MOLECULAR AND CELLULAR BIOLOGY, Nov. 1984, p. 2540–2542
0270-7306/84/112540-03$02.00/0

Vol. 4, No. 11

Transcriptional Regulation of a Tumor Promoter and Mitogen-Inducible Gene in Human Lymphocytes

SURESH K. ARYA,* FLOSSIE WONG-STAAL, AND ROBERT C. GALLO

Laboratory of Tumor Cell Biology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20205

Received 10 May 1984/Accepted 17 August 1984

Tumor-promoting phorbol esters affect a variety of cellular functions which may underlie tumor promotion. We isolated from human lymphocytes a cDNA clone whose gene is inducible by the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate as well as by the T-cell mitogen phytohemagglutinin. Nuclear transcription experiments suggested that this induction is primarily caused by the increased transcription of the gene. It is interesting that this gene is expressed constitutively in human T-cell leukemia virus-infected mature T cells. The results support the notion that 12-O-tetradecanoyl-phorbol-13-acetate can affect cellular functions by causing transcriptional activation of specific genes.

12-O-Tetradeconoyl-phorbol-13-acetate (TPA) is a potent tumor promoter in the two-stage mouse skin carcinogenesis model (21). It apparently induces a cascade of pleiotypic responses which may underlie the process of tumor promotion (4, 5). TPA also has a variety of effects on cells in culture and, specifically in hematopoietic cells, can induce or inhibit cellular differentiation (9, 17, 19, 20). In addition, tumor promotion triggered by TPA in some cells is preceded by hyperplasia (15), which may be akin to the mitogenic response of lymphocytes induced by phytohemagglutinin (PHA) or other mitogens. The molecular mechanisms of the effects of TPA on cellular functions and of lymphocyte mitogenesis by PHA have not been extensively explored. In only a few cases have the underlying genetic processes affected by TPA or PHA been identified. For example, the TPA-induced increase in the synthesis of some glycoproteins in murine cells was shown to be caused by the increase in translatable mRNAs (10, 12), and the TPA-caused increase in the production of mouse mammary tumor virus was correlated with elevated steady-state levels of virus-specific RNA (1). It was recently reported that the TPA-mediated inhibition of collagen synthesis in murine epidermal cells was caused by a reduction in collagen mRNA levels (22). It has been shown that the TPA- and PHA-induced production of T-cell growth factor by human cells is caused by the enhanced synthesis of T-cell growth factor mRNA (3, 7; S. K. Arya and R. C. Gallo, Biochemistry, in press).

We recently isolated from a cDNA library of a human cell a gene which is inducible by TPA. It is interesting that the expression of this gene, termed JD15, is also modulated by T-cell-specific mitogenic lectin PHA. With a view that the study of this gene may provide insights for understanding the molecular mechanism of TPA as well as PHA action, we have investigated the expression of this gene in some detail.

The JD15 clone was obtained by differential screening of a cDNA library of human leukemic Jurkat cell mRNA that had been enriched for inducible genes. The expression and inducibility of the JD15 gene were investigated by the Northern blot hybridization procedure as described previously (2, 3, 7). This gene was expressed as a 17S to 18S mRNA species, corresponding to about 1.8 to 2.0 kilobases. The specific mRNA species was barely detectable in uninduced Jurkat cells (less than five copies per cell) but was abundantly expressed in Jurkat cells induced by TPA (more than 100 copies per cell) (Fig. 1A). About the same level of this mRNA was detected whether the cells were treated with 10 or 50 ng of TPA per ml. It was also induced by treatment of Jurkat cells with PHA, although to a much lower level than by treatment with TPA. A combined treatment of cells with TPA and PHA increased the level of this mRNA somewhat, even when the concentration of each inducer was maximal (Fig. 1A). The induction of the JD15 gene was selective; the mRNA of another clone (termed JB1) which is constitutively expressed in Jurkat cells was not affected (Fig. 1B).

