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Protection from Cell Death by *mcl-1* Is Mediated by Membrane Hyperpolarization Induced By K^+ Channel Activation

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Abstract. *Mcl-1,* a member of the *Bcl-2* family, has been identified as an inhibitor of apoptosis induced by anticancer agents and radiation in myeloblastic leukemia cells. The molecular mechanism underlying this phenomenon, however, is not yet understood. In the present study, we report that hyperpolarization of the membrane potential is required for prevention of *mcl-1* mediated cell death in murine myeloblastic FDC-P1 cells. In cells transfected with *mcl-1,* the membrane potential, measured by the whole-cell patch clamp, was hyperpolarized more than −30 mV compared with control cells. The membrane potential was repolarized by increased extracellular K^+ concentration (56 mV per 10-fold change in K^+ concentration). Using the cell-attached patch-clamp technique, K^+ channel activity was 1.7 times higher in *mcl-1* transfected cells ($NP_o = 22.7 \pm 3.3\%$) than control cells ($NP_o = 13.2 \pm 1.9\%$). Viabilities of control and *mcl-1* transfected cells after treatment with the cytotoxin etoposide (20 μg/ml), were 37.9 ± 3.9% and 78.2 ± 2.0%, respectively. Suppression of K^+ channel activity by 4-aminopyridine (4-AP) before etoposide treatment significantly reduced the viability of *mcl-1* transfected cells to 49.0 ± 4.6%. These results indicate that as part of the prevention of cell death, *mcl-1* causes a hyperpolarization of membrane potential through activation of K^+ channel activity.

Key words: Apoptosis — 4-Aminopyridine — High K^+ concentration — Gene expression — Membrane potential

Introduction

Cell death is a normal physiological process in cell development and tissue homeostasis, where a balance is maintained between cell proliferation, cell differentiation, and cell death. Alterations in this balance are implicated in various human diseases including cancer, viral infections, autoimmune diseases, neurodegenerative disorders and AIDS (acquired immunodeficiency syndrome). Recent studies suggest that domains in *Bcl-x_L* and probably other members of the family of viability-regulation proteins *Bcl-2* are similar to pore-forming domains of bacterial toxins, which can form ion channels in intracellular membranes (Muchmore et al., 1996; Schendel et al., 1997). Analysis of the three-dimensional structure of *Bcl-x_L* revealed the existence of a pore-forming domain composed of two central hydrophobic α-helices surrounded by five amphipathic helices, as well as a sixty-residue flexible loop. This tertiary structure has homology with the membrane translocation domain of colicin and diphtheria toxins (Muchmore et al., 1996), toxins that are thought to dimerize and form a membrane pore. In an analogous fashion, *Bcl-x_L* forms pores in artificial membranes (Minn et al., 1997), and *Bcl-2* forms cation-selective channels in lipid membranes (Schendel et al., 1997). A pore-forming mechanism is involved in the homeostasis of cellular organelles, particularly in the mitochondria, and may protect cells against abnormal electrochemical changes (Gajewski & Thompson, 1996).

Mcl-1, is a viability-promoting member of the *Bcl-2* family that is expressed in immature cell as they initiate differentiation. When *mcl-1* who transfected into immature myeloid cell, it was found to have a function similar to that of *Bcl-2* in that it promoted cell survival in response to a variety of apoptotic stimuli including the antitumor drug etoposide, calcium ionphore, UV irradiation, and the withdrawal of essential growth factors (Zhou et al., 1997). *Mcl-1* also prolonged the viability of Chinese hamster ovary (CHO) cells undergoing apoptosis in response to *c-myc* overexpression (Reynolds et al.,

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1994). *Mcl-1* binds to *Bax* and inhibits *Bax*-induced death of yeast cells in both the yeast two-hybrid system and cell-free systems (Sato et al., 1994). *Mcl-1* also interacts with *Bax* in mouse cell, and this may contribute to the prolong cell viability under apoptosis-inducing conditions (Zhou et al., 1997). The mechanism through which *mcl-1* promotes cell survival, however, is still not clearly understood.

Voltage-gated K^+ channels play an important role in cell proliferation, cell differentiation, and programmed cell death (Lu et al., 1993; Xu, Wilson & Lu, 1996; Wang et al., 1997). A variety of agents that cause cell death by apoptosis are known to inhibit K^+ channel activity. Thus, reactive oxygen species (ROS) inhibit Kv channel activity (Bright, Kar & Kar, 1994; Duprat et al., 1995), and can cause cell death that is inhibited by *Bcl-2* (Hockenbery et al., 1993; Kane et al., 1993). Another apoptosis-inducing agent, TNF-α inhibits the expression of both the inward rectifying and outward K^+ channels, decreases K^+ current amplitude in oligodendrocytes, and causes membrane depolarization. This precedes and contributes to eventual cell damage (Soliven, Szuchet & Nelson, 1991), where *Bcl-2* can also prevent TNF-induced death (Pitti et al., 1996; Thompson, 1995; Szabo et al., 1996). Similarly, activation of Fas leads to inhibition of the *n*-type K channel (K1.3) in Jurkat T lymphocytes (Pitti et al., 1996).

Since a variety of apoptosis-induced agents inhibit K^+ channel activity generally as an early effect and since the effects of such agents are inhibited by *Bcl-2* family members, we wondered whether members of this family might have effects on K^+ channel activity in intact cells as they do in artificial membrane systems. Although we could not measure K^+ channel activity in the mitochondrial membrane where *Bcl-2* family members localized in normal cell, we have previously found that transfection with these gene products lead to widespread expression associated with cell membranes throughout the transfected cell. It therefore seemed possible that membrane K^+ channels might be affected in *mcl-1* transfected cells, through either direct or indirect effects.

