MOLECULAR AND CELLULAR BIOLOGY, July 1991, p. 3528–3536
0270-7306/91/073528-09$02.00/0

Vol. 11, No. 7

Negative Regulation of Globin Gene Expression during Megakaryocytic Differentiation of a Human Erythroleukemic Cell Line

NADYA L. LUMELSKY* AND BERNARD G. FORGET

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

Received 11 February 1991/Accepted 15 April 1991

The human erythroleukemic cell line K562 was used as a model for analysis of the mechanisms responsible for alterations in gene expression during differentiation. K562 cells normally synthesize fetal hemoglobin (γ-globin), but treatment with tumor-promoting phorbol esters (phorbol myristate acetate and tetradecanoyl phorbol acetate) results in the loss of the erythroid phenotype of the cells and causes a shift toward a megakaryocytic phenotype. This shift involves markedly decreased production of fetal hemoglobin and de novo synthesis of a number of proteins specific for megakaryocytes. The results of this work indicate that negative regulation of fetal hemoglobin during megakaryocytic differentiation of K562 cells occurs at the level of down regulation of γ-globin mRNA accumulation. This effect consists of at least two components: reduction in the rate of transcription of the γ-globin gene and decrease in stability of the normally very stable γ-globin mRNA. We have developed two assay systems that permit investigation of the transcriptional and posttranscriptional effects of phorbol myristate acetate independently from each other. These assay systems make use of a heterologous reporter gene for the transcriptional analysis and a marked γ-globin gene for the analysis of mRNA stability. The DNA sequences located in the 3′ flanking region of the ^{A}γ-globin gene were found to be responsible for the decrease in transcription rate.

Differentiation of eukaryotic cells is accomplished through the alteration of the expression of genes characteristic of the terminal differentiated phenotype. Not only do the appropriate genes have to be activated during this process, but the genes that are not normally expressed in a given cell lineage need to be silenced. The molecular mechanisms responsible for the complex interplay of these positive and negative regulatory events are still poorly understood in most systems. In this work, we used phorbol ester-mediated megakaryocytic differentiation of the human erythroleukemic cell line K562 as a model to investigate the molecular mechanisms associated with suppression of erythroid-specific genes during activation of megakaryocyte-specific differentiation.

Megakaryocytopoiesis, a process leading to formation of platelets, is part of multilineage hematopoietic differentiation. Hematopoiesis is organized in a hierarchical fashion. A pluripotent stem cell located in the bone marrow differentiates through a series of committed progenitors toward mature terminally differentiated cells of each blood cell lineage. In vitro purification of the natural progenitors committed to a particular blood cell lineage has not yet been accomplished in most cases. One of the major obstacles to achieving this goal is the unavailability of good assays for the identification of progenitors. Until more progress is made in the purification of natural hematopoietic progenitors, established tissue culture cell lines that mimic the differentiation patterns of progenitors can serve as model systems for studying the regulation of gene expression during hematopoietic cell differentiation.

The available data indicate that erythrocytic and megakaryocytic pathways of differentiation are closely related and that there may exist a progenitor common to these two cell lineages. Erythrocytic and megakaryocytic cells share several transcription factors which are not found in other hematopoietic lineages (27, 38). These factors are necessary for the expression of the erythrocyte- and megakaryocyte-specific genes. However, despite the presence of these common transcription factors, megakaryocytes do not express erythrocyte-specific genes. Thus, at some stage during maturation of the putative erythrocytic-megakaryocytic progenitor cell, an additional level of regulation responsible for the proper silencing of the erythroid genes is imposed in the megakaryocytes.

A cell line that can be used to mimic silencing of the erythroid genes in megakaryocytes is the human erythroleukemic cell line K562. The K562 cell line was established from a patient with chronic myelogenous leukemia (26). Under standard culture conditions, these cells express a number of erythroid-specific genes, such as those for fetal hemoglobins (^{A}γ- and ^{G}γ-globins), embryonic hemoglobins (ε- and ζ-globins), and α-globin (41), as well as erythroid-specific cell surface markers (3). However, when these cells are exposed to tumor-promoting phorbol esters such as 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and 12-myristate 13-acetate (PMA), their differentiation phenotype changes: expression of the globin genes is turned off, and the expression of megakaryocytic genes is initiated (1, 2, 50). Such a drastic transition of phenotypes makes this system an attractive model with which to investigate the molecular mechanisms of gene silencing during differentiation.

An additional attractiveness of this system stems from the fact that the biochemical effect of tumor-producing phorbol esters is well established. These agents are known to induce activation of the serine-threonine kinase, protein kinase C (PKC). PKC activation mediates most of the pleiotropic biological effects of these compounds (30). Activation of PKC by phorbol esters mimics the action of diacylglycerols, which are products of multistep phosphoinositol lipid turn-

* Corresponding author.

over initiated by the binding of some growth factors and hormones to their cell surface receptors during natural signal transduction. Phorbol esters lead to direct activation of PKC, bypassing the natural signal transduction pathway. A number of cellular substrates are phosphorylated by PKC, but their role in signal transduction is unclear (39). Through a series of still poorly understood phosphorylation events, the PKC signal reaches the nucleus of the cell and leads to an alteration in the expression of genes containing so-called TPA-responsive elements in their regulatory regions (15). Transcription factor AP-1, a heterodimer of the products of two nuclear proto-oncogenes, c-*jun* and c-*fos*, binds to the TPA-responsive element in response to phorbol esters. AP-1 binding usually results in activation of transcription of genes bearing this regulatory sequence element (11, 36).

In the work described here, we found that the phorbol ester-mediated suppression of fetal γ-globin genes in K562 cells takes place at both the transcriptional and posttranscriptional levels. We also identified the *cis*-acting element mediating the transcriptional down-regulation. Interestingly, this negative regulatory element is contained within the same DNA fragment that acts as an enhancer of γ-globin gene expression in transient transfection assays. Posttranscriptional down-regulation probably involves the destabilization of γ-globin mRNA. The destabilizing effect of phorbol esters is reversed by the protein synthesis inhibitor cycloheximide and the RNA polymerase II inhibitor actinomycin D (dactinomycin).

MATERIALS AND METHODS

Cell culture. K562 cells were passaged in RPMI 1640 medium supplemented with 15% fetal calf serum. The cells were always split before they reached the density of 10^6/ml. Incubations with PMA were carried out at the starting cell density of 4×10^5/ml for the indicated periods of time. PMA was dissolved in acetone at a concentration of 800 μM and diluted in phosphate-buffered saline (PBS), as necessary. The final concentrations in cell cultures were as follows: PMA, 20 nM; hemin, 50 μM; cycloheximide, 10 μg/ml; and actinomycin D, 5 μg/ml (all from Sigma, St. Louis, Mo.).

