0026-895X/91/0697-05$03.00/0

MOLECULAR PHARMACOLOGY, 39:697-701

ACCELERATED COMMUNICATION

Activation of the c-*jun* Protooncogene in Human Myeloid Leukemia Cells Treated With Etoposide

ERIC RUBIN, SURENDER KHARBANDA, HISATO GUNJI, and DONALD KUFE

Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

Received January 22, 1991; Accepted March 19, 1991

SUMMARY

The epipodophyllotoxin etoposide is an inhibitor of topoisomerase II. The effects of this agent on gene expression, particularly the transcriptional induction of genes implicated in growth control, are unknown. The present results demonstrate that etoposide induces expression of the c-*jun* protooncogene in HL-60 myeloid leukemia cells. This induction of c-*jun* expression was maximal at 3 hr and was transient. Similar findings were obtained in the human U-937 myeloid leukemia cell line. Nuclear run-on assays demonstrated that the induction of c-*jun* expression by etoposide is regulated at the transcriptional level. The results further demonstrate that etoposide-induced c-*jun* expression occurs in association with the appearance of c-*fos* transcripts. Moreover, the c-*jun* gene is induced by etoposide during periods of oligonucleosomal DNA cleavage, which is characteristic of programmed cell death. These findings suggest that transcriptional induction of c-*jun* expression represents a signaling pathway activated in the cellular response to etoposide-induced DNA damage.

DNA topoisomerase activity and the resultant alterations in supercoiled DNA are necessary for replication and gene transcription (1, 2). Topoisomerase II is the target of several antitumor agents, including the semisynthetic podophyllotoxin etoposide (1, 2). Treatment of mammalian cells with this agent is associated with the formation of DNA strand breaks (3). These breaks are induced as a result of cleavable complex formation between etoposide, topoisomerase II, and the 5′ terminus of the DNA strand (4, 5). Previous studies have demonstrated a correlation between etoposide-induced DNA double-strand breaks and cytotoxicity (6). The finding that these breaks are rapidly repaired in the absence of drug has suggested that additional cellular events may contribute to etoposide-induced lethality. In this context, etoposide and the other topoisomerase II inhibitors induce cell cycle arrest in G_2 phase (7, 8). However, the precise mechanisms by which etoposide induces cell lethality remain unclear.

Little is known about the effects of etoposide on the transcriptional regulation of specific genes, particularly those involved in growth control. However, recent studies have demonstrated that other cytotoxic agents activate transcription of the c-*jun* immediate early response gene (9, 10). The c-*jun* immediate early response gene is rapidly activated during stimulation of cell proliferation by growth factors and phorbol esters (11–15). The c-*jun* gene codes for the major form of the AP-1 transcription factor and is a member of a multigene family that includes *jun*-B and c-*fos* (reviewed in Refs. 16–18). The products of this family form dimers through leucine zipper motifs, while a basic domain binds to the DNA consensus sequence $TGA^G/_CTCA$ (16–18). DNA binding affinity is higher for c-Jun and c-Fos heterodimers than that of c-Jun homodimers (19, 20). The c-Jun product increases transcription of the c-*jun* gene by an autoregulatory mechanism (21). This nuclear factor also activates the transcription of a variety of other genes, including metallothionein (22).

The present results demonstrate that etoposide treatment is associated with increases in c-*jun* expression. This effect is regulated by activation of c-*jun* gene transcription. The finding that the induction of c-*jun* expression by etoposide is associated with internucleosomal DNA cleavage suggests that this agent may activate a programmed cellular response to DNA damage.

Materials and Methods

Cell culture. HL-60 human myeloid leukemia cells (American Type Culture Collection) were grown at 37° in RPMI 1640 medium containing 15% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1 mM nonessential amino acids. U-937 monoblastic leukemia cells (American Type Culture Collection) were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Cells in

This investigation was supported by United States Public Health Service Grants CA-29431 and CA-42802, awarded by the National Cancer Institute, Department of Health and Human Services.