Materials and Methods

Cell Culture

FDC-P1 murine myeloid progenitor cells were maintained at 37°C in a 5% CO_2-humidified incubator in RPMI 1640 medium (Gibco, BRL) supplemented with 25 mM HEPES buffer, 0.0004% (v/v) β-mercaptoethanol, 10% fetal bovine serum (FBS, Gibco, BRL), and 10% conditioned medium from WEHI-3b cells as a source of interleukin-3 (IL-3). To study whether *mcl-1* also forms K^+ channels, murine myeloblastic FDC-P1 cells were transfected with vectors that use a promoter of the mouse mammary tumor virus long terminal repeat (MAM-LTR) and drive inducible expression of *mcl-1*. The advantage of the inducible system was that expression of *mcl-1* could be compared in the same transfectant clone; thus, survival differences arising from clonal variation were eliminated. Clonal transfectant lines derived from FDC-P1 cells, 8.5MAM*neo* and 7.5MAM*mcl-1*, were maintained in transfectant growth medium composed of phenol red-free RPMI 1640 medium supplemented with 25 mM HEPES, 0.0004% (v/v) β-mercaptoethanol, 10% FBS, 10% conditioned medium from WEHI-3b cells as a source of IL-3, 50 U/ml penicillin, 50 μg/ml streptomycin, and 0.4 mg/ml geneticin (G-418) (Gibco, BRL).

Plasmid Construction

There were two kinds of constructions: pMAM*mcl-1* and pMAM*neo plasmids*. The pMAM*mcl-1 plasmid* was constructed by inserting the cDNA clone containing the entire *mcl-1* coding region into the pMAM-*neo* vector between *Sal* I and *Xba* I restriction enzyme cleaving sites. In this context, expression of *mcl-1* is under the control of the MAMTV-LTR promoter. pMAM*neo* plasmid, lacking the *mcl-1* insert, was used as the control.

Electroporation and Cloning of Transfectants

One day prior to electroporation, FDC-P1 cells were diluted to 2×10^5 cells/ml with standard medium. The following day cells were washed twice with, and resuspended in, ice-cold PBS at 1×10^7 cells/ml. The target DNA (10 μg) was then added to 0.5 ml of cell suspension and incubated on ice for 10 min. Electroporation was performed on a Genepulser using settings of 250 V and 500 μF. Electroporated cells (5×10^5 cells/ml) were incubated on ice for 10 min and then incubated at 37°C in enriched medium consisting of RPMI 1640 supplemented with 20% FBS, 20% WEHI-3b conditioned medium, penicillin/streptomycin, and β-mercaptoethanol as described above. After 2 days, G-418 was added to a final concentration of 0.4 mg/ml.

Patch-Clamp Studies

Both cell-attached and inside-out patch clamp configurations were used in the present study. Pipettes were manufactured with a two-stage puller (PP-83, Narishige) with a resistance of 3–4 MΩ when filled with 150 mM KCl solution. The solutions used in these experiments were: (1) KCl bath solution containing (in mM): 140 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 1 ethylene glycol-bis (β-aminoethyl ether)-N,N,N′, N′-tetraacetic acid (EGTA) and 10 HEPES, pH 7.4; and (2) pipette solution containing 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, pH 7.4. Single-channel currents were recorded by an Axonpatch 200A amplifer (Axon Instruments, Foster City, CA) and filtered with a 4-pole low-pass filter at 1 kHz and digitalized at 22 kHz by a pulse-code modulator (A.R. Vetter, Rebersburg, PA). A pCLAMP program (Axon Instruments) was used to analyze the single-channel data. Channel activity was determined as NP_o, where N represents the number of channels in the patch and P_o represents the probability of an open channel. All experiments were performed at room temperature (RT) (21–23°C).

A nystatin-perforated-patch technique was used to measure the membrane potential. Nystatin, a polyene antibiotic, partitions into cholesterol-containing lipids and forms pores in the cell membrane. These pores allow monovalent cations and anions to permeate the cell membrane but exclude multivalent ions and nonelectrolytes equal in size to, or larger than, glucose. Thus, the nystatin-perforated-patch technique provides stable measurement without disruption of the cytoplasmic concentrations of divalent cations or metabolites (Korn & Horn, 1989; Rae et al., 1991). The nystatin stock solution, 5 mg nystatin (Sigma, St. Louis, MO) in 0.1 ml dimethyl sulfoxide (DMSO), was freshly pre-

pared every week and stored at −20°C. Immediately before the pipette was filled, the stock solution was thawed by sonication. Four μl of the stock solution were diluted into 1.0 ml of pipette solution and dispersed by sonication for 30 sec. The pipette solution was stable for 2 hr. The tip of each pipette was filled with nystatin-free solution, then back-filled with the nystatin-containing solution. The pipette solution for the whole-cell patch clamp contained (in mM) 140 KCl, 2 $MgCl_2$, 0.5 $CaCl_2$, 2 Na-ATP, 1 EGTA and 10 HEPES (pH 7.2). The bath solution contained (in mM) 140 NaCl, 2 KCl, 1 $CaCl_2$, 10 HEPES (pH 7.4). The whole-cell configuration was obtained within 5 to 15 min after the formation of a gigaohm (GΩ) seal with access pipette resistances between 2 and 6 MΩ.

Suppression of K^+ Channels and Etoposide or UV Irradiation Induction

8.5 MAM*neo* and 7.5MAM*mcl-1* transfected cells, at a concentration of 3×10^5 cells/ml, were incubated in complete culture medium containing 0.1 mM dexamethasone for at least 2 hr to induce the expression of the *mcl-1* gene. Then, the K^+ channel blocker 4-AP was added to the culture medium to a final concentration of 2 mM. UV irradiation or etoposide (apoptosis inducer) was then applied to cells. For exposure to UV irradiation, cells were placed in a sterilgard tissue culture hood at a distance of 60 inches from the UV-c light and exposed to an intensity of 40 $\mu W/cm^2$ for 3 to 8 min (60 to 72 J/m^2). For exposure to etoposide, a stock solution of 10 mg/ml etoposide in DMSO was added to the culture medium at a final concentration of 20 μg/ml. Following etoposide and UV treatments, cells were incubated at 37°C in 5% CO_2 for 15 to 24 hr. Cell viability measurements were made using the trypan blue dye exclusion assay.

