Reversible Phosphorylation of Bcl2 following Interleukin 3 or Bryostatin 1 Is Mediated by Direct Interaction with Protein Phosphatase 2A*

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Interleukin 3 (IL-3) stimulates the net growth of murine factor-dependent NSF/N1.H7 and FDC-P1/ER myeloid cells by stimulating proliferation and suppressing apoptosis. Recently, we discovered that Bcl2 is phosphorylated at an evolutionarily conserved serine residue (Ser70) after treatment with the survival agonists IL-3 or bryostatin 1, a potent activator of protein kinase (Ito, T., Deng, X., Carr, B., and May, W. S. (1997) J. Biol. Chem. 272, 11671–11673). In addition, an intact Ser70 was found to be required for Bcl2's ability to suppress apoptosis after IL-3 withdrawal or toxic chemotherapy. We now show that phosphorylation of Bcl2 occurs rapidly after the addition of agonist to IL-3-deprived cells and can be reversed by the action of an okadaic acid (OA)-sensitive phosphatase. A role for protein phosphatase (PP) 2A as the Bcl2 regulatory phosphatase is supported by several observations: 1) dephosphorylation of Bcl2 is blocked by OA, a potent PP1 and PP2A inhibitor; 2) intracellular PP2A, but not PP1, co-localizes with Bcl2; 3) the purified PP2Ac catalytic subunit directly dephosphorylates Bcl2 in vitro in an OA-sensitive manner; 4) the purified PP2Ac catalytic subunit preferentially dephosphorylates Bcl2 in vitro compared with PP1 and PP2B; 5) reciprocal immunoprecipitation studies indicate a direct interaction between PP2A and hemagglutinin (HA)-Bcl2; and 6) treatment of factor-deprived cells with bryostatin 1 dramatically increases the association between PP2A and Bcl2. Increased association between Bcl2 and PP2A occurs 15 min after agonist stimulation when Bcl2 phosphorylation has peaked and immediately before dephosphorylation. An agonist-induced increased association of PP2A and Bcl2 fails to occur in cells expressing the inactive, phosphorylation-negative S70A Bcl2 mutant, which indicates that an intact Ser70 site is necessary and sufficient for the interaction to occur. Functional phosphorylation of Bcl2 at Ser70 is proposed to be a dynamic process regulated by the sequential action of an agonist-activated Bcl2 kinase and PP2A.

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§ The abbreviations used are: IL-3, interleukin 3; Bryo, bryostatin 1; OA, okadaic acid; PP, protein phosphatase; PKC, protein kinase C; ER, endoplasmic reticulum; PAGE, polyacrylamide gel electrophoresis; wt, wild type.
nism that may positively regulate the antiapoptotic function of Bcl2, at least in factor-dependent cells. However, others have reported an inability to easily detect Bcl2 phosphorylation under continuous growth conditions (14), suggesting a possible role for a Bcl2 phosphatase in reversing phosphorylation. Reversible phosphorylation is a well-understood mechanism for the rapid regulation of critical cell functions including metabolism, signal transduction, cell division, growth, and memory (15, 16). Specifically, IL-3 signaling is a dynamic process that results in signal amplification characterized by the rapid phosphorylation-dephosphorylation of critical post-receptor components such as Janus-activated kinase 2, RAF1, and mitogen-activated protein kinase (2). Whereas protein kinase C (PKC), an IL-3 activated post-receptor kinase (2), can directly phosphorylate Bcl2 in vitro and possibly in vivo (1, 11, 17), a regulatory Bcl2 phosphatase that opposes the action of an agonist-activated Bcl2 kinase has not yet been identified. Studies were designed to test whether a protein phosphatase is involved in regulating Bcl2 phosphorylation in IL-3-dependent cells and to identify the mechanism(s) involved in regulating Bcl2 dephosphorylation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bcl2 and BAX antisera were produced, characterized, and used as described previously (11). Synthetic murine IL-3 was kindly provided by Ian Clark-Lewis (University of British Columbia, Vancouver, British Columbia, Canada). Bryostatin 1 was the kind gift of G. R. Pettit (Arizona State University, Tempe, AZ). The purified catalytic subunit of PP2A was prepared as described previously (18). Rabbit polyclonal PP2A/A and PP2Ac antisera were obtained from Calbiochem-Novabiochem Corp. (San Diego, CA), and the mouse monoclonal anti-hemagglutinin (HA) antibody was obtained from BabCO (Richmond, CA). PP1, PP2A, and PP2B were purchased from Calbiochem-Novabiochem Corp. The Tβ218 Serine/Threonine Phosphatase Assay Kit was purchased from Promega (Madison, WI). All other reagents are from commercial sources, unless stated otherwise.

