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ACCELERATED COMMUNICATION

Regulation of *jun*-B Gene Expression by 1-β-D-Arabinofuranosylcytosine in Human Myeloid Leukemia Cells

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SUMMARY

The *jun*-B gene is a member of the *jun* family of immediate early response genes that regulate cellular responses to growth factors. The present studies have examined the effects of 1-β-D-arabinofuranosylcytosine (ara-C) on *jun*-B expression in human KG-1 myeloid leukemia cells. The results demonstrate that ara-C increases *jun*-B mRNA levels. The level of *jun*-B transcripts was maximal after 12 hr of exposure to 10^{-5} M ara-C and persisted through 72 hr. Nuclear run-on assays demonstrated that ara-C treatment is associated with an increased rate of *jun*-B gene transcription. The results also demonstrate that ara-C-induced *jun*-B mRNA levels are regulated by a posttranscriptional mechanism. The level of *jun*-B transcripts in ara-C-treated cells was superinduced by inhibition of protein synthesis. Moreover, cycloheximide prolonged the half-life of ara-C-induced *jun*-B transcripts. These results, thus, demonstrate that ara-C induces expression of the *jun*-B gene in KG-1 cells and that this effect is mediated by transcriptional and posttranscriptional mechanisms.

Polypeptide growth factors activate the expression of immediate early response genes involved in mediating cellular responses. For example, the c-*jun* protooncogene is induced as an immediate early event by growth factors (1–5), as well as by serum (6) and phorbol esters (3). The c-*jun* gene codes for a major form of the AP-1 transcription factor complex (7–11). This complex binds to a DNA sequence motif (TRE) that regulates the transcription of genes responsive to phorbol esters (8, 11–13). The affinity of Jun/AP-1 binding to the TRE is related to the formation of homodimers or heterodimers with the product of the c-*fos* gene (14, 15). Jun-B is another member of the Jun family that forms dimers and binds to the TRE (16, 17). However, the biologic properties of c-Jun and Jun-B differ, in that Jun-B functions as a negative regulator of genes activated by c-Jun (17, 18). Although previous studies have demonstrated that *jun*-B expression is induced by growth factors and the *ras* oncoprotein (5, 16, 19), less is known about other agents that activate this gene.

Ara-C is an inhibitor of cell proliferation that incorporates into elongating DNA strands (20, 21). The extent of ara-C incorporation into DNA correlates with inhibition of DNA synthesis and loss of self-renewal capacity (20–23). The inhibition of replication by ara-C is associated with DNA fragmentation (24). Moreover, the effects of this agent have been associated with changes in gene expression. For example, ara-C induces a differentiated human K562 erythroleukemia cell phenotype that is characterized by increased heme synthesis and accumulation of globin mRNAs (25, 26). Other studies have demonstrated that human myeloid leukemia cell lines can be induced by ara-C to differentiate along the monocytic lineage (27–29). This effect has been associated with down-regulation of c-*myc* gene expression and increased levels of c-*fos* transcripts (30). The basis for the effects of ara-C on gene expression, however, have remained unclear.

The present studies have examined the effects of ara-C on regulation of *jun*-B gene expression in human KG-1 myeloid leukemia cells. The results demonstrate that ara-C induces transcription of this gene. Moreover, the findings indicate that ara-C also regulates *jun*-B mRNA levels by a posttranscriptional mechanism.

Materials and Methods

Cell culture. KG-1 myeloid leukemia cells were obtained from the American Type Culture Collection and grown in Iscove's modified Dulbecco's medium containing 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Ara-C (Sigma Chemical Co., St. Louis, MO) was diluted in medium without serum and then added to the cell cultures. Aphidicolin (Sigma) was first dissolved in phosphate-buffered saline/methanol (2:1, v/v) and

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ABBREVIATIONS: TRE, phorbol ester-responsive element; ara-C, 1-β-D-arabinofuranosylcytosine; CHX, cycloheximide; kb, kilobases; SDS, sodium dodecyl sulfate; SSC, standard saline citrate.

then diluted in medium. Viability was determined by trypan blue exclusion.