Statistical Analysis

Data were presented as original values, or as means ± SE. Significant differences were determined by using the paired *t*-test and ANOVA, plus Tukey's range test at the confidence interval indicated.

Results

Single-channel currents were measured in symmetric 140/140 mM KCl solutions using the cell-attached configuration in 7.5MAM*mcl-1* cells, both in the absence and presence of *Mcl-1* induced by addition of dexmethasone. The current-voltage relationship (*I-V* curve) was linear for both groups, and the single-channel conductances were 33.4 ± 2.6 pS and 34. ± 0.8 pS (measured from the slope of the *I-V* curves) in dexamethasone-induced (dex-induced) and uninduced 7.5MAM*mcl-1* cells, respectively. The difference in slope of these *I-V* curves was not significant ($P > 0.5$, $n = 5$) (Fig. 1*A*). The sensitivity of K^+ channels to the K^+ channel blocker 4-aminopyridine (4-AP) was tested in dex-induced 7.5MAM*mcl-1* cells. K^+ channels in these cells were sensitive to 4-AP in a dose-dependent manner (Fig. 1*B*). These results indicate that *mcl-1* did not itself form a K^+-selective channel in the plasma membrane of dex-induced 7.5MAM*mcl-1* cells.

K^+ channel activity (NP_o) was also compared in dex-induced or uninduced 7.5MAM*mcl-1* and 8.5MAM*neo* cells using the cell-attached patch clamp technique (Fig. 2*A* and *B*). Activity of the single channel opening was significantly increased in 7.5MAM*mcl-1* cells induced to express mcl-1 compared to either uninduced 7.5MAM*mcl-1* cells or vector-transfected 8.5MAM*neo* cells. The channel activities was 22.6 ± 3.4% ($n = 4$) in *mcl-1* expressed 7.5MAM*mcl-1* cells, compared to NP_o 13.2 ± 2.0% ($n = 5$) in vector-transfected 8.5MAM*neo* cells and NP_o 9.2 ± 1.2% ($n = 5$) in uninduced 7.5MAM*mcl-1* cells (Fig. 2*C*). These results demonstrate that K^+ channel activity is increased approximately 2-fold when expression of the introduced *mcl-1* is induced in those cells.

By using the perforated whole-cell patch-clamp in a bath solution containing 2 mM K^+, the membrane potential (V_m) was found to be hyperpolarized about −30 mV in *mcl-1* expressing 7.5MAM*mcl-1* cells compared to control cells (to −96 from −68 mV) (Fig. 3*A*). To confirm that V_m changes in *mcl-1* expressed cells were caused by alteration of K^+ channel activity, the V_m in *mcl-1* expressed 7.5MAM*mcl-1* cells was measured at different concentrations of extracellular K^+. The membrane potential was repolarized as extracellular K^+ concentration was increased from 2 to 60 mM, with a slope of 56 mV per 10-fold change of extracellular K^+ concentration, as expected for K^+-dependent conductance (Fig. 3*B*). The fact that changes in V_m correlated with changes in extracellular K^+ concentration indicate that the hyperpolarized membrane potential in *mcl-1* expressing cells is due to K^+ ion movement.

The effect of suppression of K^+ channel activity by 4-AP on V_m in *mcl-1* expressing 7.5MAM*mcl-1* cells was also monitored. After the addition of 4-AP to the bath solution (2 mM), the membrane potential decreased significantly from −96.0 ± 1.0 to −66.3 ± 0.88 mV ($P < 0.001$, $n = 3$) (Fig. 3*C*). Inhibition by 4-AP of the hyperpolarization of V_m in *mcl-1* expressing cells further confirms the role of the K^+ channel in *mcl-1* associated hyperpolarization.

To confirm that *mcl-1* has the ability to inhibit apoptosis, 20 μg/ml etoposide was added to the culture for 15 hr to induce apoptosis. Viabilities of vector-transfected cells and *mcl-1*-expressed cells were 37.9 ± 3.9% and 78.2 ± 2.0%, respectively. Similar results were observed after these cells were exposed to UV-c light for 8 min (Fig. 4*A*). The channel blocker 4-AP (2 mM) was applied to cultured cells to suppress K^+ channel activity and to examine cell viabilities in both vector-transfected 8.5MAM*neo* and *mcl-1* expressed 7.5MAM*mcl-1* cells. Suppression of K^+ channel activity by 4-AP reduced the viability of *mcl-1* expressing cells in the presence of etoposide from 78.2% to 49.0%, which was nearly as low as the viability in vector transfected 8.5-MAM *neo* controls. Suppression of K^+ channel activity by 4-AP did

