BCL-2 Improves Oxidative Phosphorylation and Modulates Adenine Nucleotide Translocation in Mitochondria of Cells Harboring Mutant mtDNA

Members of the BCL-2-related antiapoptotic family of proteins have been shown previously to regulate ATP/ADP exchange across the mitochondrial membranes and to prevent the loss of coupled mitochondrial respiration during apoptosis. We have found that BCL-2/BCL-xL can also improve mitochondrial oxidative phosphorylation in cells harboring pathogenic mutations in mitochondrial tRNA genes. The effect of BCL-2 overexpression in mutated cells was independent from apoptosis and was presumably associated with a modulation of adenine nucleotide exchange between mitochondria and cytosol. These results suggest that BCL-2 can regulate respiratory functions in response to mitochondrial distress by regulating the levels of adenine nucleotides.

The proto-oncogene BCL-2 prevents apoptosis and some forms of cellular necrosis, although the exact mechanisms of its anti-cell death function are not known. BCL-2 was shown to localize to multiple cell compartments, including the outer mitochondrial membrane. A number of functions related to mitochondria have been proposed for BCL-2, including the capacity to prevent loss of mitochondrial membrane potential and opening of the mitochondrial permeability transition pore, which might be responsible for the activation of certain apoptotic pathways. In addition, BCL-xL, another member of the BCL-2 antiapoptotic family of proteins, was shown to regulate ATP/ADP exchange across the mitochondrial membranes and to prevent the loss of coupled mitochondrial respiration in response to proapoptotic stimuli. It was proposed that the ability of the BCL-2 family of proteins to maintain mitochondrial integrity after proapoptotic stimuli might be related to maintaining the voltage-dependent anion channel of the outer mitochondrial membrane in an open configuration. This would prevent the accumulation of anions, particularly ADP, ATP, and creatine-P04, in the mitochondrial intermembrane space. Moreover, BCL-xL overexpression can stimulate mitochondrial respiration in cultured cells independent of the induction of apoptosis.

Mutations in mtDNA are associated with a heterogeneous group of sporadic or maternally inherited metabolic disorders. Usually these mutations cause impairment of oxidative phosphorylation (OXPHOS) with a reduction in mitochondrial membrane potential and ATP synthesis. We studied the effect of BCL-2 and BCL-xL overexpression on mitochondrial and cytosolic ATP pools in human trans mitochondrial hybrid lines containing normal mtDNA, mutated mtDNA causing defective respiratory chains, and cells lacking mtDNA (ρ0 cells), which have no functioning respiratory chain. We found that in both wild type (WT) and mtDNA mutant (MT) cells BCL-2 and BCL-xL overexpression tended to equalize the relative ATP content in the two cell compartments, presumably promoting its exchange across the mitochondrial membranes. We investigated the effect of BCL-2 and BCL-xL expression in cells harboring pathogenic mutations in mitochondrial tRNA-coding genes to assess whether modulation of anion exchange and the protective effect on mitochondrial function could improve defects of the mitochondrial respiratory chain caused by mtDNA mutations. We showed that antiapoptotic BCL-2 family members ameliorated the defective OXPHOS phenotype in three different cell lines harboring distinct mitochondrial tRNA gene mutations. These data raise the possibility that BCL-2 might have a more general protective function againstinsulting agents on mitochondrial physiology that is not necessarily limited to its antiapoptotic properties.

