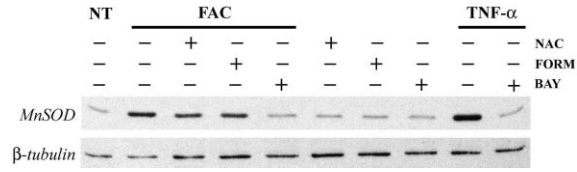

Fig. 3. Free radical scavengers partially block MnSOD induction by iron. Normal fibroblasts were pretreated (+) for 1 h with the antioxidant *N*-acetyl-L-cysteine (NAC, 30 mM), the hydroxyl radical scavenger sodium formate (FORM, 50 mM), the IκB-α phosphorylation inhibiting agent Bayer 11-7082 (BAY, 20 μM), or left untreated (−) before being exposed for 10 h to FAC (800 μg/ml). For comparison, cells were pretreated with Bayer 11-7082 or left untreated before exposure to the alternative stimulus TNF-α. As controls, some samples were treated solely with blocking agents without exposure to stressful stimuli, or received no treatment at all (NT). Direct extracts were used to make Western blots (4 μg/lane) for the analysis of MnSOD protein levels with β-tubulin serving as a loading control. The results are representative of those obtained in two independent experiments.

while minimizing interference from cytotoxic processes such as apoptosis, yet no induction was observed (Fig. 2A). Conversely, exposure to TNF-α could induce MnSOD expression to the same degree in FRDA and control cells (Fig. 2B), suggesting that some mechanisms of MnSOD regulation remained unaltered in FRDA cells. Cotreatment of cells with iron and the antioxidant *N*-acetyl-L-cysteine, the H_2O_2 scavenger reduced glutathione (not shown), or the hydroxyl radical scavenger formate, led to modest impairments of induction in all cases (approximately 50%, Fig. 3), although expression remained substantially higher than in the untreated cells.

Since NF-κB regulates induction of MnSOD by certain stimuli and since it itself is sensitive to the cellular redox state, the activity of this factor following iron loading was also studied. At low levels of iron exposure in which potentiation of MnSOD expression was observable, increased formation of NF-κB-specific binding complexes was undetectable by electromobility shift assay (EMSA; data not shown) and degradation of the inhibitory protein IκB-α that typically precedes NF-κB activation was also absent (Fig. 4A), while higher levels of iron led to increases in NF-κB activity in both control and FRDA cells to an equal extent (Fig. 4B). Treatment with the control stimulus TNF-α produced rapid loss of IκB-α and strong activation of NF-κB (Fig. 4A, B). Coincubation of iron-loaded cells with the anti-inflammatory agent Bayer 11-7082, which among other properties is capable of blocking phosphorylation of IκB-α, proved the most potent inhibitor of iron- and TNF-α-induced MnSOD expression (Fig. 3), bringing protein levels back down to that observed in untreated cells.

4. Discussion

As a whole, our results demonstrate that iron regulation of MnSOD expression involves a complicated combination of mechanisms. While low doses of iron can significantly increase MnSOD protein levels via an NF-κB-independent pathway, an NF-κB-dependent pathway also contributes synergistically to induction at higher levels of iron. Most notably, differences in the activity of the first pathway seem to be responsible for the surprising observation that MnSOD induction is impaired in FRDA cells, because this differential induction is observed