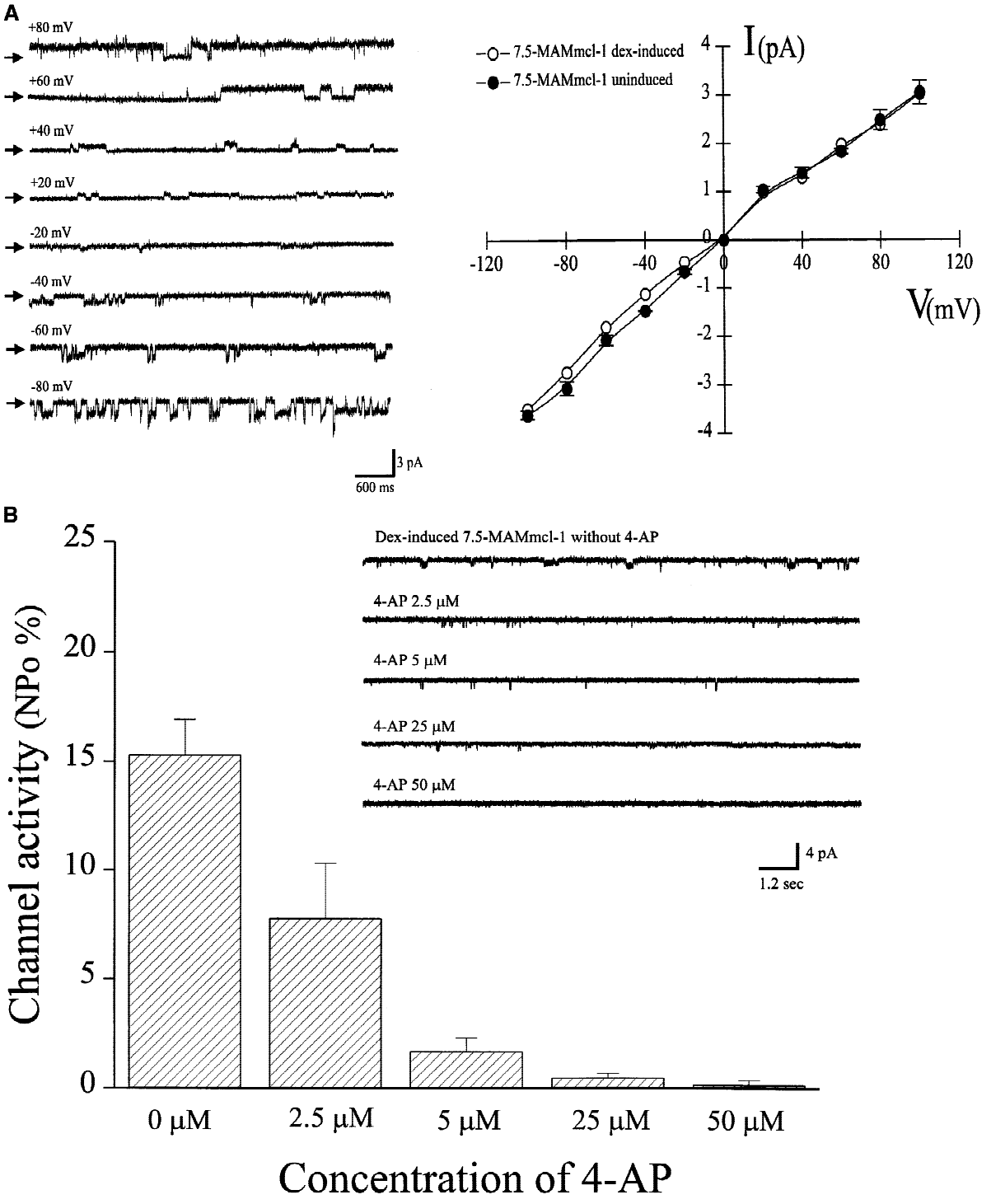

Fig. 1. Single K^+ channel current recorded from 7.5MAM*mcl-1* cells before and after dexamethasone induction (dex-induced). (*A*) Single-channel current was recorded at varied membrane potentials using the cell-attached patch clamp technique. The patch pipette contained 140 mM KCl solution. Arrows indicate the closed state of the channel. Current-voltage relationship (*I-V*) of the K^+ channel. The slopes of the *I-V* curves were 34.1 ± 0.8 pS and 33.4 ± 2.6 pS for uninduced and dex-induced 7.5MAM*mcl-1* cells, respectively. Data are plotted as mean K^+ channel activity ± SE. (*B*) Blockade of K^+ channel activity (NP_o) by 4-AP in dex-induced 7.5MAMmcl-1 cells. In cell-attached patches, 2.5, 5, 25, or 50 μM of 4-AP were added in the extracellular solution at a membrane potential of −60 mV. Top panel shows traces at different concentrations of 4-AP. Columns represent mean values of K^+ channel activity ± SE; as asterisk indicates a significant difference ($P < 0.05$, $n = 4$). Significance among different groups was examined by using one-way ANOVA and Tukey's test.

