An amount of human pro-apoptotic Bax as low as 0.01% of total protein was sufficient to cause cell death in *Escherichia coli*. The bacterial cell death was examined using a viable bacteria-specific fluorescence indicator system and loss of colony formation ability. Co-expression of anti-apoptotic Bcl-xL showed a modest inhibitory effect on the cell death caused by Bax. The trace amount of Bax elongated *E. coli* and accumulated monounsaturated fatty acids, suggesting an unusual metabolism of redox in the host. In fact, an increase of KCN-dependent O₂ consumption accompanied the expression of Bax. At the same time, a fluorescent pH indicator showed the apparent accumulation of protons outside the cell, suggesting that the membrane is intact. Bax increased the level of superoxide anion as measured by the expression of superoxide-dependent promoter. Nicked DNA was significantly generated, and the frequency of mutations resistant to rifampicin was increased by 30-fold, depending upon the expression of Bax. It is proposed that trace amounts of Bax increase oxygen consumption, triggering generation of superoxide, which affects DNA, leading to bacterial death.

Apoptosis in multicellular organisms is an active cellular self-destruction that is directed by genes. It is not only a physiologically important process in tissue homeostasis and developmental elimination but also a final defense against viral infection and the emergence of cancer (1–5).

Bcl-2 protects cells from apoptosis induced by a wide variety of stimuli, including radiation, growth factor deprivation, free radicals, alterations in Ca²⁺, viral infection, chemotherapeutic drugs, and anoxia (6). Its related proteins, the Bcl-2 family, regulate apoptosis by interacting one with each other (7). Members of the Bcl-2 family are functionally classified into two groups (8, 9). The first group, including Bcl-2, Bcl-xL, Mcl-1, and A1, suppresses apoptosis. In contrast, the second group, including Bax, Bak, Bad, Bik, Bcl-δB, and Bcl-xS, promotes apoptosis.

Bax is a 21-kDa membrane protein with a membrane anchor sequence at the C terminus and promotes apoptosis (10). Bax has three conserved motifs, BH1, BH2 and BH3, which are conserved among several members of the Bcl-2 family (11, 12). Overexpression of Bax countered the death-protecting activity of Bcl-2 and accelerated apoptosis of pro-B cells induced by interleukin-3 withdrawal, whose rates were affected by the ratio of Bax to Bcl-2 (10). Bax forms a heterodimer with Bcl-2 (10). Yeast two-hybrid assays showed that Bax can also bind to the apoptosis-suppressing factors, Bcl-xL, Mcl-1, and A1 (13, 14). Conversely, Bcl-2 and Bcl-xL can bind to the apoptosis-accelerating factors, Bcl-xL (13, 14), Bak (15), Bik (11, 12), and Bad (13, 14). A set of complex and selective interactions among apoptosis-suppressing and apoptosis-accelerating factors appears to dictate the fate of the cell, survival or death following an apoptotic stimulus (14).

Bax expression in the budding yeast *Saccharomyces cerevisiae* caused cell death (13, 16). Dimerization and targeting to mitochondrial membrane appear to be essential for Bax to exert cytotoxicity in both yeast and mammalian cells (17). Recently Bak as well as Bax induced cell death when expressed in the fission yeast *Schizosaccharomyces pombe* (18). Co-expression of anti-apoptotic proteins Bcl-2, Bcl-xL, or Mcl-1 abolished the cytotoxicity of Bax or Bcl in yeast cells (13, 16, 18, 19). Bak/Bax-induced cell death of yeast partially resembled Bax-induced apoptosis of mammalian cells regarding dead cell phenotype (18). Inducible expression of Bax in mammalian Jurkat T cells initiated apoptosis without an extra death stimulus (20). This process accompanied the generation of reactive oxygen species (ROS), and decrease of mitochondrial membrane potential (20). The cell-killing activity of Bax appears to function in biological systems ranging from eukaryotic unicellular organisms to mammalian cells.