Plasmid constructions. The *Hin*dIII-*Bam*HI DNA fragment containing the copy of the luciferase cDNA clone with the simian virus 40 small t antigen intron and the simian virus 40 polyadenylation sequences from plasmid pUC007L (a gift of Kevin McDonagh, National Institutes of Health) was introduced into the Epstein-Barr virus (EBV)-derived vector p220.2 (49) (a gift of William Sugden, University of Wisconsin) to create plasmid p220Luc. During this cloning procedure, the *Hin*dIII site of the insert and the *Xba*I site of the vector were filled in with Klenow DNA polymerase, but the *Bam*HI sites of the insert and the vector were preserved. The γ-globin promoters (the minimal [−380] and extended [−1350] forms) were introduced upstream of the luciferase gene of p220Luc into the *Sal*I site to create plasmids p220Lucγ and p220LucγL, respectively. The fragments of interest from the β-globin gene cluster were inserted upstream of the minimal γ-globin gene promoter into a *Hin*dIII site of p220Lucγ. This was accomplished by making all of the fragments blunt ended with Klenow DNA polymerase or mung bean nuclease and addition of *Hin*dIII linkers. The plasmid containing a copy of the marked γ-globin gene (p220γ*) was produced by inserting the 3.3-kb *Hin*dIII-*Hin*dIII fragment of the γ* gene insert (37) into the *Hin*dIII site of p220.2.

Transfections and selection of stable lines. Plasmids of interest were introduced into logarithmically growing K562 cells by electroporation using a Gene Pulser (Bio-Rad, Richmond, Calif.). The electroporations were carried out as follows. The cells (10^7 cells per transfection) were washed twice with PBS and resuspended in 0.8 ml of HBS buffer (20 mM *N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid [HEPES; pH 7.05], 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM glucose); 20 μg of plasmid DNA was added, and the cells were incubated on ice for 10 min. The cells were shocked at 350 mV and 500 μF (the time constant was about 10 ms), transferred on ice for 10 more min, and placed into 30 ml of prewarmed RPMI medium. After 48 h, hygromycin (Calbiochem, La Jolla, Calif.) was added to a final concentration of 250 μg/ml. The cells were diluted to a final concentration of $\sim 4 \times 10^5$ cells per ml to ensure their ability to grow actively. After 48 h, the cells were serially diluted into 24-well microtiter plates and incubated for approximately 2 weeks, until large mature colonies were formed. The cells were then pooled from the wells in which the colonies were most abundant, and the culture was split several times to free it from dead cell debris before being frozen in aliquots.

Preparation of RNA, nuclear run-on assays, and luciferase expression assays. Total cytoplasmic RNA was prepared from $\sim 10^7$ cells as described previously (7), with some modifications. The cells were washed with cold PBS and resuspended in 400 μl of cold low-salt Tris (20 mM Tris [pH 7.4], 10 mM NaCl, 3 mM magnesium acetate) containing 25 μl of 1 mM ribonucleoside-vanadyl complexes (RNase inhibitor; Sigma). All solutions were made with diethyl pyrocarbonate-treated water to prevent RNase contamination. Then 130 μl of cold lysing buffer was added (low-salt Tris containing 5% [wt/vol] sucrose and 4% Nonidet P-40), and the lysates were incubated on ice for 5 min with occasional mixing. The lysates were underlaid with 3 ml of sucrose cushion buffer (low-salt Tris containing 24% [wt/vol] sucrose and 1% Nonidet P-40) and centrifuged at 4°C for 20 min at $\sim 4{,}000 \times g$. The upper phase containing the cytoplasmic RNA was collected and extracted by vortexing with 2 ml of acetate buffer (50 mM sodium acetate [pH 5.5] with acetic acid, 10 mM Na_2EDTA), 130 μl of 10% sodium dodecyl sulfate (SDS), and 2.7 ml of phenol saturated with acetate buffer, preheated to 56°C. The mixture was centrifuged to separate the upper phase, and the upper phase was then reextracted with phenol-chloroform (1:1) and then with chloroform alone. The RNA was precipitated twice (20 min at −70°C) with 2.5 volumes of ethanol in the presence of 1/20 volume of 10% [wt/vol] potassium acetate, washed with 70% ethanol, and resuspended in diethyl pyrocarbonate-treated H_2O. The poly(A)$^+$ RNA was prepared by using the RiboSep (Collaborative Research, Inc., Bedford, Mass.) mRNA isolation kit as recommended by the manufacturer.

The nuclear run-on transcription assays were carried out as described previously (43), with some modifications. The nuclei were prepared from 10^8 logarithmically growing cells. The cells were washed in cold PBS, resuspended in 10 ml of lysing solution (10 mM Tris [pH 7.4], 2 mM $MgCl_2$, 3 mM $CaCl_2$, 3 μm dithiothreitol, 0.3 M sucrose, 0.5% Triton X-100) and disrupted with 10 strokes of a loosely fitting Dounce homogenizer, and the nuclei were centrifuged through a 20-ml sucrose cushion (see above). The nuclear pellet was suspended in 200 μl of the reaction buffer (20 mM Tris [pH 8.3], 100 mM KCl, 4.5 mM $MgCl_2$, 0.4 mM each ATP, GTP, and CTP, 2 mM dithiothreitol, 20% glycerol, 125 μCi of [α-^{32}P]UTP [3,000 Ci/mmol; Amersham, Arlington Heights, Ill.]) and incubated immediately for 30 min at 30°C.

After addition of 10 U of RQ DNase and 40 U of RNAsin (both from Promega Corp., Madison, Wis.) and incubation for 15 min at 30°C, 40 μl of 10% SDS and 25 μl of 10-mg/ml proteinase K were added, and incubation was continued for 30 min at 37°C. The labeled RNA was then isolated by adding to the transcription reactions 400 μl of 2× lysing solution (5.5 M guanidine thiocyanate, 0.35 M sodium citrate, 1% sarcosyl), 80 μl of 2 M ammonium acetate, 800 μl of water-saturated phenol, and 160 μl of chloroform. The solution was vortexed repeatedly and kept on ice for 15 min. The mixture was centrifuged at 4,000 × *g* for 20 min, the aqueous phase was removed, and the RNA was precipitated (20 min at −70°C) by addition of 2.5 volumes of ethanol and 1/10 volume of 7 M ammonium acetate. The RNA was collected by centrifugation for 30 min at 4,000 × *g*, washed with 70% ethanol, and resuspended in 100 μl of diethyl pyrocarbonate-treated water, and the amount of radioactivity in a 1-μl aliquot was determined. The typical yield was 5 × 10^6 to 1 × 10^7 cpm/10^8 cells. The labeled RNAs were hybridized to nitrocellulose strips containing the cloned DNAs of interest. The plasmid DNAs were applied in a slot blot apparatus (Schleicher & Schuell, Keene, N.H.), and the hybridization volumes were adjusted so that the concentration of the labeled RNA, in counts per minute per milliliter of the hybridization solution, was equal in all samples. The usual hybridization volume was ~1 ml. The hybridization buffer consisted of 10 mM TES buffer (Sigma), 0.2% SDS, 10 mM EDTA, 0.3 M NaCl, 1× Denhardt's solution, and 250 μg of yeast tRNA per ml. The hybridizations were carried out for 40 h at 65°C. The strips were washed twice for 5 min at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS, twice for 20 min in 0.2× SSC–0.1% SDS at 60°C, once for 30 min at 37°C in 2× SSC–10 μg of RNase A per ml, and finally for 20 min in 2× SSC at 37°C.