EXPERIMENTAL PROCEDURES

BCL-2 and BCL-xL DNA Constructs, Cell Culture and Transfection, and Generation of Hybrid Cell Lines—Human BCL-2 cDNA was cloned in the EcoRI site of the eukaryotic expression vector pcDNA3 (Invitrogen). Human BCL-xL (a gift from Lawrence Boise, University of Miami) cloned in the expression vector pIREShoe (Clontech) was obtained as described previously. The human osteosarcoma mtDNA-less cell line (143B/2069 cells) was cultured in Dulbecco’s modified Eagle’s medium containing high glucose (4.5 g/liter) supplemented with 5% fetal bovine serum, 1 mM sodium pyruvate, and 50 μg/ml uridine.
143B/206p cells were transfected with pcDNA3-BCL-2 using FuGENE 6 (Roche Molecular Biochemicals) as described by the manufacturer. G418 (Geneticin™; Invitrogen)-resistant clones were selected and screened for BCL-2 expression by Western blotting using specific monoclonal antibodies against human BCL-2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA). One 143B/206p clone with high BCL-2 expression (206p/BCL-2) was chosen for mtDNA repopulation. 206p BCL-xL cells were obtained as described previously (8).

Hybrid cell lines were obtained by fusing 206p/BCL-2 or 206p BCL-xL cells with previously described mutated (MT) T8356C and WT myoclonus epilepsy with ragged red fibers (MERRF) (10) and WT mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) (11) cybrids. Before fusion, MERRF and MELAS cybrids were treated for 2 h with 1 μg/ml actinomycin D, a DNA transcription and replication inhibitor, to limit the amount of nuclear DNA transferred from the cybrids to the BCL hybrids. Actinomycin D-treated cells (1 × 10⁶) were washed three times in Dulbecco's modified Eagle's medium and then fused to 1 × 10⁶ 206p/BCL-2 or p206p/BCL-xL cells as described previously (12). Cells were selected in a medium containing 100 μg/ml Geneticin and lacking uridine. Surviving cells were pooled in mass cultures. MERRF MT (8356) and MERRF MT (8344) (10) cells were also directly transfected with pcDNA3 BCL-2 or with pcDNA3 empty vector as described above. In this case, cells were selected in 500 μg/ml G418 and lacking uridine. Surviving cells were transfected as described previously (17).

Cell Death Studies—Apoptotic cell death induced by treatment with 0.5 μM staurosporine (STP) for 6 h was assessed by using a cell death detection enzyme-linked immunosorbent assay kit (Roche Molecular Biochemicals) on the basis of the detection of nucleosome breakdown as described previously (8).

Biochemical Studies—Oxygen consumption was measured in a 300-μl reaction chamber equipped with a Clark-type polarographic electrode as described previously (17) with a few modifications. Cells were trypsinized, counted in a 21 automed cell counter (Beckman-Coulter, Miami, FL), and resuspended at 1.5 × 10⁵ cells lysed with a luciferase assay kit (Promega Inc.) containing 500 μM ATP.

Measurements of ATPₘ and ATPₜ—WT, WT-BCL-2, 143B, 143B/BCL-2, 143B/206p, 206p/BCL-2, MERRF MT (8356), and MERRF MT (p206p) cells were transiently transfected as described previously with pcDNA3 expressing the ATP reporter luciferase targeted to the mitochondrial matrix or to the cytosol. Mitochondrial luciferase was generated by appending to the N terminus of luciferase the presequence of the mitochondrially targeted subunit VIII of cytochrome c oxidase. This construct has been shown to be efficiently imported into mitochondria (13–15). Cytosolic luciferase was obtained by disrupting the natural C-terminal peroxisomal targeting sequence with a leucine to valine amino acid substitution at position 550 (16). 48 h after transfection with cytosolic luciferase or mitochondrial luciferase, aliquots of 2 × 10⁵ cells were resuspended in 1 ml of Dulbecco’s modified Eagle’s medium containing 100 mM pyruvate with or without 4.5 mg/ml glucose and incubated in a 24-well plate with gentle agitation at 37 °C in 5% CO₂. After 1 h, cells were collected by centrifugation and resuspended in 90 μl of 25 mM Tricine, 150 mM NaCl, pH 7.4. 10 μl of 20 mM luciferin (Promega Inc., Madison, WI) was added, and luminescence was recorded in an Opticom I luminometer (MGM Instruments, Hamden, CT). To normalize for the variability of luciferase expression, the luminescence values were expressed as a ratio to the “total potential luminescence” measured on 2 × 10⁵ aliquots of the same cells lysed with a luciferase assay kit (Promega Inc.) containing 500 μM ATP.
Oligomycin-sensitive ATP synthesis was measured on digitonin-permeabilized cells using a luciferase-based kinetic assay as described previously (18) with some modifications. Cells were trypsinized and resuspended in the reaction buffer at 1 × 10⁷/mL. Digitonin (50 µg/mL) was added to 180 µL of cell suspension followed by incubation for 1 min at room temperature. Cells were washed in 1 mL of reaction buffer, and the luminescent measurement was performed as described (18) with and without the addition of 10 µg/mL oligomycin.