ABBREVIATION: kb, kilobases.

logarithmic growth phase (0.5–1.0 × 10^6 ml) were treated with varying concentrations of etoposide.

RNA isolation and Northern blot hybridization. Total cellular RNA was isolated by a modification of the guanidine isothiocyanate technique, as described (15). RNA (20 μg/lane) was separated by electrophoresis in 1% agarose/2.2 M formaldehyde gels, transferred to a nitrocellulose filter, and hybridized to the following ^{32}P-labeled DNA probes: 1) the 1.8-kb *Bam*HI/*Eco*RI insert of a human c-*jun* DNA probe containing a 1.0-kb cDNA and 0.8-kb 3′ untranslated sequences purified from a pBluescript SK(+) plasmid (23); 2) the pA1 plasmid containing a 2.0-kb *Pst*I insert of the chicken β-actin gene (24); and 3) the 0.9-kb *Sca*I/*Nco*I c-*fos* DNA consisting of exons 3 and 4 (25). Hybridizations were performed as described (15). The filters were washed and exposed to Kodak X-Omat XAR film using an intensifying screen. Autoradiograms were scanned using a laser densitometer.

Nuclear run-on assay. Labeled nuclear RNA was prepared from 10^8 HL-60 cells, as described (15), and hybridized to the following digested DNAs: 1) the 2.0-kb *Pst*I fragment of the chicken β-actin gene (24) and 2) the 1.8-kb *Bam*HI/*Eco*RI fragment of the human c-*jun* cDNA (23). The digested DNAs were run in 1% agarose gels and transferred to nitrocellulose filters. Prehybridization of the filters and hybridization to the ^{32}P-labeled nuclear RNA were performed as described (15).

Analysis of DNA. Cells were washed twice with phosphate-buffered saline and resuspended in 20 ml of 50 mM Tris·HCl (pH 8.0), 10 mM EDTA, 0.5 mg/ml proteinase K (Sigma Chemical Co., St. Louis, MO). After incubation at 50° for 1 hr, 10 ml of 0.5 mg/ml RNase A were added for an additional 1 hr. The samples were mixed with 10 ml of 10 mM EDTA (pH 8.0) containing 1% (w/v) low-melting point agarose, 0.25% bromophenol blue, and 40% sucrose, at 70°. The DNA was separated in 2% agarose gels and visualized by UV illumination after ethidium bromide staining.

Clonogenic survival. Cells were exposed to etoposide for various intervals up to 6 hr and were collected by centrifugation. After three washes with ice-cold phosphate-buffered saline, the cells were seeded (10^3/35-mm dish) in culture medium and 0.3% agarose on a base layer of 0.5% agarose. The cells were incubated, and colonies of >50 cells were counted after 7 days.

Results

HL-60 cells were exposed to varying concentrations of etoposide to determine the effects of this agent on growth. Treatment with etoposide at 0.3 μg/ml for 24 hr was associated with a partial decrease in cell number, compared with untreated cells (Fig. 1). Similar 24-hr exposures to 30 μg/ml resulted in nearly complete cessation of growth (Fig. 1). There was little detectable loss of cell viability, as determined by trypan blue exclusion, at 24 hr whereas 48-hr exposures were associated with lethality. Cells were also exposed to 30 μg/ml etoposide for varying intervals, washed, and seeded in agar. A 1-hr exposure to this agent was associated with a 98% decrease in colony formation, whereas treatment for 3 hr resulted in complete loss of clonogenicity.

