Protein Kinase A Regulates Caspase-9 Activation by Apaf-1 Downstream of Cytochrome c*

Received for publication, December 20, 2004, and in revised form, January 31, 2005
Published, JBC Papers in Press, February 9, 2005, DOI 10.1074/jbc.M414325200

Morag C. Martin‡, Lindsey A. Allan‡, Michelle Lickrish‡, Catherine Sampson‡, Nick Morrice§, and Paul R. Clarke¶

From the ‡Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY and the §Medical Research Council Protein Phosphorylation Unit, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, United Kingdom

The cyclic AMP signal transduction pathway modulates apoptosis in diverse cell types, although the mechanism is poorly understood. A critical component of the intrinsic apoptotic pathway is caspase-9, which is activated by Apaf-1 in the apoptosome, a large complex assembled in response to release of cytochrome c from mitochondria. Caspase-9 cleaves and activates effector caspases, predominantly caspase-3, resulting in the demise of the cell. Here we identified a distinct mechanism by which cyclic AMP regulates this apoptotic pathway through activation of protein kinase A. We show that protein kinase A inhibits activation of caspase-9 and caspase-3 downstream of cytochrome c in Xenopus egg extracts and in a human cell-free system. Protein kinase A directly phosphorylates human caspase-9 at serines 99, 183, and 195. However, mutational analysis demonstrated that phosphorylation at these sites is not required for the inhibitory effect of protein kinase A on caspase-9 activation. Importantly, protein kinase A inhibits cytochrome c-dependent recruitment of procaspase-9 to Apaf-1 but not activation of caspase-9 by a constitutively activated form of Apaf-1. These data indicate that extracellular signals that elevate cyclic AMP and activate protein kinase A may suppress apoptosis by inhibiting apoptosome formation downstream of cytochrome c release from mitochondria.

Apoptosis is a physiological form of cell death that is fundamental for cell selection during development and tissue homeostasis in multicellular organisms (1). Apoptosis is important for the removal of cells damaged by cellular stresses or those that have undergone oncogenic transformation (2). The apoptotic program involves activation of a group of cysteine proteases termed caspases, which are present in cells as inactive or low activity proenzymes (3). Initiation of an intrinsic apoptotic pathway by a wide variety of stimuli causes the release of cytochrome c from mitochondria (4–6). In the cytosol, cytochrome c binds the apoptotic factor Apaf-1 and induces Apaf-1 oligomerization, leading to recruitment and activation of procaspase-9 in a large complex termed the apoptosome (7, 8). As a result, procaspase-9 autoprocesses and cleaves the effector procaspases 3 and 7, which are activated to cleave key structural and regulatory proteins and thereby bring about the biochemical and morphological changes associated with apoptotic cell death (3). Signal transduction pathways activated by extracellular and intracellular stimuli can impinge on this apoptotic pathway to control cell fate.

The cyclic AMP signal transduction pathway regulates a diverse array of cellular processes, including proliferation, differentiation, and secretion. Activation of this signaling pathway occurs through specific ligation of G-protein-coupled receptors, initiating formation of cAMP from ATP through the action of adenylate cyclase. Cyclic AMP binds to the regulatory subunits of the heterotetrameric cAMP-dependent protein kinase or protein kinase A (PKA),1 dissociating the holoenzyme and releasing the free catalytic (C) subunits. The active C subunits subsequently phosphorylate target proteins on serine or threonine residues within a relatively well defined consensus sequence (9, 10). In addition, cAMP may act independently of PKA through Epac, a guanine-nucleotide exchange factor for the Rap1 GTPase that is directly activated by cAMP (11). Recently, it has emerged that one important facet of cAMP signal transduction is to regulate apoptosis. While elevation of cAMP is associated with induction of apoptosis in lymphoid cells by glucocorticoids (12), in many cell types elevated cAMP protects against apoptosis (13–21). In contrast to the well characterized molecular mechanisms by which cAMP regulates other biological processes, little is known about the mechanism by which this ubiquitous second messenger modulates apoptosis.