Mitochondrial membrane potential was estimated using the membrane potential-sensitive dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazole carboxyanide iodide (JC-1; Molecular Probes, Eugene, OR) as described (19), except that cells were grown in 6-well plates to ~80% confluence, and the JC-1 staining was performed directly on attached cells. Fluorescence was read at 535 (green monomer) and at 595 nm (orange aggregate) in an HTS 7000 plus plate reader (PerkinElmer Life Sciences). Mitochondrial membrane potential was expressed as the ratio of the absorbance at 595 nm to the absorbance at 535 nm.

Hydrogen peroxide levels were measured in cells by 2′,7′-dichloro-fluorescein diacetate (H2DCFDA; Molecular Probes) fluorescence as described elsewhere (19).

Analyses of Mitochondrial Translation Products—Steady-state levels of mitochondrial peptides were studied by Western blotting. 20 µg of total cellular protein was separated onto 15% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membrane (Bio-Rad). After transfer, membranes were incubated with monoclonal antibodies against human cytochrome c oxidase subunits I, II, and IV and the 39-kDa subunit of complex I (Molecular Probes) at 4 °C for 12 h and subsequently incubated with peroxidase-conjugated anti-mouse IgG for 1 h at room temperature. Bands were developed with Supersignal chemiluminescence substrate (Pierce) and quantified by densitometry using a Fluor-S analyzer (Bio-Rad).

RESULTS

BCL-2 Expression and mtDNA Genotype in Hybrid Cells—The human BCL-2 cDNA cloned in the eukaryotic expression vector pCDNA3 was transplanted into human osteosarcoma cells lacking mtDNA (143B/206p⁰). G418-resistant clones were screened for BCL-2 expression, and one high expressor (termed 206p⁰/BCL-2) was selected. The 206p⁰/BCL-2 line was then repopulated with exogenous WT mtDNA. Similarly, human BCL-xL, cDNA cloned in a pIREs-neo vector was transfected into 143B/206p⁰ or in parental osteosarcoma 143B cells, and G418-resistant BCL-xL overexpressing clones were selected. The 206p⁰/BCL-2 line was repopulated with exogenous mtDNA from cybrid clones derived from a patient harboring a C3256T transition in the tRNAhsu(UUR) gene and from a patient with a T8356C transition in the tRNAlys(UUR) gene. The C3256T mutation has been associated with a disease clinically characterized by MERRF. Because in both patients the mutated mtDNA was heteroplasmic (i.e. WT and mtDNA coexisted), it was possible to isolate homoplasmic hybrid clones containing either exclusively MT or WT mtDNA.

