Differential Involvement of MEK Kinase 1 (MEKK1) in the Induction of Apoptosis in Response to Microtubule-targeted Drugs versus DNA Damaging Agents

Spencer Gibson, Christian Widmann, and Gary L. Johnson

MEK kinase 1 (MEKK1) is a 196-kDa enzyme that is involved in the regulation of the c-Jun N-terminal kinase (JNK) pathway and apoptosis. In cells exposed to genotoxic agents including etoposide and cytosine arabinoside, MEKK1 is cleaved at Asp874 by caspases. The cleaved kinase domain of MEKK1, itself, stimulates caspase activity leading to apoptosis. Kinase-inactive MEKK1, expressed in HEK293 cells, effectively blocks genotoxin-induced apoptosis. Treatment of cells with taxol, a microtubule stabilizing agent, did not induce MEKK1 cleavage in cells, and kinase-inactive MEKK1 expression failed to block taxol-induced apoptosis. MEKK1 became activated in HEK293 cells exposed to taxol, but in contrast to etoposide-treatment, taxol failed to increase JNK activity. Taxol treatment of cells, therefore, dissociates MEKK1 activation from the regulation of the JNK pathway. Overexpression of anti-apoptotic Bcl2 blocked MEKK1 and taxol-induced apoptosis but did not block the caspase-dependent cleavage of MEKK1 in response to etoposide. This indicates Bcl2 inhibition of apoptosis is, therefore, downstream of caspase-dependent MEKK1 cleavage. The results define the involvement of MEKK1 in the induction of apoptosis by genotoxins but not microtubule altering drugs.

Apoptosis is a cell death process important in development and tissue homeostasis (1, 2). Dysregulation of apoptosis contributes to human diseases including cancer (1). The treatment of many cancers involves chemotherapeutic drugs that are genotoxins (damage DNA) and microtubule-interfering toxins (3–5). Examples of each are etoposide, a topoisomerase inhibitor that functions as an effective genotoxin (4), and taxol, a microtubule stabilizer that effectively arrests cells in the G2/M phase of the cell cycle (7). Both genotoxins and microtubule-interfering drugs induce apoptosis in tumor cells (6, 8–11).

It is generally believed that the caspase cleavage of specific proteins results in the irreversible commitment of cells to undergo apoptosis (12–14). Caspases are regulated in part by the anti-apoptotic protein, Bcl2 (15). Overexpression of Bcl2 inhibits the activation of caspases and blocks genotoxin and taxol-induced apoptosis (13, 15–17). The anti-apoptotic action of Bcl2 is believed to involve its interaction with Apaf1, the Ced4 homologue that binds to and controls the activation of caspase-9 (18–20). Alternatively, Bcl2 interaction with Bax prevents the pro-apoptotic action of Bax by forming Bcl2-Bax dimer (15).

We have recently demonstrated that caspases turn off survival signals in addition to activating death signals (21). MEKK1 is among the signal transduction proteins cleaved by caspases early in the apoptotic response (21, 22). Caspase-3, also referred to as CPP32, cleaves MEKK1 at Asp874, releasing a COOH-terminal 91-kDa fragment that encodes the MEKK1 kinase domain (22). Transient overexpression of MEKK1 results in the cleavage of MEKK1 generating the 91-kDa kinase fragment. The cleavage of MEKK1 and activation of its kinase activity leads further activation of caspases. Kinase inactive MEKK1 effectively reduces caspase activation induced by genotoxins (22). This suggests that MEKK1 cleavage is involved in the amplification of caspases and thus contributes to apoptosis.

Herein, we show that kinase inactive MEKK1 blocks etoposide-induced apoptosis but fails to block microtubule-interfering toxin-induced apoptosis. Treatment with etoposide resulted in cleavage of MEKK1 that was not detected following taxol or vinblastine treatment of cells. Thus, MEKK1 contributes to genotoxin-induced apoptosis, whereas microtubule-interfering toxins induce apoptosis independent of MEKK1 cleavage. Our findings illustrate the existence of two different signaling pathways, one responsive to DNA damage and the other to microtubule toxins that differentially involve caspase-dependent MEKK1 cleavage.