The mitochondrial apoptotic pathway can be regulated at multiple stages to promote or suppress cell death (22). One important point of control is the release of cytochrome c from mitochondria. This event is regulated by the pro- and antiapoptotic proteins of the Bcl-2 family (4, 6). In addition, this apoptotic pathway can be regulated downstream of cytochrome c release by caspase inhibitor proteins such as XIAP (23). The activity of XIAP may be controlled by release of other factors such as Smac/Diablo from mitochondria (24). Components of the pathway such as caspase-9 (25, 26) and XIAP (27) are also regulated post-translationally through phosphorylation by protein kinases activated by signaling pathways. Abnormal or constitutive activation of these signaling pathways may contribute to the survival of cancer cells despite initiation of upstream apoptotic responses. Nevertheless, the mechanisms by which signaling pathways reg-

1 The abbreviations used are: PKA, protein kinase A; AMC, 7-amino-4-methylcoumarin; Apaf-1, apoptotic protease-activating factor 1; Bt2cAMP, dibutyryl cyclic AMP; 8-Br-cAMP, 8-bromo-cyclic AMP; GST, glutathione S-transferase; CARD, caspase recruitment domain; HPLC, high pressure liquid chromatography; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase.
ulate caspase activation downstream of cytochrome c remain to be fully characterized.

Cell-free systems that faithfully reproduce the regulation of apoptosis have proved to be useful for dissecting the biochemical mechanisms controlling caspase activation, including regulation by signaling pathways (28). In this study, we have investigated the ability of the cAMP signaling pathway to regulate caspase activation using cell-free systems derived from *Xenopus* eggs and mammalian cultured cells. We show that activation of PKA by cAMP blocks caspase-9 activation downstream of cytochrome c. Caspase-9 is phosphorylated directly by PKA, although mutational analysis shows that this phosphorylation is not required to inhibit caspase-9 activation. We demonstrate that PKA inhibits the recruitment of procaspase-9 to Apaf-1 in response to cytochrome c, indicating that PKA controls apoptosis formation by a novel mechanism.

### MATERIALS AND METHODS

**Plasmid Constructs and Site-directed Mutagenesis—**Human Caspase-9 and Apaf-1(1–541) cDNAs were amplified from U2OS cells and subcloned into pGEX-4T-1 (Amersham Biosciences), and expression of recombinant proteins was induced in *Escherichia coli* BLR(DE3) at 30 °C for 2 h by the addition of 1 mM isopropyl-1-thio-β-galactopyranoside. Glutathione S-transferase (GST)-tagged proteins were affinity-purified with glutathione-Sepharose 4B (Amersham Biosciences), eluting with 15 mM glutathione in buffer A (10 mM Hepes-KOH at pH 7.5, 150 mM NaCl, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM Na3VO4) containing 100 mM NaCl, 10% glycerol, 1% Triton X-100, and 1 mM EDTA. Glutathione was removed by filtration through a PD-10 column (Amersham Biosciences). The caspase recruitment domain (CARD) of *X. laevis* Apaf-1 was subcloned into pET28a and expressed as a His6-tagged protein which was purified on a nickel-agarose column (Qiagen, Hilden, Germany). The protein was eluted with 250 mM imidazole in buffer B (10 mM Hepes-KOH at pH 7.5, 150 mM NaCl, 0.07% 2-mercaptoethanol), and then imidazole was removed using a PD-10 column.

**Antibodies—**Polyclonal rabbit anti-caspase-9 (Pharmingen, San Diego, CA), monoclonal mouse anti-caspase-9 (Santa Cruz Biotechnol. Santa Cruz, CA), and monoclonal mouse anti-Apaf-1 antibody (Pharmingen) were purchased from commercial suppliers. Secondary antibodies for Western blots were goat anti-rabbit or anti-mouse IgG (Pharmingen) were purchased from commercial suppliers. Secondary antibodies for Western blots were goat anti-rabbit or anti-mouse IgG (Pharmingen). The caspase recruitment domain (CARD) of *X. laevis* Apaf-1 was subcloned into pET28a and expressed as a His6-tagged protein which was purified on a nickel-agarose column (Qiagen, Hilden, Germany). The protein was eluted with 250 mM imidazole in buffer B (10 mM Hepes-KOH at pH 7.5, 150 mM NaCl, 0.07% 2-mercaptoethanol), and then imidazole was removed using a PD-10 column.

