We investigated the effect of expression of constitutively active Ga mutants on cell survival. Transfection of constitutively active Gaq and Gal3 in two different cell lines caused condensation of genomic DNA and nuclear fragmentation. Endonuclease cleavage of genomic DNA was followed by labeling the DNA fragments and subsequent flow cytometric analysis. The observed cellular phenotype was identical to the phenotype displayed by cells undergoing apoptosis. To distinguish between the apoptosis-inducing ability of the two Ga-subunits, the signaling pathways involved in this cellular function were investigated. Whereas Gaq-induced apoptosis via a protein kinaseC-dependent pathway, Gal3 caused programmed cell death through a pathway involving the activation of the small G-protein Rho. Both of the pathways leading to apoptosis were blocked by overexpression of bel-2. In contrast to other apoptosis-inducing systems, expression of constitutively active Gaq and Gal3 triggered apoptosis in high serum as well as in defined medium.

There are two distinct modes of death in cellular systems. In one, an insult leading to irreversible cellular injury causes a phenotype called necrosis that is manifested by the rupture of the cell membrane. In the other, under certain physiological situations, death of cells is triggered by metabolic or developmentally programmed events and may be required for the organisms survival or for differentiation (1). This programmed cell death (apoptosis) occurs when a cell dies by a mechanism initiated by proteins encoded by its own genome.

Apoptosis can be distinguished from necrosis through morphological characteristics including cell shrinkage, chromatin condensation, activation of specific proteases and endonucleases, and fragmentation of genomic DNA (2). Apoptosis has been implicated in many important biological processes including immune defense, growth control, and development (3). In addition some events leading to human disease involve apoptosis (4, 5).

The execution of apoptosis is triggered by intrinsic signal transduction events that link changes in physiological conditions to the cell death machinery. Signal transduction through seven pass membrane receptors represents a common mechanism of eukaryotic signaling and physiological control. During this process heterotrimeric G-proteins are responsible for transducing a ligand binding event at the membrane into a cellular response (6, 7). There are four different classes of Ga-subunits that contribute to heterotrimeric G-proteins mediated responses (8). In addition to tight regulation of activation of signal transduction pathways the cell also contains a carefully regulated system to shut down signaling after prolonged exposure to ligands. This desensitization is primarily achieved by phosphorylation induced inactivation of the receptor and subsequent internalization of the phosphorylated receptor (9). Recently a desensitization mechanism directly acting at the a-subunits was discovered. GAP proteins were cloned that accelerate the intrinsic GTPase activity of a-subunits thereby shutting off the signal (10).

In some situations permanently elevated levels of second messengers are observed inside the cells. It is believed that these elevated activities contribute to the execution of pathological phenotypes (11). Recent data point to the involvement of the Gα-subunit, Gαo, in the execution of apoptosis triggered by a mutated and presumably constitutively active amyloid precursor protein (APP) in Alzheimer’s disease (12).

To investigate the effect of other constitutively active G-protein subunits, we examined their ability to cause apoptosis in vivo. Constitutively active mutants of Gaq, Ga12, Ga13, and Ga2 were expressed in CHO and COS-7 cells, and the resulting cellular phenotypes were observed.

**EXPERIMENTAL PROCEDURES**

**Materials**—The dye Hoechst 33258 was obtained from Sigma. The Texas red conjugated goat antibodies to rabbit immunoglobulin G (IgG) and the polyclonal antibodies to β-galactosidase were received from Cappel and 5 Prime → 3 Prime, Inc., Boulder, CO, respectively. Protease Ca3(PO4)2. Transfection kit was purchased from Promega. Protein kinase C inhibitors and EGTA/AM were received from Calbiochem. Cell culture and transfection reagents including DMEM, FBS, serum free medium (OptiMEM), LipofectAMINE, and trypsin/EDTA were purchased from Life Technologies, Inc., whereas Ham’s F-12 was received from Irvine Scientific. The Apo detect kit was obtained from Phoenix Flow Systems. The peptide inhibitor, benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (z-VAD.fmk), was purchased from Enzyme Systems Products. The in situ cell death detection kit was obtained from Boehringer Mannheim, and the Vectashield mounting medium containing 4’,6-diamidino-2-phenylindole was from Vector Laboratories.