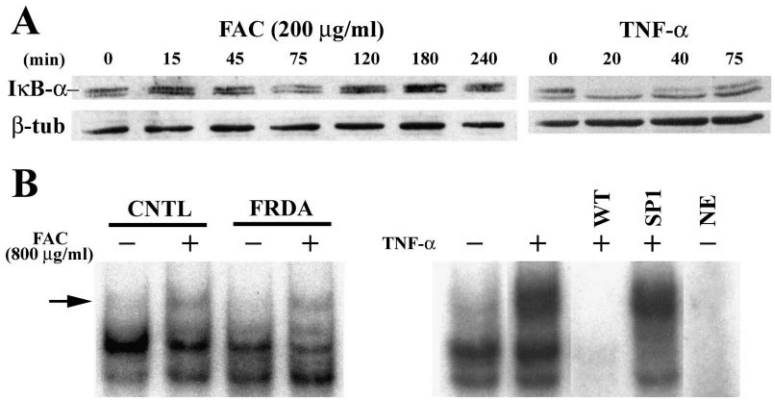

Fig. 4. NF-κB is not differentially induced by iron loading in FRDA cells. NF-κB activation in FRDA and control (CNTL) fibroblasts was determined by monitoring the disappearance of the NF-κB inhibitor, IκB-α, in cells exposed to low iron levels (FAC, 200 μg/ml) or the control stimulus TNF-α (10 ng/ml) for the times indicated (A). Cytosolic extracts were used to make Western blots (50 μg/lane) for the analysis of protein levels using β-tubulin as a loading control. The IκB-α-specific band is indicated; the lower band is non-specific and present in all extracts. EMSA (B) was also used to directly measure activation in nuclear extracts from cells exposed (+) to high iron levels (FAC, 800 μg/ml, 8 h), TNF-α (10 ng/ml, 1 h), or left untreated (−). The NF-κB-specific DNA–protein complex is indicated by an arrow and was determined by competition with excess unlabeled wild-type NF-κB (WT) or SP1 oligonucleotide; negative control with no extract (NE).

at levels of iron treatment insufficient to activate NF-κB. This is further suggested by the fact that, even at levels of exposure sufficient to potentiate NF-κB, there is no apparent difference in activity between control and patient cells. Results with free radical scavengers indicate that ROS from Fenton reactions are also important intermediaries in induction of MnSOD by iron, but their inability to completely block induction suggests that a ROS-independent regulatory mechanism also exists. Given the importance of ROS signalling in NF-κB activation and the absence of MnSOD induction by exogenous hydrogen peroxide and paraquat, it is likely that the NF-κB-independent pathway relies on this ROS-independent mechanism.

The composition of this pathway and the reason for its inhibition in FRDA cells is more difficult to explain. AP-1 and ATF-1/CREB-1 activation have been implicated in NF-κB-independent MnSOD induction by other stimuli [34,35]. Changes in cell proliferation and cell-cycle regulation can also influence MnSOD expression and in this respect it is notable that Bayer 11-7082, a compound known to not only regulate IκB-α phosphorylation but activity of the mitogen-activated protein kinases JNK-1 and p38 as well [36], was the only substance tested that was capable of inhibiting both pathways of iron-mediated MnSOD induction.

Limiting MnSOD activity may serve as a novel defense mechanism that allows cells overloaded with mitochondrial free iron to minimize production of highly toxic hydroxyl radicals by upstream inhibition of the reaction that produces the Fenton cofactor hydrogen peroxide. Normal cells would not require downregulation presumably, because they remain capable of sequestering most excess iron in an unreactive form. Suppression of hydroxyl radical production provides a direct route for the NF-κB-independent pathway to influence the NF-κB-dependent pathway, and this secondary effect might explain our failure to observe the anticipated greater potentiation of NF-κB activation in FRDA cells following treatment with high levels of iron. Our results also differ from those obtained with YFH-deficient yeast [37], which express the same level of MnSOD as wild-type cells when grown in iron replete media containing the fermentable carbon source glucose. The lack of differential induction in these cells might be a species-specific effect reflecting the absence of the NF-κB-independent regulatory pathway in yeast. Alternatively, this pathway may be present but inactive when cells are grown in glucose, since differences in expression appear to be heavily influenced by the cell respiratory activity and the type of carbon source used.