**Antibodies—**Polyclonal rabbit anti-caspase-9 (Pharmingen, San Diego, CA), monoclonal mouse anti-caspase-9 (Santa Cruz Biotechnol. Santa Cruz, CA), and monoclonal mouse anti-Apaf-1 antibody (Pharmingen) were purchased from commercial suppliers. Secondary antibodies for Western blots were goat anti-rabbit or anti-mouse IgG (Pharmingen). The caspase recruitment domain (CARD) of *X. laevis* Apaf-1 was subcloned into pET28a and expressed as a His6-tagged protein which was purified on a nickel-agarose column (Qiagen, Hilden, Germany). The protein was eluted with 250 mM imidazole in buffer B (10 mM Hepes-KOH at pH 7.5, 150 mM NaCl, 0.07% 2-mercaptoethanol), and then imidazole was removed using a PD-10 column.

**Immunodepletion of Caspase-9—**For immunodepletion of caspase-9, 40 μl of washed protein A beads (Sigma) were incubated with 5 μl of sheep polyclonal antibody against caspase-9 in 200 μl wash buffer containing 100 μl of 25 mM Hepes-KOH, 2 mM EDTA, 1 mM dithiothreitol. Alternatively, caspase-9 processing was induced using 1 μl of *in vitro* translated caspase-9 incubated for 90 min at 30 °C with 2–8 ng of Apaf-1(1–541) or 300 ng of Apaf-1 CARD in the same reaction buffer. Caspase-9(D330A) was used for the incubation with Apaf-1(1–541). Samples were subjected to SDS-PAGE and visualized by autoradiography.

**RESULTS**

**Activation of Caspase-9 and Caspase-3 Is Inhibited by cAMP Analogues through PKA—**To investigate the role of the cAMP signal transduction cascade in apoptotic regulation, we sought to determine whether this signaling pathway modulated activation of the caspase-3/caspase-9 pathway. We made use of cell-free systems that allow dissection of the biochemical mechanisms by which signal transduction cascades may impinge on this pathway. *Xenopus* egg extracts supplemented with an ATP-regenerating system undergone caspase activation upon prolonged incubation as cytochrome c is released from mitochondria (6, 29). The addition of a stable cAMP analogue, 8-Br-cAMP, to egg extracts at the start of the incubation completely blocked caspase-3 activation detected by cleavage of a fluorogenic substrate, AcDEVD-AMC (Fig. 1A). When egg extracts were fractionated to yield a post-mitochon-
Phosphorylation of caspase-9 is a mechanism by which PKA regulates caspase-9 activation and processing (30). Inhibition of both caspase-3 activation and processing to a p37 form that is caspase-9 specific (Fig. 4C) was observed when Ser-183, Ser-99, and Ser-195 were mutated to nonphosphorylatable alanines. Phosphorylation was abolished in GST-caspase-9S99A/S183A/S195A (Fig. 4D), confirming that these were indeed the only sites targeted directly by PKA.

Phosphorylation of Caspase-9 at Ser-183 in Cell Extracts—To test whether caspase-9 was phosphorylated in response to PKA activation in cell extracts, we raised an antibody against one phosphorylatable site, Ser-183. The purified antibody recognized caspase-9 only when phosphorylated at this site, since GST-caspase-9S183A was not recognized even after incubation with GST-caspase-9. Using the phosphorylatable alanine residues. As found previously, PKA phosphorylated alanine residues. As found previously, PKA phosphorylated wild-type caspase-9. Strikingly, however, mutation of these alanines was not sufficient to prevent phosphorylation by PKA.

PKA Inhibits Caspase-9 Activation Independently of Direct Phosphorylation—To test whether phosphorylation of caspase-9 is the principal mechanism by which PKA controls activation of the protease in cell extracts, we examined the ability of PKA to regulate caspase-9 activation and processing when Ser-183, Ser-99, and Ser-195 were mutated to nonphosphorylatable alanine residues. As found previously, PKA strongly inhibited cytochrome c-induced processing of in vitro translated wild-type caspase-9. Strikingly, however, mutation
of all three sites to alanine did not prevent the inhibition of caspase-9 processing by PKA in reticulocyte lysate (Fig. 6A).