**Cell Culture and Maintenance**—Cells were grown in 5% CO2 and DMEM, 10% FBS (COS-7), or Ham’s F-12, 10% FBS (CHO-K1), respectively. Transfection protocols for COS-7 and CHO-K1 cells with LipofectAMINE were described previously (13). Cells were kept in serum-free medium during LipofectAMINE transfection for 5 h and followed by the addition of an equal volume of DMEM, 20% FBS. Transfection of COS-7 cells with the Perfection kit was performed according to the manufacturer’s instructions in DMEM, 10% FBS. Treatment of COS-7 cells with z-VAD.fmk was performed according to the peptide manufacturer’s directions. Immediately after transfection of cells using lipo-

---

*This work was supported by National Institutes of Health Grant GM 34236. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Div. of Biology, 147-75, California Institute of Technology, Pasadena, CA 91125. Tel.: 818-395-3944; Fax: 818-796-7066; E-mail: simonm@starbase1.caltech.edu.

‡ The abbreviations used are: CHO, Chinese hamster ovary; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; G-protein, guanosine nucleotide-binding protein; PKC, protein kinase C; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling; z-VAD.fmk, benzoyloxycarbonyl-Val-Ala-Asp-fluoromethylketone.
fectAMINE, z-VAD.fmk was added to a final concentration of 50 μM. Cells were maintained in medium containing 50 μM z-VAD.fmk for approximately 41 h. New aliquots of the peptide inhibitor were added 4 times during the course of the incubation period to ensure that the effectiveness of the inhibitor was maximized.

Expression Plasmids—Expression plasmids for Ga13QL, GaqRC, LacZ, rasN19, and rhoAN17 were previously described (14–16). The cDNA of the constitutively active Ga12QL mutant or of bcl-2 was inserted into the pcDNA expression vector (Invitrogen) or the Pac expression vector to yield an expression plasmid for Ga12QL or bcl-2, respectively (17).

Analysis of Apoptotic Phenotypes—Cells were grown on glass cover slips and fixed at 48 h after transfection by incubation in methanol/acetone for 2 min. Immunostaining of β-galactosidase and staining of the genomic DNA were performed as described (18). Cells expressing Ga13QL display a fragmented nucleus and condensed DNA, whereas only LacZ transfected cells do not. Expression of GoqRC in CHO cells or Ga13QL and GoqRC expression in COS-7 cells causes a similar phenotype (data not shown). Transfections were performed with LipofectAMINE.

FIG. 1. Nuclear fragmentation caused by expression of constitutively active Ga-subunits. Approximately 1 × 10⁶ CHO and COS-7 cells were transiently cotransfected with 0.5 μg of pCisLacZ and pCisGa13 or pCis. After 48 h, cells were fixed and stained as described under “Experimental Procedures.” A representative field is shown through the respective filters. A, Hoechst staining of cell nuclei; B, Texas Red staining of LacZ (Ga13QL/LacZ) expressing cells. Cells expressing Ga13QL display a fragmented nucleus and condensed DNA, whereas only LacZ transfected cells do not. Expression of GaqRC in CHO cells or Ga13QL and GaqRC expression in COS-7 cells causes a similar phenotype (data not shown). Transfections were performed with LipofectAMINE.