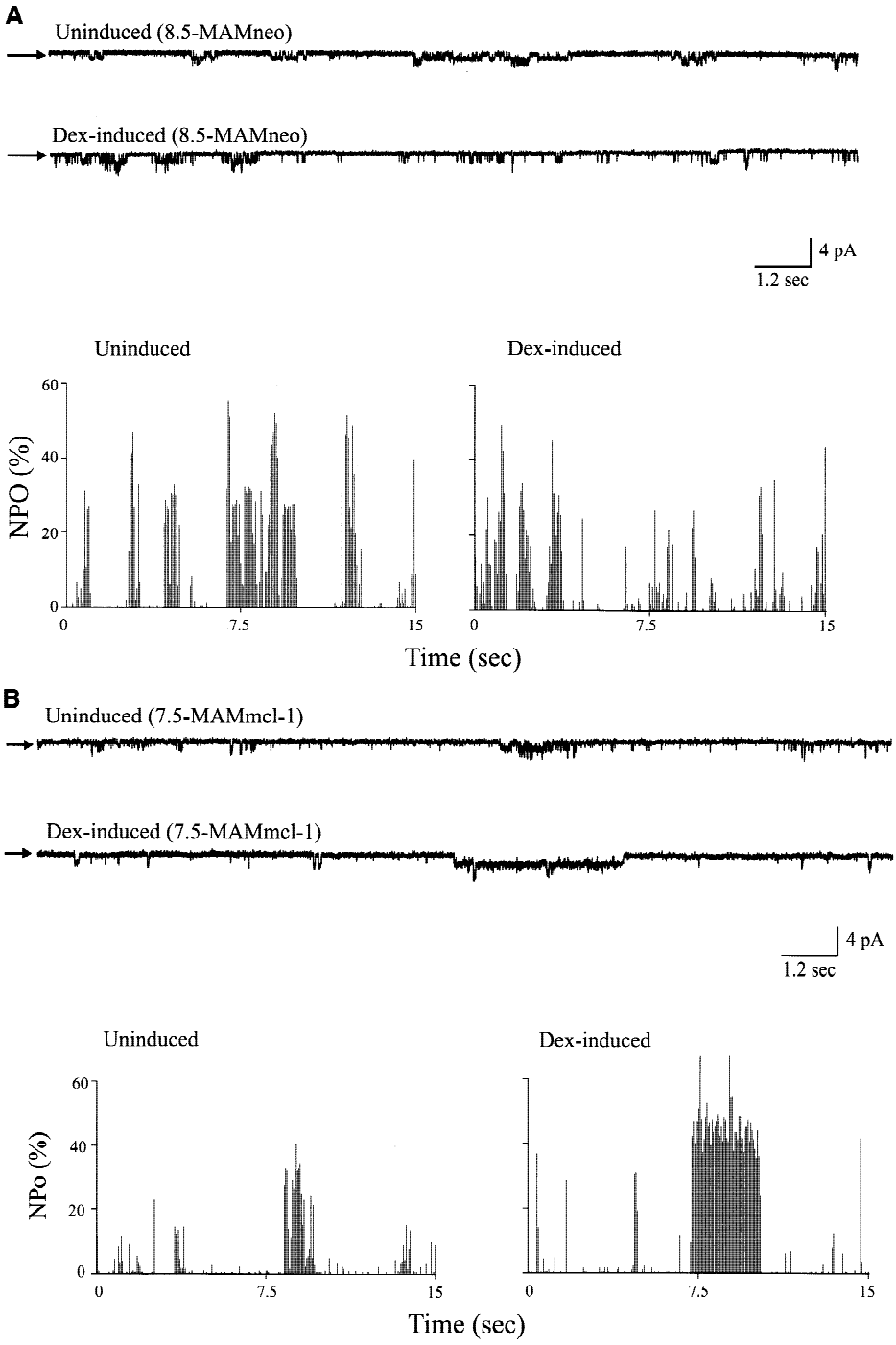

Fig. 2. Increased of K^+ channel activity in cells induced to expressing *mcl-1*. (*A*) K^+ channel activity in dex-induced and uninduced 8.5MAM*neo* cells (vector transfected). (*B*) K^+ channel activity in dex-induced and uninduced 7.5MAM*mcl-1* cells. Single current conductances were measured using the cell-attached patch clamp technique. Cell-attached patch clamp experiments were performed in symmetrical 140 mM K^+ solutions at a membrane potential of −60 mV. Histograms represent K^+ channel activity in the experimental paradigms shown in respective traces in the top panels. (*C*) Columns represent mean values of K^+ channel activity ± SE for both 8.5MAM*neo* and 7.5MAM*mcl-1* cells. An asterisk indicates a significant difference ($P < 0.01$). Data were collected from four to five independent experiments.

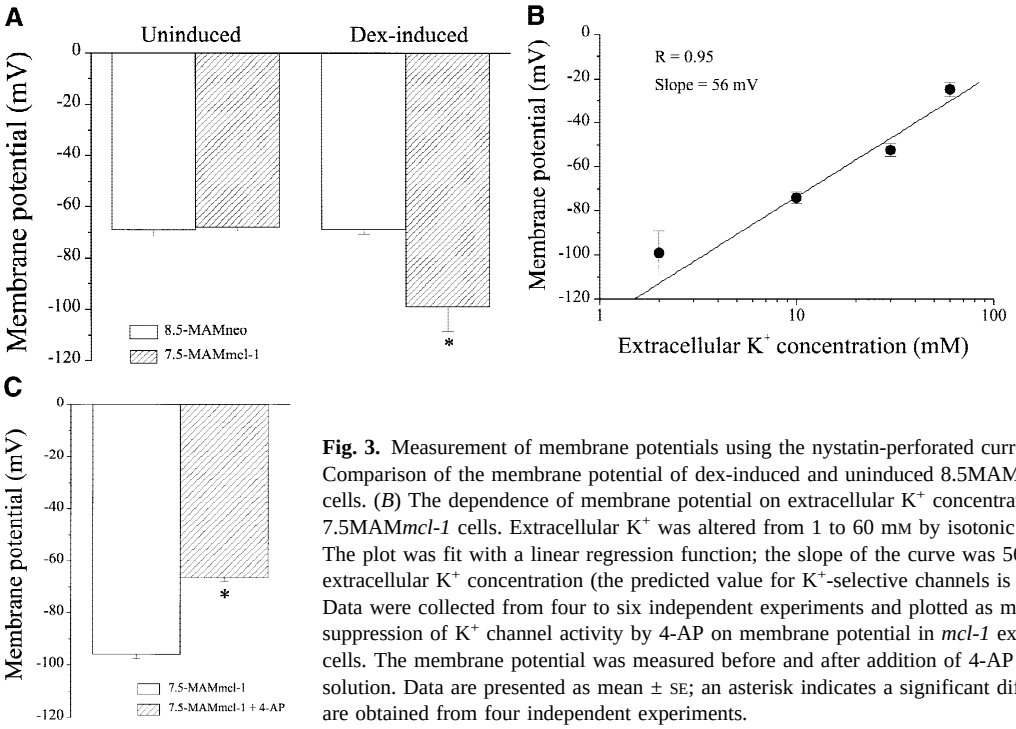

Fig. 3. Measurement of membrane potentials using the nystatin-perforated current patch clamp. (*A*) Comparison of the membrane potential of dex-induced and uninduced 8.5MAM*neo* and 7.5MAM*mcl-1* cells. (*B*) The dependence of membrane potential on extracellular K^+ concentration in dex-induced 7.5MAM*mcl-1* cells. Extracellular K^+ was altered from 1 to 60 mM by isotonic replacement of Na^+ ions. The plot was fit with a linear regression function; the slope of the curve was 56 mV/10-fold change in extracellular K^+ concentration (the predicted value for K^+-selective channels is 58 mV/10-fold change). Data were collected from four to six independent experiments and plotted as mean ± SE. (*C*) Effect of suppression of K^+ channel activity by 4-AP on membrane potential in *mcl-1* expressing 7.5MAM*mcl-1* cells. The membrane potential was measured before and after addition of 4-AP (2 mM) to the bath solution. Data are presented as mean ± SE; an asterisk indicates a significant difference ($P < 0.01$). Data are obtained from four independent experiments.