We found that an *Escherichia coli* cell transformed with a prokaryote expression vector carrying mammalian bax or bak cDNAs grew poorly in solid and liquid media. To biochemically investigate the function(s) of Bax in detail, we have chosen this organism as a Bax-expressing host, because there is no report that *E. coli* has endogenous bcl-2-related genes for interaction with Bax. Here, we present that a trace expression of Bax kills *E. coli* cells and that this process includes many physiological changes with regard to monounsaturated fatty acid composition, dioxygen consumption, generation of reactive oxygen species, and nicked DNA.
Cloning of Mammalian bax and bak cDNAs and Expression of Mammalian Antiapoptotic Factors in E. coli Cells—Human bax cDNA was synthesized and amplified from poly(A)+ RNA (QuickPrep Micro mRNA purification kit, Pharmacia Biotech). The PCR product was ligated into the vector, pGEX-3X, which was digested with a combination of EcoRI/BglII. Following transformation of DH5α by DFC-DA—A membrane-permeable fluorescent dye, DCF-DA (LAMBDA, Graz, Austria) was applied to cells as follows. DH5αMCR cells harboring the vector or pHis-bax were cultured in a M9CA medium containing Ap with shaking at 37 °C. At A600 = 0.1, IPTG (30 μM) was added to induce Bax expression, and immediately an aliquot of the culture (1 ml) was removed for measuring O2 consumption.

Oxygen Consumption—DH5αMCR cells harboring the vector or pHis-bax were inoculated in L-broth containing Ap at A600 of 0.01 and grown with vigorous shaking at 37 °C. Aliquots of the culture were removed for measuring A600 and oxygen (O2) consumption at various times. O2 consumption was determined by an oxygen electrode method using MD-1000 (ijima Electronics Mfg. Co. Ltd., Tokyo) with a water jacket at 37 °C. In each experiment, the recorder was adjusted using a sodium sulfite solution and air-saturated water (Milli-Q, Millipore) at 37 °C. The decreasing rate of oxygen in the medium was normalized against bacterial number at each measuring point. A normalized value was represented as relative O2 consumption.

Detection of Nicked DNA—DH5αMCR cells carrying pHis-bax or the vector were cultured in L-broth containing Ap at A600 of 0.01 and grown with vigorous shaking at 37 °C. Denatured single-stranded DNA was electrophoresed on 1% agarose gel and the gel was stained with ethidium bromide. After autoradiography, the 35S-labeled nicked DNA band was excised from the gel, and the DNA was eluted from the gel and precipitated with ethanol.

Detection of Superoxide by a sod-bacZ Fusion Gene—SOD mutant C7754bacA-bacZ sodB-bac cells containing pHis-bax were anaerobically cultured at 37 °C in the container used for O2 consumption experiments, while monitoring O2 levels. The cells were inoculated into L-broth supplemented with 50 μM glucose, 20 μM KNO3, and ampicillin at 0.004 of A600. The cell suspension was then blown with nitrogen gas to remove O2. Following a lag time of around 1 h, the cells grew with a doubling time of around 20 min until 0.08 of A600, during which O2 levels were undetectable. IPTG (10 μM) was added, and after 30 min, the cells (1 ml) were cooled, harvested, and washed. The cells were resuspended in 0.1 n potassium phosphate buffer, pH 7.8, containing 1 mM diithiothreitol. After addition of lysozyme, the cells were disrupted by freezing and thawing. Following DNase I treatment, the cell extracts (50 μl) were prepared for centrifugation to precipitate debris. Two milliliters of 10-fold diluted cell extracts were subjected to Galacto-Light chemiluminescent assay for β-galactosidase (Tropix, Bedford, MA) using a luminometer, Lumat LB9507 (Berthold). Under aerobic conditions, the cells with pHis-bax were grown in L-broth containing 10 μg/ml glucose and ampicillin with vigorous shaking. IPTG (1 μM) was added at 0.2 of A600 followed by the same procedure mentioned above.