A

B

Gα13QL/LacZ

Gα13QL/LacZ

LacZ

LacZ

RESULTS

Expression of Constitutively Active Ga13 and Goq but not Goa2 Triggers Apoptosis—Mutations in the catalytic domain of Ga-subunits have been described which inhibit their intrinsic GTPase activity and therefore convert these proteins into constitutively active α-subunits (19). To investigate the phenotype of cells transfected with constitutively active Ga-subunits, the nuclear morphology of the transfected cells was analyzed. The characteristic nuclear phenotype exhibited by apoptotic cells (nuclear fragmentation and condensation of genomic DNA) was used to distinguish between normal and apoptotic cells (Fig. 1). To eliminate the background of non-transfected cells, cells were cotransfected with expression plasmids for the constitutively active Ga12QL and LacZ. Only cells expressing LacZ were counted during the experiment. Expression of the various proteins was verified by Western blot analysis (data not shown). In general, a large fraction of singly transfected cells are also cotransfected with a second plasmid. Thus, there is a high probability that LacZ positive cells are also expressing the cotransfected G protein expression plasmid.

Expression of constitutively active Ga2QL in COS-7 cells does not increase the number of apoptotic cells over that observed in control experiments where LacZ alone was expressed. In contrast, expression of constitutively active GoqRC and Ga13QL increased the appearance of the apoptotic phenotype dramatically. An approximate three-fold rise in the percentage of apoptotic cells was observed. Both GoqRC and Ga13QL expression triggered programmed cell death in nearly 30% of the transfected cells (Fig. 2A). Varying the amount of expression plasmid (0.05–1 μg) revealed no differences in the occurrence of the apoptotic phenotype in our assays (data not shown). Thus, differences in the ability to trigger apoptosis does not appear to be dependent on expression levels of the
different Gα-subunits.

To rule out the effect of cell line specific mutations that could lead to differences in cell survival, CHO cells were also transfected with expression plasmids for GaqRC, Ga13QL, and Gai2QL. Again in the CHO cell system the expression of GaqRC and Ga13QL led to significant apoptotic cell death, whereas Gai2QL expression did not (Fig. 2B). Thus, the results with the CHO cells correspond to the data obtained using the COS-7 cells and, together they suggest that the ability of Ga13 and Goq to induce programmed cell death in tissue culture is cell type independent.

A second assay system was employed to further confirm the correlation between the expression of GaqRC and Ga13QL and apoptosis. The Apo-Direct assay (Phoenix Flow Systems) was used to detect increased activity of an endonuclease, an additional characteristic of apoptotic cell death, in the transfected cells. In this assay fluorescently labeled nucleotides are incorporated into the ends of the fragmented, genomic DNA via terminal transferase. Therefore an increase in fluorescence intensity indicates an increase in the amount of apoptotic cells. COS-7 cells expressing GaqRC and Ga13QL exhibit an increase in incorporation of fluorescently labeled nucleotides (Fig. 3). This is consistent with the observed morphological nuclear changes in the transfected cells and confirms the notion that Ga13QL and GoqRC expression causes apoptosis.

**Fig. 2.** GaqRC and Ga13QL expression triggers programmed cell death in transiently transfected COS-7 and CHO cells. Cells were cotransfected with GaqRC, Ga13QL, or Gai2QL and LacZ. 48 h after LipofectAMINE transfection, cells were stained as described under “Experimental Procedures.” Cells expressing LacZ and cells expressing both LacZ and showing an apoptotic nuclear phenotype were counted. The percentage of transfected cells showing fragmented nuclei are presented in these figures. Vertical bars represent ± S.D. A, transfection of COS-7 cells; B, transfection of CHO cells.

**Fig. 3.** Increased fragmentation of genomic DNA due to GaqRC and Ga13QL expression. COS-7 were transfected with GaqRC, Ga13QL, or LacZ. 48 h after transfection with LipofectAMINE, cells were fixed and treated according to the Apo-Direct staining protocol. Treated cells were analyzed in the Coulter Elite flow cytometer at 488 nm excitation. Data were plotted as a histogram. Relative fluorescence intensities are marked on the x axis.
was used to keep the cells in high serum throughout the experiment. Therefore, to determine if the expression of Ga13QL and GaqRC also cause an imbalance in cell cycle signaling, transfections were performed with Ca$_3$(PO$_4$)$_2$ in COS-7 cells in high serum. Even at continuous culture in 10% FBS, expression of Ga13QL and GaqRC increased the appearance of the apoptotic nuclear phenotype 2–3-fold compared with the amounts observed in control experiments (Fig. 4). These data confirm that constitutive activation of Ga13 and Gaq can activate pathways directly targeting the cell death machinery.