RNA isolation and Northern blot hybridization. Total cellular RNA was isolated by a modification of the guanidine-isothiocyanate technique, as described (31, 32). The RNA (20 μg) was subjected to electrophoresis in a 1% agarose/2.2 M formaldehyde gel, transferred to nitrocellulose paper, and hybridized to the following ^{32}P-labeled DNA probes: 1) the 1.8-kb *Eco*RI fragment of a murine *jun*-B cDNA purified from the p465.20 plasmid (16) and 2) the pA1 plasmid containing a 2.0-kb *Pst*I insert of the chicken β-actin gene (33). Hybridizations were performed as described (32). The filters were washed and exposed to Kodak X-Omat XAR film using an intensifying screen. Autoradiograms were scanned with a laser densitometer.

Nuclear run-on assays. Nuclei were isolated from 10^8 KG-1 cells and suspended in 100 μl of glycerol buffer (50 mM Tris·HCl, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, and 0.1 mM EDTA). An equal volume of reaction buffer (10 mM Tris·HCl, pH 8.0, 5 mM $MgCl_2$, 100 mM KCl, 1 mM ATP, 1 mM CTP, 1 mM GTP, and 5 mM dithiothreitol) was added to the nuclei suspension and incubated at 26° for 30 min with 250 μCi of [α-^{32}P]UTP (3000 Ci/mmol; Dupont, Boston, MA). The nuclear RNA was isolated as described (32) and hybridized to the following digested DNAs: 1) the 1.8-kb *Eco*RI fragment of the murine *jun*-B cDNA (16), 2) the 1.1-kb *Bam*HI insert of the human β-globin gene (34), and 3) the 2.0-kb *Pst*I fragment of the chicken β-actin gene (33). The digested DNAs were run in 1% agarose gels and transferred to nitrocellulose filters. Hybridizations were performed with 10^7 cpm of ^{32}P-labeled RNA/ml in 10 mM Tris·HCl, pH 7.4, 4× SSC (0.15 M sodium chloride, 0.15 M sodium citrate), 1 mM EDTA, 0.1% SDS, 2×