EXPERIMENTAL PROCEDURES

Materials—1-β-D-Arabinofuranosylcytosine (Ara-c), etoposide, taxol, and vinblastine were purchased from Sigma. Ara-c was dissolved in phosphate-buffered saline, whereas etoposide, taxol, and vinblastine were dissolved in dimethyl sulfoxide (Me2SO). Anti-MEKK1 antisera (c-22) was purchased from Santa Cruz, and anti-poly(A) ribose polymerase (PARP) antisera was purchased from Upstate Biotechnology.

Cells—Jurkat cells were cultured in RPMI 1640 medium (Life Technologies Inc.) supplemented with 100 units/ml penicillin/streptomycin (Gemini Bio-Products) and containing 10% fetal bovine serum (Summit Biotechnology) (RPMI-c). Human embryonic kidney 293 cells (HEK293) were grown in Dulbecco’s modified Eagle’s medium (Life Technologies Inc.) supplemented with 100 units/ml penicillin/streptomycin (Gemini Bio-Products) and containing 10% bovine calf serum (HyClone).

Immunoblots—Jurkat cells were incubated with 10 μM Ara-c, 10 μM etoposide, 10 μM taxol, or 1 μM vinblastine. HEK293 cells were incubated...

The abbreviations used are: MEKK1, MEK kinase 1; JNK, c-Jun N-terminal kinase; SEKI 1-K, inactive stress-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase 1; Ara-c, 1-β-D-arabinofuranosylcytosine; GST, glutathione S-transferase; MAPK, mitogen-activated protein kinase; DEVE-AMC, Asp-Glu-Val-Glu-7-amino-4-methyl coumarin.
bated with 100 μM etoposide or 10 μM taxol. Lower concentrations showed reduced apoptosis in a dose-response curve (data not shown). The cells were lysed as described previously (29).

Measurement of Caspase Activities—Cells were lysed in 50 mM Tris, pH 7.4, 1 mM EDTA, 10 mM EGTA, and 10 μM digitonin for 10 min at 37 °C. Lysates (120 μg) were incubated with 5 μM DEVD-AMC (Bachem) in 1 ml of 50 mM Tris, pH 7.4, 1 mM EDTA, 10 mM EGTA for 30 min at 37 °C. Fluorescence was monitored with an excitation wavelength of 380 nm and an emission wavelength of 460 nm.

Measurement of Apoptosis—Jurkat cells (1–2×10^6) were resuspended in 100 μl of incubation buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 5 mM CaCl_2) containing 1 μg/ml propidium iodide (Sigma) and a dilution of 1:50 Annexin V-Flos solution (Roche Molecular Biochemicals) and incubated on ice for 15 min. Four hundred μl of incubation buffer was then added, and the cells were sorted on a flow cytometer using 488-nm excitation and a 515-nm band-pass filter for fluorescence detection and a filter >560 nm for propidium iodide detection. Apoptotic cells were defined as green fluorescent positive and propidium iodide negative. HEK293 cells were stained with acridine orange (100 μM) and ethidium bromide (100 μM) in phosphate-buffered saline as described previously (22). The percentage of apoptotic cells was determined from cells containing normal DNA staining compared with cells with condensed DNA.

MEKK1 Involvement in Apoptosis—In HEK293 cells, the genotoxic etoposide, but not the microtubule toxin taxol, induced the cleavage of the 196-kDa MEKK1 protein at concentrations that maximize the apoptotic response indicated by the loss of full-length MEKK1 (Fig. 1A). The cleavage fragments of endogenous MEKK1 were not detected consistent with the other cleaved proteins in HEK293 cells (21, 22). Indeed, total 196-kDa MEKK1 protein levels actually increased in response to taxol over the same 48-h time course where etoposide treatment caused a loss of MEKK1 protein. Differences in MEKK1 protein levels was not because of differences in general protein amounts because protein levels of MAPK phosphatase 1 (MKP1), that is not cleaved in response to apoptotic stimuli,...
remained similar in HEK293 cells following treatment with taxol or etoposide. Caspase activation and apoptotic response by taxol, however, was similar in magnitude to that for etoposide (Fig. 1B). A second microtubule-disrupting agent, vinblastine, also failed to induce cleavage of full-length MEKK1 in HEK293 cells (data not shown) but induced caspase activity and an apoptotic response similar to taxol and etoposide (Fig. 1B).