In order to determine whether the antiproliferative effects of etoposide were associated with changes in c-*jun* gene expression, we performed Northern analyses of HL-60 cells treated with varying concentrations of etoposide for 3 hr. There were low to undetectable levels of c-*jun* transcripts in untreated HL-60 cells (Fig. 2). However, treatment with 0.3–300 μg/ml etoposide resulted in an increase in c-*jun* mRNA levels relative to untreated cells (Fig. 2). A more pronounced effect was observed at the higher dose levels (Fig. 2). In contrast, actin transcripts were decreased after treatment for 3 hr. Additional experiments utilizing cells treated for intervals from 1 to 24 hr demonstrated a maximal increase in c-*jun* mRNA levels at 3 hr (Fig. 3). Longer exposures were associated with a decline in these transcripts (Fig. 3).

Nuclear run-on experiments were performed to determine whether the increase in c-*jun* mRNA levels was related to an increase in transcription. There was no detectable change in constitutive actin gene transcription after treatment of HL-60 cells with 30 μg/ml etoposide for 3 hr (Fig. 4). In contrast, the rate of c-*jun* gene transcription was at low to undetectable levels in untreated HL-60 cells and increased after exposure to etoposide (Fig. 4). Similar findings were obtained in three separate experiments. These data indicate that the change in c-*jun* expression after etoposide treatment is at least in part related to an increase in transcription.

In order to determine whether the effects of etoposide on c-*jun* expression were limited to HL-60 cells, similar experiments

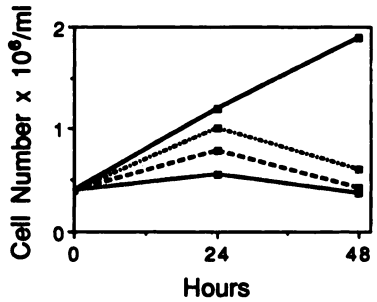

Fig. 1. Effects of etoposide on HL-60 cell growth. HL-60 cells were cultured in the presence of 0 (——), 0.3 (-·-), 3 (— —), or 30 μg/ml (·····) etoposide. Viable cell number was determined at the indicated times by trypan blue exclusion. Viability at 24 hr was >90%. At 48 hr, the viability was >90%, 75%, and 74% at 0.3, 3, and 30 μg/ml, respectively.

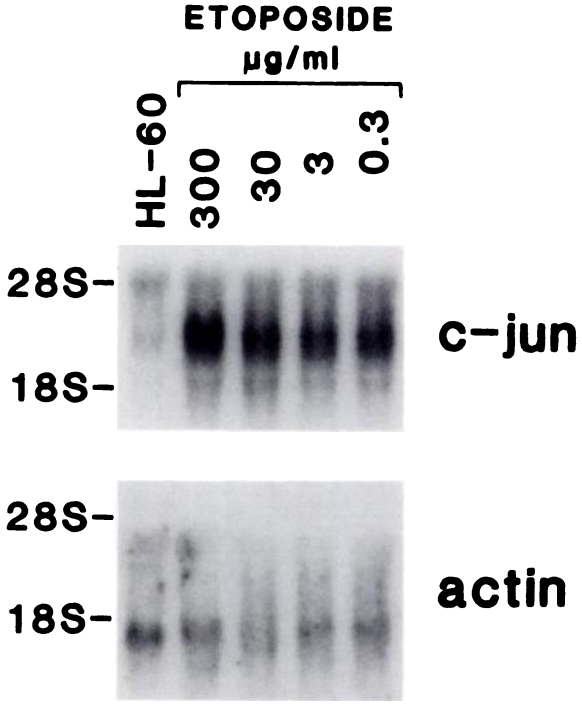

Fig. 2. Effects of etoposide on c-*jun* and actin mRNA levels. HL-60 cells were treated with the indicated concentrations of etoposide for 3 hr. Total cellular RNA (20 μg/lane) was isolated for Northern blot analysis, with hybridization to ^{32}P-labeled c-*jun* and actin DNA probes. *HL-60*, RNA from untreated cells.