**Signaling Pathways Activated by Gaq and Ga13 Leading to Apoptosis Are Different but Converge at a Step Controlled by bcl-2**—To investigate the signaling mechanism connecting the expression of Ga13QL and GaqRC to apoptosis, the effects of inhibitors of different pathways on apoptotic cell morphology were examined in COS-7 cells. Although some of the cellular responses caused by activation of Ga13 and Gaq are similar, Gaq and Ga13 activate different pathways. Whereas the target of Gaq signaling is the family of phospholipase C-$\beta$ enzymes, Ga13 does not activate this class of enzymes (21–24). In contrast, several groups have established that a major target of Gaq signaling is the small G-protein Rho (25). Therefore, to test the involvement of the Rho and the phospholipase C-$\beta$ pathways in the induction of apoptosis by GaqRC and Ga13QL, two different approaches were employed. First cells were cotransfected with expression plasmids for Ga13QL or GaqRC and a dominant interfering RhoA mutant (RhoAN19) (Fig. 5A). Coexpression of RhoAN19 with Ga13QL compared with GaqRC expression yielded a significant reduction in the amount of transfected cells displaying an apoptotic nuclear phenotype. This reduction was not observed when GaqRC and RhoAN19 were coexpressed (Fig. 5A).

To rule out a general effect of the expression of dominant negative small G-proteins, the effect of coexpression of a dominant negative Ras mutant (RasN17) in this assay system was investigated (19). Neither GaqRC- nor Ga13QL-induced apoptosis was reduced by coexpression of RasN17 demonstrating that the effect of RhoAN19 on Ga13QL-triggered apoptosis was specific for Rho.

In the second approach toward analyzing these pathways, protein kinase C inhibitors were added to cells expressing constitutively active GaqRC and Ga13QL mutants. Calphostin C and Ro31–8220 were shown to inhibit all isoforms of PKC at IC$_{50}$ of 50 or 10 nM, respectively. Thus, 50–100-fold higher concentrations of the respective inhibitors were used in these assays. Inhibition of other kinases by these inhibitors is effective only at approximately 500–1000-fold higher concentrations (26, 27). Whereas both Calphostin C and Ro31–8220 diminished the amount of apoptotic cells caused by GaqRC expression 2–3-fold, Ga13QL-induced programmed cell death was not significantly altered by the addition of the PKC inhibitors (Fig. 5B). This indicates that the PKC inhibitors specifically interfere with Gaq signaling which leads to apoptosis.

Although Ga13 and Gaq obviously activate different signaling mechanisms, these pathways may converge to initiate the apoptotic cellular response. Signal transduction pathways triggering the activation of the cell death machinery share common steps, which in many cases are inhibited by expression of bcl-2 (28, 29). Expression of bcl-2 was shown to inhibit apoptosis in several cell systems by sequestering molecules of the apoptotic pathway. To test the effect of bcl-2 on Gaq and Ga13 activated pathways, COS-7 cells were cotransfected with expression plasmids for Ga13QL or GaqRC and bcl-2. Both Ga13QL- and GaqRC-induced apoptosis were greatly reduced by overexpressed bcl-2. The observed 3-fold reduction again confirms the connection of pathways activated by Ga13 and Gaq to the cell death machinery (Fig. 6). This also reveals that both pathways must converge upstream of a point controlled by bcl-2 (Fig. 7).