The luciferase expression assays were carried out as described previously (16).

Nuclease protection assays. The radioactive RNA probe used in the nuclease protection assays was synthesized from the SP6 phage promoter, using the Promega in vitro transcription kit as recommended by the manufacturer. Nuclease protection assays were carried out essentially as described previously (42), using 10 μg of cytoplasmic RNA and 300 U of S1 nuclease (Sigma) per reaction.

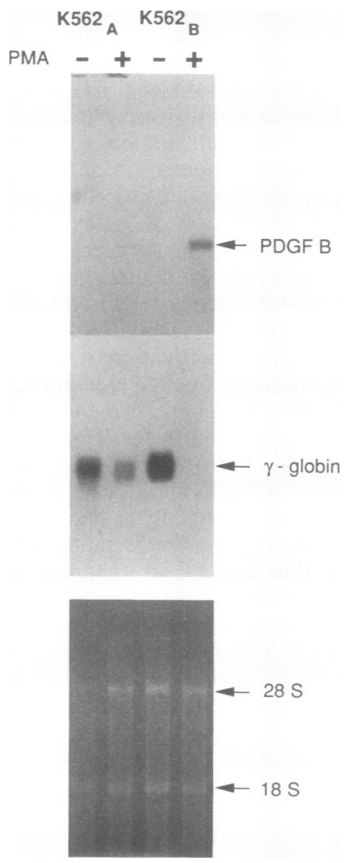

FIG. 1. Northern blot analysis of poly(A)$^+$ RNA from K562 cells following treatment with PMA. A total of 10^8 cells were exposed to PMA (20 nM) for 24 h. Poly(A)$^+$ RNA was purified by a single cycle of oligo(dT)-cellulose column chromatography (see Materials and Methods), and 5-μg samples were fractionated by electrophoresis in a 1% agarose-formaldehyde denaturing gel. The gel was stained with ethidium bromide to visualize the 18S and 28S rRNAs (bottom panel), and then the RNA was transferred onto a nitrocellulose membrane and hybridized to a γ-globin or PDGF B gene probe. Shown are the results of successive hybridization of the same filter to the γ-globin and PDGF B gene probes. The blot was exposed to X-ray film for either 16 h (PDGF B gene probe) or 30 min (γ-globin gene probe).

RESULTS

PMA suppresses the steady-state level of γ-globin mRNA in K562 cells. The first question we addressed was whether the down-regulation of γ-globin gene expression during megakaryocytic differentiation of K562 cells (33) occurs at the level of mRNA. Figure 1 shows Northern (RNA) blot analysis of the poly(A)$^+$ RNA isolated from K562 cells exposed to PMA for 24 h. Two different K562 cell isolates were used in this experiment; the $K562_A$ line was continuously maintained in culture for at least 4 years, whereas the $K562_B$ line was maintained in culture only for approximately 2 months prior to exposure to PMA. The Northern blot was probed with a γ-globin-specific probe (this probe detects both $^A\gamma$ and $^G\gamma$ transcripts) as well as with a probe specific for platelet-derived growth factor (PDGF B; a marker of megakaryocytic differentiation). This experiment clearly demonstrates that PMA induces a dramatic erythrocyte-to-megakaryocyte switch in $K562_B$ cells; moreover, this switch takes place at the level of steady-state mRNA. In $K562_A$ cells, the switch is minimal; the reduction in the amount of γ-globin mRNA and the increase in the amount of the PDGF B mRNA are small. This result suggests that that the *trans*-acting factor(s) that mediates the erythrocyte-to-megakaryocyte switch in K562 cells becomes depleted or cannot be induced after prolonged maintenance of the cells in culture. Therefore, all of the experiments described below, unless specifically indicated, were conducted with $K562_B$ cells. The expression of other erythrocyte-specific markers of K562 cells, such as ε-globin and α-globin, are also down-regulated by PMA at the level of mRNA accumulation (not shown).

The negative effect of PMA on γ-globin gene expression is also observed when hemin, the activator of γ-globin gene transcription in K562 cells (13), is present in the tissue culture medium (Fig. 2A). The positive effect of hemin on γ-globin gene transcription does not become apparent until after approximately 90 h of incubation with this agent, which is the reason for the long incubation time in this experiment (96 h). However, the effect of PMA is observed as soon as

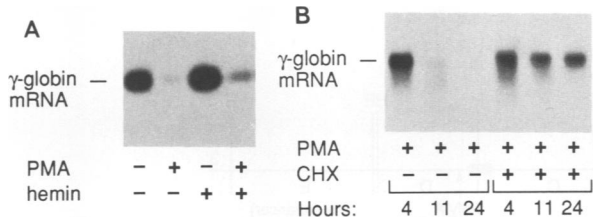

FIG. 2. Northern blot analysis of total cytoplasmic RNA from K562 cells. Cells were treated with PMA in the absence or presence of hemin (50 μM) for 96 h (A) or treated with PMA in the absence or presence of cycloheximide (10 μg/ml) for the indicated times (B). Northern blot analysis was carried out as described in the legend to Fig. 1; 10 μg of total cytoplasmic RNA was applied in each lane. The blots were exposed to X-ray film for 16 h.

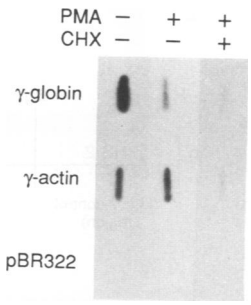

FIG. 3. Nuclear run-on transcription of γ-globin genes in K562 cell nuclei. Cells were treated with PMA in the absence or presence of cycloheximide for 24 h. Nuclei were prepared, and run-on transcription assays were carried out as described in Materials and Methods. The plasmids used for this experiment were $p^{A}\gamma KT$ (3.3-kb *Hin*dIII fragment of the $^{A}\gamma$-globin gene), pγ-actin (1.5-kb *Xho*I fragment of the human γ-actin gene), and pBR322. The amount of plasmid DNA applied per slot was equivalent to approximately 1 μg of target DNA for each probe.

11 h after its addition. Interestingly, when an inhibitor of protein synthesis, cycloheximide (Fig. 2B), or an inhibitor of RNA polymerase II, actinomycin D (not shown), is present during incubation, the effect of PMA is significantly blocked.

PMA affects both the rate of transcription and the stability of the γ-globin gene mRNA. The reduction in the amount of steady-state γ-globin gene mRNA seen in Fig. 2A could be due to transcriptional or posttranscriptional effects of PMA. Globin mRNAs are among the most stable eukaryotic mRNAs, their half-life in erythroid cells exceeding 60 h (52). Thus, even if γ-globin gene transcription is completely inhibited by PMA, after 11 h of incubation with the drug, there still should be large amounts of γ-globin mRNA present in these cells as long as mRNA stability remains unaffected. Therefore, the results shown in Fig. 2B indicate that destabilization of γ-globin gene mRNA is at least partially responsible for the observed effect of PMA.