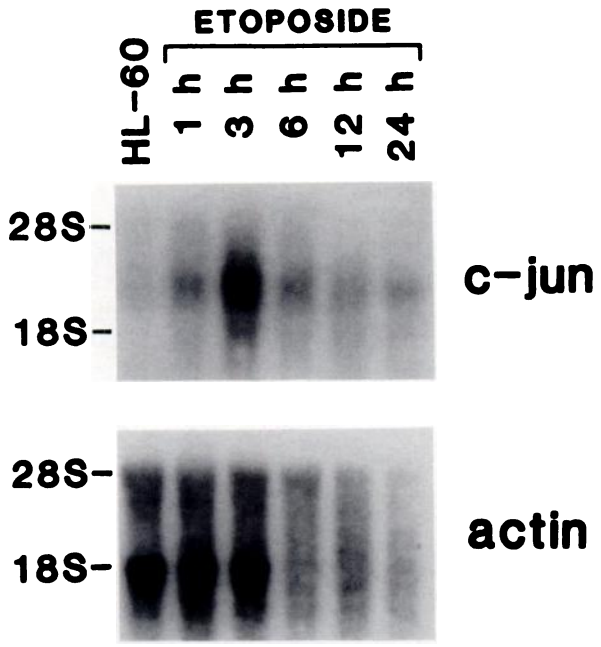

Fig. 3. Effects of etoposide exposure time on c-*jun* and actin mRNA levels. HL-60 cells were treated with 30 μg/ml etoposide for the indicated times. Total cellular RNA was isolated and hybridized as described for Fig. 2.

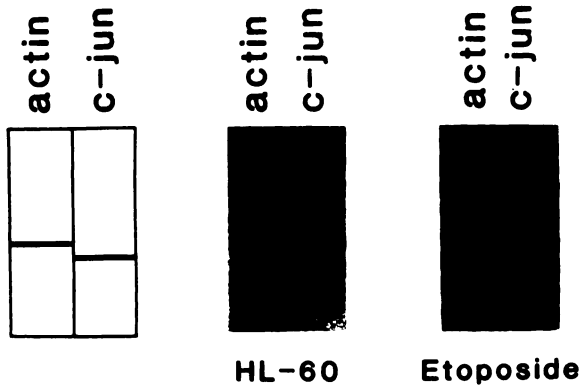

Fig. 4. Effects of etoposide on rates of c-*jun* and actin gene transcription. HL-60 cells were treated with 30 μg/ml etoposide for 3 hr. Nuclei were isolated, and the newly synthesized ^{32}P-labeled RNA was hybridized to actin and c-*jun* inserts that had been subjected to restriction enzyme digestion and Southern blotting. *Solid lines in the schematic*, insert DNA fragments.

were performed with the U-937 cell line. c-*jun* mRNA expression was at low levels in untreated U-937 cells, whereas treatment with etoposide for 3 hr was associated with increases in these transcripts (Fig. 5). These results indicate that etoposide has similar effects on c-*jun* expression in different cell lines. Because the affinity of c-*jun* for DNA binding is increased by the c-*fos* gene product, we also studied the effects of etoposide on c-*fos* mRNA levels. The results demonstrate that this agent similarly increased c-*fos* expression. Maximal levels of these transcripts were also detectable at 3 hr of drug exposure (Fig. 5).

Recent studies have demonstrated an association between c-*jun* expression and DNA cleavage after treatment of cells with certain cytotoxic agents (26). DNA from HL-60 cells treated with 3 or 30 μg/ml etoposide was, therefore, subjected to analysis in agarose gels. There was no evidence of fragmentation in