Activation of the interleukin-1$\beta$-converting enzyme-like family of cysteine proteases is thought to play a central role in the programmed cell death pathway (for review see Ref. 5). Proteolysis of their many target substrates may be responsible for the eventual demise of the cell. The interleukin-1$\beta$-converting enzyme-like family of proteases or caspases can be inhibited by specific peptide inhibitors (30). To determine if activation of caspases is involved in the observed increase in cell death induced by Ga13QL and GaqRC, COS-7 cells were cotransfected with LacZ and Ga13QL or GaqRC in the presence of 50 $\mu$M of z-VAD.fmk, an irreversible inhibitor of the caspase family of cysteine proteases. The presence of the peptide inhibitor prevented the induction of cell death by both Ga13QL and GaqRC when measured using the TUNEL assay, supporting the notion that activation of a caspase(s) is involved in Ga13QL- and GaqRC-induced apoptosis (Table I). Although, the peptide inhibitor prevented DNA fragmentation, nuclear integrity still seemed to be affected. Many cells appeared to display some of the morphological characteristics of apoptosis, nuclear fragmentation and DNA condensation. These results are similar to findings previously reported demonstrating that in BAX-induced programmed cell death inhibition of interleukin-1$\beta$-converting enzyme-like proteases with z-VAD.fmk prevented DNA fragmentation but not many of the other morphological changes associated with apoptosis (31).

**DISCUSSION**

Collectively, our results suggest that expression of constitutively active Ga13QL or GaqRC mutants triggers apoptosis in higher eukaryotic cell lines. Different cell lines contain different mutations that interfere with the cell death machinery and render these lines immortal. Ga13QL and GaqRC expression caused programmed cell death in two different cell lines, thus affecting a common pathway leading to apoptosis. In addition, the observed increase in apoptotic cell number correlates with data obtained with the expression of other proteins or with extracellular stimuli causing apoptosis, e.g. with expression of
MEKK1 in PC12 cells or tumor necrosis factor-α stimulus in U937 cells (18, 32). The ability of GaqRC and Ga13QL to activate the cell death machinery also in high serum distinguish our data from other studies where apoptosis was only achieved by simultaneous expression of the respective protein and incubation in low serum. Therefore, Ga13QL and GaqRC seem to activate signaling pathways directly linked to the cell death machinery.

Activation of Gaq can be correlated with an increase in PKC activity and elevation of internal Ca²⁺ concentration as a result of phospholipase C-β activation. The role of PKC activation in apoptosis is not completely clear. In some cell systems PKC activation inhibits apoptosis, whereas in others PKC activation can be correlated with the onset of apoptosis (33–35). In our assay system, the PKC inhibitors totally abolished the apoptosis-inducing ability of Gaq. This reveals that PKC signaling is an essential step in Gaq triggered apoptosis. Elevation of internal Ca²⁺ levels is often observed in cells dying from apoptosis (36). Therefore, to determine if GaqRC induced an increase in cytoplasmic Ca²⁺ levels that may be associated with apoptosis, COS-7 cells expressing GaqRC were incubated with the Ca²⁺ chelator EGTA/AM. Incubation with 10 μM of this Ca²⁺ chelator provokes only a slight decrease in the number of apoptotic cells, presumably due to the low concentration of EGTA/AM used (data not shown). Higher concentrations of the Ca²⁺ chelator could not be used because of its cytotoxic action at higher doses during prolonged incubation (37). Interestingly, a neural disorder, amyotrophic lateral sclerosis, causing neural cell death presumably by apoptosis is accompanied by an elevation in PKC activity and cytoplasmic Ca²⁺ levels (11). The cause of this disease is currently unknown.