206p⁰ cells are strictly auxotrophic for uridine. Thus, hybrid cells harboring the mitochondrial genotypes described above and expressing BCL-2 (termed MERRF MT-BCL-2, MERRF WT-BCL-2, MELAS MT-BCL-2, MELAS WT-BCL-2) were obtained by the fusion of 206p⁰/BCL-2 cells with WT or MT MERRF and MELAS cybrids. We note that in all the experiments involving hybrids, we deliberately avoided isolating individual clones of repopulated cells and chose instead to study pools of clones to minimize artifacts caused by potential interclonal variability. As expected, all the newly produced hybrid cell lines expressing BCL-2 contained either MT or WT mtDNA (Fig. 1A). In an independent set of experiments, 206p⁰ cells expressing BCL-xL were fused with MERRF and MELAS MT cybrids. The levels of BCL-2 in the expressor lines were ~10–20-fold higher than the endogenous levels in untransfected cells (Fig. 1B). The antiapoptotic function of BCL-2 was tested by measuring nucleosome breakdown as described above and before STP induction. BCL-2-overexpressing cells showed a marked decrease in the apoptotic response to STP (Fig. 1C). In addition, we observed that both untransfected MT cell lines displayed some degree of natural resistance against STP-induced apoptosis compared with their WT counterparts (Fig. 1C). It is possible that reduced mito-
Mitochondrial ATP synthesis and membrane potential in MT cells could lead to decreased apoptotic response to STP as described previously in 143B/206/H9267 cells (8) and in Jurkat T cells depleted of ATP by glucose deprivation combined with rotenone treatment (20).

Mitochondrial and Cytosolic ATP Levels in BCL-2- and BCL-xL-Overexpressing Cells—To measure ATP content, cells were transfected transiently with pcDNA3 containing the ATP reporter luciferase and engineered to be targeted to the mitochondrial matrix or to the cytosol. Cytosolic luciferase or mitochondrial luciferase-transfected cells were incubated in culture medium containing pyruvate with or without glucose. Relative ATP content was estimated by measuring luminescence after the addition of luciferin.

In untransfected cells containing normal mtDNA (WT hybrids and 143B parental cells), the ratio between ATP content in the mitochondria (ATPm) and in the cytosol (ATPc) was lower in the presence of glucose than when pyruvate was the only substrate available. Overexpression of BCL-2 and BCL-xL significantly increased the ATPm:ATPc ratio in the presence of glucose but did not affect it in the presence of pyruvate alone (Fig. 2). Therefore, when cells competent for respiration were grown in pyruvate, medium mitochondrial ATP became predominant over cytosolic ATP. However, overexpression of BCL-2 or BCL-xL appeared to equalize the relative ATP concentrations between the two compartments in cells grown in glucose medium (Fig. 2). As expected, 206/H9267 cells, which are OXPHOS-incompetent, were completely depleted of ATP in pyruvate medium (not shown). In glucose medium, untransfected 206/H9267 cells had an equal ATPm:ATPc ratio as BCL-2-overexpressing 206/H9267. MERRF MT cybrids transfected with BCL-2 also showed an increased ATPm:ATPc ratio in the presence of glucose compared with mock-transfected MERRF MT cybrids. In MERRF MTBCL-2 cells the ATPm:ATPc ratio was also increased when pyruvate was the only substrate in the medium (Fig. 2). In fact, in pyruvate medium the absolute ATP contents in the two compartments were ~10-fold higher in MERRF MT cells expressing BCL-2 than in mock transfected cells (not shown). These findings support the concept that BCL-2 and

![Fig. 3. ATP synthesis, cell respiration, respiratory chain activities, and mitochondrial membrane potential. Data are averages plus S.D. of three or more independent measurements. *p-values of significant differences between untransfected and BCL-2-overexpressing cells determined by unpaired Student’s t-test are shown. A, oligomycin-sensitive ATP synthesis measured in permeabilized cells using malate plus pyruvate as substrates. ATP synthesis values are expressed as the percentages of ATP synthesis in untransfected WT cells. B, cell respiration with endogenous substrates in intact (−FCCP) and in uncoupled (+FCCP) cells. Cell respiration is expressed as femtomoles of molecular oxygen consumed in 1 min per cell. C, mitochondrial membrane potential measurement using the potential-sensitive dye JC-1. Membrane potential is expressed as the ratio of the two readings at 595 and 535 nm. D, complex I (NADH-coenzyme Q reductase) activity was measured by polarography in intact cells with and without 1 μM rotenone. Activities are expressed as rotenone-sensitive oxygen consumption (nmol O2/min/mg protein). Cybrid and hybrid cells are denoted as in Fig. 1.](http://www.jbc.org)
BCL-x\textsubscript{L} may play a role in modulating the exchange of ATP across the mitochondrial membranes, probably by promoting its equilibration between the intramitochondrial and extramitochondrial milieu. These results also suggest that BCL-2 may improve respiratory functions in mtDNA mutant cells.