The difference between DNA-damaging drugs and microtubule toxins inducing MEKK1 cleavage was also observed in Jurkat cells. The DNA-damaging drugs Ara-c and etoposide induce MEKK1 cleavage, shown by loss of the 196-kDa enzyme (Fig. 2A), activation of caspases, and apoptosis (Fig. 2B); however, Ara-c was less effective at inducing caspase activity and apoptosis as compared with etoposide. We have demonstrated previously that MEKK1 cleavage is caspase-dependent (22). In contrast, taxol and vinblastine treatment of Jurkat cells resulted in an initial increase in MEKK1 expression similar to that observed with HEK293 cells. Over time, MEKK1 protein levels in response to taxol diminished to that or somewhat lower levels from that of control cells. This contrasts to HEK293 cells where MEKK1 protein remained elevated following exposure of cells to taxol. Taxol and vinblastine stimulated caspase activity and induced apoptosis in Jurkat cells (Fig. 2B) similar to that observed in HEK293 cells. These findings demonstrate a difference in the cleavage and loss of the 196-kDa MEKK1 enzyme during the apoptotic response of two cell types to DNA damage or microtubule poisoning.

Although MEKK1 is not significantly proteolized in response to taxol or vinblastine, other known caspase substrates were cleaved in response to these drugs. PARP is a well-characterized caspase substrate that is cleaved early in the apoptotic response (24). Both the genotoxins (Ara-c and etoposide) and microtubule toxins (taxol and vinblastine) induced PARP cleavage (Fig. 2A) corresponding to increased caspase activity and apoptosis (Fig. 2B) in Jurkat cells. Similar results were found in HEK293 cells (data not shown). The findings indicated that, in contrast to PARP, the degradation of MEKK1 is differen-
tially regulated by DNA damage versus microtubule poisoning.

We have demonstrated that HEK293 cells that express a kinase-inactive mutant of MEKK1 have a significantly reduced apoptotic response to genotoxins such as etoposide (22). Treatment of HEK293 cells expressing the kinase-inactive inhibitory mutant of MEKK1 with microtubule toxins gave an apoptotic response similar to control HEK293 cells, measured by acridine orange (Fig. 3A) or propidium iodide staining (Fig. 3B). In contrast, etoposide-induced apoptosis was significantly inhibited \((p < 0.05)\) in the HEK293 cells expressing kinase-inactive MEKK1 relative to control cells (Fig. 3).

Morphological analysis substantiates the differences in apoptotic potential of cells expressing kinase-inactive MEKK1 (Fig. 3C). In control cells, transfected with vector alone, treatment with etoposide, taxol, or vinblastine caused membrane blebbing and cell rounding. In cells expressing the mutant kinase-inactive MEKK1 protein, the morphological changes induced by etoposide but not taxol or vinblastine were inhibited. This finding demonstrates that kinase-inactive MEKK1 inhibits morphological changes associated with apoptosis induced by etoposide but not taxol or vinblastine. The morphological findings are consistent with the biochemical results that MEKK1 cleavage does not significantly contribute to microtubule toxin-induced apoptosis.

**Dissociation of Taxol-stimulated MEKK1 Activity and JNK Activation**—MEKK1 has been shown to regulate JNK activity in a variety of cell types (25–28). Exposure of HEK293 cells to taxol-activated MEKK1 (Fig. 4A), as measured by MEKK1 autophosphorylation and MEKK1 catalyzed phosphorylation of recombinant SEK1 (22, 23, 25, 28, 29). Etoposide activates MEKK1 to an extent similar to that of taxol (Fig. 4A). However, taxol treatment of HEK293 cells did not significantly activate JNK (Fig. 4B); etoposide in the same experiment activated JNK. Jurkat cells gave a similar result where etoposide and taxol activated MEKK1 (data not shown), but only etoposide significantly activated JNK (Fig. 4B). In human breast carcino-
noma epidermal T47D cells and mouse embryonic stem cells that we have similarly characterized, taxol activates the JNK pathway but to a significantly lower magnitude than other stress stimulants including DNA damaging drugs, irradiation, and microtubule-disrupting drugs such as nocodazole (data not shown). Thus, taxol stabilization of microtubules appears to significantly dissociate the activation of MEKK1 from the JNK pathway.