**Cell Lines, Plasmids, and Transfections**—Murine IL-3-dependent NSF/N1.H7 cells were the kind gift of H. Scott Boswell (19). Clones were selected for their expression of quantitatively similar amounts of exogenous wild type, mutant, or HA-tagged Bcl2 and characterized as described previously (12). IL-3-dependent FDC-P1/ER cells expressing high levels of endogenous wild type Bcl2 were maintained and grown as described previously (11, 20).

**Metabolic Labeling, Immunoprecipitation, Western Blot, and Tryptic Peptide Map Analysis**—Cells were labeled with [32P]orthophosphoric acid or [35S]methionine as described previously (11). Cells were washed, incubated in 10% fetal calf serum-RPMI 1640 media deprived of IL-3 for 2 h, and incubated in phosphate and/or methionine-free RPMI 1640 medium for 1 h before the addition of IL-3 or bryostatin 1 (11). Inhibitors or other agents were added as indicated in the text or figure legends. After incubation, the cells were washed and lysed in detergent buffer, and selective immunoprecipitation was carried out as described previously (11). The samples were subjected to 10—20% gradient SDS-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and exposed to Kodak X-OMAT film for the times indicated at −80 °C as described previously (11). The same membrane was also used for Western blot analysis to quantify Bcl2 or phosphatases. Films were developed using the ECL kit (Amersham) by exposure as described previously (12). For PP2Ac, Bcl2, or HA epitope-tagged Bcl2, co-immunoprecipitation analysis and immunoprecipitations were performed on cell lysates in 0.25% instead of 1.0% Nonidet P-40 detergent buffer. Comparative peptide mapping was performed as described previously (11).

**In Vitro Bcl2 Dephosphorylation Assay**—Cells were metabolically labeled with [32P]orthophosphoric acid and incubated with 100 nM bryostatin 1 for 15 min. [32P]labeled Bcl2 was immunoprecipitated as described above, and the beads were washed three times in detergent buffer and suspended in 100 μl of phosphate buffer containing 50 μM Tris-HCl, pH 7.0, 20 mM β-mercaptoethanol, 2 mM MmCl2, 0.1% bovine serum albumin (for PP1 and PP2A) or 50 mM Tris-HCl, pH 7.0, 20 mM β-mercaptoethanol, 1.0 mM CaCl2, and 1 μM calmodulin (for PP2B). Purified PP2Ac or 2 units each of commercial PP1, PP2A, or PP2B (Calbiochem) were added, and the samples were incubated for the indicated times at 30 °C as described previously (21, 22). The reaction was terminated by the addition of 2× SDS-sample buffer, and the sample was boiled for 5 min before loading onto SDS-PAGE as described above. The activity of PP2A, PP1, and PP2B was verified by the serine/threonine phosphatase assay kit, following the recommended protocol (Propel). **Cell Fractionation and Immunolocalization Studies**—Subcellular localization of Bcl2 and PP2A was performed essentially as described previously (23). Bcl2 was labeled with [32P]orthophosphoric acid and transported into the nuclei, the supernatant was centrifuged at 150,000× g to pellet the heavy membrane fraction that contains the mitochondria. The resulting supernatant was centrifuged over a 2 m sucrose cushion as described previously (24). Fifty μg of total protein from each fraction were subjected to SDS-PAGE and analyzed by Western blotting as described above.