**BCL-2 Overexpression Improves Mitochondrial ATP Synthesis, Respiration, and Mitochondrial Membrane Potential in Cybrids with Mutated mtDNA—**Mitochondrial ATP synthesis was measured in permeabilized cells with malate plus pyruvate as substrates. The rate of ATP synthesis was \( \sim 10 \) -fold lower in both MERRF MT and MELAS MT cells compared with the respective WT lines. In M\textsubscript{BCL-2} cells, ATP synthesis was restored to \( \sim 40\% \) of WT levels (i.e. 4-fold increase; Fig. 3A). On the contrary, a moderate reduction (\( \sim 40\% - 50\% \)) of ATP synthesis was observed in WT\textsubscript{BCL-2} cells as compared with their untransfected counterparts (Fig. 3A).

Mitochondrial respiration measured in MT cells (Fig. 3B) was reduced to \( \sim 15\% \) of WT. However, in M\textsubscript{BCL-2} cells it was restored to \( \sim 60\% \) of WT values (i.e. a 4-fold increase). The addition of the protonophore FCCP, which completely uncouples mitochondria, increased cell respiration 2-fold in WT cells and to a lower degree in MT cells. The proportion by which respiration was induced by FCCP was substantially unchanged in BCL-2-expressing cells, suggesting that the increased respiration in intact cells was unlikely to be caused by the uncoupling of mitochondria by BCL-2.

Mitochondrial membrane potential measured by the potentiometric mitochondrial dye JC-1 in medium containing glucose and pyruvate was significantly increased in both M\textsubscript{BCL-2} cell lines compared with untransfected cells but was still lower than in WT cells (Fig. 3C).

The activity of respiratory chain complex I, measured as rotenone-sensitive cell respiration (Fig. 3D), was severely decreased in MERRF MT and to an even greater extent in MELAS MT. In both MT cell lines, overexpression of BCL-2 partially restored complex I activity (\( \sim 40\% \) of WT levels).

To exclude the possibility that selection in medium lacking uridine (during the production of the different cell lines used in this study) might have favored the growth of spontaneous revertants, MERRF MT cybrids were also directly transfected with pcDNA3-BCL-2 or mock-transfected with pcDNA3 (neo) and selected in medium containing G418 and high glucose and supplemented with uridine. In addition, directly transfected cybrids served as controls for the extra dose of nuclear genes presumably present in the hybrids derived from fusion of cybrids with 206\textsubscript{p}BCL-2 cells. MERRF MT\textsubscript{BCL-2} and WT\textsubscript{BCL-2} as well as mock-transfected clones were selected and mitochondrial respiration was measured. In this case, we could not use pools of clones because many G418-resistant cells might not express high levels of BCL-2. MERRF MT\textsubscript{BCL-2} clones had higher respiratory rates than mock-transfected clones: 2.9 \( \pm \) 0.4 and 1.7 \( \pm \) 0.8 fmol of O\textsubscript{2}/min/cell, respectively (number of clones \( = 6 \); \( p < 0.01 \) (Fig. 3B)), confirming the results described previously on MERRF MT\textsubscript{BCL-2} hybrids created by fusion and selection in medium containing G418 and lacking uridine. In addition, to further confirm these findings, cybrids containing homoplasmic levels of a different, more severe, \( tRNA^\text{Lys} \) mutation at nucleotide 8344 also associated with MERRF (21) were transfected with pcDNA3-BCL-2 or mock-transfected with pcDNA3 (neo) and selected in G418. MERRF 8344 MT clones overexpressing BCL-2 also showed improved cell respiration and ATP synthesis. Oxygen consumption in BCL-2 and mock-transfected MERRF 8344 MT clones was 1.2 \( \pm \) 0.3 and 0.6 \( \pm \) 0.3 fmol of O\textsubscript{2}/min/cell, respectively (number of BCL-2 expressing clones \( = 6 \); number of mock-transfected clones \( = 8 \); \( p < 0.002 \) as determined by unpaired Student’s \( t \) test (Fig. 3B)). ATP synthesis was increased 5.5-fold in BCL-2 MERRF 8344 MT over the mock transfected (number of clones \( = 5 \) in each group; \( p < 0.03 \) (not shown)).