To determine whether the reduction in the amount of γ-globin mRNA in K562 cells after treatment with PMA is also due to a decrease in the rate of transcription, nuclear run-on transcription assays were carried out with cells treated with PMA and with PMA in the presence of cycloheximide. For those assays, labeled nascent RNAs were isolated from nuclei prepared from the treated cells and were hybridized with a recombinant plasmid carrying γ-globin gene sequences (the γ-actin gene was used as an internal control; pBR322 was used as a negative control). The results are shown in Fig. 3. Quantitation of the signal by scanning densitometry revealed that after 24 h of PMA exposure, the rate of transcription of the γ-globin gene is reduced 4.5-fold; in the presence of both PMA and cycloheximide, it is reduced 4-fold (values were corrected for the γ-actin signal). Therefore, inhibition of protein synthesis does not prevent the negative effect of PMA on γ-globin gene transcription. This result suggests that the partial reduction in the amount of steady-state γ-globin mRNA observed in the presence of PMA and cycloheximide (Fig. 2B) is due to the transcriptional effect of PMA, whereas the posttranscriptional effect of PMA is reversed by cycloheximide.

Another possible mechanism for PMA-induced suppression of γ-globin mRNA accumulation is a block in transcription elongation. This phenomenon is responsible for control of the steady-state mRNA levels of two nuclear proto-oncogenes, c-*myc* and c-*fos* (6, 8). This possibility was examined by hybridizing the products of nuclear run-on transcriptions to separate subcloned fragments corresponding to different regions of the γ-globin gene in the 5′-to-3′ direction. If PMA caused a block in transcription elongation of the γ-globin mRNA, the hybridization signal obtained with fragments located at the 3′ end of the gene would have been lower than that obtained with fragments located closer to the 5′ end of the gene. The results (not shown) did not follow this pattern; thus, a block in elongation does not appear to contribute to the observed effect of PMA on γ-globin mRNA accumulation.

Sequences located in the 3′-flanking region of the $^{A}\gamma$-globin gene are responsible for transcriptional down-regulation. In the next series of experiments, we searched for *cis*-acting regulatory elements that mediate the transcriptional down-regulation of the γ-globin genes. In numerous transcriptional regulatory systems, the positive and negative regulatory elements are found in the immediate upstream regions of the promoter. In a number of systems, however, additional regulatory elements are located downstream of the site of transcription initiation (in the introns and in the 3′-flanking regions of the genes) and in the far-upstream regions of the gene clusters (4, 5, 20, 51). Specifically, in the β-globin gene cluster on chromosome 11, both downstream and far-upstream regulatory elements have been identified (19, 24, 51). Therefore, the $^{A}\gamma$-globin gene promoter, the region of the $^{A}\gamma$-globin gene located downstream from the cap site, and the far-upstream region of the β-globin gene cluster were examined for their possible roles in mediating the negative effect of PMA on γ-globin gene transcription.

To separately analyze the transcriptional effect of PMA and its effect on γ-globin mRNA stability, we made use of a heterologous reporter gene, i.e., the gene encoding firefly luciferase, an enzyme that catalyzes oxidation of D-luciferin in the presence of ATP, Mg^{2+}, and O_2. During this reaction, a flash of visible light is generated that is proportional to the quantity of luciferase in the reaction mixture (16). The firefly luciferase mRNA would not be expected to contain the same instability determinant as the γ-globin mRNA. In addition, expression of the reporter gene is easy to simultaneously and independently assay with the expression of the γ-globin genes. A series of recombinant plasmids was constructed in which the luciferase reported gene was fused to the γ-globin gene promoter; both minimal (−380) and extended (−1350) γ-globin gene promoters were analyzed. Also, other plasmids were constructed in which various portions of the $^{A}\gamma$-globin gene to be tested for regulatory functions were positioned upstream of the minimal promoter (Fig. 4). In one of the constructs, the so-called hypersensitive site 2 (HS 2)

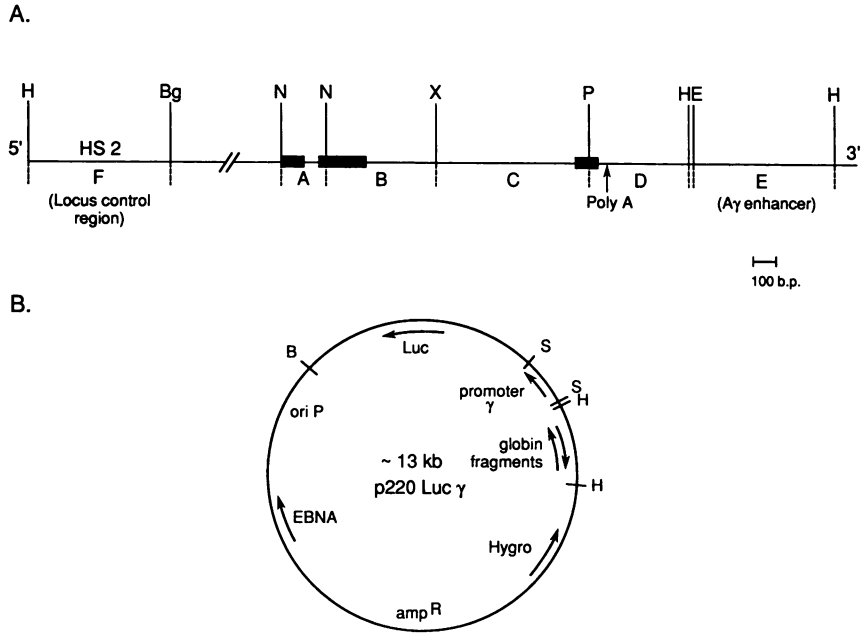

FIG. 4. Diagrams of the human $^{A}\gamma$-globin gene (A) and firefly luciferase expression vector (B). (A) The γ-globin gene exons are shown as solid boxes. The DNA fragments tested for their role in mediating the PMA effect on luciferase gene expression are designated A through F. Poly A, polyadenylation site of the γ-globin gene; HS 2, hypersensitive site 2 of the locus control region (see text). (B) The EBV-derived plasmid p220Lucγ was used in the functional analysis of γ-globin gene fragments. Luc, firefly luciferase structural gene; ori P, EBV origin of replication; EBNA, gene for EBNA 1; Hygro, gene conferring resistance to the antibiotic hygromycin B; amp^{R}, ampicillin resistance gene. Restriction enzyme sites: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; N, *Nco*I; P, *Pst*I; S, *Sal*I; X, *Xba*I.

was introduced upstream of the minimal promoter. The HS 2 element is normally located approximately 23 kb upstream of the $^{A}\gamma$-globin gene in the far-upstream region of DNase I hypersensitivity of the β-globin gene cluster that is called the locus control region (31). This region confers position-independent and high-level expression of human globin genes in transgenic mice (19) and functions as a potent erythroid-specific enhancer in gene transfer experiments (51). The reporter gene plasmids containing cloned sequences to be analyzed for their regulatory function were transfected into K562 cells, stable transfectants were selected, and expression of the reported gene was measured.

