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Transcriptional Modulation of Human T-Cell Growth Factor Gene by Phorbol Ester and Interleukin 1†

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ABSTRACT: T-cell growth factor (TCGF) is a protein that is required for the continuous proliferation of activated normal T lymphocytes. It is produced by a subset of T lymphocytes upon appropriate stimulation. A human leukemic T-cell line (Jurkat) can be induced with the lectin phytohemagglutinin and the phorbol ester tetradecanoylphorbolacetate (TPA) to produce T-cell growth factor. This production was enhanced by including the lymphokine interleukin 1 in the induction medium. Interleukin 1 alone did not substantially increase T-cell growth factor production by cells treated only with phytohemagglutinin. These effects were preceded by and correlated with the induction of T-cell growth factor mRNA. Northern blot experiments with cloned TCGF DNA as a probe showed that TCGF mRNA was induced rapidly in cells treated with TPA and phytohemagglutinin, and this induction was augmented by interleukin 1. Thus, the production of T-cell growth factor was regulated at the level of its mRNA. Nuclear transcription experiments suggested that the TCGF gene was more actively transcribed in cells treated with TPA and phytohemagglutinin than in cells treated with phytohemagglutinin alone. The transcription of the TCGF gene was further increased when interleukin 1 was included along with TPA and phytohemagglutinin. When continued synthesis of RNA in induced cells was blocked with actinomycin D and cells were subsequently cultured in the presence or absence of inducing agents, the steady-state levels of TCGF mRNA declined in all cultures. This decline was roughly equivalent in cells incubated without the inducers and those incubated with phytohemagglutinin. The decline in mRNA levels was slightly greater for cells incubated with TPA or TPA and phytohemagglutinin as compared with control cells. The inducers apparently did not increase the half-life of T-cell growth factor mRNA. Thus, elevated steady-state levels of T-cell growth factor mRNA in induced cells were due to the increased transcriptional activity of the T-cell growth factor gene. Both TPA and interleukin 1 appeared to act synergistically with phytohemagglutinin in increasing the synthesis of growth factor mRNA and thus the production of the protein.

The protein T-cell growth factor (TCGF),[1] also termed interleukin 2, is required for the proliferation of activated normal T cells (Morgan et al., 1976; Smith et al., 1979; Ruscetti & Gallo, 1981) but not necessarily of neoplastic T cells (Arya et al., 1984a). It is produced by a subset of mature T cells upon stimulation with antigens or lectins, such as phytohemagglutinin (PHA) (Ruscetti et al., 1980; Mier & Gallo, 1980). Normal human lymphocytes can be activated by PHA in vitro to produce TCGF. This activation requires a macrophage-produced lymphokine, termed interleukin 1 (IL-1), in addition to PHA (Larsson et al., 1980; Smith et al., 1980; Oppenheim et al., 1980). Some of the human neoplastic T cells can also be induced in vitro to produce TCGF (Gillis & Watson, 1980) while some mature neoplastic T cells produce TCGF constitutively (Gootenberg et al., 1982; Arya et al., 1984a). Induction in some cases such as with human leukemic cel line Jurkat or JM requires both PHA and phorbol ester tetradecanoylphorbolacetate (TPA) (Gillis & Watson, 1980), suggesting that TPA can substitute for IL-1 in these leukemic cells but only IL-1 has this effect with normal lymphocytes (Farrar et al., 1980; Stadler et al., 1981). However, it is not known if TPA and IL-1 act by identical or similar mechanism(s) for the two cell types.

We have previously shown that the production of TCGF by human cells is regulated at the mRNA level in that the TCGF producer cells contain TCGF mRNA but nonproducer cells do not (Arya et al., 1984a; Clark et al., 1984). The exact mechanism of induction of TCGF mRNA and hence of TCGF production has not yet been explored. The inducing agents, for example, could increase the transcription of the TCGF gene and/or could affect some posttranscriptional maturation step and half-life of mRNA. In addition, it is possible that the secondary inducing agents, such as TPA, may affect some posttranslational step resulting in an increased release of TCGF by the induced cells. Here, we show that the induction of TCGF production by TPA and IL-1 in combination with PHA is preceded by and correlated with the synthesis of TCGF

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[1] Abbreviations: TCGF, T-cell growth factor; IL-1, interleukin 1; PHA, phytohemagglutinin; TPA, tetradecanoylphorbolacetate; SSC, 0.15 M NaCl–0.015 M sodium citrate, pH 7; SDS, sodium dodecyl sulfate; Gdn-HCl, guanidine hydrochloride; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; MOPS, 4-morpholinepropanesulfonic acid.

mRNA and that the induction of TCGF mRNA is due to the increased transcriptional activity of the TCGF gene.