not by itself reduce cell viability, nor did it further decrease the viability of etoposide treated 8.5 MAM*neo* cells (Fig. 4*B*).

Because the preliminary data showed that the hyperpolarized V_m of *mcl-1*-expressed cells was altered following changes in extracellular K^+ concentration, the effect of high $[K^+]_{out}$ on prevention of cell death was examined (Fig. 4*C*). An increase of extracellular K^+ to 60 mM significantly reduced the viability of *mcl-1* expressing cells from 72.4 ± 1.6% to 52.0 ± 2.6% ($P < 0.05$, $n = 8$) after the induction of apoptosis by etoposide. Viability of control cells was not affected by high K^+ treatment. These results reveal that suppression of K^+ channel activity and inhibition of V_m hyperpolarization can abolish the enhancement effect of *mcl-1* mediated cell survival in programmed cell death.

Discussion

K^+ channels maintain a stable membrane potential for cell survival. The findings reported here demonstrate that the membrane potential of *mcl-1* expressing 7.5MAM*mcl-1* cells is hyperpolarized due to increased activity of a K^+ channel that is sensitive to 4-AP. *Mcl-1* did not appear to itself create this K^+ channel, but latter caused an increase in the opening probability of the endogenous K^+ channel. This hyperpolarization appeared important for the effect of *mcl-1*, as prevention of the hyperpolarization (with 4-AP or increased K^+ concentration) reversed the protection from viability associated with this gene product. These results are consistent with reports from other groups indicating that *Bcl-2* can hyperpolarize the resting membrane potential in *bcl-2*-transfected PW (human B cell lymphoma) and HL60 cells (Gilbert et al., 1996). Similar to our results, this hyperpolarization was associated with increased radioresistance, and depolarization by increasing extracellular K^+ concentrations from 40 to 140 mM significantly decreased the radioresistance of the *Bcl-2*-transfected cells (Gilbert et al., 1996).

It is widely accepted that K^+ channels play important roles in maintaining membrane potential. The striking similarity of the three-dimensional structure of *Bcl-*$_{xL}$, and *Bcl-2* homologue, to the pore-forming domains of diphtheria toxin and bacterial colicins suggests that it may function as a pore-forming protein in the intracellular membranes where it has been found (Muchmore et al., 1996). *Bcl-2* also exhibits pore-forming activity and can form discrete ion-conducting cation channels in lipid membranes (Schendel et al., 1997). It is unknown whether *mcl-1* forms a channel pore in the membrane. In the present study, we use the single channel patch clamp to measure the single channel conductance and pharmacological properties of the K^+ channel in transfectants constitutively expressing *mcl-1* (7.5MAM*mcl-1*) and vector-only controls (8.5MAM*neo*). Our results reveal that the properties of two K^+ channels are identical, indicating that *mcl-1* did not form a exogenous cation channel in the membrane of transfectant cells. In addi-