Using HeLa cytosolic extracts, we immunodepleted endogenous caspase-9 and abrogated activation of caspase-3 by cytochrome c (Fig. 6B and C). Caspase-3 activity assayed at the times shown by release of AMC from the tetrapeptide DEVD-AMC. B, caspase-3 activity assayed after a 2-h incubation. Values represent mean ± S.E. of n = 3 experiments. Inhibition of caspase activation by 8-Br-cAMP is significant (**, p < 0.01). C, Western blot analysis after 2-h incubation using a polyclonal antibody that recognizes the 37- and 35-kDa cleavage products of caspase-9 as well as the proenzyme.

PKA Inhibits Apoptosome Formation—Cytochrome c induces the ATP/dATP-dependent assembly of Apaf-1 and the recruitment of procaspase-9 into a large oligomeric complex, the apoptosome (7, 8). PKA could therefore potentially inhibit caspase-9 activation by regulating the interaction of caspase-9 with Apaf-1. To study the formation of native apoptosome complexes, we used immunoprecipitation of endogenous caspase-9 with detection of caspase-9 and Apaf-1 by Western blotting of the immunoprecipitates (31). We confirmed that incubation of HeLa cytosolic extracts with cytochrome c induced the association of Apaf-1 with caspase-9 in the presence of ATP (Fig. 7A). Precipitation of active PKA or Bt2cAMP strongly reduced the association between caspase-9 and Apaf-1 and also blocked the activation of caspase-9 detected by its processing. These inhibitory effects were reversed by the PKA inhibitor H89 (Fig. 7B). This demonstrates that PKA inhibits the assembly of the apopto-
some in response to cytochrome c, thereby preventing the subsequent activation of caspase-9 and downstream activation of caspase-3.

Cytochrome c is thought to induce the exposure of the caspase recruitment domain of Apaf-1 that interacts with caspase-9 (3). We found that processing of caspase-9 or caspase-9S99A/S183A/S195A induced by Apaf-1 CARD (32) was not inhibited by PKA, in striking contrast to the effect of PKA on cytochrome c-induced processing (Fig. 8A). These data point to a mechanism by which PKA inhibits Apaf-1 activation and exposure of the CARD, thereby preventing the subsequent recruitment of caspase-9 to the apoptosome independently of caspase-9 phosphorylation. To test further whether PKA acts at the stage of Apaf-1 oligomerization and activation, we examined the ability of PKA to affect caspase-9 processing induced by Apaf-1-(1–541), a constitutively activated form. Apaf-1-(1–541) induces caspase-9 processing to the p35 form, whereas cytochrome c results in formation of both p35 and p37 forms because of downstream caspase-3 activity (30, 33). To ensure that comparable activation of caspase-9 was being induced by these two stimuli, we used caspase-9D330A, which cannot be cleaved by caspase-3 to the p37 form, and titrated the amount of Apaf-1-(1–541) that was added. We found that PKA had no effect on caspase-9 processing induced by Apaf-1-(1–541), even when the extent of caspase-9 processing was similar to that induced by cytochrome c. This indicates that PKA cannot inhibit Apaf-1 function once Apaf-1 has been oligomerized and activated in response to cytochrome c. One possible mechanism is through phosphorylation of Apaf-1 by PKA; indeed, we found that purified GST-Apaf-1-(1–541) was phosphorylated directly when incubated with PKA (Fig. 8C).

**DISCUSSION**

The ubiquitous second messenger cyclic AMP regulates apoptosis in a diverse range of cells, but the mechanism by which this occurs has remained elusive. In many cell types, elevation of cAMP is associated with prevention of apoptosis. This report provides evidence that cAMP acts through PKA to inhibit a major apoptotic pathway at a point downstream of cytochrome c release from mitochondria. We have shown that PKA inhibits caspase-9 activation by Apaf-1 in response to cytochrome c. PKA phosphorylates purified caspase-9 at three sites, although PKA can strongly inhibit caspase-9 activation independently of caspase-9 phosphorylation.