Materials and Methods

Materials. PHA-M and TPA were obtained respectively from Gibco, Grand Island, NY, and Sigma Chemical Co., St. Louis, MO. IL-1 was purchased from Genzyme, Boston, MA, and was devoid of mitogenic (TCGF) and interferon activity.

Cell Culture and TCGF Assays. Human Jurkat cells were cultured at 37 °C under 5% CO_2 humidified atmosphere at a density of (1–2) × 10^6 cells/mL in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. They were treated with the indicated concentrations of the inducing agents (PHA, TPA, IL-1) for 4–6 or 20–24 h. For TCGF assays, the conditioned medium from cultures treated for 20–24 h was serially diluted and assayed for TCGF activity by measuring thymidine incorporation by TCGF-dependent mouse CTLL cells as described (Gillis et al., 1978).

RNA Isolation and Blot Hybridization. RNA was isolated from freshly harvested cells by repeated Gdn-HCl extraction (Chirgwin et al., 1979) followed by cesium chloride centrifugation (Glisin et al., 1974) as described by Arya (1982). Briefly, cells were suspended in 10 volumes of 8 M Gdn-HCl (0.02 M sodium acetate, pH 5.2) and quickly homogenized with a Polytron tissue disrupter (Brinkman Instruments). One-half volume of ethanol was added, and the mixture was stored at −20 °C for 2 h. It was centrifuged at 8000*g* for 15 min, the pellet was dissolved in half the original volume of 8 M Gdn-HCl (50 mM Tris-HCl, pH 7.2–5 mM EDTA–5 mM dithiothreitol), and RNA was precipitated by the addition of 0.5 volume of ethanol. The above step was repeated once more. The final pellet was dissolved in 5 M Gdn-HCl (50 mM Tris-HCl, pH 7.2–5 mM EDTA–5 mM dithiothreitol), layered over a step gradient of CsCl consisting of 5.7 and 4.7 M CsCl in 10 mM Tris-HCl–1 mM EDTA (pH 7.2)–0.2% sodium lauroyl sarcosinate, and centrifuged at 35000 rpm for 16–18 h in a Beckman SW50 or SW41 rotor at 12 °C. The pelleted RNA was dissolved in water and ethanol precipitated. Poly(A) selection was attained by oligo(dT)–cellulose chromatography (Aviv & Leder, 1972; Arya, 1982).

For Northern blot hybridization, poly(A)-selected RNA was denatured by incubation at 65 °C for 5 min in 10 mM MOPS (pH 7.2)–50% formamide–6% formaldehyde, electrophoresed in a 1% agarose slab gel containing 6% formaldehyde (10 μg of RNA/lane), and transferred to a Zeta Probe membrane (Bio-Rad Laboratories) by electroelution. Dot blots were prepared by denaturing poly(A)-selected RNA in 6× SSC–6% formaldehyde (65 °C, 10 min) and applying serial dilutions (1:1) of RNA in 15× SSC to a sheet of nitrocellulose. The first sample in a series contained 10 μg of RNA. The filters were baked for 2 h at 80 °C under vacuum. The filter with RNA was prehybridized at 37 °C for 2–4 h in a mixture containing 50% formamide, 5× SSC, 5× PM [PM = 0.02% each of bovine serum albumin, poly(vinylpyrolidone), and Ficoll 400], 0.05 M sodium phosphate (pH 7), 100 μg/mL yeast RNA, 20 μg/mL denatured salmon DNA, and 0.1% SDS. Hybridization with ^{32}P-labeled probe was performed at 37 °C for 16–18 h in the above mixture plus 10% dextran sulfate. The probe consisted of a full-length TCGF cDNA clone (Clark et al., 1984) labeled in vitro by nick translation. The filter was subsequently washed repeatedly with SSC containing 0.1% SDS at 65 °C, air-dried, and exposed to a Kodak XAR film with intensifying screens.