**BCL-2 Overexpression Does Not Affect Free Radical Production in Mutated Cells in Nonapoptotic Conditions**—It has been suggested that members of the BCL-2 antiapoptotic protein family can protect cells from the potentially deleterious effects of mitochondrial reactive oxygen species production that follow apoptotic stimuli (22, 23). Therefore, it could be hypothesized that if mitochondrial tRNA mutations cause increased reactive oxygen species production, BCL-2 overexpression could prevent damage to mitochondria by scavenging reactive oxygen species. However, hydrogen peroxide levels measured by H\textsubscript{2}DCFDA dye in MT and WT cells growing in normal conditions without apoptotic stimuli were not increased in MT cells as compared with WT cells, and BCL-2 overexpression did not seem to affect hydrogen peroxide levels (not shown).

To verify whether the effect of BCL-2 overexpression on MT cells could be mimicked by exogenous antioxidants, MERRF...
MT cells were grown for a prolonged period of time in the presence of 10 μM dihydroliroic acid. This antioxidant agent has been shown to protect PC12 rat cells from cell death induced by mitochondrial respiratory chain inhibitors (24). Cells treated with dihydroliroic acid for 1 or 2 weeks did not show any improvement in respiratory chain activities and cell respiration (not shown), further suggesting that protection from free radical damage is unlikely to play a major role in the biochemical improvement induced by BCL-2 overexpression.

**BCL-2 Overexpression Does Not Affect Respiratory Chain Subunit Steady-state Levels**—The steady-state levels of respiratory chain complex IV subunits I and II (cytochrome c oxidase I and II), which are encoded by the mtDNA, were markedly decreased in MT cells. BCL-2 overexpression did not modify the steady-state levels of these subunits in MERRF and MELAS MT cells (not shown). The levels of complex IV subunit IV (cytochrome c oxidase IV) and complex I 39-kDa subunit (CO I 39-kDa) that are both encoded by the nuclear DNA were similar in all cells and were unmodified by BCL-2 overexpression (not shown). This finding suggests that BCL-2 did not exert its effects on mitochondrial respiration by increasing the levels of mitochondrial enzymes, but rather by modulating their activities.

**DISCUSSION**

It was suggested that BCL-x<sub>S</sub> is able to regulate mitochondrial membrane potential and ATP/ADP exchange across the mitochondrial membranes in cells deprived of growth factors (5, 6). BCL-2 has also been shown to promote H<sup>+</sup> efflux from the mitochondrial matrix into the intermembrane space of isolated mitochondria as a mechanism to maintain membrane potential in response to uncoupling agents (3). These observations suggest that the antiapoptotic BCL-2 family of proteins might have a role in maintenance of mitochondrial homeostasis that is not limited to their antiapoptotic properties. These findings also seem to point to adenine nucleotide translocation and transmembrane proton flux as potential critical sites for the action of BCL-2 proteins.