Sustained activation of Gaq was recently shown to cause transformation of NIH3T3 cells (38). This study also showed that a large fraction of the transfected cells died due to expression of constitutively active Gaq. These data can now be explained by the correlation between GaqRC and apoptosis that we found. The transformed phenotype might occur in cells that accumulate mutations affecting the cell death machinery.
Recently it was demonstrated that sustained activation of Rho triggers programmed cell death in low serum (39). In contrast, our data show that coexpression of RhoAN19 and Rho triggers programmed cell death in low serum (39). In addition, null mutation of Gα13 might activate an additional pathway that contributes to the Rho signaling in inducing apoptosis in high serum.

Our data clearly demonstrate that Ga13QL- and GaqRC-triggered apoptosis can be blocked by overexpression of bcl-2. Thus, these pathways enter the cell death signaling cascade upstream of a point controlled by bcl-2. In conclusion, our results demonstrate the ability of Gα-subunits to induce programmed cell death through different pathways that, however, converge before the final execution of cell death.

We also performed experiments where constitutively active Ga12QL was expressed in COS-7 cells. A significant fraction of the cells showed the apoptotic phenotype; however, the amount was well below the fraction of apoptotic cells obtained with expression of GaqRC and Ga13QL (20% ± 6.08; data not shown). Recently it was demonstrated that Ga13 and Ga12 recruit different signaling pathways to activate Na+/H+ exchangers (40). In addition, null mutation of Ga13 in mice displays a distinct phenotype, which also argues against redundant functions of Ga13 and Ga12 (41). Therefore, it is likely that activated Ga13 transmits signals to the cell death machinery through pathways not recruited by Ga12.

Our study clearly demonstrates that sustained activation of Gaq or Go13 can trigger apoptosis in different cell systems. Several human diseases are thought to be caused by a sustained stimulus that cannot be shut down through desensitization processes. The role of sustained signaling through Gaq or Go13 in development and in disease remains to be more clearly defined.

**Acknowledgments—**We thank Dr. J. Heller Brown for the gift of the RasN17 expression plasmid. We also thank S. Diamond for professional help with flow cytometric analysis and Dr. T. Wieland for helpful discussion. CHO cells were generously provided by Dr. M. C. Jasek.