Nuclear Transcription. Nuclei were prepared essentially according to Mulvihill & Palmiter (1977), including the additional purification by centrifugation through 2 M sucrose. Freshly harvested cells were washed with 10 mM Tris-HCl (pH 7.2)–10 mM KCl–3 mM $MgCl_2$–3 mM dithiothrietol and suspended in lysis buffer [10 mM Tris (pH 7.5)–2 mM $MgCl_2$–3 mM $CaCl_2$–0.5 mM β-mercaptoethanol–0.3 M sucrose]. The suspension was made 0.02% in NP40, vortexed vigorously but briefly, and centrifuged at 8000*g* for 10 min. The pellet was resuspended in lysis buffer, diluted 1:1 with 2 M sucrose, layered on a column of 2 M sucrose in 10 mM Tris-HCl (pH 7.5)–5 mM $MgCl_2$–0.5 mM β-mercaptoethanol, and centrifuged at 20000 rpm for 40 min in a Beckman SW50.1 rotor. The pelleted nuclei were suspended in 40% glycerol–50 mM Tris-HCl (pH 8.2)–5 mM $MgCl_2$–0.1 mM EDTA at a density of about 1 × 10^8 nuclei/mL and stored at −80 °C when not immediately used. The quality of nuclei was monitored by phase-contrast microscopy after staining the suspension with trypan blue.

About 1 × 10^7 nuclei were incubated at 26 °C for 40 min in a 200-μL reaction mixture containing 20% glycerol, 50 mM Tris-HCl (pH 75.), 0.1 M KCl, 4.5 mM $MgCl_2$, 2 mM dithiothrietol, 0.4 mM each of ATP, GTP, and CTP, and 200 μCi of [^{32}P]UTP (3000 Ci/mmol). The mixture was made 0.5 unit/μL in RNasin (Boehringer-Mannheim Biochemicals), and RNA was purified as described by McKnight & Palmiter (1979). About (10–20) × 10^6 cpm of [^{32}P]RNA was hybridized to cloned TCGF DNA and vector pBR 322 DNA (1 and 0.1 μg) immobilized on a nitrocellulose filter. Each filter received the same amount of [^{32}P]RNA in a given experiment, and hybridization was carried at 37 °C for 3 days in 0.8 mL of hybridization mixture used for Northern blotting described above. The filters were subsequently washed with 2× SSC–0.1% SDS at 60 °C and incubated at 37 °C for 40 min in 2× SSC with 10 μg/mL RNase A and 10 units/mL RNase T_1. They were further incubated for 40 min with 50 μg/mL proteinase K, washed again with several changes of 2× SSC–0.1% SDS at 60 °C, air-dried, and exposed to a Kodak XAR film with intensifying screens.

Actinomycin D Treatment. Cells were induced with PHA (1%) and TPA (50 ng/mL) for 4 h. An aliquot of cells was harvested, and the rest were cultured in aliquots with fresh medium containing 5 μg/mL actinomycin D and (i) no additive, (ii) PHA, (iii) TPA, and (iv) PHA plus TPA. They were incubated for additional 4 h and processed for RNA isolation as described above. This protocol of treatment resulted in more than 95% inhibition of total RNA synthesis.

Results

We have previously reported the kinetics of induction of TCGF mRNA in Jurkat cells (Clark et al., 1984). The TCGF mRNA reaches its maximal intracellular level in 4–6 h postinduction. Extracellular TCGF production by Jurkat cells is maximal at about 12 h and is maintained at a high level for at least 20–24 h (Gillis & Watson, 1980; our unpublished results). Thus, for this study, TCGF mRNA levels and extracellular TCGF were measured at 4–6 and 20–24 h, respectively.

Induction of TCGF Production. Figure 1 shows the production of TCGF by Jurkat cells induced by PHA in combination with TPA, IL-1, or both. Neither TPA nor IL-1 alone induced significant TCGF production. PHA alone induced some production, which was increased somewhat by IL-1. The addition of TPA along with PHA resulted in marked enhancement in TCGF production, confirming an earlier report (Gillis & Watson, 1980). The level of TCGF in the medium was significantly increased by the inclusion of IL-1 with PHA and TPA. The inset in Figure 1 details the IL-1-caused enhancement in the production of TCGF. The aliquots of me-