**RESULTS**

**IL-3 and Bryostatin Induce Rapid and Reversible Phosphorylation of Bcl2 That Is Sensitive to Okadaic Acid**—IL-3 and Bryo may inhibit apoptosis in IL-3-dependent myeloid NSF/N1.H7 and FDC-P1/ER cells by inducing the serine phosphorylation of Bcl2 (11). However, little is known about the mechanism(s) of regulation of Bcl2 phosphorylation and whether a Bcl2 protein phosphatase might also be involved. Therefore, studies were performed to determine the kinetics and stability of Bcl2 phosphorylation after agonist addition to factor-deprived cells. The cells were metabolically labeled with [32P]orthophosphoric acid and stimulated with various combinations of agonists for 30 min, sheared by repeated aspiration through a 25-gauge needle, and the supernatant was centrifuged at 10,000 × g to pellet the light membrane fraction that contains the plasma membranes and endoplasmic reticulum (ER). The resulting supernatant contains the cytosolic fractions. The nuclei were further purified by centrifugation over a 2 m sucrose cushion as described previously (24). Fifty μg of total protein from each fraction were subjected to SDS-PAGE and analyzed by Western blotting as described above.

**PP2A Can Directly and Rapidly Dephosphorylate Bcl2**—PP2A is the most abundant serine/threonine-specific protein phosphatase expressed in mammalian cells (25). PP2A enzymatic activity is reported to be more potent than Bcl2. However, the hyperphosphorylation of Bcl2 as described above and resulted in a prolonged hyperphosphorylation of Bcl2 (26). Interestingly, however, IL-3- or Bryo-induced hyperphosphorylation was found to be rapidly modulated, returning to a lower, steady-state baseline level by 60 min. Because the level of expression of Bcl2 does not vary significantly during agonist treatment, the transient nature of phosphorylation is concluded to result from the sequential action of a Bcl2 kinase and phosphatase.

**To assess whether the ubiquitously expressed PP1 and/or PP2A protein serine/threonine phosphatases may be involved, the cells were treated with okadaic acid (OA), a potent PP1 and PP2A inhibitor. After treatment for 120 min in the presence of IL-3, OA had completely prevented the dephosphorylation of Bcl2 as described above and resulted in a prolonged hyperphosphorylation of Bcl2 (Fig. 1a).** However, the hyperphosphorylation observed was temporally delayed compared with that seen after agonist addition (Fig. 1b). Interestingly, both Bryo- and OA-induced Bcl2 hyperphosphorylation occurred at ser70 because stimulated Bcl2 was unable to be phosphorylated (Fig. 2a). Comparative tryptic peptide maps from Bryo- or OA-stimulated phosphorylation confirmed that the site was identical to Tyr164 and was different from Tyr160 (Fig. 2b). These findings suggest that an IL-3-independent kinase may have been involved in the treatment with OA. Alternatively, OA, possibly by inhibiting a phosphatase, might indirectly activate a Bcl2 kinase.
of 10–15 nM for PP1 (26, 27), and PP2Ac can dephosphorylate Bcl2 in vitro in an OA-sensitive manner (Fig. 3a). It has been shown in some cell types that even relatively high concentrations of OA can cause a selective inhibition of PP2A with little or no effect on PP1 (28). Because a relatively low concentration of OA (5 nM) was used in the experiments, the results are consistent with but do not prove a primary role for PP2A or a PP2A-like phosphatase in dephosphorylating Bcl2 in intact myeloid cells. Therefore, additional studies were performed to determine which purified protein phosphatase could directly and most efficiently dephosphorylate Bcl2 in vitro. 32P-labeled Bcl2 was again used as a substrate for the commercial phosphatase in dephosphorylating Bcl2 in intact myeloid cells. Therefore, additional studies were performed to determine which purified protein phosphatase could directly and most efficiently dephosphorylate Bcl2 in vitro. 32P-labeled Bcl2 was again used as a substrate for the commercial phosphatases tested. 2 units each of PP1, PP2A, or PP2B were added for the times indicated. Results reveal that only purified PP2A could more rapidly and completely dephosphorylate 32P-labeled Bcl2 (Fig. 3b, compare lanes 2–5 with lanes 6–9 and lanes 10–13). The findings indicate that PP2A is more efficient than either PP1 or PP2B and indicate that phosphorylated Bcl2 is a potential physiologic substrate for PP2A.