Figure 2 shows the kinetics of induction of JD15 mRNA and depicts the results of two separate experiments. The synthesis of JD15 mRNA was induced rather rapidly. It reached its maximal level by ca. 2 to 3 h postinduction, persisted at high levels for ca. 8 h, and then declined to about one-third of the maximal level by ca. 20 h postinduction. The kinetics of induction were similar whether the cells were treated with PHA alone or TPA alone (data not shown), although the maximal levels reached were dependent upon the inducer used, consistent with the results presented in Fig. 1.

To determine if the inducing agents acted primarily by influencing JD15 gene expression or by some other mechanisms, we performed nuclear transcription or "run off" experiments. Such transcription systems support the elongation of RNA polymerase II transcripts initiated in vivo but do not allow the reinitiation of transcription. Thus, the incorporation of radiolabeled nucleotides by nuclei in vitro into specific RNA provides an estimate of the number of polymerase molecules in the process of transcribing a specific gene (6, 11). Jurkat cells were treated for 4 h with TPA (10 or 50 ng/ml), PHA (1%), or TPA plus PHA, and nuclei were prepared and allowed to elongate RNA chains in vitro in the presence of [^{32}P]UTP essentially by the methods of McKnight and Palmiter (16) and Mulvihill and Palmiter (18). The labeled RNA was hybridized with pJD15 DNA, pBR322 DNA, or pJB1 or pJH8 DNA (Fig. 3). Some transcripts corresponding to the JD15 gene could be detected in uninduced Jurkat cells. The amount of such transcripts was markedly increased in cells treated with TPA or PHA. The

* Corresponding author.

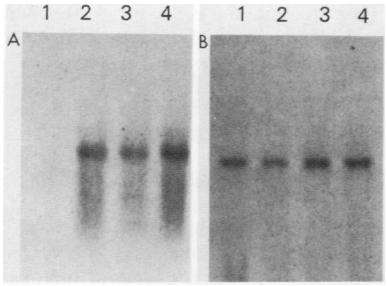

FIG. 1. Induction of (A) JD15 and (B) JB1 gene expression in human Jurkat cells. Cells were treated with TPA (50 ng/ml), PHA (1%), or TPA (50 ng/ml) plus PHA (1%) for 4 h. Polyadenylic acid-selected RNA was isolated and analyzed for JD15-specific and JB1-specific sequences by Northern blot hybridization with nick-translated cloned DNA probes. Lanes: 1, RNA from control cells; 2, RNA from cells treated with TPA; 3, RNA from cells treated with PHA; 4, RNA from cells treated with TPA plus PHA.

amounts of these transcripts induced by the combined treatment with TPA and PHA were not additive; they appeared to be slightly higher than that induced by TPA or PHA alone. These effects were specific for the JD15 gene. The transcripts corresponding to two other genes (JB1 and JH8) that are constitutively expressed in Jurkat cells were not modulated by either TPA or PHA. The majority of the transcripts synthesized by nuclei in vitro were apparently initiated by RNA polymerase II, as the synthesis was sensitive to α-amanitin inhibition in a dose-dependent manner (data not shown). These results thus showed that the induction of JD15 mRNA was at least in part caused by the transcriptional activation of the gene.

It was possible that an inducer-mediated increase in the half-life of JD15 mRNA contributed to the induction. To test this, we induced Jurkat cells with PHA and TPA for 4 h and blocked further RNA synthesis with actinomycin D. The cells were then incubated with or without inducers for an additional 4 h, and steady-state levels of JD15 mRNA were analyzed by dot blot hybridization (Fig. 4). The JD15 mRNA levels in actinomycin D-treated cells cultured with or without inducers were roughly equivalent, showing that neither inducer caused significant stabilization of JD15 mRNA. The induction of JD15 mRNA was thus primarily caused by the increased transcription of the gene. As we also detected the transcripts of this gene in uninduced cells, it is likely that TPA and PHA do not activate the transcription of the gene that was otherwise inactive but enhance the rate or extent of its transcription or both.