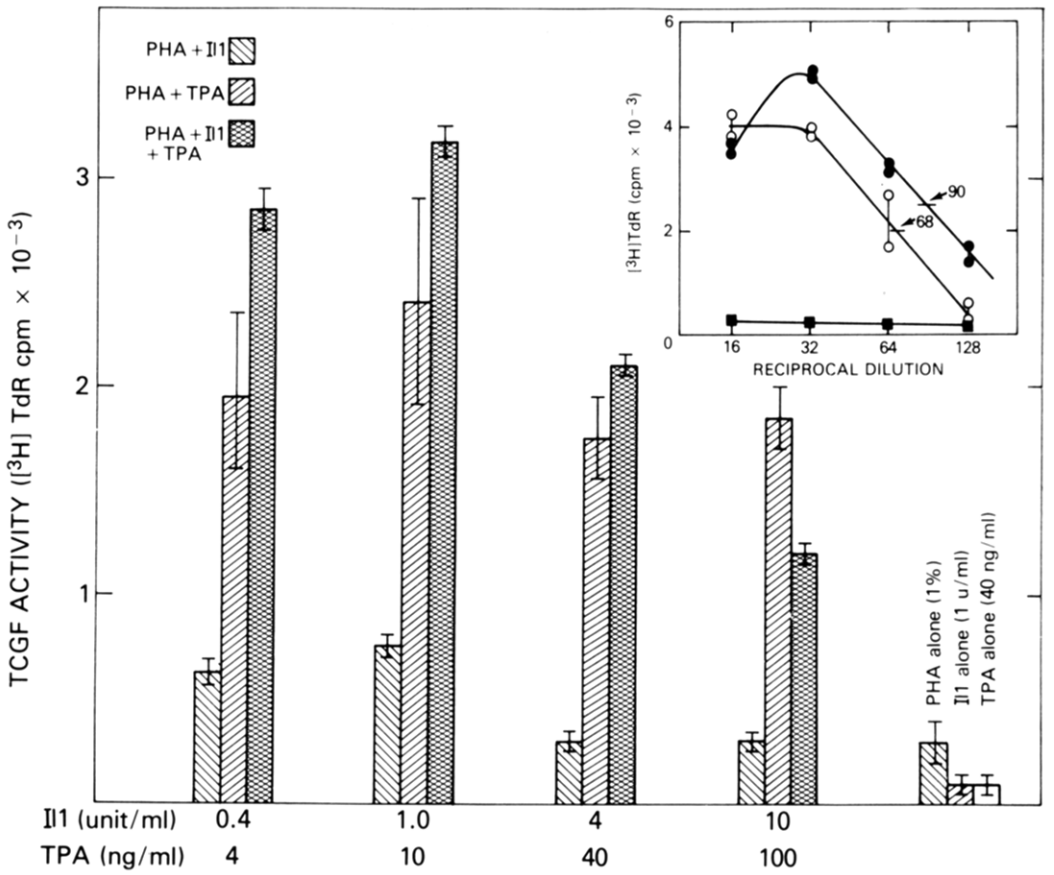

FIGURE 1: PHA-, TPA-, and IL-1-induced production of TCGF by Jurkat cells. The cells were treated for 20 h with the indicated inducing agent, alone or in combination. The conditioned medium was harvested, diluted 8-fold, and assayed for TCGF activity by measuring [^{3}H]thymidine incorporation by murine CTLL cells. (Inset) Result of an experiment where medium harvested from cultures treated with IL-1 (1 unit/mL) (■), PHA (1%) plus TPA (10 ng/mL) (○), and PHA (1%) plus TPA (10 ng/mL) plus IL-1 (1 unit/mL) (●) was serially diluted and assayed for TCGF activity.

dium from cultures treated with IL-1 (1 unit/mL), PHA (1%) plus TPA (10 ng/mL), and PHA (1%) plus TPA (10 ng/mL) plus IL-1 (1 unit/mL) were serially diluted and assayed for TCGF activity (see Materials and Methods). The dilution that gave half-maximal TCGF activity for cultures induced with PHA plus TPA was about 68 and for those induced with PHA plus TPA plus IL-1 was about 90. As noted by Gillis & Watson (1980), the maximal stimulation by TPA was obtained when its concentration was about 10 ng/mL, and this level of stimulation was maintained up to a concentration of at least 50 ng/mL. The concentration of IL-1 that gave maximal response in combination with PHA and TPA was 1 unit/mL.