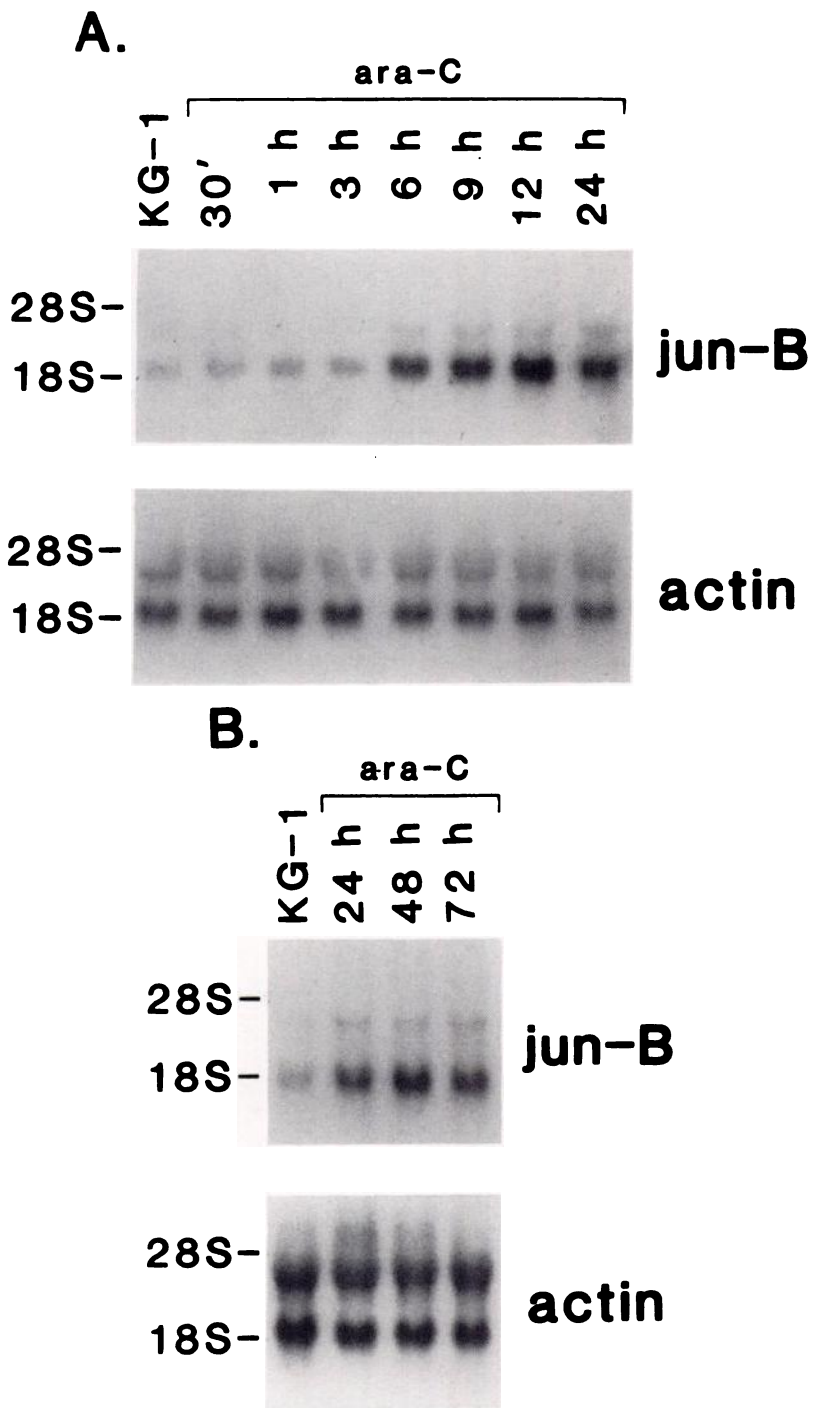

Fig. 1. Effects of 10^{-5} M ara-C on *jun*-B and actin mRNA levels in KG-1 cells. KG-1 cells were treated with 10^{-5} M ara-C for the indicated times. Total cellular RNA (20 μg/lane) was monitored by Northern analysis, with hybridization to the ^{32}P-labeled *jun*-B and actin DNA probes. *KG-1*, RNA from untreated cells.

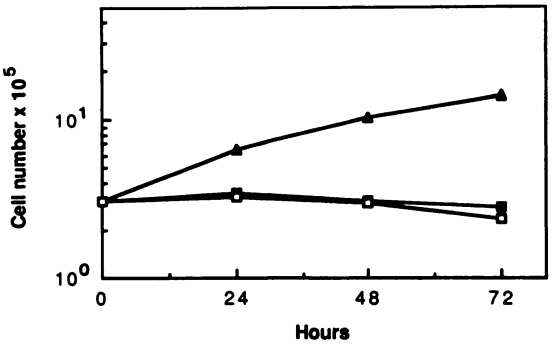

Fig. 2. Effects of ara-C and aphidicolin on KG-1 cell growth. KG-1 cells in logarithmic growth phase were seeded at 3 × 10^5/ml. Ara-C (■) and aphidicolin (□) were added at 10^{-5} M and cell number was monitored for control (△) and treated cells at the indicated times.

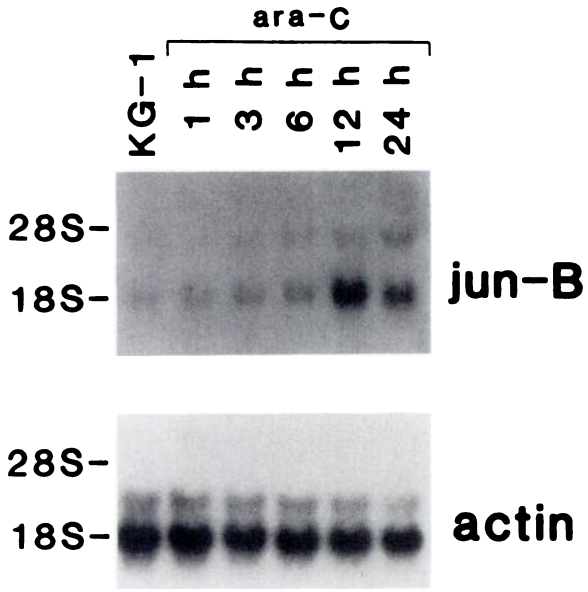

Fig. 3. Effects of 10^{-6} M ara-C on *jun*-B and actin mRNA levels. KG-1 cells were treated with 10^{-6} M ara-C for the indicated times. Total cellular RNA (20 μg) was hybridized to the ^{32}P-labeled *jun*-B and actin DNA probes.