Detection of Nicked DNA—DH5αMCR cells carrying pHis-bax or the vector were cultured in L-broth at 37 °C. The cells were incubated for indicated periods at the stationary phase. Harvested cells were lysed with 1 n NaOH. During incubation at room temperature, DNA was denatured, and DNA strands with nicks were separated from intact DNA by ultrafiltration (100,000 × g, 1 h). Denatured single strands of DNA were precipitated by ethanol and dissolved in TE buffer. After RNase A treatment, DNA samples were precipitated with isopropanol alcohol and dissolved in TE buffer (10 mM Tris·Cl, pH 7.4, 1 mM EDTA, pH 8.0 (1 μl per 0.1 A600 unit). One microliter was subjected to alkaline-agarose gel electrophoresis followed by blotting to a nylon membrane Hybrid-N+ (Amersham Pharmacia Biotech). A 32P-labeled probe was prepared by an oligo-random labeling kit (Amersham Pharmacia Biotech). Hybridization was performed in a Rapid-hyb buffer (Amersham Pharmacia Biotech) at 65 °C. The membrane was washed twice with 0.1 × SSC containing 0.1% SDS at 65 °C and exposed to an imaging plate for analysis by a bioimaging analyzer BAS1500 (Fuji Film, Ltd., Tokyo). The image was visualized by Pictrography (Fuji Film, Ltd.).

Frequency of Rifampicin-resistant Mutants—DH5αMCR cells were barking the vector or pHis-bax was cultured in L-broth. The cells were applied on isopore track-etched membrane filters (Millipore) at A600 = 0.3 (1.4 × 107 cells/ml). After washing with phosphate-buffered saline, the filters with cells were immersed in 2.5% glutaraldehyde. The cells were dried by a critical point drying method after dehydration with ethanol. The cells were dried by a critical point drying method after dehydration with ethanol.
**RESULTS**

A Trace Amount of Bax Causes Cell Death in E. coli—We constructed inducible expression plasmids to overexpress the mammalian apoptosis regulatory factors, Bax, Bak, and Bcl-xL in *E. coli*. During the studies, the cDNAs of *bax* and *bak* were found to make smaller colonies than the vectors alone, even in the absence of an inducer, IPTG in *E. coli* (Fig. 1A). On the other hand, the introduction of *bcl-xL* cDNA, the anti-apoptotic factor, resulted in the same sized colonies as transformants of vectors alone. Regardless of the expression vector, pHis (pProEX-1; see “Materials and Methods”) or pGST (pGEX-3X; see “Materials and Methods”), the same results were obtained. These results indicate that small colonies are formed by the expression of the mammalian pro-apoptotic factors. Since the vector harbors the *lacI* gene, the expression should be limited in the absence of the inducer. It was difficult to maintain the transformants forming the small colony, because suppressor mutant cells easily appeared. Thus, in each experiment, the transformants were freshly obtained by introduction of each plasmid into competent cells.
It was confirmed by two methods that the small colony size was due to cell death. The first method involved colony formation ability, the second, a viable bacteria specific fluorescent indicator system as an indicator of dead or living cells. An *E. coli* cell DH5αMCR was transformed with the vector or pHis-bax. Cells from these colonies were mixed and enumerated using a microscope. Diluted cell suspension was then plated on L-plates to determine the number of viable cells forming colonies. The total cell number per colony of the cells with pHis-bax was one-sixth of that of the cells with the empty vector, and inoculated into L-broth with Ap. The growth curve was taken with vigorous shaking at 37 °C. At each culture time, the cell number was counted under a microscope in a hemacytometer of 0.02 mm in depth. B. Western blotting of total proteins from the transformants at points marked as ①, ②, and ③ in A. The proteins (20 μg) were subjected to Western blot analysis using anti-Bax polyclonal antibody, and bands corresponding to Bax were visualized by a chemiluminescence method with a peroxidase-conjugated secondary antibody. In the right three lanes, indicated amounts of His-Bax purified by nickel-nitrilotriacetic acid resin and gel filtration were immunoblotted.