Steady-State Levels of TCGF mRNA. The levels of TCGF mRNA were evaluated by Northern blot and dot blot hybridization. Figure 2 shows the results of two separate experiments. As previously noted (Arya et al., 1984a,b; Clark et al., 1984), TCGF mRNA in human cells exists as an 11–12S species. Untreated control cells contained essentially undetectable, if any, TCGF mRNA. Similarly, cells treated with TPA alone (Figure 2) or IL-1 alone (not shown) did not contain detectable TCGF mRNA. The cells treated with PHA contained low but detectable levels of TCGF mRNA, and these levels were markedly increased by including TPA (10 ng/mL) in the induction medium (Figure 2a). IL-1 further increased the concentration of TCGF mRNA above that induced by PHA and TPA when it was included along with PHA and TPA (Figure 2b). To determine if IL-1-induced enhancement in TCGF mRNA levels could be observed when the TPA concentration in the induction medium was in excess of the minimal required for the maximal TCGF induction (10

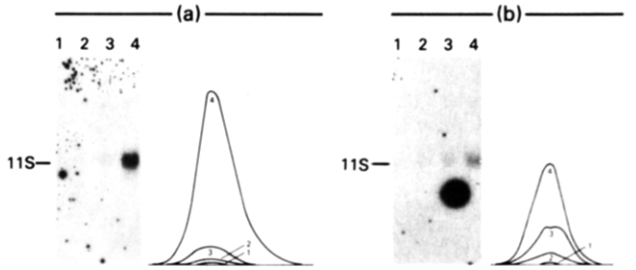

FIGURE 2: Induction of TCGF mRNA in Jurkat cells by PHA, TPA, and IL-1. Cels were treated with PHA (1%), TPA (10 ng/mL), and IL-1 (1 unit/mL) for 4 h. Poly(A)-selected RNA was isolated and analyzed for TCGF-specific sequences by Northern blot hybridization with labeled cloned TCGF DNA as a probe. (a) (Lane 1) Control cells; (lane 2) cells treated with TPA; (lane 3) cells treated with PHA; (lane 4) cells treated with PHA plus TPA. (b) (Lane 1) Control cells; (lane 2) cells treated with PHA plus IL-1; (lane 3) cells treated with PHA plus TPA; (lane 4) cells treated with PHA plus TPA plus IL-1. On the right are the relative intensities of the bands traced by a laser-sourced densitometer.

ng/mL), cells were incubated with IL-1 along with PHA in the presence of 40 ng/mL TPA instead of 10 ng/mL as for the previous experiment. The TCGF mRNA levels in this case were analyzed by dot blot hybridization. The results, presented in Figure 3, confirmed the results presented in Figure 2 and further showed that IL-1-induced enhancement in TCGF mRNA levels occurred whether the concentration of TPA in the induction medium containing PHA was 10 or 40 ng/mL. Some variation in the relative levels of TCGF mRNA was observed from experiment to experiment, presumably related to the time Jurkat cells had been maintained in culture, but

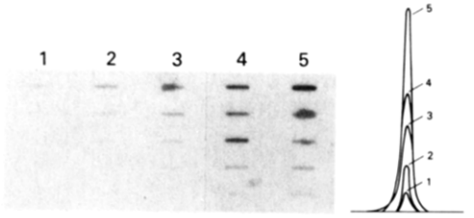

FIGURE 3: Induction of TCGF mRNA in Jurkat cells by PHA, TPA, and IL-1. Cells were treated with PHA (1%), TPA (40 ng/mL), and IL-1 (1 unit/mL) for 6 h. Poly(A)-selected RNA was analyzed for TCGF-specific sequences by dot blot hybridization with labeled cloned TCGF DNA as a probe. (Lane 1) Control cells; (lane 2) cells treated with PHA; (lane 3) cells treated with PHA plus IL-1; (lane 4) cells treated with PHA plus TPA; (lane 5) cells treated with PHA plus TPA plus IL-1. (The relative greater intensity of the third band from top in lane 4 is an artifact; it was not observed in other experiments.) Curves on the right are the respective densitometer tracings of the top bands shown on left.

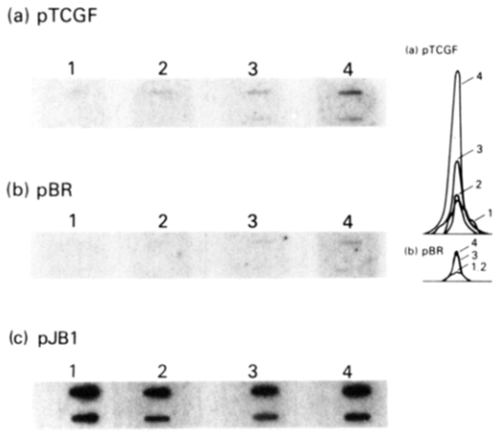

FIGURE 4: Analysis of TCGF mRNA synthesized by nuclei from induced and uninduced Jurkat cells. Cells were induced with PHA (1%) and TPA (40 ng/mL) for 4 h, nuclei from uninduced and induced cells were allowed to elongate RNA chains in vitro, and labeled RNA thus obtained was hybridized with cloned TCGF DNA, cloned JB1 DNA, and vector pBR DNA immobilized on a nitrocellulose sheet. (Lane 1) Control cells; (lane 2) cells treated with TPA; (lane 3) cells treated with PHA; (lane 4) cells treated with PHA plus TPA. Curves on the right are the respective densitometer tracings of the top bands shown on left.

this affected only the magnitude of the observed induction in a particular experiment.