Denhardt's solution (0.02% bovine serum albumin, 0.02% ficoll, and 0.02% polyvinylpyrrolidone), 40% formamide, 100 μg/ml yeast tRNA, for 72 hr at 42°. The filters were washed in: 1) 2× SSC, 0.1% SDS, at 37° for 30 min; 2) 200 ng/ml RNase A in 2× SSC at room temperature for 5 min; and 3) 0.1× SSC, 0.1% SDS, at 42° for 30 min.

Results

The effects of ara-C on *jun*-B gene expression were studied in KG-1 myeloid leukemia cells. Low but detectable levels of 1.8-kb *jun*-B transcripts were present in untreated KG-1 cells (Fig. 1A). In contrast, treatment with 10^{-5} M ara-C was associated with an increase in *jun*-B expression that was apparent by 6 hr and reached maximum levels by 12 hr (Fig. 1A). Longer ara-C exposures demonstrated persistent elevation in *jun*-B mRNA levels through at least 72 hr (Fig. 1B). These effects of ara-C were associated with little if any change in levels of actin gene transcripts (Fig. 1). Similar studies were performed with aphidicolin, an inhibitor of DNA polymerase α (35). Although 10^{-5} M aphidicolin also inhibited KG-1 cell proliferation (Fig. 2), there was no detectable effect of this agent on *jun*-B expression in these cells (data not shown). In contrast, other experiments with 10^{-6} M ara-C, which partially inhibited KG-1 cell growth, similarly demonstrated increases in *jun*-B gene expression. However, this effect was not detectable until 12 hr (Fig. 3).

Run-on assays were performed to determine whether the effects of ara-C on *jun*-B expression involve an increase in the transcriptional rate of this gene. Nuclear RNA was isolated from cells treated with 10^{-5} M ara-C for 6 hr and was hybridized to actin, β-globin, and *jun*-B DNAs. The actin gene was constitutively transcribed in untreated KG-1 cells (Fig. 4). Moreover, the rate of actin gene transcription was unaffected by ara-C treatment (Fig. 4). There was not detectable transcription of the β-globin gene (negative control) in untreated or ara-C-treated cells (Fig. 4). In contrast, a low level of *jun*-B transcription was observed in untreated cells and this rate was increased 3.0-fold in ara-C-treated cells (Fig. 4). Transcription of the *jun*-B gene was increased 1.6-fold by ara-C in a similar but separate experiment. Taken together, these results indicate that ara-C increases *jun*-B expression and that this effect is related, at least in part, to a transcriptional mechanism.

Other studies were performed to determine whether ara-C regulates *jun*-B expression by a posttranscriptional mechanism. KG-1 cells were treated with CHX to inhibit protein synthesis. CHX alone increased *jun*-B mRNA levels at 2, 4, and 8 hr (Fig. 5A). Moreover, the combination of ara-C and CHX demonstrated superinduction of *jun*-B transcripts at 8 hr, compared with that obtained with either agent alone (Fig. 5A). These findings suggested that ara-C-induced *jun*-B transcripts may be stabilized by inhibition of protein synthesis. Consequently, KG-1 cells were treated with ara-C for 6 hr to induce *jun*-B expression and the half-life of *jun*-B mRNA was determined after inhibition of transcription with actinomycin D. Using these experimental conditions, the half-life of *jun*-B mRNA was approximately 56 min (Fig. 5B). In contrast, this half-life was increased to 86 min when the ara-C-treated cells were exposed to both actinomycin D and CHX (Fig. 5B). These results suggested that the increase in *jun*-B mRNA levels during ara-C treatment is also mediated by a posttranscriptional mechanism involving the synthesis of a labile protein.