The fact that the combined effect of TPA and PHA on JD15 gene transcription was not additive in relation to their individual effects suggests that the two inducing agents may act by the same mechanism. However, there was a slight and reproducible enhancement of mRNA levels induced by the combined treatment, even when the concentration of each inducer was optimal. It is possible that these inducers act by binding to the specific receptors on the cellular membrane and that their effects on mRNA synthesis are brought about by secondary mediators. The saturating concentration of a given inducer used here may pertain to its capacity to bind

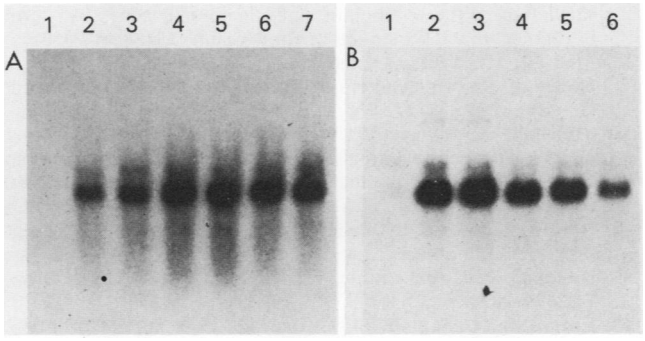

FIG. 2. Kinetics of induction of JD15 mRNA in Jurkat cells. Cells were incubated with TPA (50 ng/ml) plus PHA (1%) for various times, and polyadenylic acid-selected RNA was analyzed for JD15-specific sequences by Northern blot hybridization with nick-translated cloned pJD15 DNA as the probe. (A) Lanes 1 through 7, RNA isolated at 0, 1, 1.5, 2, 2.5, 3, and 4 h postinduction, respectively. (B) Lanes 1 through 6, RNA isolated at 0, 2, 4, 6, 8, and 20 h postinduction, respectively.

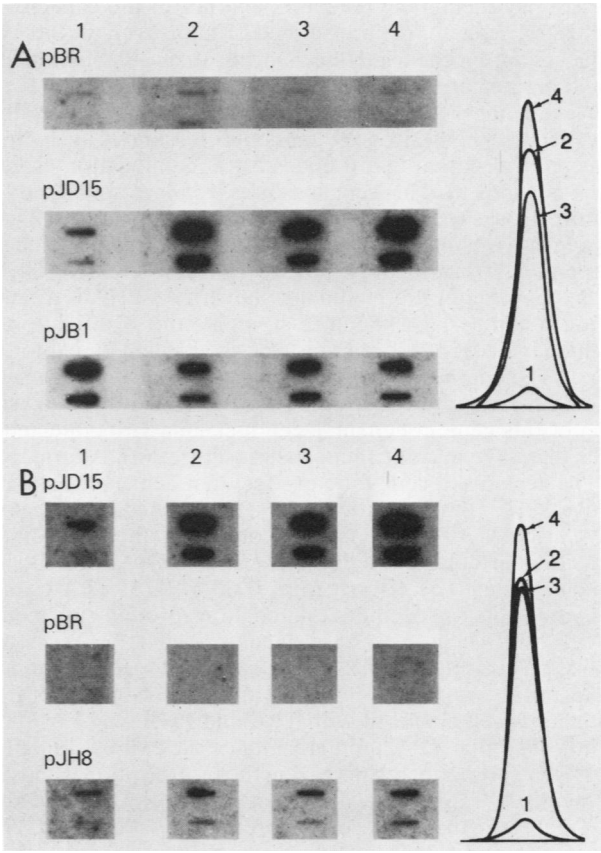

FIG. 3. Analysis of JD15 mRNA synthesized by nuclei from uninduced and induced Jurkat cells. Nuclei from uninduced cells and cells induced for 4 h were allowed to elongate RNA chains in vitro, and labeled RNA thus obtained was hybridized with cloned pJD15 DNA, pBR322 DNA, pJB1 DNA, or pJH8 DNA. (A) Cells were treated with 10 ng of TPA per ml and 1% PHA. (B) Cells were treated with 50 ng of TPA per ml and 1% PHA. Lanes: 1, uninduced cells; 2, cells induced with TPA; 3, cells induced with PHA; 4, cells induced with TPA plus PHA. Curves to the right of each panel represent the relative intensities of the bands for pJD15 DNA traced by a laser source densitometer.