Nuclear Transcription. To determine if the inducing agents acted by primarily influencing TCGF gene transcription, nuclear transcription, or "run-off", experiments were performed. In vitro nuclear transcription assays have been used to study transcriptional activation (McKnight & Palmiter, 1979; Groudine et al., 1981). Such nuclear transcription systems support elongation of RNA polymerase II transcripts initiated in vivo but do not permit reinitiation. Thus, 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 (Groudine et al., 1981; Chao et al., 1983). For these experiments, cells were treated with inducing agents alone or in combination, and the nuclei from each culture were prepared and allowed to elongate the preinitiated RNA chains in vitro. Figures 4 and 5 show the results of hybridization of labeled RNA thus obtained with TCGF DNA (cloned in pBR322) (pTCGF) and vector pBR322 DNA (pBR). The treatment of cells with PHA resulted in some increase in the transcription of the TCGF gene, and this was further enhanced by TPA (Figure 4). When the cells were treated with IL-1 in addition to PHA and TPA, transcription of the TCGF gene was increased above

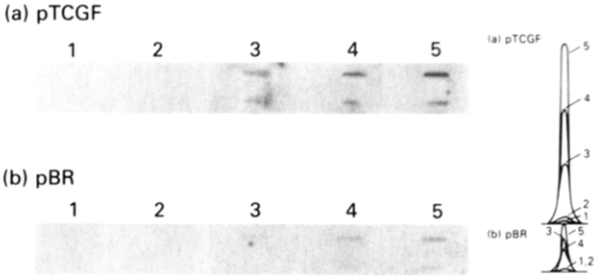

FIGURE 5: Analysis of TCGF mRNA synthesized by nuclei from induced and uninduced Jurkat cells. Cells were induced with IL-1 (1 unit/mL), PHA (1%), and TPA (40 ng/mL) for 6 h, nuclei from uninduced and induced cells were allowed to elongate RNA chains in vitro, and labeled RNA thus obtained was hybridized with cloned TCGF DNA and vector pBR DNA immobilized on a nitrocellulose sheet. (Lane 1) Uninduced cells; (lane 2) cells treated with IL-1; (lane 3) cells treated with PHA plus IL-1; (lane 4) cells treated with PHA plus TPA; (lane 5) cells treated with PHA plus TPA plus IL-1. Curves on the right are the respective densitometer tracings of the top bands shown on the left.

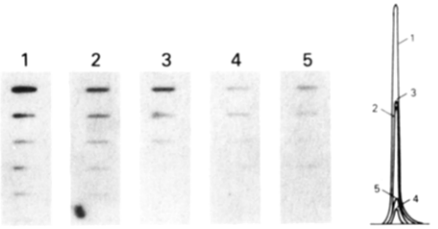

FIGURE 6: Effect of actinomycin D on the persistence of TCGF mRNA in induced Jurkat cells. Cells were induced with PHA (1%) and TPA (40 ng/mL) for 4 h, washed, and recultured for an additional 4 h in medium containing actinomycin D (5 μg/mL) with or without inducers. RNA was isolated and hybridized with labeled cloned TCGF probe by dot blot hybridization. (Lane 1) Cells induced for 4 h with no further treatment; (lane 2) induced cells incubated with actinomycin D without inducer; (lane 3) induced cells incubated with actinomycin D and PHA; (lane 4) induced cells incubated with actinomycin D and TPA; (lane 5) induced cells incubated with actinomycin D and PHA plus TPA. Curves on the right are the respective densitometer tracings of the top bands shown on the left.

that induced by PHA and TPA (Figure 5). Whether TPA alone (Figure 4) or IL-1 alone (Figure 5) activated gene transcription could not be determined because of the low magnitude of the response obtained. The RNA synthesized by nuclei from induced cells generally gave a slightly higher background hybridization with pBR322 DNA, but the induction of TCGF gene transcription was selective. The transcripts corresponding to another cloned gene, termed JB1, were not induced by either PHA or TPA or a combination of the two agents (Figure 4). The JB1 clone was obtained from a Jurkat cell cDNA library, and its mRNA is constitutively synthesized in Jurkat cells (data not shown). The transcription by nuclei in vitro was sensitive to α-aminitin inhibition in a dose-dependent manner; at a concentration of α-aminitin of 0.5 μg/mL or more, a transcription was inhibited by 75–80% (data not shown).