Discussion

AP-1 has been identified as a complex composed of products of the *fos* and *jun* gene families (7–15). These proteins bind to the TRE and, thereby, regulate the transcription of target genes. There are several known members of the Jun family that interact with Fos and the TRE (14, 36, 37). However, the physiologic roles of the Jun proteins appear to differ. In this regard, Jun-B acts as a negative regulator of the c-*jun* gene (17, 18). The balance between members of the AP-1 complex may be disrupted in cell transformation. Mutations in the coding regions of the c-*fos* and c-*jun* genes have been associated with their activation to oncogenes (38, 39). Moreover, deregulated expression of normal Fos and Jun proteins can also transform cells (40, 41). Thus, although these genes normally regulate the cellular response to growth factors, their aberrant expression may contribute to transformation.

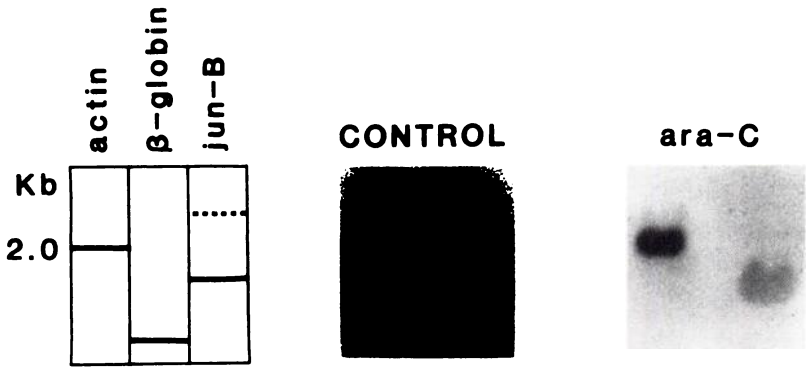

Fig. 4. Effects of ara-C on rates of *jun*-B gene transcription. KG-1 cells were treated with 10^{-5} M ara-C for 6 hr. Nuclei were isolated and the newly synthesized ^{32}P-labeled RNA was hybridized to actin, β-globin, and *jun*-B inserts subjected to restriction enzyme digestion and Southern blotting. *Solid lines* in the schematic, insert DNA fragments; *dashed line*, vector sequences.

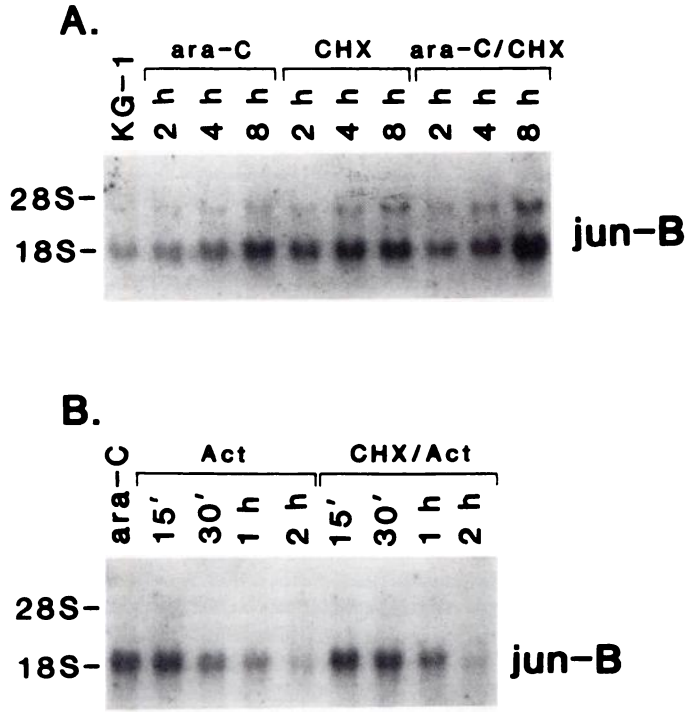

Fig. 5. Effects of CHX and actinomycin D on *jun*-B mRNA levels in ara-C-treated cells. A, KG-1 cells were treated with 10^{-5} M ara-C, 10 μg/ml CHX, or both agents for the indicated times. B, Cells were treated with 10^{-5} M ara-C for 6 hr followed by the addition of 5 μg/ml actinomycin D (*Act*), alone and in combination with CHX, for the indicated times. Total cellular RNA (20 μg) was hybridized to the ^{32}P-labeled *jun*-B probe. The half-life of the *jun*-B transcript was determined by laser densitometry and was normalized to actin mRNA levels. Hybridization to the actin probe demonstrated equal loading of the lanes.