PP2A Specifically Co-localizes with Cellular Structures That Contain Bcl2—Bcl2 is an integral membrane protein located mainly on the outer mitochondrial membrane (6, 29), ER, and nuclear membrane (30). Because a suppression of apoptosis by

![Fig. 1. IL-3 and bryostatin induce reversible phosphorylation of Bcl2, whereas okadaic acid induces sustained hyperphosphorylation of Bcl2. a. IL-3-dependent NSF.N1/H-7 cells were metabolically labeled with [32P]orthophosphoric acid for 1 h and treated with 1 μg/ml IL-3 or 5 nM okadaic acid or both for the indicated times. Bcl2 was immunoprecipitated from cell lysate using Bcl2 antisera and processed as described under “Experimental Procedures.” Nitrocellulose filters containing immunoprecipitated proteins were exposed to Kodak X-Omat film for 24 h. The same membrane was reprobed with Bcl2 antisera and developed using the ECL method as described. b. IL-3-dependent FDC-P1/ER cells were labeled as described in a and treated with 100 nM bryostatin 1 or OA for the indicated times. Cells were lysed, and Bcl2 was immunoprecipitated and analyzed as described above. c, quantitative Bcl2 phosphorylation was determined by an electronic autoradiography analysis of the samples in a and b.]
Regulation of Bcl2 Phosphorylation Involves PP2A

Subunits of the PP2A core complex (33) are co-localized in the heavy membrane fraction, which contains mitochondrial membranes, as well as in the ER and nuclear membrane fractions (Fig. 4). By contrast, PP1 is not present in either the heavy or nuclear membrane fractions. Furthermore, whereas PP2B has been reported to be an interacting protein (34), this OA-insensitive protein phosphatase was found to be primarily localized in the cytoplasmic and ER fractions of IL-3-dependent cells, which contain very little Bcl2. Only very low levels of PP2B were associated with the mitochondrial membrane fraction (~3% of total PP2B as determined by densitometry; Fig. 4) as previously reported (35, 36). These findings further support a role for PP2A as a physiologic Bcl2 phosphatase in vivo, at least in murine factor-dependent myeloid cells.

PP2Ac but Not PP1 Directly Associates with Bcl2—Studies were performed to determine whether an OA-sensitive phosphatase may interact directly with Bcl2 to potentially bring about dephosphorylation after agonist addition. First, reciprocal immunoprecipitation with anti-HA or anti-PP2A was performed. Parental NSF/N1.H7 IL-3-dependent cells that stably express HA-Bcl2 were produced as described under “Experimental Procedures.” The HA-Bcl2-expressing cells were metabolically labeled with [35S]methionine and treated with Bryo for 30 min, a time at which Bcl2 phosphorylation is maximal (Fig. 1b). After treatment, HA-Bcl2 or PP2Ac was immunoprecipitated and analyzed by SDS-PAGE. Results reveal that the HA antibody selectively immunoprecipitated [35S]-labeled HA-Bcl2 (28 kDa) and a [35S]-labeled 36-kDa band that was identified by Western analysis as the catalytic subunit of PP2A (Fig. 5, lane 3). The same membrane filter was probed with BAX antisera (11) to serve as a control, because BAX is known to heterodimerize with Bcl2 under agonist lysis conditions (Fig. 5, lane 3; Ref. 7). Interestingly, BAX could only be co-immunoprecipitated with an anti-HA antibody, but not when the PP2A antisera was used, suggesting that the PP2A antibody may interrupt the Bcl2-BAX interaction under these conditions (Fig. 5, lane 2). Therefore, the finding that PP2Ac is co-immunoprecipitated with Bcl2 suggests a functional relationship. To confirm a specific interaction, native non-epitope-tagged p26 Bcl2 was labeled with [35S] and tested. Results reveal that native Bcl2 was also co-immunoprecipitated by the PP2Ac antisera, confirming the specificity of the interaction (Fig. 5, lane 2).

Second, a similar study was performed with cell lysates to test for an interaction between PP1 and Bcl2. Again, results indicate that HA-Bcl2 and PP2Ac, but not HA-Bcl2 and PP1, can be co-immunoprecipitated from detergent lysates of growing cells (Fig. 6, a and b). Because PP1 is expressed, as detected by the Western blotting of cell lysates (data not shown), these findings confirm that PP2A but apparently not PP1 may directly bind to Bcl2 and regulate phosphorylation. Interestingly, PP2A was also found to be associated with Bcl2, even after IL-3 deprivation for 2 h, although at significantly reduced levels. In this case, the addition of Bryo stimulated a rapid and dramatic increase in the association of Bcl2 and PP2A only after 15 min, a time by which Bcl2 phosphorylation has become maximal (Fig. 7). Because similar results were obtained for two different IL-3-dependent myeloid cell lines, these findings indicate that the Bcl2-PP2A interaction is not limited to a specific cell type (Fig. 7, a and b). This finding eliminates the possibility that the observed association results from an artifact of exogenous HA-Bcl2 expression.