Actinomycin D Treatment. To determine if PHA or TPA affected the half-life of TCFG mRNA, the induced cells were incubated with actinomycin D in the presence or absence of inducers. The results are shown in Figure 6. PHA plus TPA as expected induced significant TCGF mRNA during the first 4 h of induction. The levels of TCGF mRNA declined in all actinomycin D treated cultures during the next 4 h of incubation. These levels in actinomycin D treated cells cultured with or without PHA were roughly equivalent, showing that actinomycin D treatment effectively blocked further induction of TCGF mRNA by PHA. Somewhat unexpectedly, the levels

of TCGF mRNA in treated cells cultured with PHA plus TPA were lower than the control-treated cultures. Similar results were obtained for treated cells cultured with TPA alone.

Discussion

TPA has been shown to affect a variety of cellular functions, inducing some in certain cells and depressing them in others (Hecker et al., 1982). The molecular mechanisms of these effects have not been extensively investigated. The availability of cloned TCGF DNA and unambiguous induction of TCGF by TPA along with PHA provided a model system to study its effects at the molecular level. The TPA-caused enhancement of TCGF production by cells induced with PHA could be due to transcriptional or posttranscriptional effects. For example, TPA could increase the release of TCGF by the induced cells rather than increase its synthesis. TPA is known to alter the cytoskeleton and morphology of cells (Rifkin et al., 1979; Rovera et al., 1979) and reportedly induces the release of such agents as prostaglandins (Weinstein et al., 1979) and plasminogen activator (Quigley, 1979). Our results, however, show that TPA selectively increases the steady-state levels of TCGF mRNA in induced cells (Figures 2 and 3). Similarly, IL-1 also increase the accumulation of TCGF mRNA in cells induced with PHA and TPA (Figures 2 and 3). Both of these inducers appear to act synergistically with PHA. These effects are brought about at least in part by enhanced transcriptional activity of the TCGF gene. Nuclear transcription experiments suggest that TCGF gene transcription is increased in cells induced with TPA plus PHA and in cells induced with IL-1 plus PHA (Figures 4 and 5). We were unable to determine if TPA or IL-1 activates TCGF gene transcription in the absence of PHA. Cells treated with TPA alone (Figure 2) or IL-1 alone (not shown) did not contain detectable steady-state levels of TCGF mRNA. The effects on steady-state mRNA levels and RNA transcribed by nuclei in vitro were selective for TCGF. These parameters for another gene (JB1), isolated from a Jurkat cell cDNA library, were not modulated by PHA and TPA. Further, the gene transcription was sensitive to α-aminitin inhibition, showing that up to 80% of RNA molecules labeled by nuclei in vitro were initiated by RNA polymerase II.

It has been suggested that TPA acts with leukemic Jurkat cells in a manner analogous to IL-1 with normal lymphocytes in inducing TCGF production (Farrar et al., 1980; Stadler et al., 1981). Our results show that IL-1 increases the synthesis of TCGF mRNA in Jurkat cells even in cultures maximally induced with TPA plus PHA. This implies that TPA and IL-1 act by different mechanisms in enhancing the activity of the TCGF gene induced with PHA. It is likely that both of these inducers act by binding to specific reeptors on the cellular membrane (Sando et al., 1981), and their effect on mRNA synthesis is brought about by secondary mediators. The saturating concentration of a given inducer (e.g., TPA) used here may pertain to its capacity to bind the specific membrane receptors and not to the capacity of the TCGF gene to be induced by secondary mediators activated by that inducer. Thus, TPA and IL-1 could individually generate different or the same secondary mediators, which could affect TCGF gene transcription by acting in concert on the same basic process such as the rate of transcription of the gene.