**Bcl2 Blocks Apoptosis Downstream of MEKK1 Cleavage**—An indicator of microtubule toxin treatment is the phosphorylation of Bcl2 (30–33) because of either interference with microtubule structure or blockage of cells in the G2/M checkpoint of the cell cycle. In HEK293 cells, taxol and vinblastine but not etoposide stimulated the phosphorylation of Bcl2 as characterized by the shift in mobility by SDS-polyacrylamide gel electrophoresis of the phosphorylated Bcl2 species (Fig. 5A). Expression of the mutant kinase-inactive MEKK1, which suppressed etoposide but not taxol-induced apoptosis (Fig. 3), had no effect on taxol or vinblastine-stimulated Bcl2 phosphorylation (Fig. 5A). In addition to Bcl2 phosphorylation, taxol also activates Cdc2 kinase activity that has been proposed to be involved in taxol-induced apoptosis (34). Expression of the kinase-inactive MEKK1 failed to inhibit taxol-induced Cdc2 kinase activity as assessed by Cdc2 phosphorylation of histone (data not shown). Thus MEKK1 is not upstream of either Cdc2 kinase or Bcl2 phosphorylation.

Stable overexpression of Bcl2 in HEK293 cells effectively inhibited apoptosis and caspase activation in response to taxol (Fig. 5, B and C). Bcl2 overexpression also inhibited etoposide-induced apoptosis (not shown). Interestingly, Bcl2 overexpression also inhibited apoptosis induced by overexpression of full-length MEKK1 or the 91-kDa MEKK1 kinase fragment (Fig. 5D). The increased caspase activity observed with MEKK1 expression was also inhibited by Bcl2 overexpression (Fig. 5E). The findings demonstrate that Bcl2 inhibits MEKK1-dependent and independent apoptosis in HEK293 cells.

Surprisingly, in Bcl2 overexpressing HEK293 cells, exposure to etoposide induced the cleavage and loss of endogenous MEKK1. In contrast, the cleavage of Cbl or PARP, both caspase substrates, was inhibited by Bcl2 overexpression (21, 24) (Fig. 6A). The cleavage of MEKK1 was dependent on caspases in control and Bcl2 overexpressing cells because p35, the baculovirus inhibitor of caspases (35), blocked MEKK1 cleavage. These finding suggest that Bcl2 blocks MEKK1-induced apoptosis and that Bcl2 functions downstream of MEKK1 in the pathway leading to MEKK1-dependent apoptosis.

**DISCUSSION**

Because of their different mechanisms of action, genotoxins and microtubule-interfering toxins are used in combination therapies for the treatment of several different cancers (1, 9, 11). Both genotoxins and microtubule toxins generally induce death of cells by apoptosis (8). Based on the markedly different mechanisms of action, the pro-apoptotic pathways regulated by...
genotoxins and microtubule toxins to initiate apoptosis are predicted to be different. Our findings demonstrate that both types of chemotherapeutic drugs (22) activate MEKK1. Strikingly, only genotoxins like etoposide, and not the microtubule toxins taxol or vinblastine, significantly induce the cleavage of MEKK1 even though treatment of cells with each drug activates the kinase activity of 196-kDa MEKK1 (data not shown). Thus, genotoxins and microtubule drugs selectively and differentially regulate the cleavage of specific caspase substrates. These results define the differential regulation of caspase-dependent cleavage of proteins during the apoptotic response to clinically relevant chemotherapeutic drugs. A likely explanation for this observation is that either MEKK1 or the caspase that cleaves MEKK1 is differentially localized in the cell with etoposide versus taxol treatment. The caspase that cleaves MEKK1 in response to etoposide treatment is unable to cleave MEKK1 in taxol-treated cells even though other proteins including Cbl and PARP are cleaved.

Several reports suggest that taxol-induced apoptosis is because of JNK activation. In OVCA 420 cells, expression of MEKK1 kinase inactive mutant effectively blocked JNK activity and blocked taxol-induced apoptosis (36–38). In contrast, both Jurkat and HEK293 cells activate MEKK1 activity following taxol treatment but failed to activate JNK. In addition, expression of the kinase inactive MEKK1 in HEK293 cells failed to block taxol-induced apoptosis. Murine embryonic stem cells activate JNK following treatment with the microtubule toxin nocodazole. This response is absent in embryonic stem cells lacking MEKK1; however, the cells still undergo apoptosis (38). Thus JNK activation in response to taxol is not required to induce apoptosis.