When an *E. coli* cell, DH5αMCR, harboring pHis-bax was grown in L-broth aerobically at 37 °C, cell growth was stopped at 3 × 10⁸ cells/ml, corresponding to A₆₀₀ = 0.6 (a stationary phase) (Fig. 3A). The density of the bacteria was determined by direct counting under a microscope, while DH5αMCR carrying the empty vector stopped growth at 6 × 10⁸ cells/ml. Again, when bcl-x was tandemly located downstream of bax, co-expression of Bcl-xL somewhat inhibited the Bax cytotoxicity (Fig. 2, top). Two B3H mutant Bax proteins were examined, because Bax requires BH3 to form heterodimers with Bcl-xL (25). The first mutant BaxGD67–68 has two alanine substitutions of glycine 67 and aspartic acid 68 in BH3 and still maintains the ability to interact with Bcl-xL (25). The cytotoxicity due to this Bax mutant (BaxGD67–68) was antagonized by Bcl-xG196 (Fig. 2, middle). In contrast, the second mutant BaxΔ63–71, which deletes amino acids 63 to 71 in BH3, cannot interact with Bcl-xL (25). BaxΔ63–71 was shown to be cytotoxic to the cells, regardless of the expression of Bcl-xG196 (Fig. 2, bottom). Thus, Bcl-xL modestly inhibits the cell death by antagonizing Bax in bacterial cells as well as mammalian cells.

When an *E. coli* cell, DH5αMCR, harboring pHis-bax was grown in L-broth aerobically at 37 °C, cell growth was stopped at 3 × 10⁸ cells/ml, corresponding to A₆₀₀ = 0.6 (a stationary phase) (Fig. 3A). The density of the bacteria was determined by direct counting under a microscope, while DH5αMCR carrying the empty vector stopped growth at 6 × 10⁸ cells/ml. Again, when bcl-x was tandemly located downstream of bax, co-expression of Bcl-xL somewhat inhibited the Bax cytotoxicity (Fig. 3A). A Western blot analysis revealed that the cells carrying pHis-bax expressed a trace of Bax before reaching the station-
ary phase (Fig. 3B), although these cells were not treated with IPTG to induce an expression of Bax. The amount of Bax expressed was calculated to be 0.01% of total E. coli protein by comparing the bands with those of the purified Bax protein. This content corresponds to approximately 1,000 molecules of Bax in one E. coli cell. These results showed that a trace of Bax is cytotoxic to E. coli cells, resulting in death.

The morphology of the cells with pHis-bax at 1.4 × 10^8 cells/ml, corresponding to the 3 in Fig. 3A, were observed by scanning electron microscopy. The cells expressing the trace amount of Bax were unusually elongated compared with the control cells (Fig. 4).

**Fatty Acid Composition**—To explore the mechanism of cell death, fatty acid composition was examined. After a 2.5-h incubation at the stationary phase, the cells with pHis-bax or the vector were harvested. After hexane extracts of the cells following methanolysis, fatty acid methyl esters were analyzed by gas-mass spectrometry (Fig. 5). Fragmentation patterns of each compound separated by gas chromatography were searched in the National Institute of Standard and Technology reference data bases. The cells with pHis-bax increased C16 = 1 and C18 = 1 fatty acids by 8- and 4-fold, respectively, compared with those of the cells with the empty vector. Searches in the libraries identified C16 = 1 to be palmitoleic methyl ester, but C18 = 1 methyl ester is unknown with regard to the position of the double bond. On the whole, the Bax expression increased monounsaturated fatty acid composition by 6-fold from 6 to 36%. These findings suggest a specific change of the bacterial physiology was induced by the trace amount of Bax, probably due to the unusual reduction-oxidation system.

**Increased Oxygen Consumption by Bax Expression**—As the next step, oxygen consumption was examined during cell growth. The cells with pHis-bax showed that a relative increase in O_2 consumption by 60% at the late log phase and then a rapid decrease just before entering the stationary phase (Fig. 6A). The cells with the empty vector consumed oxygen at a constant rate in the log phase, and this rate gradually decreased following entrance into the late log and stationary phases (Fig. 6B). O_2 consumption was inhibited completely by addition of 1 mM KCN (data not shown), indicating that Bax activated an electron transport system directly or indirectly.