The present studies demonstrate that ara-C regulates expression of the *jun*-B gene in KG-1 myeloid leukemia cells. Similar results have been obtained in ara-C-treated HL-60 and KG-1a leukemia cells (data not shown), thus suggesting that these findings are not limited to a certain cell line. The effects of ara-C on *jun*-B expression appear to involve at least two mechanisms. The nuclear run-on assays demonstrate that ara-C increases the rate of *jun*-B gene transcription. Moreover, the results indicate that levels of ara-C-induced *jun*-B transcripts are regulated at the posttranscriptional level by a labile protein. The finding that inhibition of protein synthesis is associated with superinduction of *jun*-B transcripts is in concert with the demonstration that immediate early response genes have AT-rich regions in their 3′ untranslated regions, which presumably serve as recognition sites for degradation by labile RNases (42). However, the precise mechanisms responsible for regulation of *jun*-B expression at the transcriptional and posttranscriptional levels remain unknown.

Previous studies have demonstrated that ara-C increases expression of the c-*fos* gene in human myeloid leukemia cells (30). More recent work indicates that this effect on c-*fos* expression is in part related to transcriptional activation of the c-*fos* gene.[1] Thus, induction of both the c-*fos* and *jun*-B genes in ara-C-treated cells could result in the formation of c-Fos-Jun-B heterodimers and, thereby, the regulation of certain target genes. However, in contrast to the rapid (within 15 min) induction of immediate early response genes by growth factors, the effects of ara-C on *jun*-B expression were detectable between 6 and 12 hr. The physiologic significance of this delay in *jun*-B expression is unclear. Nonetheless, induction of meyloid leukemia cell differentiation with phorbol esters is associated with induction of the *jun*-B gene at 6 hr.[1] In view of previous findings that ara-C induces differentiation of myeloid leukemia cells (25–30), the present results could reflect regulation of *jun*-B expression that occurs during a process of terminal differentiation.

Finally, there are few insights regarding mechanisms by which ara-C might regulate gene transcription. The present results with aphidicolin, an inhibitor of DNA polymerase α that does not incorporate into DNA (35), would suggest that the effects of ara-C are unrelated to cell cycle arrest. In contrast, previous studies have demonstrated that ara-C induces DNA strand breaks in a concentration- and time-dependent manner (24). Moreover, recent work has demonstrated that DNA strand breakage, and not inhibition of DNA replication, is associated with increased intracellular levels of c-*myc* protein (43). Other studies have also shown that c-*fos* transcripts are increased in cells treated with DNA-damaging agents (44–46). Taken together, expression of immediate early response genes may, thus, represent a physiologic response to damage of genomic DNA.

References

1. Ryder, K., and D. Nathans. Induction of protooncogene c-*jun* by serum growth factors. *Proc. Natl. Acad. Sci. USA* **85**:8464–8467 (1988).
2. Quantin, B., and R. Breathnach. Epidermal growth factor stimulates transcription of c-*jun* protooncogene in rat fibroblasts. *Nature* (*Lond.*) **334**:538–539 (1988).
3. 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).
4. Wu, B.-Y., E. J. Fodor, R. H. Edwards, and W. J. Rutter. Nerve growth factor induces the protooncogene c-*jun* in PC12 cells. *J. Biol. Chem.* **264**:9000–9003 (1989).
5. Petrovaara, L., L. Sistonen, T. J. Bos, P. K. Vogt, J. K. Oja, and K. Alitalo.

[1] Datta, R., M. Sherman, R. Stone, and D. Kufe. Unpublished data.