Ironically, while reducing hydroxyl radical damage, the impairment of MnSOD induction is likely to lead to other problems, including an increase in superoxide levels, which might explain the inactivation of mitochondrial Fe-S proteins seen in FRDA cells. Given the anti-apoptotic effects of MnSOD expression and the phenotypic similarities between MnSOD null mice and FRDA patients, this impairment might also explain the increased susceptibility of FRDA cells to killing by iron [9] and prove to be a major underlying factor in some aspects of FRDA pathology. In the end, by reducing MnSOD upregulation, FRDA cells may only be prolonging an inevitable death by exchanging overproduction of one toxic ROS for another.

Acknowledgements: We thank Manuela Santos and Laura Montermini for research planning. S.J. was the recipient of a McGill University fellowship. This work was supported by grants from NIH-NINDS (NS34192), CIHR (FRN14689), and MDA-USA to M.P.

References

[1] Campuzano, V., Montermini, L., Molto, M.D., Pianese, L., Cossee, M., Cavalcanti, F., Monros, E., Rodius, F., Duclos, F., Monticelli, A., Zara, F., Canizares, J., Koutnikova, H., Bidichandani, S.I., Gellera, C., Brice, A., Trouillas, P., de Michele, G., Filla, A., de Frutos, R., Palau, F., Patel, P.I., di Donato, S., Mandel, J.L., Cocozza, S., Koenig, M. and Pandolfo, M. (1996) Science 271, 1423–1427.
[2] Pandolfo, M. (2001) in: The Cerebellum and Its Disorders (Manto, M.U. and Pandolfo, M., Eds.), pp. 505–518, Cambridge University Press, Cambridge.
[3] Casazza, F. and Morpurgo, M. (1996) Am. J. Cardiol. 77, 895–898.
[4] Ben Hamida, M., Belal, S., Sirugo, G., Ben Hamida, C., Panayides, K., Ionannou, P., Beckmann, J., Mandel, J.L., Hentati, F., Koenig, M. and Middleton, L. (1993) Neurology 43, 2179–2183.