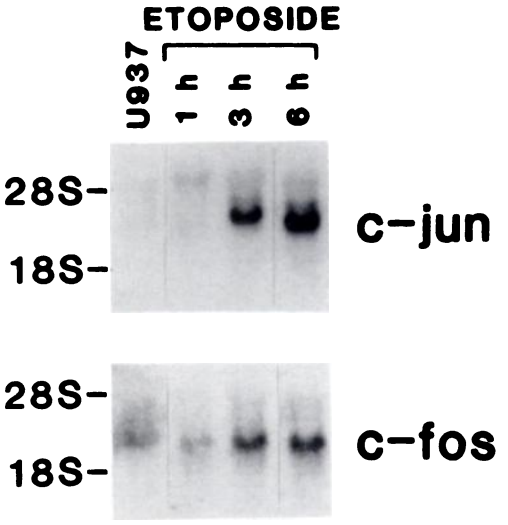

Fig. 5. Effects of etoposide on c-*jun* and c-*fos* mRNA levels in U-937 cells. U-937 cells were treated with 30 μg/ml etoposide for the indicated times. Total cellular RNA was prepared for Northern blot analysis, with hybridization to ^{32}P-labeled c-*jun* and c-*fos* DNA probes. *U937*, RNA from untreated cells.

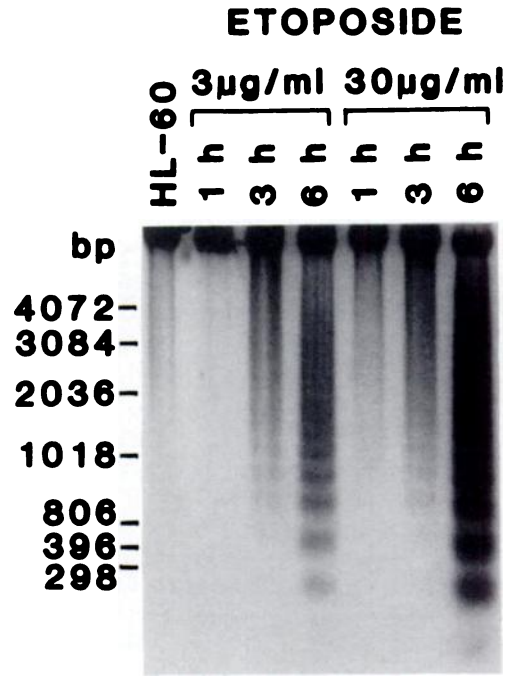

Fig. 6. Effects of etoposide on DNA fragmentation. HL-60 cells were treated with 3 or 30 μg/ml etoposide for 1–6 hr. Cellular DNA was separated in a 2% agarose gel and visualized by UV illumination after ethidium bromide staining.

untreated cells (Fig. 6). Moreover, 1-hr exposures to this agent had little if any effect. In contrast, treatment for 3 hr was associated with the appearance of DNA fragments at multiples of approximately 200 base pairs (Fig. 6). This fragmentation of DNA into multiple nucleosome-sized pieces was, thus, detectable during maximal expression of the c-*jun* gene. Similar findings were obtained with U-937 cells (data not shown).

Discussion

The available evidence indicates that topoisomerase II is involved in the regulation of gene transcription (27–30). Indeed,

inhibition of topoisomerase II activity has been associated with decreases in transcription of certain genes (30–32). In contrast, there are no previous reports that treatment of cells with etoposide or other topoisomerase II inhibitors is associated with activation of specific genes. The present results demonstrate that exposure of HL-60 cells to growth-inhibitory concentrations of etoposide results in expression of the c-*jun* gene. Run-on assays further demonstrate that the increase in c-*jun* mRNA levels is related at least in part to activation at the transcriptional level. This induction of c-*jun* transcripts by etoposide was transient, with maximal levels at 3 hr and then subsequent down-regulation. Taken together, these results suggest that the cellular response to etoposide induces transient activation of specific genes.

The transcriptional regulation of c-*jun* expression has been described for certain growth factors and phorbol esters (11–15). This immediate early response gene is activated in the absence of protein synthesis and codes for the AP-1 DNA binding protein. Jun/AP-1 regulates the transcription of a variety of genes, including that of c-*jun* itself (21, 22). The affinity of Jun/AP-1 binding and presumably the pattern of induced genes is controlled by complex formation of c-Jun with the c-*fos* gene product (19, 20). In this regard, treatment of myeloid leukemia cells with etoposide was also associated with increased expression of the c-*fos* gene. The kinetics of c-*fos* expression were similar to those for c-*jun*, with transient increases that were maximal at 3 hr. These results indicate that etoposide induces at least two members of this gene family that code for leucine zipper DNA binding proteins. The findings also suggest that activation of these transcription factors, and thereby the expression of other genes, could represent a programmed cellular response to this agent.