Overexpression of Bcl2 did not block the caspase-dependent cleavage of MEKK1. In contrast, cleavage of Cbl and PARP was inhibited. Western blotting of caspase-3 in Bcl2 overexpressing cells indicates that caspase 3 is activated determined by the loss of the inactive procaspase form of caspase 3 following etoposide treatment. Caspase-9 activation, however, was blocked by overexpression of Bcl2. Taken together, the data show that cleavage of MEKK1 possibly by caspase 3 in the absence of Bcl2 overexpression does not induce apoptosis. Thus, the ability of Bcl2 overexpression to block MEKK1-induced apoptosis, and amplification of caspase activity implies that caspases activated in response to MEKK1 activation and

\[ \text{S. Gibson, C. Widmann, and G. L. Johnson, unpublished observation.} \]
subsequent cleavage are functionally downstream of Bcl2.

Our results show that altered regulation of Bcl2, Cdc2 kinase activity, or related regulatory proteins in response to microtubule toxins effectively bypasses the role of MEKK1 in amplifying caspase activation. The different apoptotic signaling pathways for etoposide and taxol probably contribute to their greater effectiveness in chemotherapy in combination than either alone.

The importance of these findings is that we have identified for the first time the differential involvement of a pro-apoptotic kinase, activated 91-kDa MEKK1, in chemotherapy-induced apoptosis. Thus, we have defined a kinase whose regulation influences the action of specific classes of chemotherapeutic drugs. This implies that it will be possible to define pro- and anti-apoptotic components of the cell death pathway, such as MEKK1, that selectively regulate the apoptotic potential of specific drugs. In the future, it may be possible to alter the activity of such kinases to enhance the apoptotic potential of tumor cells. Such advances in apoptotic signal transduction therapy will allow chemotherapy and anti-angiogenic therapy to have significantly greater efficacy in ablating human tumors.