An Intact Serine 70 Site Is Required for Increased Association of BCL2-PP2A—Increased PP2A association may functionally dephosphorylate Bcl2. Because agonist-induced Bcl2 phosphorylation occurs on Ser70 (12), a study was performed to assess whether an intact Ser70 phosphorylation site is required for the observed increase in Bcl2-PP2A association after agonist-induced Bcl2 phosphorylation. Cells stably expressing either endogenous wt (FDG-P1/ER) or recombinant wt (wt-Bcl H7) Bcl2 or the phosphorylation-negative S70A Bcl2 mutant (S70A-Bcl2-H7) were grown and deprived of IL-3 for 2 h, as described previously (12). Bryostatin was added to induce Bcl2 phosphorylation, and any change in the interaction between Bcl2 and PP2A was assessed by immunoprecipitation after detergent lysis of the cells. Results indicate that both endogenous and recombinant wt Bcl2, but not the phosphorylation-negative S70A Bcl2 mutant, are able to support a time-dependent increase in association between Bcl2 and PP2Ac (Fig. 7). Increased association between Bcl2 and PP2A, however, does not occur until Bcl2 phosphorylation is maximal (i.e. 15–30 min). Importantly, the increased association noted clearly precedes wt Bcl2 dephosphorylation, suggesting that phosphorylated Ser70 may be required for any increase in func-
Bcl2 phosphatase. Previously, we determined that activated phosphorylation by 60–120 min, which indicates the effect of a incomplete dephosphorylation to a lower steady-state level of imal by 15–30 min. Bcl2 then undergoes an appreciable but min) after the addition of IL-3 or bryostatin and becomes max-

Functionally, Bcl2 phosphorylation occurs rapidly (within 5 ings. We have now discovered that reversible phosphorylation of Bcl2 may occur through the action of PP2A. Moreover, because agonist-induced Bcl2 phosphorylation may be important for cell survival, whereas OA-induced irreversible phosphorylation may interfere with Bcl2 function as suggested recently (37, 38). Presumably, a prolonged exposure of cells to OA would not only inhibit a Bcl2 phosphatase but would also inhibit other critical PP2A-regulated processes. This supports our earlier finding that Bcl2 phosphorylation, although required, is not sufficient for cell survival (11, 12). Furthermore, in support of a necessary role for the dynamic regulation of Bcl2 phosphorylation in survival, it has recently been found that nerve growth factor-induced Bcl2 phosphorylation can be reversed by angiostatin binding to rat PC12W pheochromocytoma cells that depend on nerve growth factor for survival (13). Significantly, angiostatin was discovered to induce apoptosis even in the presence of nerve growth factor, apparently by activating a mitogen-activated protein kinase phosphatase (MKP-1; Ref. 13) that may also induce Bcl2 dephosphorylation either directly or indirectly. Collectively, these results confirm that reversing growth factor-induced Bcl2 phosphorylation and/or interfering with dynamic Bcl2 phosphorylation may have a negative effect on cell survival.

The relative activity and/or access of a phosphatase will presumably determine the steady-state level of Bcl2 phosphorylation and function. Mechanistically, an intact Ser70 phosphorylation site, but not PP2Ac activity, seems to be required (Fig. 7). The enhanced interaction between PP2A and Bcl2 may result from either an increased availability of phosphorylated Ser70 or a potential conformational change in Bcl2 resulting from phosphorylation. Whatever the mechanism, the increased association between Bcl2 and PP2A may function to ensure the dynamic nature of Bcl2 phosphorylation and thereby maintain the appropriate physiological level of phosphorylation that is required under steady-state growth conditions.

The results also suggest the potential involvement of a agonist-independent Bcl2 kinase in addition to IL-3 or Bryoactivated PKC (11, 12). Bcl2 can become hyperphosphorylated when OA is added in the absence of exogenous agonist (Fig. 1b), which suggests that the inhibition of OA-sensitive phosphatase activity may allow a constitutively active protein kinase to phosphorylate Bcl2 (Fig. 1b). However, the identity of this potential agonist-independent Bcl2 kinase is not yet clear.