In normal cells, ATP is generated both by glycolysis in the cytosol and by OXPHOS in mitochondria, from which it is exported to the other cellular compartments where most of it is used. We found that in untransfected WT cells the ATP<sub>m</sub>/ATP<sub>c</sub> ratio was higher in a medium without glucose (in which cells produce ATP mainly through oxidative phosphorylation) than when cells were grown with a high concentration of glucose. BCL-2 and BCL-x<sub>S</sub> appeared to increase the ATP<sub>m</sub>/ATP<sub>c</sub> ratio in glucose medium, presumably by promoting the ATP exchange across the mitochondrial membranes. Accordingly, we found that WT cells overexpressing BCL-2/BCL-x<sub>S</sub> had a significantly reduced mitochondrial ATP synthesis, possibly as a response to the increased ATP<sub>m</sub>/ATP<sub>c</sub> ratios. In WT cells, which have a normal respiratory chain, an increased ATP<sub>m</sub>/ATP<sub>c</sub> ratio may have an inhibitory effect on ATP synthesis. On the contrary, we showed that BCL-2 expression increased the ATP<sub>m</sub>/ATP<sub>c</sub> ratio in cells harboring the MERRF mutation not only in glucose but also in the oxidative substrate pyruvate, and it stimulated mitochondrial respiration and ATP synthesis.

It was suggested that ATP/ADP translocation is the most efficient mechanism to maintain mitochondrial membrane potential in cells with a defective respiratory chain, such as p<sup>60</sup> cells (25). To maintain membrane potential in respiration-deficient cells, ATP is imported inside mitochondria and hydrolyzed by the F1-ATPase (Fig. 4). Maintenance of a mitochondrial membrane potential, even if reduced, is necessary for cell survival, because crucial functions such as mitochondrial protein import and processing are membrane potential-dependent (26, 27). This behavior could explain the differences between ATP production in wild type and in mutant cells.

Cells harboring homoplasmic levels of mitochondrial tRNA mutations must rely primarily on glycolysis, because their mitochondrial ATP synthesis is extremely low. Therefore, it is likely that similar mechanisms to those described in p<sup>60</sup> cells, which involve ATP/ADP exchange to maintain membrane potential, operate in mutated cells as well. The events through which BCL-2 and BCL-x<sub>S</sub> facilitate the exchange of creatine-PO<sub>4</sub> and adenine nucleotides between cytosol and mitochondria in response to perturbations of cellular metabolism seem to occur at the level of the voltage-dependent anion channel in the outer mitochondrial membrane voltage-dependent anion channel (5). When mitochondrial production of ATP becomes low, as in the initial phases of apoptosis, or in cells that harbor mutations of the mtDNA, BCL-x<sub>S</sub> and BCL-2 might promote the exchange of ATP and ADP between mitochondria and cytosol. Mutant cells, which unlike p<sup>60</sup> cells have some residual capability to maintain a functioning respiratory chain, could benefit from the higher mitochondrial membrane potential and higher intramitochondrial supplies of adenine nucleotides. At this point it is unclear how BCL-2 improves oxidative phosphorylation activity, but our results suggest that this effect is associated with increased ATP flux into mitochondria. This increased flux could stimulate oxidative phosphorylation by leading to the formation of ADP (through ATP-hydrolyzing reactions), which could directly stimulate the respiratory chain. Alternatively, BCL-2 could increase ATP-dependent functions of mitochondria containing mutated mtDNA, thereby increasing OXPHOS efficiency. This result could be accomplished, for instance, by improved stability of certain components of the mitochondrial respiratory chain such as cytochrome c leading to more efficient electron transfer and OXPHOS. Improved OXPHOS efficiency may then increase membrane potential and ATP synthesis.

In conclusion, we believe that antiapoptotic BCL-2-related proteins may have a role in the homeostasis of mitochondrial metabolism not only in response to apoptotic stimuli but also in a variety of conditions that require mitochondrial adaptation to metabolic stresses. BCL-2/BCL-x<sub>S</sub> can modulate the ATP gradient between mitochondria and cytosol, and their increased expression may not only protect cells during noxious stimuli but also improve energy metabolism within mitochondria with defective respiratory chain functions.

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