To examine the effect by the activation of the electron transport system, a membrane-permeable fluorescent dye, DCF-DA (29, 30), was applied to the cells expressing Bax by IPTG induction. DCF-DA in cells is deacetylated and converted to DCF by esterase(s). DCF gives a strong fluorescent signal at 525 nm in a range of alkaline but not acidic pH. DH5αMCR with pHis-bax, which was incubated with IPTG for 15 min, expressed Bax at a detectable level, and the expression level of Bax increased to be maximized after a 45-min incubation with IPTG (Fig. 7A, inset). After IPTG treatment for indicated time, the cells were incubated with DCF-DA for 15 min. The relative fluorescence intensity of the cells at 525 nm became stronger depending on the expression levels of Bax (Fig. 7A). Under this condition, O_2 consumption increased by 40–50% for an hour (data not shown). The cells carrying the empty vector did not give any increase of the fluorescent signal at 525 nm after the incubation with IPTG (Fig. 7B). These results indicated that the inside of the cells became more alkaline by the expression of Bax.

A simple explanation for this is that Bax accelerated the electron transport system to pump protons out and that the membrane remained intact even on accumulation of unusual fatty acids.

**Increase of Superoxide Radicals by Bax Expression**—The increment of O_2 consumption by Bax expression led us to speculate enhanced production of superoxide. Superoxide is converted to hydrogen peroxide by SOD (superoxide dismutase) in cells as the first step in protection cells from oxygen radicals. E. coli has two different genes responsible for SOD, sodA and sodB, which encode Mn-SOD and Fe-SOD, respectively. The sodA gene is inducible by superoxide (31, 32). A sodA double mutant QC774 is completely devoid of SOD activity (21). Paraquat, a potential generator of superoxide, increases the sodA expression anaerobically when E. coli is cultured with nitrate as a terminal electron acceptor (33–35). QC774 has a mutation in the sodA gene where the endogenous promoter of the gene is fused with lacZ (21). QC774 cells harboring pHis-bax were anaerobically grown in L-broth supplied with glucose and potassium nitrate. β-Galactosidase activity was enhanced 2-fold in cells treated with IPTG for 30 min compared with cells untreated with IPTG (Fig. 8A). Under aerobic conditions, induced Bax enhanced the promoter activity to some extent (Fig. 8B), probably because the background is relatively high. These results suggested that expression of Bax caused an increase of superoxide in cells.

**Nicked DNAs and Mutants**—Generation of superoxide results in generation of other ROS. Damage of DNA was examined by two methods: by extent of degraded DNA and by the increase of the mutation frequency. The cells with pHis-bax or the empty vector were grown in L-broth. After various periods of incubation at the stationary phase, the cells were solubilized in a alkali solution. Denatured single-stranded DNAs were
subjected to alkaline-agarose gel electrophoresis and detected by Southern blot analysis (Fig. 9). The cells with pHis-bax had markedly increased amounts of nicked DNA after 1 nt h e stationary phase, while the cells with the empty vector did not. As the next criteria of damage of DNA, the frequency of mutations of the cells with pHis-bax was compared with that of the cells with the vector (Fig. 10). DH5αMCR cells carrying pHis-bax gave 63 rifampicin-resistant (Rif r) colonies per 10^{10} cells, while the cells harboring the empty vector gave 2.1 Rif r colonies per 10^{10} cells. Thus, Bax expression increased the frequency of DNA mutation by 30-fold. Taken together, trace amounts of Bax appear to kill bacteria by damaging the DNA.

DISCUSSION

We reported here that a trace amount of the human Bax protein halted the growth and then caused the death of E. coli cells accompanied by some physiological changes, including increases in monounsaturated fatty acids, O2 consumption, superoxide radicals, nicked DNA, and the frequency of mutations. Bax easily mutated the host and plasmid DNA to give colonies of normal size. Therefore, so far, it might be difficult to find these phenomena.