Whereas PP2A has been closely associated with the regulation of various fundamental cell processes (16, 33), virtually nothing is known about how mitochondrial PP2A may affect apoptosis. Our results suggest a mechanism for regulation of Bcl2 that involves PP2A. The findings also provide the first evidence for a novel population of PP2A that is associated with the mitochondrial membrane, possibly through an interaction

**Fig. 6. PP2Ac but not PP1 co-immunoprecipitates with HA-Bcl2.** Cells expressing HA-Bcl2 growing in IL-3-containing media were lysed, and immunoprecipitations were performed with preimmune (lane 1), HA, or PP2Ac antisera (lane 2), as indicated. After separation and purification by SDS-PAGE and transfer to nitrocellulose membranes as indicated under "Experimental Procedures," the filters were probed with the antisera indicated (Blot).
with Bcl2. Targeting PP2A by direct protein interaction may specify PP2A function and play an important role in Bcl2 phosphorylation and function. In support of this notion, another example in which targeting specifies PP2A function involves the dephosphorylation of the microtubule-associated protein tau (39).

Interestingly, the Ca\(^{2+}\) calmodulin serine/threonine phosphatase PP2B/calcineurin has also been reported to interact directly with and be sequestered by Bcl2 in Jurkat T cells (34). This interaction apparently prevents the nuclear localization of NF-AT and facilitates T-cell survival. However, there is no evidence that PP2B is involved in the regulation of Bcl2 phosphorylation and function. Moreover, because PP2B is not sensitive to OA at the concentrations used and PP2B is not co-immunoprecipitated with Bcl2 after the agonist treatment of cells, a role for PP2B in Bcl2 phosphorylation seems unlikely, at least in factor-dependent myeloid cells. Therefore, the binding of either PP2A or PP2B to Bcl2 may have different functional consequences and points out the potential versatility of Bcl2 in interacting with and regulating other components involved in apoptosis.

Finally, it has been reported that phosphorylation of Bcl2 is not detected in some tumor cells (14). Given our findings, it seems plausible that a failure to detect Bcl2 phosphorylation in some cells may reflect, at least in part, the increased activity of a Bcl2-associated phosphatase or the absence of a mitochondrial Bcl2 kinase (17). Thus, a low steady-state level of Bcl2 phosphorylation in some growing cells may be difficult to detect experimentally without also inhibiting an associated Bcl2 phosphatase activity.

In summary, agonist-induced phosphorylation of Bcl2 at Ser\(^{70}\) can now be appreciated in dynamic terms, with the relative steady-state level of phosphorylation being tightly regulated by the concerted action of a Bcl2 kinase and PP2A. These findings help to advance our understanding of the mechanisms underlying the regulation of Bcl2 phosphorylation, which is required for full and potent Bcl2 function (12). Furthermore, this regulatory mechanism identifies a poten-
tional novel therapeutic target for antineoplastic drug development strategies.