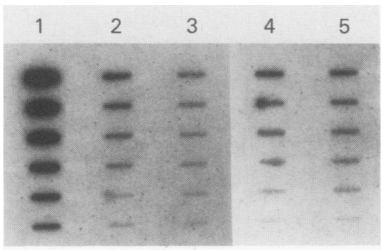

FIG. 4. Effect of actinomycin D on the persistence of JD15 mRNA in induced Jurkat cells. Cells were induced with TPA (50 ng/ml) and PHA (1%) for 4 h, washed, and recultured for an additional 4 h in medium containing actinomycin D (5 μg/ml) with or without inducers. RNA was isolated and hybridized with cloned pJD15 DNA as the probe by dot blot hybridization. Lanes: 1, cells induced for 4 h with no further treatment; 2, induced cells incubated with actinomycin D and TPA plus PHA; 3, induced cells incubated with actinomycin D and neither inducer; 4, induced cells incubated with actinomycin D and TPA; 5, induced cells incubated with actinomycin D and PHA.

the specific membrane receptors and not to the capacity of the JD15 gene to be transcribed. Thus, even if the two inducers act by the same mechanism, their combined effect could be greater than their individual effects.

We do not yet know the identity of the gene product encoded by the JD15 gene, although we know by hybrid-selected translation that it codes for a 32-kilodalton protein (data not shown). This gene is expressed more abundantly in mature T cells (peripheral blood lymphocytes, human T-cell leukemia virus-infected T cells) or nearly mature T cells (Jurkat cells) than in immature T cells (Molt-4, CCRF-CEM) or B cells (Daudi, Raji) (data not shown). As PHA is a T-cell mitogen and as TPA may also stimulate the proliferation of lymphocytes (15), it is possible that the protein coded for by the JD15 gene serves as a proliferation signal for some T cells. This may be consistent with the constitutive expression of this gene in human T-cell luekemia virus-infected T cells that have become immortal in cultures (3). Alternatively, the gene could code for a product that defines the state of activation or differentiation of T cells. Other investigators have also described the mitogen- or growth factor-mediated induction in cultured cells of specific mRNAs (8, 13, 14) which appear to be distinct from JD15 mRNA. TPA-caused induction of a specific 32-kilodalton protein in mouse BALB/c-3T3 cells was recently described (12). We have, however, not detected mRNA species homologous to human JD15 mRNA in mouse NIH-3T3 fibroblasts or C57Bl/EL4 lymphoma cells induced with TPA and PHA.

It is clear that TPA and PHA induce the expression of the JD15 gene primarily by increasing the transcriptional activity of the gene. Combined with recent results on the regulation of T-cell growth factor gene expression by PHA and TPA (S. K. Arya and R. C. Gallo, Biochemistry, in press), these studies show that TPA and PHA alone or in combination can cause transcriptional activation of specific genes. Such an activation may be associated with mitogenesis and hyperplasia, leading to neoplastic transformation.

We thank E. P. Gelmann for advice and assistance.