[5] Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M. and Kaplan, J. (1997) Science 276, 1709–1712.
[6] Rotig, A., de Lonlay, P., Chretien, D., Foury, F., Koenig, M., Sidi, D., Munnich, A. and Rustin, P. (1997) Nat. Genet. 17, 215–217.
[7] Foury, F. (1999) FEBS Lett. 456, 281–284.
[8] Delatycki, M.B., Camakaris, J., Brooks, H., Evans-Whipp, T., Thorburn, D.R., Williamson, R. and Forrest, S.M. (1999) Ann. Neurol. 45, 673–675.
[9] Wong, A., Yang, J., Cavadini, P., Gellera, C., Lonnerdal, B., Taroni, F. and Cortopassi, G. (1999) Hum. Mol. Genet. 8, 425–430.
[10] Bradley, J.L., Blake, J.C., Chamberlain, S., Thomas, P.K., Cooper, J.M. and Schapira, A.H. (2000) Hum. Mol. Genet. 9, 275–282.
[11] Rustin, P., von Kleist-Retzow, J.C., Chantrel-Groussard, K., Sidi, D., Munnich, A. and Rotig, A. (1999) Lancet 354, 477–479.
[12] Halliwell, B. and Gutteridge, J.M. (1984) Biochem. J. 219, 1–14.
[13] Stohs, S.J. and Bagchi, D. (1995) Free Radic. Biol. Med. 18, 321–336.
[14] Keyer, K. and Imlay, J.A. (1996) Proc. Natl. Acad. Sci. USA 93, 13635–13640.
[15] Puccio, H., Simon, D., Cossee, M., Criqui-Filipe, P., Tiziano, F., Melki, J., Hindelang, C., Matyas, R., Rustin, P. and Koenig, M. (2001) Nat. Genet. 27, 181–186.
[16] van Loon, A.P., Pesold-Hurt, B. and Schatz, G. (1986) Proc. Natl. Acad. Sci. USA 83, 3820–3824.
[17] Guidot, D.M., McCord, J.M., Wright, R.M. and Repine, J.E. (1993) J. Biol. Chem. 268, 26699–26703.
[18] Pani, G., Bedogni, B., Anzevino, R., Colavitti, R., Palazzotti, B., Borrello, S. and Galeotti, T. (2000) Cancer Res. 60, 4654–4660.
[19] Bernard, D., Quatannens, B., Begue, A., Vandenbunder, B. and Abbadie, C. (2001) Cancer Res. 61, 2656–2664.
[20] Drane, P., Bravard, A., Bouvard, V. and May, E. (2001) Oncogene 20, 430–439.
[21] Lebovitz, R.M., Zhang, H., Vogel, H., Cartwright Jr., J., Dionne, L., Lu, N., Huang, S. and Matzuk, M.M. (1996) Proc. Natl. Acad. Sci. USA 93, 9782–9787.
[22] Melov, S., Schneider, J.A., Day, B.J., Hinerfeld, D., Coskun, P., Mirra, S.S., Crapo, J.D. and Wallace, D.C. (1998) Nat. Genet. 18, 159–163.
[23] Visner, G.A., Dougall, W.C., Wilson, J.M., Burr, I.A. and Nick, H.S. (1990) J. Biol. Chem. 265, 2856–2864.
[24] Ono, M., Kohda, H., Kawaguchi, T., Ohhira, M., Sekiya, C., Namiki, M., Takeyasu, A. and Taniguchi, N. (1992) Biochem. Biophys. Res. Commun. 182, 1100–1107.
[25] Poswig, A., Wenk, J., Brenneisen, P., Wlaschek, M., Hommel, C., Quel, G., Faisst, K., Dissemond, J., Briviba, K., Krieg, T. and Scharffetter-Kochanek, K. (1999) J. Invest. Dermatol. 112, 13–18.
[26] Franco, A.A., Odom, R.S. and Rando, T.A. (1999) Free Radic. Biol. Med. 27, 1122–1132.
[27] Warner, B.B., Stuart, L., Gebb, S. and Wispe, J.R. (1996) Am. J. Physiol. 271, L150–L158.
[28] Kiningham, K.K., Xu, Y., Daosukho, C., Popova, B. and St. Clair, D.K. (2001) Biochem. J. 353, 147–156.
[29] Zhu, C.H., Huang, Y., Oberley, L.W. and Domann, F.E. (2001) J. Biol. Chem. 276, 14407–14413.
[30] Schulze-Osthoff, K., Beyaert, R., Vandevoorde, V., Haegeman, G. and Fiers, W. (1993) EMBO J. 12, 3095–3104.
[31] Shi, X., Dong, Z., Huang, C., Ma, W., Liu, K., Ye, J., Chen, F., Leonard, S.S., Ding, M., Castranova, V. and Vallyathan, V. (1999) Mol. Cell. Biochem. 194, 63–70.
[32] Golub, T.R., Slonim, D.K., Tamayo, P., Huard, C., Gaasenbeek, M., Mesirov, J.P., Coller, H., Loh, M.L., Downing, J.R., Caligiuri, M.A., Bloomfield, C.D. and Lander, E.S. (1999) Science 286, 531–537.
[33] Schreiber, E., Matthias, P., Muller, M.M. and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419.
[34] Borrello, S. and Demple, B. (1997) Arch. Biochem. Biophys. 348, 289–294.
[35] Kim, H.P., Roe, J.H., Chock, P.B. and Yim, M.B. (1999) J. Biol. Chem. 274, 37455–37460.
[36] Pierce, J.W., Schoenleber, R., Jesmok, G., Best, J., Moore, S.A., Collins, T. and Gerritsen, M.E. (1997) J. Biol. Chem. 272, 21096–21103.
[37] Foury, F. and Talibi, D. (2001) J. Biol. Chem. 276, 7762–7768.