All of the recombinant plasmids were based on an EBV-derived vector, p220.2 (49). The EBV origin of replication, the gene encoding EBNA 1, and a selectable marker conferring resistance to the antibiotic hygromycin B were incorporated into this vector (Fig. 4B) to ensure that it can be maintained stably and extrachromosomally after transfection into tissue culture cells. The extrachromosomal maintenance of the plasmid permits the avoidance of possible effects on expression of the reporter gene due to the site of integration in the host genome (47). The stably transfected cultures were chosen over the transiently transfected ones because of the strength and the reproducibility of the signal provided by stable cultures. All stable cultures represented pools of 50 to 100 individual clones. The plasmid copy number per cell varied from 20 to 100 in different pools, as determined by Southern blot analysis of DNA from Hirt lysates (22) (not shown).

Nuclear run-on transcription assays were carried out with nuclei prepared from the stably transfected K562 cells to compare the effects of PMA on the transcription rates of the endogenous γ-globin genes and the transfected firefly luciferase reporter gene. As shown in Fig. 5, neither the minimal (−380) or the extended (−1350) promoter alone contains the regulatory element(s) sufficient to confer a pattern of transcriptional down-regulation of the reporter gene by PMA. When the transfected plasmid contained HS 2 inserted upstream of the γ-globin promoter (fragment F; Fig. 4A), transcription of the reporter was increased in the presence of PMA (Fig. 5B5). The only cells in which the pattern of the expression of the reporter gene was similar to that of the endogenous gene were those transfected with the plasmid containing the fragment derived from the 3′-flanking region of the $^{A}\gamma$-globin gene (fragment E; Fig. 4A) placed upstream of the γ-globin promoter (Fig. 5B3). In different run-on transcription experiments, the degree of inhibition of luciferase gene transcription by PMA in such stably transfected cells varied from two- to sixfold, whereas the degree of inhibition of endogenous γ-globin gene transcription by PMA was somewhat stronger, four- to eightfold. The same 3′-flanking fragment of the $^{A}\gamma$-globin gene was found by Bodine and Ley (10) to act as a transcriptional enhancer in transient transfection assays. None of the other examined fragments (Fig. 4A) had any effect on luciferase gene transcription (not shown).

When expression of the functional luciferase protein was measured in various stable transfectants (not shown), the signal in the presence of PMA was always considerably higher than in the absence of the drug. The luciferase signal was also increased by PMA when the γ-globin promoter was replaced by the herpes simplex virus thymidine kinase gene promoter. Thus, the increased luciferase activity appears to be independent of the *cis*-acting elements of the transcriptional machinery and probably takes place at the posttranscriptional level. For example, PMA could increase the

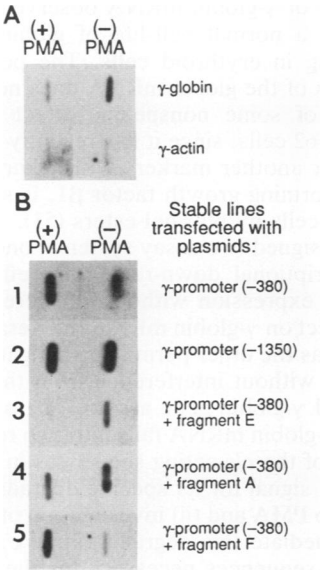

FIG. 5. Nuclear run-on transcription of endogenous genes (A) and transfected luciferase genes (B) in K562 cells. Cells were stably transfected with plasmids of the p220Lucγ series in which the luciferase gene was driven by γ-globin gene promoters of different lengths (−380 bp or −1350 bp from the cap site). Constructs containing the minimal (−380 bp) promoter also contained different DNA fragments (A, E, and F; see Fig. 4A) of the γ-globin gene region. Fragment F (locus control region HS 2) was in the genomic orientation, whereas the elements that originate from the region downstream of the γ-globin gene cap site (fragments A and E) were in the reverse genomic orientation. Similar results were obtained when fragments A and E were in the genomic orientation. The endogenous γ-globin and γ-actin genes were used as internal controls.

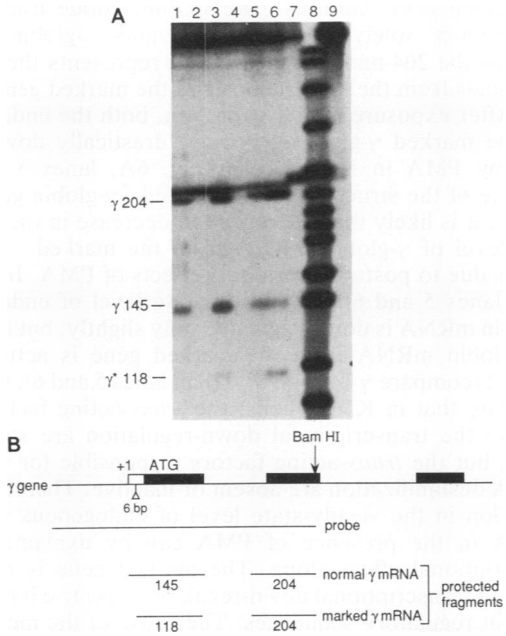

FIG. 6. (A) Nuclease protection analysis of RNA from transfected K562 cells. K562 cells (isolates A and B; see text) untransfected or stably transfected with the marked γ-globin gene were treated with PMA for 24 h. RNA was isolated, and the nuclease protection assay was carried out as described in Materials and Methods. The sizes (in nucleotides) of the protected fragments are shown. γ* indicates the protected fragment from the marked γ-globin gene. (B) Diagram of the marked ^{A}γ-globin gene, showing the sizes (in nucleotides) of the protected mRNA fragments of the normal and marked γ-globin genes expected in the nuclease protection assay. The 6-bp deletion of the marked γ-globin gene extends from bases +21 to +26 relative to the cap site of the ^{A}γ-globin gene and is a part of the 5′ untranslated region. The RNA probe used in the nuclease protection assay was generated from plasmid JEMγBSPA, in which the *Apa*I (−202)-*Bam*HI (+481) fragment of the ^{A}γ-globin gene was cloned downstream from the SP6 promoter. Lanes: 1, $K562_B$, untransfected, no PMA; 2, $K562_B$, untransfected, with PMA; 3, $K562_B$, transfected, no PMA; 4, $K562_B$, transfected, with PMA; 5, $K562_A$, transfected, no PMA; 6, $K562_A$, transfected, with PMA; 7, control with yeast tRNA substituted for K562 RNA; 8, DNA marker; 9, probe alone, not protected.

stability of the luciferase mRNA, since tumor-producing phorbol esters have been demonstrated to have such an effect in some systems (46, 53). Also, PMA could possibly exert its effect on the level of translation. Whatever the mechanism is, the posttranscriptional effect of PMA on luciferase gene expression invalidates the results of the assays that measure the functional amount of the protein in cellular extracts. The nuclear run-on transcription assays, on the other hand, directly measure the parameter of interest, i.e., the transcription rate.