Much of the previous focus of research on the regulation of apoptosis has been on the expression or activity of proteins of the Bcl-2 family, which can control the release of cytochrome c from mitochondria into the cytosol. The cAMP signal transduction pathway has been suggested to regulate apoptosis at this
stage by targeting the pro-apoptotic Bcl-2 family protein Bad, which is phosphorylated on certain serine residues by PKA. These phosphorylated residues cause sequestration of Bad by 14-3-3, with the resultant dissociation of Bad from its anti-apoptotic partner, Bcl-xL (34–38). However, expression of Bad is restricted to certain cell types, and Bad-deficient mice appear to display no developmental abnormality. In most cell types, cell death by growth factor withdrawal proceeds normally in the absence of Bad. Thus, Bad cannot account exclusively for the induction of apoptosis in response to loss of extracellular signals such as those operating through elevation of cAMP (39, 40).

Our results demonstrate that the cAMP signaling pathway acting through PKA can directly inhibit the mitochondrial apoptotic pathway downstream of cytochrome c. This role appears to be conserved in vertebrates. In Xenopus, PKA also plays roles in the cell division cycle during early embryonic development when it is periodically activated (41) and may play a role in the timing of M-phase through inhibition of Cdc25 phosphatase (42). Direct suppression of caspase-9 activation by

![Regulation of Caspase-9 Activation by Protein Kinase A](image)

**Fig. 6.** Inhibition of caspase-9 activation by PKA does not require direct phosphorylation of caspase-9. A, caspase-9 (WT, wild type) or caspase-9(AAA) (AAA) translated in vitro and incubated with active PKA catalytic subunit before addition of cytochrome c. Samples were analyzed for caspase-9 processing by SDS-PAGE and autoradiography. B, HeLa cytosolic extracts depleted of caspase-9 incubated with in vitro translated wild-type caspase-9 (WT) or caspase-9(AAA) (AAA) and PKA catalytic subunit as shown. Control incubations lacked cytochrome c or were without in vitro translated caspase-9. Caspase-3 activity induced by cytochrome c addition was assayed by measuring cleavage of DEVD-AMC. C, shows caspase-9 detected by Western blot in each of the samples assayed in B compared with S100 HeLa cytosolic extract prior to depletion.

![PKA inhibits apoptosis](image)

**Fig. 7.** PKA inhibits apoptosome assembly. A, HeLa cytosolic extract was incubated with or without cytochrome c. Samples were removed for Western blotting using rabbit polyclonal antibodies against Apaf-1 and caspase-9 (input). Caspase-9 was immunoprecipitated from the remainder of the incubation using a sheep polyclonal antibody to caspase-9, and immunoprecipitates (caspase-9 IP) were immunoblotted with antibodies to Apaf-1 or caspase-9 as indicated. A nonspecific band in the immunoprecipitates detected by the caspase-9 antibody migrates above processed caspase-9 at about 40 kDa. B, caspase-9 immunoprecipitates from incubations containing cytochrome c, H89, PKA, and db-cAMP (db-cAMP) were analyzed by Western blotting with antibodies to Apaf-1 or caspase-9.

![PKA does not block caspase-9 activation](image)

**Fig. 8.** PKA does not block caspase-9 activation by activated Apaf-1-(1–541) or Apaf-1 CARD. A, caspase-9 (WT, wild type) or caspase-9(AAA) (AAA) translated in vitro and incubated with active PKA catalytic subunit and cytochrome c or Apaf-1 CARD to induce caspase activation, analyzed by SDS-PAGE, autoradiography, and detection of processed caspase-9. B, caspase-9(AAA) translated in vitro and incubated with active PKA catalytic subunit and cytochrome c or differing amounts of GST-Apaf-1-(1–541) to induce caspase activation, analyzed by SDS-PAGE, autoradiography, and detection of processed caspase-9. C, phosphorylation of GST-Apaf-1-(1–541) by PKA using [γ-32P]ATP, detected by autoradiography.
PKA could therefore couple control of apoptosis with cell cycle progression at this stage of development. More generally, activation of PKA by cAMP elevation in response to extracellular signals may play an important role in the regulation of caspase-9 activation in somatic cells.