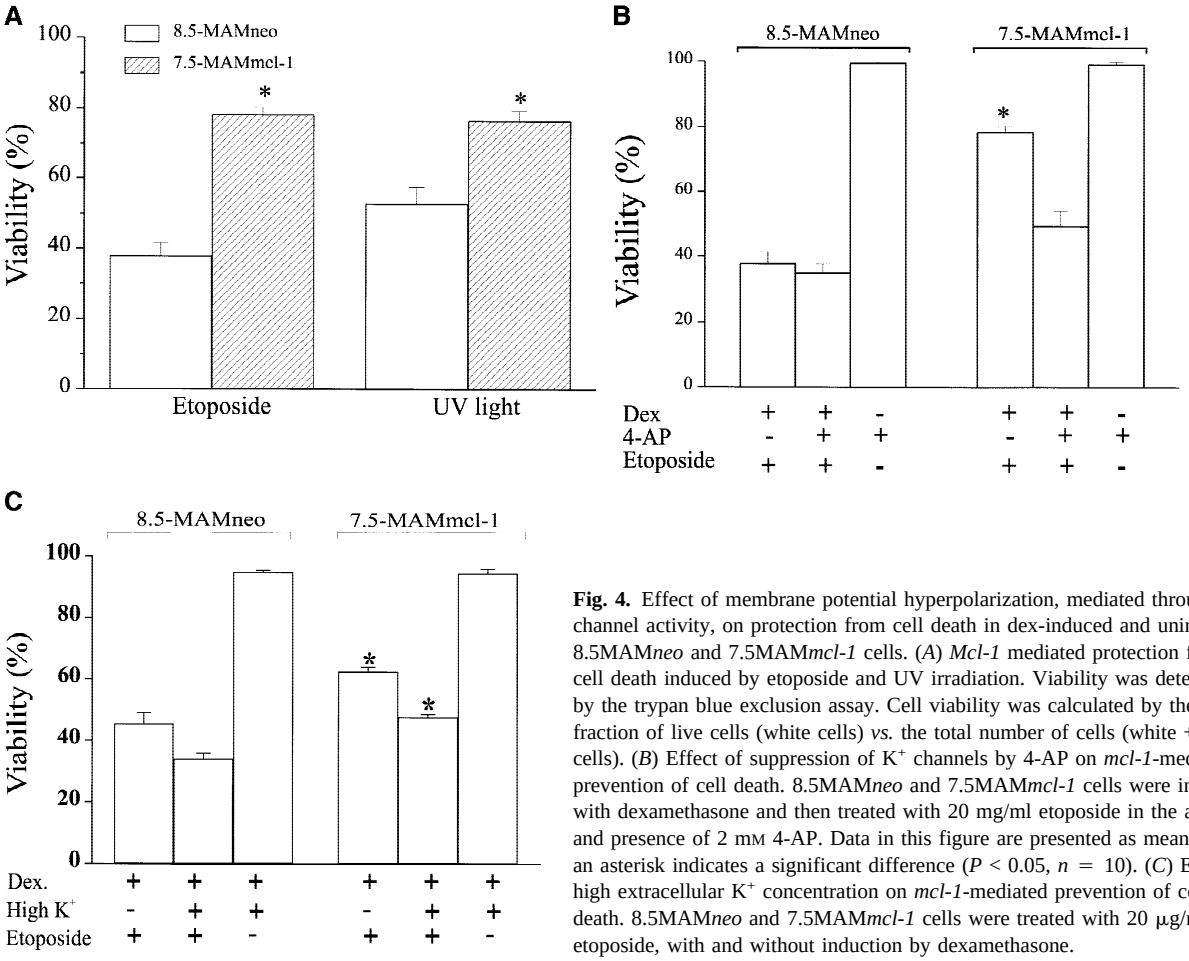

Fig. 4. Effect of membrane potential hyperpolarization, mediated through K^+ channel activity, on protection from cell death in dex-induced and uninduced 8.5MAM*neo* and 7.5MAM*mcl-1* cells. (*A*) *Mcl-1* mediated protection from cell death induced by etoposide and UV irradiation. Viability was determined by the trypan blue exclusion assay. Cell viability was calculated by the fraction of live cells (white cells) *vs.* the total number of cells (white + blue cells). (*B*) Effect of suppression of K^+ channels by 4-AP on *mcl-1*-mediated prevention of cell death. 8.5MAM*neo* and 7.5MAM*mcl-1* cells were induced with dexamethasone and then treated with 20 mg/ml etoposide in the absence and presence of 2 mM 4-AP. Data in this figure are presented as mean ± SE; an asterisk indicates a significant difference ($P < 0.05$, $n = 10$). (*C*) Effect of high extracellular K^+ concentration on *mcl-1*-mediated prevention of cell death. 8.5MAM*neo* and 7.5MAM*mcl-1* cells were treated with 20 μg/ml etoposide, with and without induction by dexamethasone.

tion, K^+ channel activity was markedly increased in *mcl-1* transfected cells suggesting that product proteins of *mcl-1* may play a role in regulating membrane channel activity.

As noted earlier, *mcl-1* is a member of the *Bcl-2* family, and its expression is stimulated at early stages of differentiation. Similar to *Bcl-2*, *mcl-1* prolongs the viability of CHO cells undergoing apoptosis mediated by *c-myc* overexpression (Reynolds et al., 1994) and inhibits cell apoptosis induced by exposure to cytotoxic agents (such as etoposide and calcium ionphore), UV irradiation, and the withdrawal of required growth factors (Zhou et al., 1997). Distribution of *mcl-1* in cells is very similar to *Bcl-2*, which is located in intracellular membranes, including those of mitochondria, the endoplasmic reticulum, and the nucleus (Chen et al., 1989; Hockenbery et al., 1990; Alnemri et al., 1992; Yang et al., 1996). These studies indicate that, for the prevention of apoptosis, *Bcl-2* must attach to the membrane. However, recent results indicate that free forms of *Bcl-2* also inhibit cell death; a truncated *Bcl-2* mutant lacking the C-terminal anchor has been shown to inhibit apoptosis in both neurons deprived of NGF and fibroblasts treated with TNF-α (Borner et al., 1994; Oltvai, Milliman & Korsmeyer, 1993). A similar result was found in $Bcl\text{-}_{xL}$-transfected cells (Muchmore et al., 1996). Conceivably, anti-apoptosis gene products of the *Bcl-2* family may directly or indirectly interact with proteins bound to cell membranes, including proteins that are structural components of ion channels.

The mechanism of action of the *Bcl-2* family members is still largely unknown, mechanisms being considered include effect on mitochondrial membrane, interactions with pro-apoptotic member of this family such as Bax and Bad (Muchmore et al., 1996; Yang et al., 1996; Schendel et al., 1997), and interactions with other proteins in the apoptotic pathway such as Apf1, which can bind to cytochrome c when not bound to *Bcl-2*, activating caspass responsible for apoptosis (Liu et al., 1996; Liu et al., 1997). Our results demonstrating the increase in K^+ channel activity and the hyperpolarization of the membrane potential in the presence of *mcl-1* suggest that *mcl-1* indirect effects on K^+ channel activity contribute to its prevention of cell death. We conclude that voltage-gated K^+ channels may play a role in the prevention of cell death mediated by expression of the *mcl-1* gene.