PMA regulation of a marked γ-globin gene in K562 cells. To investigate the mechanism of the destabilizing effect of PMA on γ-globin mRNA, we stably introduced a marked ^{A}γ-globin gene into K562 cells on the same EBV-derived vector that was used for the luciferase gene constructions described above. The marked gene contains a 6-bp deletion in its 5′ untranslated region, and its mRNA can easily be distinguished from that of the endogenous γ-globin genes in nuclease protection assays (37) (Fig. 6). Thus, the effect of PMA on the marked and on the endogenous genes can be monitored simultaneously. Since the mRNAs of the marked gene and the endogenous gene are practically identical, they may be expected to be influenced by the same putative *trans*-acting destabilizing determinant induced by PMA. The copy of the marked ^{A}γ-globin gene is contained within a 3.3-kb *Hin*dIII DNA fragment (−1350 to +1950 relative to the cap site), which includes the extended (−1350) γ-globin promoter fragments but does not contain fragment E that mediates the negative PMA effect on γ-globin gene transcription (Fig. 4A and 5). Also, it is noteworthy that the −1350 promoter is not repressed transcriptionally by PMA (Fig. 5). Because the marked γ-globin gene is missing all of the regulatory elements involved in transcriptional down-regulation, it represents a useful system for investigation of the *cis*-acting regulatory sequences that mediate the destabilizing effect of PMA on globin mRNAs, separately from its effect on γ-globin gene transcription. Negative regulation of the marked globin gene, if observed, should then be solely due to a posttranscriptional effect.

The results of the nuclease protection assay are shown in Fig. 6A. The results with untransfected K562 cells are shown in lanes 1 and 2. The protected fragments of 145 and 204 nucleotides in length are the products of the endogenous γ-globin genes. In lanes 3 to 6, the nuclease protection assays were carried out with RNA prepared from $K562_A$ and $K562_B$ cells (see above) stably transfected with the marked γ-globin gene and exposed to PMA for 24 h. A protected fragment of 118 nucleotides corresponds to the transcript of the marked ^{A}γ-globin gene (γ*); it appears only in the stably

transfected K562 cultures. The 145-nucleotide fragment is the product solely of the endogenous γ-globin genes, whereas the 204-nucleotide fragment represents the sum of the signals from the endogenous and the marked genes (Fig. 6B). After exposure to PMA for 24 h, both the endogenous and the marked γ-globin genes are drastically down-regulated by PMA in $K562_B$ cells (Fig. 6A, lanes 3 and 4). Because of the structure of the marked ^{A}γ-globin gene (see above), it is likely that the observed decrease in the steady-state level of γ-globin mRNA from the marked ^{A}γ-globin gene is due to posttranscriptional effects of PMA. In $K562_A$ cells (lanes 5 and 6), the steady-state level of endogenous γ-globin mRNA is down-regulated only slightly, but the level of γ-globin mRNA from the marked gene is actually increased (compare γ 145 and γ* 118 in lanes 5 and 6). One can speculate that in $K562_A$ cells, the *trans*-acting factors that provide the transcriptional down-regulation are still functional, but the *trans*-acting factors responsible for γ-globin mRNA destabilization are absent or inactive. Therefore, the reduction in the steady-state level of endogenous γ-globin mRNA in the presence of PMA can by explained by a transcriptional effect alone. The marked gene is not subjected to transcriptional down-regulation because it lacks the relevant regulatory sequences. The cause of the increase in the steady-state level of mRNA from the marked ^{A}γ-globin gene following exposure of $K562_A$ cells (but not $K562_B$ cells) to PMA is unknown.

DISCUSSION

PMA drastically decreases the steady-state level of γ-globin mRNA despite active transcription of the gene. In this work, we have demonstrated that the human erythroleukemia cell line K562 undergoes a dramatic erythrocyte-to-megakaryocyte shift after exposure to the tumor-promoting phorbol ester PMA. This shift is associated with suppression of the steady-state level of globin mRNA and an increase in the steady-state level of the mRNA for the megakaryocyte-specific protein PDGF B. We chose to study the mechanism of the negative regulation of the most abundant erythroid protein of K562 cells, γ-globin, as a model for gene silencing during differentiation. The mechanisms employed by these cells for the down-regulation of globin gene expression may be similar to the mechanisms associated with suppression of globin gene expression in normal megakaryocytes.

We have found that the negative regulation of γ-globin genes by PMA takes place at both the transcriptional and posttranscriptional levels. PMA reduces the rate of γ-globin gene transcription approximately four- to eightfold, whereas the posttranscriptional effect appears to be even more dramatic. The posttranscriptional effect results in disappearance of the steady-state γ-globin mRNA from PMA-treated K562 cells despite relatively active transcription of the gene (compare Fig. 1 and 3). We propose that the posttranscriptional down-regulation involves destabilization of γ-globin mRNA. The RNA polymerase II inhibitor actinomycin D and the protein synthesis inhibitor cycloheximide strongly suppressed the effect of PMA on γ-globin mRNA (Fig. 2B). This result suggests that the putative factor(s) that mediates the PMA effect is an unstable protein. Although at present we cannot formally rule out other possible posttranscriptional PMA effects, such as aberrant mRNA processing or interference with mRNA transport from the nucleus, these possibilities appear less likely. Even if an additional posttranscriptional effect does contribute to this down-regulatory phenomenon, it would be difficult to reconcile the complete disappearance of γ-globin mRNA observed in the presence of PMA with a normal half-life of globin mRNA that is extremely long in erythroid cells. The posttranscriptional destabilization of the globin mRNA does not appear to be a consequence of some nonspecific effect of PMA on all mRNAs in K562 cells, since it has recently been shown that the mRNA for another marker of megakaryocytic differentiation, transforming growth factor β1, is specifically stabilized in K562 cells by phorbol esters (53).

We have designed two assay systems; one allows analysis of the transcriptional down-regulatory effect of PMA on γ-globin gene expression without interference from its destabilizing effect on γ-globin mRNA (luciferase reporter gene assay), whereas the other permits investigation of the destabilizing effect without interference from the transcriptional effect (marked γ-globin gene assay). The problem of destabilization of γ-globin mRNA falls into two research areas: (i) investigation of the *cis*-acting sequences in γ-globin mRNA that provide a signal for its specific degradation after exposure of cells to PMA and (ii) investigation of the *trans*-acting factors that mediate this degradation. We have found that the *cis*-acting sequences necessary for the degradation are contained within the marked ^{A}γ-globin mRNA. In the majority of previously described cases, the signal determining the stability of a given mRNA is localized within the 3′ untranslated region of the mRNA (23, 32, 46, 55). Therefore, work is currently under way to produce nested 3′-end deletions of the marked gene in order to precisely identify the relevant sequences.