It was shown that the Bax protein is lethal in S. cerevisiae and S. pombe (13, 16, 18). However, the amount of Bax expressed was not described. In the case of E. coli, a trace amount (0.01% of total protein) was sufficient to kill the host. Two criteria were applied to distinguish death from growth arrest: a viable bacteria-specific fluorescence dye system and a decrease of colony formation ability (Fig. 1). These results indicated that E. coli cells carrying pHis-bax are easily killed, and the process does not involve cell lysis. This death appears to be specifically caused by the mammalian pro-apoptotic factors, because Bcl-xL did not affect E. coli growth, unlike Bax and Bak. Bcl-xL had a modest inhibitory effect on Bax in E. coli. When Bcl-xL was expressed in excess of Bax, Bcl-xL effectively abolished the Bax cytotoxicity (Fig. 2). On the other hand, Bcl-xL antagonized the cytotoxicity to the less extent, when bcl-xL was tandemly located...
zyme activities were normalized against the cell density.

IPTG was added to 10 mM to the cell to avoid association with Bcl-xL. Again, it was observed that Bax protein molecules in the cell could be recovered by ethanol precipitation and applied to an alkaline-agarose gel (1% gel). After blotting to a nylon membrane, single-stranded DNAs in the supernatants were recovered by ethanol precipitation and applied to an alkaline-agarose gel. Two microliters of 10- and 100-fold diluted cell extracts were subjected to chemiluminescent assays for β-galactosidase as described under “Materials and Methods.” The enzyme activities were normalized against the cell density.

**Fig. 8. Increased generation of superoxide radicals by Bax expression.** A, SOD mutant QC744aodZ-lacZ sodB-kan cells containing pHis-bax were anaerobically cultured at 37 °C in L-broth supplemented with 50 mM glucose, 20 mM KNO3, and ampicillin and then IPTG was added to 10 μM. B, under aerobic condition, the cells were cultured in L-broth containing 10 mM glucose and ampicillin with vigorous shaking. IPTG was added to 1 μM. In each case, the cells were harvested after 30 min and disrupted by freezing and thawing. The crude extracts of the cells treated with or without IPTG were prepared by centrifugation to precipitate debris. Two microliters of 10- and 100-fold diluted cell extracts were subjected to chemiluminescent assays for β-galactosidase as described under “Materials and Methods.” The enzyme activities were normalized against the cell density.

**Fig. 9. Increase in nicked DNAs by Bax expression.** DH5αMCR cells were transformed with pHis-bax or pHis. After an overnight culture at room temperature on L-plates, the transformants were cultured in L-broth for 0, 30, and 60 min after reaching the stationary phase. The harvested cells were solubilized in 1 N NaOH. After ultracentrifugation at 100,000 × g for 1 h, denatured and short DNAs in the supernatants were recovered by ethanol precipitation and applied to an alkaline-agarose gel (1% gel). After blotting to a nylon membrane, single-stranded DNAs were detected by α-32P-labeled probe prepared by random priming using genomic DNA of E. coli as a template. Signals were visualized by an imaging analyzer, BAS 1500, and Pictrography.

downstream of bax (Figs. 1 and 3). In the latter, the expression of Bcl-xL was nearly the same as that of Bax and the trace amount (data not shown). Most of the Bcl-xL protein molecules in the cell could avoid association with Bcl-xL. Again, it was underscored that Bcl-xL inhibited the Bax cytotoxicity through an interaction with BH3 of Bax (Fig. 2) as shown in mammalian cells (25). In addition, we have recently identified the region of Bax lethal to E. coli and found that the region is responsible for inducing apoptosis (22).

During culture in L-broth, a trace of Bax protein was detected at 2 h before cessation of growth and the amount increased gradually with vigorous shaking (Fig. 3). At the point at which cell growth stopped, the amount of Bax expressed in a single cell corresponded to no more than 0.01% total E. coli protein. It is well known that the depletion of glucose in medium elevates cAMP levels in cells to make a complex with a cAMP receptor protein. In turn, this complex can activate lac promoter. It is likely that depletion of glucose contained in yeast extract results in the expression of a trace amount of Bax protein.