REFERENCES

1. Reed, J. C. (1998) *Cancer J. Sci. Am.* 4, Suppl. 1, S8–S14
2. Kuan, N. K., and Passaro, E., Jr. (1998) *Arch. Surg.* 133, 773–775
3. Bhalla, K., Ibrado, A. M., Tourkina, E., Tang, C., Mahoney, M. E., and Huang, Y. (1998) *Leukemia* 7, 563–568
4. Jensen, P. B., and Sehested, M. (1997) *Biochem. Pharmacol.* 54, 755–759
5. Jones, N. A., Turner, J., McIver, A. J., Brown, R., and Dive, C. (1998) *Mol. Pharmacol.* 53, 819–826
6. Capranico, G., Giaccone, G., Zunino, F., Garattini, S., and D’Incalci, M. (1997) *Cancer Chemother. Biol. Response Modif. Annu.* 17, 114–131
7. Donaldson, K. L., Goolsby, G. L., Kiener, P. A., and Wahl, A. F. (1994) *Cell Growth Diff.* 5, 1041–1050
8. Lokich, J., Anderson, N., Moore, C., Bern, M., Coco, F., Sonneborn, H., Dow, E., and Strong, D. (1998) *Eur. J. Cancer* 34, 664–667
9. Staunton, M. J., and Galfrey, E. F. (1998) *Arch. Pathol. Lab. Med.* 122, 310–319
10. Zaffaroni, N., Silvestrini, R., Orlandi, L., Bearzatto, A., Gornati, D., and Villa, R. (1998) *Br. J. Cancer* 77, 1378–1385
11. Currea, P., and Miller, M. J. (1998) *Br. Med. Bull.* 54, 151–162
12. Thornberry, N. A., and Lazebnik, Y. (1998) *Science* 281, 1312–1316
13. Allen, R. T., Cluck, M. W., and Agrawal, D. K. (1998) *Cell Mol. Life Sci.* 54, 427–445
14. Cyrys, V., and Yuan, J. (1998) *Genes Dev.* 12, 1551–1570
15. Chao, D. T., and Korsmeyer, S. J. (1998) *Ann. Rev. Immunol.* 16, 395–419
16. Ibrado, A. M., Huang, Y., Fang, G., and Bhalla, K. (1998) *Cell Growth Diff.* 7, 1087–1094
17. Ibrado, A. M., Liu, L., and Bhalla, K. (1997) *Cancer Res.* 57, 1109–1115
18. Pan, G., O’Rourke, K., and Dixit, V. M. (1998) *J. Biol. Chem.* 273, 5841–5845
19. Zou, H., Henzel, W. J., Liu, X., Lutschg, A., and Wang, X. (1997) *Cell* 90, 405–413
20. Vaux, D. L. (1997) *Cell* 90, 389–390
21. Widmann, C., Gibson, S., and Johnson, G. L. (1999) *J. Biol. Chem.* 273, 7141–7147
22. Widmann, C., Gerwins, P., Johnson, N. L., Jarpe, M. B., and Johnson, G. L. (1999) *Mol. Cell. Biol.* 18, 2416–2429
23. Johnson, N. L., Gardner, A. M., Diener, K. M., Lange-Carter, C. A., Gleavy, J., Jarpe, M. B., Minden, A., Karin, M., Zon, L. I., and Johnson, G. L. (1996) *J. Biol. Chem.* 271, 3229–3237
24. Gu, Y., Sarnecki, C., Aldape, R. A., Livingston, D. J., and Su, M. S.-S. (1995) *Cell Growth Diff.* 7, 67–74
25. Fanger, G. R., Gerwins, P., Widmann, C., Jarpe, M. B., and Johnson, G. L. (1997) *J. Biol. Chem.* 272, 18715–18718
26. Fanger, G. R., Gerwins, P., Widmann, C., Jarpe, M. B., and Johnson, G. L. (1997) *Carr. Optin. Genet. Dev.* 7, 67–74
27. Karin, M., and Delhase, M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 9067–9069
28. Lange-Carter, C. A., Pleiman, C. M., Gardner, A. M., Blumer, K. J., and Johnson, G. L. (1998) *J. Biol. Chem.* 273, 5841–5845
29. Fanger, G. R., Johnson, N. L., and Johnson, G. L. (1997) *EMBO J.* 16, 4961–4972
30. Widmann, C., Jarpe, M. B., and Johnson, G. L. (1997) *Cancer Chemother. Biol. Response Modif. Annu.* 17, 563–568
31. Kroning, R., and Lichtenstein, A. (1998) *Leukemia* 12, S8–S14
32. Huang, Y., and Strong, D. (1998) *Annu. Rev. Immunol.* 16, 4507–4511
33. Shen, S. C., Huang, T. S., Jee, S. H., and Kuo, M. L. (1998) *Cell. Biol. Growth Differ.* 9, 23–29
34. Gong, M., and Guarino, L. A. (1994) *Science* 260, 1001–1004
35. Guo, Y., Sarnecki, C., Aldape, R. A., Livingston, D. J., and Su, M. S.-S. (1995) *J. Biol. Chem.* 270, 18715–18718
36. Fanger, G. R., Gerwins, P., Widmann, C., Jarpe, M. B., and Johnson, G. L. (1997) *Carr. Optin. Genet. Dev.* 7, 67–74
37. Karin, M., and Delhase, M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 9067–9069
38. Lange-Carter, C. A., Pleiman, C. M., Gardner, A. M., Blumer, K. J., and Johnson, G. L. (1998) *Science* 281, 315–319
39. Fanger, G. R., Johnson, N. L., and Johnson, G. L. (1997) *EMBO J.* 16, 4961–4972
40. Widmann, C., Jarpe, M. B., and Johnson, G. L. (1997) *Cancer Chemother. Biol. Response Modif. Annu.* 17, 563–568
41. Kroning, R., and Lichtenstein, A. (1998) *Leukemia* 12, 227–236
42. Haldar, S., Jena, N., and Croce, C. M. (1997) *Arch. Pathol. Lab. Med.* 121, 1911–1914
43. Shen, S. C., Huang, T. S., Jee, S. H., and Kuo, M. L. (1998) *Cell. Biol. Growth Differ.* 9, 23–29
44. Gong, M., and Guarino, L. A. (1994) *Virology* 204, 38–44
45. Lee, L.-F., Li, G., Templeton, D. J., and Ting, J. P.-Y. (1998) *J. Biol. Chem.* 273, 28253–28260
46. Wang, T. H., Wang, H. S., Ichijo, H., Giannakakou, P., Foster, J. S., Fojo, T., and Wimalasena, J. (1998) *J. Biol. Chem.* 273, 4928–4936
47. Yujiri, T., Sather, S., Fanger, G. R., and Johnson, G. L. (1998) *Science* 282, 1911–1914