Enhanced *jun* gene expression is an early genomic response to transforming growth factor β stimulation. *Mol. Cell. Biol.* **9**:1255–1262 (1989).
6. Ryseck, R. P., H. I. Hirai, M. Yaniv, and R. Bravo. Transcriptional activation of c-*jun* during the G_0/G_1 transition in mouse fibroblasts. *Nature* (*Lond.*) **334**:535–537 (1988).
7. Bohmann, D., T. J. Bos, A. Admon, T. Nishimura, P, K. Vogt, and R. Tijan. Human protooncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science (Washington D. C.)* **238**:1386–1392 (1987).
8. Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Ramsdorf, C. Jonat, P. Herrlich, and M. Karin. Phorbol ester-inducible genes contain a common *cis*-element recognized by a TPA-modulated *trans*-acting factor. *Cell* **49**:729–739 (1987).
9. Angel, P., E. A. Allegretto, S. T. Okino, K. Hattori, 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).
10. Chiu, R., W. J. Boyle, J. Meek, T. Smeal, T. Hunter, and M. Karin. The c-fos protein interacts with c-*jun*/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* **54**:541–552 (1988).
11. Lee, W., P. Mitchell, and R. Tijan. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell* **49**:741–752 (1987).
12. Chiu, R., M. Imagawa, R. J. Imbra, J. R. Bockoven, and M. Karin. Multiple *cis*- and *trans*-acting elements mediate the transcriptional response to phorbol esters. *Nature* (*Lond.*) **329**:648–651 (1987).
13. Imagawa, M., R. Chiu, and M. Karin. Transcription factor AP-2 mediates induction by two different signal transduction pathways: protein kinase C and cAMP. *Cell* **51**:251–260 (1987).
14. Nakabeppu, Y., K. Ryder, and D. Nathans. DNA binding activities of three murine jun proteins: stimulation by fos. *Cell* **55**:907–915 (1988).
15. Halazonetis, T. D., K. Georgopoulos, M. E. Greenberg, and P. Leder. c-jun dimerizes with itself and with c-fos, forming complexes of different DNA binding affinities. *Cell* **55**:917–924 (1988).
16. Ryder, K., L. F. Lau, and D. Nathans. A gene activated by growth factors is related to the oncogene v-*jun*. *Proc. Natl. Acad. Sci. USA* **85**:1487–1491 (1988).
17. Chiu, R., P. Angel, and M. Karin. *jun*-B differs in its biological properties from, and is a negative regulator of, c-*jun*. *Cell* **59**:979–986 (1989).
18. Schutte, J., J. Viallet, M. Nau, S. Segal, J. Fedorko, and J. Minna. *jun*-B inhibits and c-*fos* stimulates the transforming and *trans*-activating activities of c-*jun*. *Cell* **59**:987–997 (1989).
19. Sistonen, L., E. Holtta, T. P. Makela, J. Keski-Oja, and K. Alitalo. The cellular response to induction of the $p21^{c\text{-}Ha\text{-}ras}$ oncoprotein includes stimulation of *jun* gene expression. *EMBO J.* **8**:815–822 (1989).
20. Kufe, D., P. Major, E. M. Egan, and P. Beardsley. Correlation of cytotoxicity with incorporation of ara-C into DNA. *J. Biol. Chem.* **255**:8997–9000 (1980).
21. Major, P., E. M. Egan, G. Beardsley, M. Minden, and D. Kufe. Lethality of human myeloblasts correlates with the incorporation of ara-C into DNA. *Proc. Natl. Acad. Sci. USA* **78**:3235–3239 (1981).
22. Major, P., E. M. Egan, D. Herrick, and D. Kufe. The effect of ara-C incorporation on DNA synthesis in cells. *Biochem. Pharmacol.* **31**:2937–2940 (1982).
23. Kufe, D., D. Munroe, D. Herrick, E. Egan, and D. Spriggs. Effects of 1-β-D-arabinofuranosylcytosine incorporation on eukaryotic DNA template function. *Mol. Pharmacol.* **26**:128–134 (1984).
24. Fram, R., and D. Kufe. DNA strand breaks caused by inhibitors of DNA synthesis: 1-β-D-arabinofuranosylcytosine and aphidicolin. *Cancer Res.* **42**:4050–4053 (1982).
25. Luisi-DeLuca, C., T. Mitchell, D. Spriggs, and D. Kufe. Induction of terminal differentiation in human K562 erythroleukemia cells by arabinofuranosylcytosine. *J. Clin. Invest.* **74**:821–827 (1984).