Bax expression enhanced O2 consumption of E. coli cells by 60% for 45 min just before it completely stopped the growth (Fig. 6). Since KCN inhibited the O2 consumption, a respiratory chain was activated (data not shown). It is not likely that the Bax protein directly binds to oxygen to consume oxygen, because computer analysis showed that Bax does not share any motif with proteins which bind to dioxygen. It is unknown how Bax activates the respiratory chain. Enhanced generation of superoxide by Bax expression can be also explained by activation of the respiratory chain. Otherwise, a redox imbalance due to Fe2+ or NADPH depletion in cells may take place in response to Bax expression (36, 37).

Generation of superoxide results in high levels of ROS, including hydrogen peroxide and hydroxyl radical, which are known to cause damage to DNA, proteins, and membranes. Enhanced conversion of superoxide to other ROS probably accounts for the increase of nicked DNA caused by Bax expression, although we could not detect any substantially damaged products such as 8-hydroxydeoxyguanosine and peroxidized lipid and any radicals by electron spin resonance spectrometry (data not shown).

It is very interesting that in cells expressing Bax, the composition of monounsaturated fatty acids increased 6-fold (Fig. 5). Some bacteria such as E. coli and Pseudomonas synthesize monounsaturated fatty acids via an anaerobic pathway, whereas in others, such as Bacillus and Corynebacterium, and in animals, synthesis is via an aerobic pathway (38). In the aerobic pathway, desaturase(s) uses oxygen molecules in a monooxygenase-type reaction. Bax expression may activate the enzymes involved in the synthesis of monounsaturated fatty acids in the anaerobic pathway. It is well known that the higher the content of unsaturated fatty acids, the more flexible the cell membrane. The cells expressing Bax were expected to be more sensitive to osmotic pressure. Scanning electron mi-
croscopy revealed that the cells expressing Bax enlarged along both axes (Fig. 4).

Recently the x-ray structures of renatured (39) and native (27) truncated Bcl-xL protein were resolved. The three-dimen-
sional fold of Bcl-x is similar to that of the bacterial toxins diphtheria toxin and colicin A (27, 39). Like these bacterial toxins, Bcl-xL was also shown to form an ion channel in synthetic lipid membrane with selectivity for K+ and Na+ (40). These proteins appear to insert and be internalized into a membrane by a very similar multistep mechanism. Bax may insert into a membrane more easily than Bcl-xL, because Bax has a high sequence homology with Bcl-xL, and its expected hydrophobic cleft, formed by BH1, BH2 and BH3, is bigger than that of Bcl-xL (27). In addition, Bax structure lacks two hydro-
gen bonds stabilizing the central helices, suggesting that Bax possesses a greater potential for membrane insertion than either Bcl-2 or Bcl-xL (27). As expected, Bax has recently been shown to form an ion channel at neutral pH in synthetic lipid membrane, whose activity was inhibited by Bcl-2 (41). The results of DCF-DA should be interpreted with caution. One plausible explanation is as follows: Bax formed an ion channel and enhanced the transport of specific cations (not proton) or anions, which diminished the membrane potential as a result. To compensate the membrane potential, the respiratory chain may be activated to enhance the proton pumping activity, leading the inside of the cell to become alkaline. The activated respiration may lead to oxygen radical formation.

Bcl-2 inhibits the release of cytochrome c from mitochondria (42, 43) and the loss of mitochondrial membrane potential (44–46). It can be explained as that Bax associates with Bcl-2 and Bcl-xL to abolish their activities, resulting in cell death. It has been proposed that Bcl-2 functions as an inhibitor of the anti-oxidant pathway. This is also explained as that Bax generates superoxide and that Bcl-2 antagonizes Bax. In fact, Xiang et al. (20) reported that inducible expression of Bax causes apoptosis without the need for stimulus and increases ROS. Recently, we have isolated an E. coli mutant that suppresses cell death even on expression of Bax, in which the RNase E gene is split into 5′- and 3′-regions. The truncated RNase E also made cells resistant to paraquat, a generator of superoxide. Therefore, it is likely that Bax induces the oxygen radical in E. coli. We show the region lethal to bacteria is common to a region inducing apoptosis in mammalian cells (22). The findings described in this study offer a useful approach using E. coli for investigating the molecular mechanism of apoptosis.

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