REFERENCES

1. Williams, G. T., Smith, C. A., Spooner, E., Dexter, T. M., and Taylor, D. R. (1990) Nature 343, 76–79
2. Ihle, J. N. (1996) Cell 84, 331–334
3. Vaux, D. L., Cory, S., and Adams, T. M. (1988) Nature 335, 440–442
4. Yang, E., and Korsmeyer, S. J. (1996) Blood 88, 886–401
5. Krajewski, S., Tanaka, S., Takayama, S., Schüll, M. J., Fenton, W., and Need, J. C. (1993) Cancer Res. 53, 4701–4719
6. Yang, J., Liu, X., Bhalla, K., Peng, T., Jones, D. P., and Wang, X. (1997) Science 275, 1129–1130
7. Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1993) Cell 74, 609–619
8. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmed, M., Alnermi, E. S., and Wang, X. (1997) Cell 91, 479–489
9. Zou, H., Henzel, W. J., Lin, A., Lutschg, A., and Wang, X. (1997) J. Exp. Med. 183, 1533–1544
10. Zamzami, N., Susin, S. A., Marchetti, P., Hirsch, T., Gomez-Monterrey, I., Castedo, M., and Kroemer, G. (1996) J. Biol. Chem. 271, 1353–1354
11. May, W. S., Tyler, P. G., Ito, T., Armstrong, D. K., Qatsha, K. A., and Davidson, N. E. (1994) J. Biol. Chem. 269, 11671–11673
12. Horiiuchi, M., Hayashida, W., Kambe, T., Yamada, T., and Dzau, V. J. (1997) J. Biol. Chem. 272, 11671–11673
13. Gajewski, T., and Thompson, C. B. (1996) Cell 87, 589–592
14. Cohen, P., Klumpp, S., and Schelling, D. L. (1989) FEBS Lett. 250, 601–606
15. Mumby, M. (1995) Semin. Cancer Biol. 6, 229–237
16. Krajewski, S., Tanaka, S., Takayama, S., Schüll, M. J., Fenton, W., and Need, J. C. (1993) Cancer Res. 53, 4701–4719
17. Yang, E., and Korsmeyer, S. J. (1996) Blood 88, 886–401
18. Fields, A. P., Pincus, S. M., Kraft, A. S., and May, W. S. (1989) J. Biol. Chem. 264, 21896–21901
19. Hunter, T. (1995) Cell 80, 225–236
20. Nagase, T., Murakami, T., Nozaki, H., Inoue, R., Nishito, Y., Tanabe, O., Usui, H., and Takeda, M. (1997) J. Biochem. (Tokyo) 122, 178–187
21. Halder, S., Jena, N., and Croce, C. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4507–4511
22. Srivastava, R. K., Srivastava, A. R., Korsmeyer, S. J., Nesterova, M., Chou-Chung, Y. S., and Longo, D. L. (1998) Mol. Cell. Biol. 18, 3509–3517
23. Sontag, E., Nunhabehdi-Craig, V., Lee, G., Bloom, G. S., and Mumby, M. C. (1996) Neuron 17, 1201–1207
24. Carroll, M. P., Spivak, J. L., McMahon, M., Weich, N., Rapp, U. R., and May, W. S. (1991) J. Biol. Chem. 266, 14964–14969
25. Gong, C. X., Singh, T. J., Iqbal, I. G., and Iqbal, K. (1993) J. Neurochem. 61, 921–926
26. Wang, H. G., Rapp, U. R., and Reed, J. C. (1996) Cell 87, 629–638
27. Fields, A. P., Pincus, S. M., Kraft, A. S., and May, W. S. (1989) J. Biol. Chem. 264, 21896–21901
28. Mumby, M. C., Green, D. D., and Hemings, B. A. (1995) J. Biol. Chem. 272, 13856–13863
29. Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R., and Korsmeyer, S. J. (1990) Nature 348, 334–336
30. Nguyen, M., Millar, D. G., Yong, V. W., Korsmeyer, S. J. and Shore, G. C. (1993) J. Biol. Chem. 268, 25281–25288
31. Zhu, W., Cowin, A., Wasy, G. W., Penn, L. Z., Leber, B., and Andrews, D. W. (1996) EMBO J. 15, 4130–4141
32. Petit, P. X., Susin, S. A., Zamzami, N., Minotte, B., and Kroemer, G. (1996) FEBS Lett. 390, 7–13
33. Mumby, M. C., and Walter, G. (1993) Physiol. Rev. 73, 673–700
34. Shibasaki, F., Kondo, E., Akagi, T., and Mekeen, F. (1997) Nature 386, 728–721
35. Hunter, T. (1995) Cell 80, 225–236
36. Srivastava, R. K., Srivastava, A. R., Korsmeyer, S. J., Nesterova, M., Chou-Chung, Y. S., and Longo, D. L. (1998) Mol. Cell. Biol. 18, 3509–3517
37. Sontag, E., Nunhabehdi-Craig, V., Lee, G., Bloom, G. S., and Mumby, M. C. (1996) Neuron 17, 1201–1207
38. Srivastava, R. K., Srivastava, A. R., Korsmeyer, S. J., Nesterova, M., Chou-Chung, Y. S., and Longo, D. L. (1998) Mol. Cell. Biol. 18, 3509–3517
39. Sontag, E., Nunhabehdi-Craig, V., Lee, G., Bloom, G. S., and Mumby, M. C. (1996) Neuron 17, 1201–1207