**REFERENCES**

1. Darzynkiewicz, Z., Li, X., and Gong, J. (1994) Methods Cell Biol. 41, 15–38
2. Kerr, J. F. R., Wyllie, A. H., and Currie, A. R. (1972) Br. J. Cancer 26, 239–257
3. Neiman, D. R., Thomas, S. J., and Loring, G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5857–5861
4. Arends, M., and Wyllie, A. (1991) Int. Rev. Exp. Pathol. 32, 223–254
5. Vaux, D. L., and Strasser, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2239–2244
6. Watson, S., and Arkinstall, S. (1994) The G-protein Linked Receptor Facts Book, Academic Press, Orlando, FL
7. Simon, M. I., Strathmann, M., and Gautam, N. (1991) Science 252, 802–808
8. Strathmann, M. P., and Simon, M. I. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5582–5586
9. Freedman, N. J., Liggett, S. B., Drachman, D. E., Pei, G., Caron, M. G., and Lefkowitz, R. J. (1995) J. Biol. Chem. 270, 17953–17961
10. Druey, K. M., Blumer, V. H., Kang, J., and Kehrl, M. (1996) Nature 379, 742–746
11. Krieger, C., Lanius, R. A., Pelech, S. L., and Shaw, C. A. (1996) Trends Pharmacol. Sci. 17, 114–120
12. Yamatoguchi, T., Matsui, T., Okamoto, T., Kamatsu, K., Takeda, S., Fukumoto, H., Iwatsubo, T., Suzuki, N., Asami-Odaka, A., Ireland, S., Kinane, B., Giambarella, U., and Nishimoto, I. (1996) Science 272, 1349–1352
13. Slepek, V. Z., Katz, A., and Simon, M. I. (1995) J. Biol. Chem. 270, 4037–4041
14. Jiang, H., Wu, D., and Simon, M. I. (1993) FEBS Lett. 330, 319–322
15. Sah, V. P., Hoshijima, M., Chien, K. R., and Brown, J. H. (1996) J. Biol. Chem. 271, 31185–31190
16. Collins, L. R., Minden, A., Karin, M., and Brown, J. H. (1996) J. Biol. Chem. 271, 17349–17353
17. Paez-Gomez, A. M., Coats, W. S., Baque, S., Alan, T., Gerard, R. D., and Newgard, C. C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 25129–25134
18. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) Science 270, 1326–1331
19. Bourne, H. R., Sanders, D. A., and McCormick F. (1990) Nature 348, 125–132
20. Evan, G. I., Brown, L., Whyte, M., and Harrington E. (1995) Curr. Opin. Cell Biol. 7, 825–834
21. Dhankskar, N., Heasley, L. E., and Johnson, G. L. (1995) Endocr. Rev. 16
22. Lee, C. H., Park, D., Wu, D., Rhee, S. G., and Simon M. I. (1992) *J. Biol. Chem.* **267,** 16944–16947
23. Hepler, J. R., Kozasa, T., Smrcka, A. V., Simon, M. I., Rhee, S. G., Sternweis, P. C., and Gilman, A. G. (1993) *J. Biol. Chem.* **268,** 14367–14375
24. Taylor, S., Chae, H., Rhee, S., and Exton, J. (1991) *Nature* **350,** 516–518
25. Buhl, A. M., Johnson, N. L., Dhanasekaran, N., and Johnson, G. L. (1995) *J. Biol. Chem.* **270,** 24631–24634
26. McKenna, J. P., and Hanson, P. J. (1993) *Biochem. Pharmacol.* **46,** 583–588
27. Tamaoki, T (1990) *Bio/Technology* **8,** 732
28. Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1994) *Cell* **79,** 189–192
29. Armstrong, R. C., Aja, T., Xiang, J., Gaur, S., Krebs, J. P., Hoang, K., Bai, X., Korsmeyer, S. J., Karanewska, D. S., Fritz, L. C., and Tomaselli, K. J. (1996) *J. Biol. Chem.* **271,** 16850–16855
30. Xiang, J., Chao, D. T., and Korsmeyer, S. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14559–14563
31. Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M., Haimovitz-Friedman, A., Fuks, Z., and Kolesnick, R. N. (1996) *Nature* **380,** 75–79
32. Emoto, Y., Manome, Y., Meinhardt, G., Kisaki, H., Kharbanda, S., Robertson, M., Ghayur, T., Wong, W. W., Kamen, R., Weichselbaum, R., and Kufe, D. (1995) *EMBO J.* **14,** 6148–6156
33. Diaz-Meco, M., Muniesa, M. M., Frutos, S., Sanchez, P., Llorente, J., Sanz, L., Moscat, J. (1996) *Cell* **86,** 777–786
34. Ohkusu, K., Isebe, K., Hidaka, H., and Nakashima, I. (1995) *Eur. J. Immunol.* **25,** 3180–3186
35. Orrenius, S., and Nicotera, P. (1994) *J. Neurochem.* **60,** 1–11
36. Togo, Y., and Matsumoto, Y. (1990) *Biochem. Pharmacol.* **39,** 1775–1778
37. Kalincic, G., Nazarali, A. J., Hermoute, S., Xu, N., and Gutkind, S. (1992) *Mol. Cell. Biol.* **12,** 4687–4693
38. Jimenez, B., Arends, M., Esteve, P., Persano, R., Sanchez, R., Ramon y Cajal, S., Wyliff, A., and Lacal, J. C. (1995) *Oncogene* **10,** 811–816
39. Dhanasekaran, N., Prasad, M. V. V. S. V., Wadsworth, S. J., Dermott, J. M., and van Rossum, G. (1994) *J. Biol. Chem.* **269,** 11892–11896
40. Offermanns, S., Mancino, V., Revel, J. P., and Simon, M. I. (1997) *Science* **275,** 533–536