LITERATURE CITED

1. **Arya, S. K.** 1980. Phorbol ester mediated stimulation of the synthesis of mouse mammary tumour virus. Nature (London) **284**:71–72.
2. **Arya, S. K.** 1982. Sequence complexity of polyadenylated RNA in human breast carcinoma cells. Int. J. Biochem. **14**:19–24.
3. **Arya, S. K., F. Wong-Staal, and R. C. Gallo.** 1984. T cell growth factor gene: lack of expression in human T-cell leukemia-lymphoma virus-infected cells. Science **223**:1086–1087.
4. **Blumberg, P. M.** 1980. *In vitro* studies on the mode of action of the phorbol esters, potent tumor promotors. Crit. Rev. Toxicol. **8**:153–197.
5. **Boutwell, R. K., A. K. Verma, C. L. Ashendel, and E. Astrup.** 1982. Mouse skin: a useful model system for studying mechanism of chemical carcinogenesis, p. 1–12. *In* E. Hecker, N. E. Fusenig, W. Kunz, F. Manks, and H. W. Thielmann (ed.), Carcinogenesis and biological effects of tumor promoters. Raven Press, Publishers, New York.
6. **Chao, M. V., P. Mellon, P. Charnay, T. Maniatis, and R. Axel.** 1983. The regulated expression of β-globin genes introduced into mouse erythroleukemia cells. Cell **32**:483–493.
7. **Clark, S. C., S. K. Arya, F. Wong-Staal, M. Matsumoto-Kobayaski, R. M. Kay, R. J. Kaufman, E. L. Brown, C. Shoemaker, T. Copland, S. Oroszlan, K. Smith, M. G. Sarngadharan, S. G. Lindmer, and R. C. Gallo.** 1984. Human T-cell growth factor: partial amino acid sequence, cDNA cloning, and organization and expression in normal and leukemic cells. Proc. Natl. Acad. Sci. U.S.A. **81**:2543–2547.
8. **Cochran, B. H., A. C. Reffel, and C. D. Stiles.** 1983. Molecular cloning of gene sequences regulated by platelet-derived growth factor. Cell **33**:939–947.
9. **Fibach, E. R., P. A. Gambari, G. Shaw, R. C. Maniatis, S. Reuben, S. Sassa, R. A. Rifkind, and P. A. Marks.** 1979. Tumor promoter-mediated inhibition of cell differentiation: suppression of the expression of erythroid functions in murine erythroleukemia cells. Proc. Natl. Acad. Sci. U.S.A. **76**:1906–1910.
10. **Gottesman, M. M., and M. E. Sobel.** 1980. Tumor promoters and Kirsten sarcoma virus increase synthesis of a secreted glycoprotein by regulating levels of translatable mRNA. Cell **19**:449–455.
11. **Groudine, M., M. Peretz, and H. Weintraub.** 1981. Transcriptional regulation of hemoglobin switching in chicken embryos. Mol. Cell. Biol. **1**:281–288.
12. **Hiwasa, T., S. Fujimura, and S. Sakiyama.** 1982. Tumor promoters increase the synthesis of a 32,000 dalton protein in Balb/c 3T3 cells. Proc. Natl. Acad. Sci. U.S.A. **79**:1800–1804.
13. **Kelley, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
14. **Linzer, D. I. H., and D. Nathans.** 1983. Growth-related changes in specific mRNAs of cultured mouse cells. Proc. Natl. Acad. Sci. U.S.A. **80**:4271–4275.
15. **Mastro, A. M.** 1982. Phorbol esters: tumor promotion, cell regulation, and immune response. Lymphokines **6**:253–313.
16. **McKnight, G. S., and R. D. Palmiter.** 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct. J. Biol. Chem. **254**:9050–9058.
17. **Miao, R. M., A. H. Fiedsteel, and D. W. Fodge.** 1978. Opposing effects of tumor promoters on erythroid differentiation. Nature (London) **274**:271–272.
18. **Mulvihill, E. R., and R. D. Palmiter.** 1977. Relationship of nuclear estrogen receptor levels to induction of ovalbumin and conalbumin mRNA in chick oviduct. J. Biol. Chem. **252**:2060–2068.
19. **Rovera, G., T. G. O'Brien, and L. Diamond.** 1977. Tumor promoters inhibit spontaneous differentiation of Friend erythroleukemia cells in culture. Proc. Natl. Acad. Sci. U.S.A. **74**:2894–2898.
20. **Rovera, G., D. Santoli, and C. Damsky.** 1979. Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. Proc. Natl. Acad. Sci. U.S.A. **76**:2779–2783.
21. **Scribner, J. D., and R. Suss.** 1978. Tumor initiation and promotion. Int. Rev. Exp. Pathol. **18**:137–198.
22. **Sobel, M. E., L. D. Dion, J. Vuust, and N. H. Colburn.** 1983. Tumor-promoting phorbol esters inhibit procollagen synthesis at a pretranslational level in JB-6 mouse epidermal cells. Mol. Cell. Biol. **3**:1527–1532.