26. Watanabe, T., T. Mitchell, E. Sariban, K. Sabbath, J. Griffin, and D. Kufe. Effects of 1-β-D-arabinofuranosylcytosine and phorbol ester on differentiation of human K562 erythroleukemia cells. *Mol. Pharmacol.* **27**:683–688 (1985).
27. Griffin, J., D. Munroe, P. Major, and D. Kufe. Induction of differentiation of human myeloid leukemic cells by inhibitors of DNA synthesis. *Exp. Hematol.* **10**:776–783 (1982).
28. Takeda, K., J. Minowada, and A. Bloch. Kinetics of appearance of differentiation-associated characteristics in ML-1, a line of human myeloblastic leukemia cells, after treatment with 12-*O*-tetradecanoylphorbol-13-acetate, dimethyl sulfoxide, or 1-β-D-arabinofuranosylcytosine. *Cancer Res.* **42**:5152–5185 (1982).
29. Chomienne, C., N. Balitrand, and J. Abita. Effects of 1-β-D-arabinofuranosylcytosine on differentiation of U-937 cells. *Med. Sci.* **11**:731–732 (1983).
30. Mitchell, T., E. Sariban, and D. Kufe. Effects of 1-β-D-arabinofuranosylcytosine on proto-oncogene expression in human U-937 cells. *Mol. Pharmacol.* **30**:398–402 (1986).
31. Chirgwin, J. M., A. E. Pryzbyla, R. J. MacDonald, and W. J. Rutter. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5924–5929 (1979).
32. Sherman, M., R. Stone, R. Datta, S. Bernstein, and D. Kufe. Transcriptional and posttranscriptional regulation of c-*jun* expression during induction of monocytic differentiation. *J. Biol. Chem.* **265**:3320–3323 (1990).
33. Cleveland, D. W., M. Lopata, R. MacDonald, N. Cowan, W. Rutter, and M. Kirschner. Number and evolutionary conservation of *alpha*- and *beta*-tubulin and cytoplasmic *beta*- and *gamma*-actin genes using specific cloned cDNA probes. *Cell* **20**:95–105 (1980).
34. Wilson, J. T., L. B. Wilson, J. K. deRiel, L. Villa-Komaroff, A. Efstratiadis, B. G. Forget, and S. M. Weissman. Insertion of synthetic copies of human globin genes into bacterial plasmids. *Nucleic Acids Res.* **5**:563–580 (1978).
35. Huberman, J. New views on the biochemistry of eukaryotic DNA replication by aphidicolin, an unusual inhibitor of DNA polymerase *alpha*. *Cell* **23**:647–648 (1981).
36. Zerial, M., L. Toschi, R.-P. Ryseck, M. Schuermann, R. Muller, and R. Bravo. The product of a novel growth factor activated gene, *fos*B, interacts with JUN proteins enhancing their DNA binding activity. *EMBO J.* **8**:805–813 (1989).
37. Hirai, S.-I., R.-P. Ryseck, F. Mechta, R. Bravo, and M. Yaniv. Characterization of *jun*D: a new member of the *jun* protooncogene family. *EMBO J.* **8**:1433–1439 (1989).
38. Curran, T., G. Peters, C. Van Beveren, N. Teich, and I. Verma. FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. *J. Virol.* **44**:674–682 (1982).
39. Maki, Y., T. Bos, C. Davis, M. Starbuck, and P. Vogt. Avian sarcoma virus 17 carries the *jun* oncogene. *Proc. Natl. Acad. Sci. USA* **84**:2848–2852 (1987).
40. Miller, A., T. Curran, and I. Verma. c-fos protein can induce cellular transformation: a novel mechanism of activation of a cellular oncogene. *Cell* **36**:51–60 (1984).
41. Schutte, J., J. Minna, and M. Birrer. Deregulated expression of human c-*jun* transforms primary rat embryo cells in cooperation with an activated c-Ha-*ras* gene and transforms Rat-1a cells as a single gene. *Proc. Natl. Acad. Sci. USA* **86**:2257–2261 (1989).
42. Shaw, G., and R. Kamen. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**:659–667 (1986).
43. Sullivan, N. F., and A. E. Willis. Elevation of c-myc protein by DNA strand breakage. *Oncogene* **4**:1497–1502 (1989).
44. Hollander, M. C., and A. J. Fornace, Jr. Induction of c-*fos* RNA by DNA-damaging agents. *Cancer Res.* **49**:1687–1692 (1989).
45. 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 metallothionien. *Mol. Cell. Biol.* **9**:5169–5181 (1989).
46. Futscher, B. W., and L. C. Erikson. 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).

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