The experiments with actinomycin D suggest that TPA may affect other processes involved in TCGF production in addition to TCGF gene transcription. The steady-state level of TCGF mRNA in actinomycin D blocked, TPA-treated cultures was lower than that of the actinomycin D blocked control cultures. This may suggest an increased degradation of TCGF mRNA caused by TPA. Alternatively, TPA could increase the utilization of TCGF mRNA by increasing the rate of its translation into TCGF. These alternatives have yet to be investigated. In either case, this would be secondary to its affect on the synthesis of TCGF mRNA because the steady-state levels of TCGF mRNA are elevated in TPA-treated cultures. It is of course possible that this effect of TPA on TCGF mRNA turnover is an artifact introduced by actinomycin D treatment and is not a significant factor in the normal induction process.

The TPA- and IL-1-enhanced production of TCGF by PHA-induced cultures is at least in part due to their effect on the transcriptional activity of the TCGF gene. This is among the few known cases where TPA induction has been demonstrably related to the transcription of specific gene(s). Other examples include preliminary evidence showing TPA-caused stimulation of mouse mammary tumor virus is probably due to the increased synthesis of virus-specific RNA (Arya, 1980) and the induction of a 32-kDa protein in mouse cells is also correlated with the appearance of a specific mRNA (Hiwasa et al., 1982). In addition, TPA-mediated inhibition of cellular differentiation of murine erythroleukemia cells is accompanied by decreased accumulation of globin mRNA (Fibach et al., 1979).

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Characteristics of Tyrosinate Fluorescence Emission in α- and β-Purothionins†

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ABSTRACT: The CD, absorption, and fluorescence spectra and fluorescence lifetimes of three highly homologous, basic cytotoxic proteins isolated from wheat (α_1-, α_2-, and β-purothionins) and a moderately homologous protein isolated from *Crambe abyssinica* (crambin) have been measured. The purothionins each contain a single tyrosine, while crambin has two tyrosine residues. At neutral pH in buffered solution or in water, β-purothionin showed a single fluorescence emission peak maximal at 345 nm; α_1- and α_2-purothionins gave a double-humped emission (λ_{max} 308 and 345 nm), while crambin emitted only at 303 nm. Under acid pH conditions (<pH 3) or when denatured in 6 M guanidine hydrochloride, the spectra of the α- and β-purothionins showed predominantly the 303-nm emission (τ = 3.1 ns) while at pH >10.0 only the 345-nm emission was evinced by all three proteins. Crambin showed typical tyrosine emission in the pH range 3-9 and weak tyrosinate fluorescence at pH >10.5. From these features, and from the absorption and CD spectra, we infer that the 345-nm fluorescence emission of either α_1- or β-purothionin is from tyrosinate moieties. The purothionin emission spectra appear to be generated by excited-state proton transfer rather than from tyrosinate species in the ground state.

Protein fluorescence maximal at wavelengths greater than 310 nm is usually ascribed to the presence of tryptophan residues. In general, tyrosine fluorescence is considerably more blue shifted (λ_{max} occurs at approximately 303 nm) (Teale & Weber, 1957; Longworth, 1971; Cowgill, 1976). Recently, however, a component of tyrosine fluorescence that apparently derives from the excited state of the ionized *p*-hydroxyphenyl moiety (Cornog & Adams, 1963) has been described in several proteins containing tyrosine but no tryptophan residues (Kimura & Ting, 1971; Kimura et al., 1972; Graziani et al., 1974; Rayner et al., 1976; Szabo et al., 1978; Lim & Kimura, 1980; Jordano et al., 1983; Pundak & Roche, 1983). The characteristic feature of this fluorescence emission is that it is maximal at λ >310 nm, usually at 345 nm. Not surprisingly, such emission is likely to be masked in proteins containing both tryptophan and tyrosine residues, either by the predominance of tryptophan emission or by the occurrence of energy transfer (at rates faster than the rate of proton transfer) from tyrosine to tryptophan moieties. Despite the problems inherent in identifying tyrosinate fluorescence in the presence of tryptophan emission, its presence has been demonstrated in at least one protein, albumin (Longworth, 1981).

For tyrosinate fluorescence to occur, there is an obvious requirement for proton transfer from the phenolic moieties of tyrosine (either in the ground state or in the excited state) to an appropriate acceptor. Proton transfer from the *p*-hydroxyphenyl moiety in the excited state is far more likely because of the increased acidity of the excited state relative to the ground state. Szabo et al. (1979) quotes values of 5.4 and 10.4 for pK_a's of the phenolic hydroxyl of the excited and ground states, respectively. The proton acceptor can, in principle, be buffer species in the solvent providing they are sufficiently basic (e.g., phosphate or acetate; Szabo et al., 1979). In proteins in which tyrosinate fluorescence is evinced,

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