Is AP-1 directly involved in transcriptional down-regulation of γ-globin gene expression by PMA? The alteration in gene expression following administration of the tumor-promoting phorbol esters can be accomplished through the action of the transcription factor AP-1 (see above). However, the involvement of other transcription factors in this phenomenon has also been documented (14, 17, 45). Although it has been shown by others (2) that expression of both the c-*fos* and c-*jun* proto-oncogenes is activated upon the administration of TPA to K562 cells, it still remains to be determined whether AP-1 is directly involved in the down-regulation of γ-globin gene expression. This could be studied by introducing the c-*jun* and c-*fos* proto-oncogenes into K562 cells by using mammalian expression vectors with the hope of reproducing the negative effect of PMA on γ-globin expression in these gene transfer experiments.

The sequences that mediate the transcriptional down-regulation of γ-globin genes by PMA map to a 750-bp-long fragment previously designated as the ^{A}γ-globin gene enhancer. It remains to be determined whether the positive and negative regulatory sequences of this element overlap. The CGAGTCA sequence located in the 750-bp-long fragment, 1,028 bp downstream of the ^{A}γ-globin gene polyadenylation site, could potentially serve as an AP-1 binding site (the AP-1 consensus sequence is TGAGTCA). One could speculate that the negative effect of PMA on γ-globin gene transcription is the result of competition between AP-1 (induced by PMA) and some erythroid-specific factor that contributes to positive regulation of γ-globin genes in the erythroid environment (in the absence of PMA). Interestingly, the erythroid-specific factor NFE-2 recognizes a consensus sequence (TGAGTCA) that overlaps that of AP-1 (29). When the mechanism of the negative regulatory effect of PMA on γ-globin gene transcription is fully understood, it will be important to determine whether the same mechanism is used for the coordinate suppression of other erythroid-specific genes during megakaryocytic differentiation of K562 cells.

Stabilizer versus destabilizer model of regulation of stability of γ-globin mRNA. One can envision two models to explain the differential stability of γ-globin mRNA in the absence or in the presence of PMA. The first model assumes that in the erythroid environment, γ- (and other) globin mRNAs are specifically stabilized by some putative *trans*-acting factor. This factor may extend their half-life severalfold over the average half-life of poly(A)$^+$ mRNA in erythroid cells (52). PMA, or the megakaryocytic environment, would inactivate this stabilizer and cause degradation of globin mRNA by the means of some ubiquitous cellular nuclease. The second model postulates that globin mRNAs are intrinsically stable in erythroid cells because of a stabilizing *cis*-acting element that, for example, can form a protective secondary structure. PMA would then induce a specific nuclease (destabilizer) capable of overcoming the protective influence of this *cis*-acting element. This destabilizer would not recognize megakaryocyte-specific mRNAs in PMA-treated K562 cells (Fig. 1; 53).

In accordance with the proposed models, AP-1 could act as a positive regulator in inducing a specific nuclease (second model) or as a negative regulator by interfering with the expression or function of a putative stabilizer of globin mRNAs (first model). In this regard, the recently described functional antagonism between c-*jun* and the glucocorticoid receptor might be relevant (44) because steroid hormones are known to stabilize mRNAs in a number of different systems (9, 35). It is noteworthy that the c-*erbA* proto-oncogene (thyroid hormone receptor), which is a member of the steroid hormone receptor superfamily (54), is preferentially expressed in chicken erythroid cells during differentiation (21) and may be important in erythroid differentiation (56). One could speculate that if the thyroid hormone receptor plays a role in extending the half-life of globin mRNAs in erythroid cells, then the overexpression of c-*jun* induced by PMA could interfere with its stabilizer function.

Several successful attempts to design differential cell-free mRNA degradation systems have been made (12, 34, 40, 48, 53). Development of such a cell-free system from extracts of K562 cells untreated and treated with PMA could be useful in distinguishing between the models described above. The availability of the $K562_A$ and $K562_B$ cell lines that respond to PMA in a different fashion will also be valuable for this purpose.

Is globin mRNA stability differentially regulated in vivo in erythroid and nonerythroid tissues? The phorbol ester-mediated destabilization of γ-globin mRNA in K562 cells can be viewed in the larger framework of tissue-specific regulation of globin gene expression. Do globin mRNAs have different stabilities in erythroid versus nonerythroid tissues in vivo? Does lower mRNA stability act in concert with transcriptional silencing to prevent the expression of these genes in nonerythroid tissues? The advantage of this kind of dual control over a single effect is that it would provide a more flexible and precise way to regulate gene expression in response to extracellular stimuli. There are a number of examples in which both transcriptional and posttranscriptional effects are involved in the regulation of a given gene in a specific cell lineage (25, 28, 35). Destabilization of the mRNA for macrophage colony-stimulating factor receptor by the dominant action of granulocyte-macrophage colony-stimulating factor has recently been implicated in suppression of the differentiation of a bipotential myeloid progenitor along the monocytic lineage (18). This specific mRNA destabilization results in differentiation of the progenitor along the granulocytic pathway (18). From the available data, it is becoming clear that it is a combination of transcriptional and posttranscriptional effects that accounts for the tremendous complexity of eukaryotic gene regulation. Phorbol ester-mediated megakaryocytic differentiation of K562 cells provides a good model system for the detailed molecular analysis of this complex process.

ACKNOWLEDGMENTS

We thank Allen Bale for the PDGF B probe, Richard Gelinas for the plasmid carrying the marked γ-globin gene, Kevin McDonagh for the plasmid carrying the firefly luciferase gene, and William Sugden for the EBV-derived vector p220.2. We thank Paula Kavathas for helpful discussions and critical reading of the manuscript.

This work was supported in part by grants from the National Institutes of Health and the Cooley's Anemia Foundation.