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References

Alnemri, E.S., Fernandes, T.F., Haldar, S., Croce, C.M., Litwack, G. 1992. Involvement of Bcl-2 in glucocorticoid-induced apoptosis of human pre-B-leukemias. *Cancer Res.* **52:**491–495

Borner, C., Martinou, I., Mattmann, C., Irmler, M., Schaerer, E., Martinou, J.C., Tschopp, J. 1994. The protein bcl-2 alpha does not require membrane attachment, but two conserved domains to suppress apoptosis. *J. Cell. Biol.* **126:**1059–1068

Bright, J., Khar, J., Khar, A. 1994. Apoptosis: programmed cell death in health and disease. *Biosci. Reports* **14:**67–81

Chen, P.L., Scully, P., Shew, J.Y., Wang, J.Y., Lee, W.H. 1989. Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. *Cell* **58:**1193–1198

Duprat, F., Guillemare, E., Romey, G., Fink, M., Lesage, F., Lazdunski, M., Honore, E. 1995. Susceptibility of cloned K^+ channels to reactive oxygen species. *Proc. Natl. Acad. Sci. USA* **92:**11796–11800

Gajewski, T.F., Thompson, C.B. 1996. Apoptosis meets signal transduction: elimination of a BAD influence. *Cell* **87:**589–592

Gilbert, M.S., Saad, A.H., Rupnow, B.A., Knox, S.J. 1996. Association of Bcl-2 with membrane hyperpolarization and radioresistance. *J. Cell. Physiol.* **168:**114–122

Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R.D., Korsmeyer, S.J. 1990. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* **348:**334–336

Hockenbery, D.M., Oltvai, Z.N., Yin, X.M., Milliman, C.L., Korsmeyer, S.J. 1993. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75:**241–251

Kane, D.J., Sarafian, T.A, Anton, R., Hahn, H., Gralla, E.B., Valentine, J.S., Ord, T., Bredesen, D.E. 1993. Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* **262:**1274–1277

Korn, S.J., Horn, R. 1989. Influence of sodium-calcium exchange on calcium current rundown and the duration of calcium-dependent chloride currents in pituitary cells, studied with whole cell and perforated patch recording. *J. General Physiol.* **94:**789–812

Liu, X., Kim, C.N., Yang, J., Jemmerson, R., Wang, X. 1996. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* **86:**147–157

Liu, X., Zou, H., Slaughter, C., Wang, X. 1997. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* **89:**175–184

Lu, L., Yang, T., Markakis, D., Guggino, W.B., Craig, R.W. 1993. Alterations in a voltage-gated K^+ current during the differentiation of ML-1 human myeloblastic leukemia cells. *J. Membrane Biol.* **132:**267–274.

Minn, A.J., Velez, P., Schendel, S.L., Liang, H., Muchmore, S.W., Fesik, S.W., Fill, M., Thompson, C.B. 1997. Bcl-x(L) forms an ion channel in synthetic lipid membranes. *Nature* **385:**353–357

Muchmore, S.W., Sattler, M., Liang, H., Meadows, R.P., Harlan, J.E., Yoon, H.S., Nettesheim, D., Chang, B.S., Thompson, C.B., Wong, S.L., Ng, S.L., Fesik, S.W. 1996. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. *Nature* **381:**335–341

Oltvai, Z.N., Milliman, C.L., Korsmeyer, S.J. 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74:**609–619

Pitti, R.M., Marsters, S.A., Ruppert, S., Donahue, C.J., Moore, A., Ashkenazi, A. 1996. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J. Biol. Chem.* **271:**12687–12690

Rae, J., Cooper, K., Gates, P., Watsky, M. 1991. Low access resistance perforated patch recordings using amphotericin B. *J. Neurosci. Meth.* **37:**15–26

Reynolds, J.E., Yang, T., Qian, L., Jenkinson, J.D., Zhou, P., Eastman, A., Craig, R.W. 1994. Mcl-1, a member of the Bcl-2 family, delays apoptosis induced by *c-Myc* overexpression in Chinese hamster ovary cells. *Cancer Res.* **54:**6348–6352

Sato, T., Hanada, M., Bodrug, S., Irie, S., Iwama, N., Boise, L.H., Thompson, C.B., Golemis, E., Fong, L., Wang, H.G., et al. 1994. Interactions among members of the Bcl-2 protein family analyzed with a yeast two-hydrid system [published erratum appears in *Proc. Natl. Acad. Sci. USA* 1995 Feb 28;92(5):2016]. *Proc. Natl. Acad. Sci. USA* **91:**9238–9242

Schendel, S.L., Xie, Z., Montal, M.O., Matsuyama, S., Montal, M., Reed, J.C. 1997. Channel formation by antiapoptotic protein Bcl-2. *Proc. Natl. Acad. Sci. USA* **94:**5113–5118

Soliven, B., Szuchet, S., Nelson, D.J. 1991. Tumor necrosis factor inhibits K^+ current expression in cultured oligodendrocytes. *J. Membrane Biol.* **124:**127–137

Szabo, I., Gulbins, E., Apfel, H., Zhang, X., Barth, P., Busch, A.E., Schlottmann, K., Pongs, O., Lang, F. 1996. Tyrosine phosphorylation-dependent suppression of a voltage-gated K^+ channel in T lymphocytes upon Fas stimulation. *J. Biol. Chem.* **271:**20465–20469

Thompson, C.B. 1995. Apoptosis in the pathogenesis and treatment of disease. *Science* **267:**1456–1462

Wang, L., Xu, B., White, R.E., Lu, L. 1997. Growth factor-mediated K^+ channel activity associated with human myeloblastic ML-1 cell proliferation. *Am. J. Physiol.* **273:**C1657–C1665

Xu, B., Wilson, B.A., Lu, L. 1996. Induction of human myeloblastic ML-1 cell G1 arrest by suppression of K^+ channel activity. *Am. J. Physiol.* **271:**C2037–C2044

Yang, T., Buchan, H.L., Townsend, K.J., Craig, R.W. 1996. MCL-1, a member of the BLC-2 family, is induced rapidly in response to signals for cell differentiation or death, but not to signals for cell proliferation. *J. Cell. Physiol.* **166:**523–536

Zhou, P., Qian, L., Kozopas, K.M., Craig, R.W. 1997. Mcl-1, a Bcl-2 family member, delays the death of hematopoietic cells under a variety of apoptosis-induced conditions. *Blood* **89:**630–643