Etoposide blocks cells in late S/G_2 phase (7, 8). This effect is associated with inhibition of $p34^{cdc2}$ kinase activity and may play a role in etoposide-induced cell death (33, 34). Etoposide also induces DNA fragmentation (3, 6). However, the precise mechanisms responsible for etoposide-induced cytotoxicity remain unclear. Moreover, it is not clear whether the activation of c-*jun* expression by etoposide represents part of the cellular response to the lethal effects of this agent. Of potential relevance to the present findings is the recent demonstration that cytosine arabinoside activates transcription of the c-*jun* gene (9). Other studies have demonstrated that ionizing radiation induces c-*jun* expression (10). Taken together with the present results, these findings suggest that activation of the c-*jun* gene is associated with exposure to distinct classes of DNA-damaging agents. Of interest, similar findings have been described for the c-*fos* gene. A variety of agents that damage DNA, including UV light, transiently activate c-*fos* transcription (35–37). Thus, both c-*jun* and c-*fos* expression appear to represent at least one component of the cellular response to alterations in DNA structure. This response of mammalian cells may be analogous to SOS induction in bacteria after exposure to DNA-damaging agents (38).

Previous studies have demonstrated that treatment of cells with topoisomerase II inhibitors is associated with internucleosomal DNA cleavage (39). This pattern of DNA damage has been described in apoptosis or programmed cell death (40). The same pattern of oligonucleosomal DNA fragmentation has been identified in cells exposed to ionizing irradiation or cytosine arabinoside (26, 41). Recent findings have also indicated that activation of c-*jun* transcription occurs during periods of endonucleolytic DNA cleavage (26). Similar results were obtained in the present study. c-*jun* expression was detectable in association with etoposide-induced oligonucleosomal DNA cleavage. Moreover, these events occurred in a terminal cell population that had undergone complete loss of survival in colony-forming assays. The induction of specific genes has been described during apoptosis of ventral prostate cells (42). Thus, activation of Jun/AP-1 in response to DNA-damaging agents may constitute at least part of a program that commits these cells to terminal events. However, the relationship, if any, between c-*jun* expression and induction of programmed cell death will require further study.