We have suggested previously that phosphorylation of caspase-9 at Thr-125 by ERK MAPK inhibits activation of the procaspase through inhibition of a conformational change induced by binding to Apaf-1 that activates the catalytic activity of caspase-9 (26). Inhibition of caspase-9 activation by PKA might therefore operate through a similar mechanism in which PKA would directly phosphorylate the proenzyme and prevent its activation. However, although we found that PKA does indeed phosphorylate purified caspase-9 at three serine residues, we also found that mutation of these residues to nonphosphorylatable alanine did not abolish the inhibition of caspase-9 activation by PKA in cell extracts. By contrast, mutation of Thr-125 significantly abrogates the inhibitory effect of ERK MAPK on caspase-9 activation (26). Although it remains possible that phosphorylation of one or more of the sites in caspase-9 that are targeted by PKA could play a role in its regulation, it is clear that PKA can operate through a distinct mechanism to prevent caspase-9 activation.

Importantly, we have found that PKA strongly inhibits the recruitment of procaspase-9 to Apaf-1 in response to cytochrome c. This step involves the oligimerization of Apaf-1 to form a large complex and then recruitment of procaspase-9 through interactions between the CARD domain of Apaf-1 and the structurally related prodomain of caspase-9. The inability of PKA to inhibit caspase-9 processing by constitutively activated Apaf-1-(1–541) or Apaf-1 CARD suggests that PKA would directly phosphorylate the proenzyme and prevent its activation. PKA could therefore couple control of apoptosis with cell cycle progression.

One obvious candidate substrate for PKA is Apaf-1 itself, and we have found that PKA is phosphorylated by PKA in vitro. To determine the possible function of Apaf-1 phosphorylation in the response to PKA activation, it will be necessary to identify the phosphorylation sites and to test their roles. Interestingly, Kornbluth and colleagues (43) have recently also provided evidence for the control of apoptosis formation and the phosphorylation of Apaf-1 in cell extracts in response to the Bcr-Abl fusion tyrosine kinase, although the mechanism is likely to be indirect, since no tyrosine phosphorylation of Apaf-1 was detected. Alternatively, phosphorylation of an as yet unidentified regulatory factor by PKA could control the activation of Apaf-1 and the recruitment of caspase-9. Elucidation of this novel level of control of the apoptosome is likely to be important in understanding the control of apoptosis by protein kinase signaling pathways.

Acknowledgement—We thank Prof. Seamus Martin (Trinity College, Dublin, Ireland) for advice on caspase-9 immunoprecipitation.