References

1. Glisson, B. S., and W. E. Ross. DNA topoisomerase II: a primer on the enzyme and its unique role as a multidrug target in cancer chemotherapy. *Pharmacol. Ther.* **32**:89–106 (1987).
2. Liu, L. F. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* **58**:351–375 (1989).
3. Long, B. H., S. T. Musial, and M. G. Brattain. Single- and double-strand DNA breakage and repair in human lung adenocarcinoma cells exposed to etoposide and teniposide. *Cancer Res.* **45**:3106–3112 (1985).
4. Liu, L. F., T. C. Rowe, L. Yang, K. M. Tewey, and G. L. Chen. Cleavage of DNA by mammalian DNA topoisomerase II. *J. Biol. Chem.* **258**:15365–15370 (1983).
5. Ralph, R. K. Chromosomal DNA fragments from mouse cells exposed to an intercalating agent contain a 175-kdalton terminal polypeptide. *Can. J. Biochem. Cell Biol.* **63**:780–783 (1985).
6. Long, B. H., S. T. Musial, and M. G. Brattain. DNA breakage in human lung carcinoma cells and nuclei that are naturally sensitive or resistant to etoposide and teniposide. *Cancer Res.* **46**:3809–3816 (1986).
7. Barlogie, B., B. Drewinko, D. A. Johnston, and E. J. Freireich. The effect of Adriamycin on the cell cycle traverse of a human lymphoid cell line. *Cancer Res.* **36**:1975–1979 (1976).
8. Drewinko, B., and B. Barlogie. Survival and cycle-progression delay of human lymphoma cells in vitro exposed to VP-16-213. *Cancer Treat. Rep.* **60**:1295–1306 (1976).
9. Kharbanda, S. M., M. L. Sherman, and D. W. Kufe. Transcriptional regulation of c-*jun* gene expression by arabinofuranosylcytosine in human myeloid leukemia cells. *J. Clin. Invest.* **86**:1517–1523 (1990).
10. Sherman, M. L., R. Datta, D. E. Hallahan, R. R. Weichselbaum, and D. W. Kufe. Ionizing radiation regulates expression of the c-*jun* protooncogene. *Proc. Natl. Acad. Sci. USA* **87**:5663–5666 (1990).
11. Ryder, K., and D. Nathans. Induction of protooncogene c-*jun* by serum growth factors. *Proc. Natl. Acad. Sci. USA* **85**:8464–8467 (1988).
12. Quantin, B., and R. Breathnach. Epidermal growth factor stimulates transcription of c-*jun* proto-oncogene in rat fibroblasts. *Nature (Lond.)* **334**:538–539 (1988).
13. Brenner, D. A., M. O'Hara, P. Angel, M. Chojikier, and M. Karin. Prolonged activation of *jun* and collagenase genes by tumor necrosis factor-α. *Nature (Lond.)* **337**:661–663 (1989).
14. Wu, B.-Y., E. J. Fodor, R. H. Edwards, and W. J. Rutter. Nerve growth factor induces the proto-oncogene *jun* in PC12 cells. *J. Biol. Chem.* **264**:9000–9003 (1989).
15. Sherman, M. L., R. Stone, R. Datta, S. Bernstein, and D. Kufe. Transcriptional and posttranscriptional regulation of c-*jun* expression during monocytic differentiation of human myeloid leukemic cells. *J. Biol. Chem.* **265**:3320–3323 (1990).
16. Bohmann, D., A. Admon, D. R. Turner, and R. Tjian. Transcriptional regulation by the AP-1 family of enhancer-binding proteins: a nuclear target for signal transduction. *Cold Spring Harbor Symp. Quant. Biol.* **53**:695–700 (1988).
17. Mitchell, P. J., and R. Tjian. Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science (Washington, D.C.)* **245**:371–378 (1989).
18. Johnson, P. F., and S. L. McKnight. Eukaryotic transcriptional regulatory proteins. *Annu. Rev. Biochem.* **58**:799–839 (1989).
19. Nakabeppu, Y., K. Ryder, and D. Nathans. DNA binding activities of three murine Jun proteins: stimulation by Fos. *Cell* **55**:907–915 (1988).
20. Halazonetis, T. D., K. Georgopoulos, M. E. Greenberg, and P. Leder. c-*jun* dimerizes itself and with c-*fos*, forming complexes of different DNA binding affinities. *Cell* **55**:917–924 (1988).
21. Angel, P., K. Hattori, T. Smeal, and M. Karin. The *jun* protooncogene is positively autoregulated by its product, Jun/AP-1. *Cell* **55**:875–885 (1988).
22. Lee, W., P. Mitchell, and R. Tjian. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell* **49**:741–752 (1987).
23. Angel, P., E. A. Allegretto, S. T. Okino, K. Hatton, W. J. Boyle, T. Hunter,

and M. Karin. Oncogene *jun* encodes a sequence specific transactivator similar to AP-1. *Nature (Lond.)* **332**:166–171 (1988).
24. Cleveland, D. W., M. A. Lopata, R. J. MacDonald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. Number and evolutionary conservation of α- and β-tubulin and cytoplasmic β- and γ-actin genes using specific cloned cDNA probes. *Cell* **20**:95–105 (1980).
25. Curran, T., W. MacConnell, F. van Straaten, and I. Verma. Structure of the FBJ murine osteosarcoma virus genome: molecular cloning of its associated helper virus and the cellular homolog of the v-*fos* gene from mouse and human cells. *Mol. Cell. Biol.* **3**:914–921 (1983).
26. Gunji, H., S. Kharbanda, and D. Kufe. Induction of internucleosomal DNA fragmentation in human myeloid leukemia cells by 1-β-D-arabinofuranosylcytosine. *Cancer Res.* **51**:741–743 (1991).
27. Sanzay, B. Modulation of gene expression by drugs affecting DNA gyrase. *J. Bacteriol.* **138**:40–47 (1979).
28. Kmiec, E. B., and A. Worcel. The positive transcription factor of the 5S RNA gene induces a 5S DNA-specific gyration in *Xenopus* oocyte extracts. *Cell* **41**:945–953 (1985).
29. Weintraub, H., P. F. Chena, and K. Conrad. Expression of transfected DNA depends on DNA topology. *Cell* **46**:115–122 (1986).
30. Glikin, G. C., and D. Blangy. *In vitro* transcription by *Xenopus* oocytes RNA polymerase II requires a DNA topoisomerase II activity. *EMBO J.* **5**:151–155 (1986).
31. Han, S., A. Udvardy, and P. Schedl. Novobiocin blocks the *Drosophila* heat shock response. *J. Mol. Biol.* **183**:13–29 (1985).
32. Ryoji, M., and A. Worcel. Chromatin assembly in *Xenopus* oocytes: *in vivo* studies. *Cell* **37**:21–32 (1984).
33. Lock, R. B., and W. E. Ross. Inhibition of $p34^{cdc2}$ kinase activity by etoposide or irradiation as a mechanism of G_2 arrest in Chinese hamster ovary cells. *Cancer Res.* **50**:3761–3766 (1990).
34. Lock, R. B., and W. E. Ross. Possible role for $p34^{cdc2}$ kinase in etoposide-induced cell death of Chinese hamster ovary cells. *Cancer Res.* **50**:3767–3771 (1990).
35. Stein, B., H. J. Rahmsdorf, A. Steffen, M. Litfin, and P. Herrlich. UV-induced DNA damage is an intermediate step in UV-induced expression of human immunodeficiency virus type 1, collagenase, c-*fos*, and metallothionein. *Mol. Cell. Biol.* **9**:5169–5181 (1989).
36. Hollander, M. C., and A. J. Fornace, Jr. Induction of *fos* RNA by DNA-damaging agents. *Cancer Res.* **49**:1687–1692 (1989).
37. Futscher, B. W., and L. C. Erickson. Changes in c-*myc* and c-*fos* expression in a human tumor cell line following exposure to bifunctional alkylating agents. *Cancer Res.* **50**:62–66 (1990).
38. Little, J. W., and D. W. Mount. The SOS regulatory system of *Escherichia coli*. *Cell* **29**:11–22 (1982).
39. Kaufmann, S. H. Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. *Cancer Res.* **49**:5870–5878 (1989).
40. Wyllie, A. H. Glucocorticoid induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature (Lond.)* **284**:555–556 (1980).
41. Ijiri, K. Apoptosis (cell death) induced in mouse bowel by 1,2-dimethylhydrazine, methylazoxymethanol acetate, and γ-rays. *Cancer Res.* **49**:6342–6346 (1989).
42. Buttyan, R., C. A. Olsson, J. Pintar, C. Chang, M. Bandyk, P.-Y. Ng, and I. S. Sawczuk. Induction of the TRPM-2 gene in cells undergoing programmed death. *Mol. Cell. Biol.* **9**:3473–3481 (1989).

Send reprint requests to: Donald W. Kufe, Dana-Farber Cancer Institute, Laboratory of Clinical Pharmacology, Department of Medicine, 44 Binney St., Boston, MA 02115.