REFERENCES
1. Jacobson, M. D., Weil, M., and Raff, M. C. (1997) Cell 88, 347–354
2. Lowe, S. W., Cepedo, E., and Evan, G. (2004) Nature 432, 307–315
3. Fuentes-Prior, P., and Salvesen, G. S. (2004) Biochem. J. 384, 201–232
4. Yang, J., Liu, X., Bhatla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P., and Wang, X. (1997) Science 275, 1129–1132
5. Liu, X., Kim, C. N., Yang, J., Jemmerren, R., and Wang, X. (1996) Cell 86, 147–157
6. Klueck, R. M., Bosozy-Wetzel, E., Green, D. R., and Newmeyer, D. D. (1997) Science 275, 1132–1136
7. Zou, H., Li, Y., Liu, X., and Wang, X. (1999) J. Biol. Chem. 274, 11549–11556
8. Aebach, D., Jiang, X., Morgan, D. G., Heuser, J. E., Wang, X., and Akey, C. W. (2002) Mol. Cell 9, 423–432
9. Francis, S. H., and Corbin, J. D. (1994) Annu. Rev. Physiol. 56, 237–272
10. Taylor, S. S., Yang, J., Wu, J., Haste, M. N., Radzio-Andzelm, E., and Anand, G. (2004) Biochem. Biophys. Acta 1697, 259–269
11. de Roij, J., Zwarthuis, P. J., Verheijen, M. H., Cool, R. H., Nijman, S. M., Wittinghofer, A., and Bos, J. L. (1998) Nature 396, 474–477
12. Zhang, L., and Insel, P. A. (2004) J. Biol. Chem. 279, 20858–20865
13. Rossi, A. G., Cousin, J. M., Dransfield, I., Lawson, M. F., Chilvers, E. R., and Haslett, C. (1995) Biochem. Biophys. Res. Commun. 217, 892–899
14. Rossi, A. G., McCutcheon, J. C., Roy, N., Chilvers, E. R., Haslett, C., and Dransfield, I. (1998) J. Immunol. 160, 3562–3568
15. Orlov, S. N., Thorin-Trevesse, N., Dulin, N. O., Dam, T. V., Fortuno, M. A., Tremblay, J., and Hamet, P. (1999) Cell Death Differ. 6, 661–672
16. Yusta, B., Boushey, R. P., and Drucker, D. J. (2000) J. Biol. Chem. 275, 35345–35352
17. von Kneiden, A., and Brune, B. (2000) Mol. Cell. Biochem. 212, 35–43
18. Boucher, M. J., Duchesne, C., Laine, J., Morisset, J., and Rivard, N. (2001) Biochem. Biophys. Res. Commun. 285, 207–216
19. Martin, M. C., Dransfield, I., Haslett, C., and Rossi, A. G. (2001) J. Biol. Chem. 276, 45041–45050
20. Nishihara, H., Hwang, M., Kizaka-Kondoh, S., Eckmann, L., and Insel, P. A. (2004) J. Biol. Chem. 279, 26176–26183
21. Kwon, G., Poppain, K. L., Marshall, C. A., Schaffer, J. E., and McDaniel, M. L. (2004) J. Biol. Chem. 279, 8938–8945
22. Danial, N. H., and Korsmeyer, S. J. (2004) Cell 116, 205–219
23. Deveraux, Q. L., Takahashi, R., Salvesen, G. S., and Reed, J. C. (1997) Nature 388, 900–904
24. Shiozaki, E. N., and Shi, Y. (2004) Trends Biochem. Sci. 29, 486–484
25. Cardone, M. H., Roy, N., Stennicke, H. R., Salvesen, G. S., Franke, T. F., Stanbridge, E., Frusch, S., and Reed, J. C. (1998) Science 282, 1318–1321
26. Allan, L. A., Merrice, N., Bradly, S., Magee, G., Pathaki, S., and Clarke, P. R. (2003) Nat. Cell Biol. 5, 647–654
27. Dan, H. C., Sun, M., Kaneko, S., Feldman, R. I., Nicosia, S. V., Wang, H. G., Tsang, B. K., and Cheng, J. Q. (2004) J. Biol. Chem. 279, 25862–25869
28. Tan, Y., Demeter, M. R., Ruan, H., and Comb, M. J. (2000) J. Biol. Chem. 275, 25860–25869
29. Zhou, X. M., Liu, Y., Payne, G., Lutz, R. J., and Chittenden, T. (2000) J. Biol. Chem. 275, 25046–25051
30. Virdee, R., Parone, P. A., and Tolkovsky, A. M. (2000) Curr. Biol. 10, 1151–1154
31. Datta, S. R., Ranger, A. M., Lin, M. Z., Sturgill, J. P., Ma, Y. C., Cowan, C. W., Dikkes, P., Korsmeyer, S. J., and Greenberg, M. E. (2002) Dev. Cell 3, 631–643
32. Ranger, A. M., Zha, J., Harada, H., Datta, S. R., Daniel, N. N., Gilmore, A. P., Kurok, J. L., LeBeau, M. M., Greenberg, M. E., and Korsmeyer, S. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9324–9329
33. Grecco, D., Porcellini, A., Avvedimento, E. V., and Gottesman, M. E. (1996) Science 271, 1718–1723
34. Duckworth, B. C., Weaver, J. S., and Ruderman, J. V. (2003) Proc. Natl. Acad. Sci. U. S. A. 99, 10784–10789
35. Deming, P. B., Schafer, Z. T., Tashker, J. S., Potts, M. B., Deshmukh, M., and Kornbluth, S. (2004) Mol. Cell. Biol. 24, 10289–10299