REFERENCES

1. **Alitalo, R., T. P. Mäkelä, P. Koshinen, L. C. Andersson, and K. Alitalo.** 1988. Enhanced expression of transforming growth factor β during megakaryoblastic differentiation of K562 leukemia cells. Blood **71**:899–906.
2. **Alitalo, R., J. Partanen, L. Petrovaara, E. Hölttä, L. Sistonen, L. Andersson, and K. Alitalo.** 1990. Increased erythroid potentiating activity/tissue inhibitor of metalloproteinases and *jun/fos* transcription factor complex characterize tumor promoter-induced megakaryoblastic differentiation of K562 leukemia cells. Blood **75**:1974–1982.
3. **Andersson, L. C., K. Nilsson, and C. G. Gahmberg.** 1979. K562-A human erythroleukemic cell line. Int. J. Cancer **23**:143–147.
4. **Antoniou, M., E. deBoer, G. Habets, and F. Grosveld.** 1988. The human β-globin gene contains multiple regulatory regions: identification of one promoter and two downstream enhancers. EMBO J. **7**:377–384.
5. **Banerji, J., L. Olson, and W. Schaffner.** 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell **33**:729–740.
6. **Bentley, D. L., and M. Groudine.** 1986. A block to elongation is largely responsible for decreased transcription of c-*myc* in differentiated HL-60 cells. Nature (London) **321**:702–706.
7. **Berger, S. L., D. M. Wallace, G. P. Siegal, J. M. Hitchcock, C. S. Birkenmeier, and S. N. Reber.** 1981. Preparation of interferon messenger RNA with the use of ribonucleoside-vanadyl complexes. Methods Enzymol.**79**:59–67.
8. **Blanchard, J. M., M. Piechaczyk, P. Fort, A. Bonniev, N. Mechti, J. Rech, M. Cuny, B. Lebleu, and P. Jeanteur.** 1988. The regulatory strategies of c-*myc* and c-*fos* proto-oncogenes share some common mechanisms. Biochimie **70**:877–884.
9. **Block, M. L., and D. J. Shapiro.** 1983. Estrogen stabilizes vitellogenin messenger RNA against cytoplasmic degradation. Cell **34**:207–214.
10. **Bodine, D. M., and T. J. Ley.** 1987. An enhancer element lies 3′ to the human fetal Aγ-globin gene. EMBO J. **6**:2997–3004.
11. **Bohmann, D., T. J. Bos, A. Admon, T. Nishimura, P. K. Vogt, and R. Tjian.** 1987. Human proto-oncogene c-*jun* encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science **238**:1386–1392.
12. **Brewer, G., and J. Ross.** 1989. Regulation of c-*myc* mRNA stability in vitro by a labile destabilizer with an essential nucleic acid component. Mol. Cell Biol. **9**:1996–2006.
13. **Charnay, P., and T. Maniatis.** 1983. Transcriptional regulation of globin gene expression in the human erythroid cell line K562. Science **220**:1281–1283.
14. **Chiu, R., M. Imagawa, J. Imbra, J. R. Bockoven, and M. Karin.** 1987. Multiple *cis*- and *trans*-acting elements mediate the transcriptional response to phorbol esters. Nature (London) **329**: 648–651.
15. **Curran, T., and B. R. Franse.** 1988. *Fos* and *jun*: the AP-1 connection. Cell **55**:395–397.
16. **DeWet, J. R., K. V. Wood, M. DeLuca, D. R. Helinski, and S. Subramani.** 1987. Firefly luciferase gene: structure and expres-

sion in mammalian cells. Mol. Cell. Biol. 7:725–737.
17. **Elsholtz, H., H. J. Mangalam, E. Potter, V. R. Albert, S. Supowit, R. M. Evans, and M. G. Rosenfeld.** 1986. Two different *cis*-active elements transfer the transcriptional effects of both EGF and phorbol esters. Science **234**:1552–1557.
18. **Gliniac, B. C., and L. R. Rohrschneider.** 1990. Expression of the M-CSF receptor is controlled post-transcriptionally by the dominant action of GM-CSF and multi-CSF. Cell **63**:1073–1083.
19. **Grosveld, F., G. B. van Assendelft, D. R. Greaves, and G. Kollias.** 1987. Position independent, high level expression of the human β-globin gene in transgenic mice. Cell **51**:975–985.
20. **Hayashi, S., K. Goto, T. S. Okada, and H. Kondoh.** 1987. Lens-specific enhancer in the third intron regulates expression of the chicken δ1-crystallin gene. Genes Dev. **1**:818–828.
21. **Hentzen, D., A. Renucci, D. LeGuellec, M. Benchaibi, P. Jurdic, O. Gandrillon, and J. Samarut.** 1987. The chicken c-*erbA* proto-oncogene is preferentially expressed in erythrocytic cells during late stages differentiation. Mol. Cell. Biol. **7**:2416–2424.
22. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell culture. J. Mol. Biol. **26**:365–369.
23. **Jones, T. R., and M. D. Cole.** 1987. Rapid cytoplasmic turnover of c-*myc* mRNA: requirement for the 3′ untranslated sequences. Mol. Cell. Biol. **7**:4513–4521.
24. **Kollias, G., J. Hurst, E. deBoer, and F. Grosveld.** 1987. The human β-globin gene contains a downstream developmental specific enhancer. Nucleic Acid. Res. **15**:5739–5747.
25. **Levine, R. A., J. E. McCormack, A. Buckler, and G. E. Sonenshein.** 1986. Transcriptional and post-transcriptional control of c-*myc* gene expression in WEHI231 cells. Mol. Cell. Biol. **6**:4112–4116.
26. **Lozzio, C. B., and B. B. Lozzio.** 1975. Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. Blood **45**:321–334.
27. **Martin, D. I. K., L. I. Zou, G. Mutter, and S. Orkin.** 1990. Expression of erythroid transcription factor in megakaryocytic and mast cell lineages. Nature (London) **344**:444–447.
28. **Marzluff, W. F., and N. B. Pandey.** 1988. Multiple regulatory steps control histone mRNA concentrations. Trends Biochem. Sci. **13**:49–52.
29. **Mignotte, V., J. F. Eleouet, N. Raich, and P. H. Romeo.** 1989. *Cis*- and *trans*-acting elements involved in the regulation of the erythroid promoter in the human porphobilinogen deaminase gene. Proc. Natl. Acad. Sci. USA **86**:6548–6552.
30. **Nishizuka, Y.** 1986. Studies and perspectives of protein kinase C. Science **233**:305–312.
31. **Orkin, S. H.** 1990. Globin gene regulation and switching: *circa* 1990. Cell **63**:665–672.
32. **Pandey, N. B., and W. Marzluff.** 1987. The stem-loop structure at the 3′ end of histone mRNA is necessary and sufficient for regulation of histone mRNA stability. Mol. Cell. Biol. **7**:4557–4559.
33. **Papayannopoulou, T., B. Nakamoto, T. Yokochi, A. Chait, and R. Kannagi.** 1983. Human erythroleukemia cell line (HEL) undergoes a drastic macrophage-like shift with TPA. Blood **62**:832–845.
34. **Pei, R., and K. Calame.** 1988. Differential stability of c-*myc* mRNAs in a cell-free system. Mol. Cell. Biol. **8**:2860–2868.
35. **Petersen, D. D., S. R. Koch, and D. K. Granner.** 1989. 3′ noncoding region of phosphoenolpyruvate carboxykinase mRNA contains a glucocorticoid response mRNA-stabilizing element. Proc. Natl. Acad. Sci. USA **86**:7800–7804.
36. **Rauscher, J. R., III, L. C. Sambucetti, T. Curran, R. J. Distel, and B. M. Spiegelman.** 1988. Common DNA binding site for *fos* protein complexes and transcription factor AP-1. Cell **52**:471–480.
37. **Rixon, M. W., and R. E. Gelinas.** 1988. A fetal globin gene mutation in $^{A}\gamma$ nondeletion hereditary persistence of fetal hemoglobin increases promoter strength in a nonerythroid cell. Mol. Cell. Biol. **8**:713–721.
38. **Romeo, P. H., M. H. Prandini, V. Joulin, V. Mignotte, M. Prenawt, W. Vainchenker, G. Marguerie, and G. Uzan.** 1990. Megakaryocytic and erythrocytic lineages share specific transcription factors. Nature (London) **344**:447–449.
39. **Rosengurt, E., M. Rodriguez-Pena, and K. A. Smith.** 1983. Phorbol esters, phospholipase C, and growth factors rapidly stimulate phosphorylation of a Mr 80,000 protein in intact quiescent 3T3 cells. Proc. Natl. Acad. Sci. USA **80**:7244–7248.
40. **Ross, J., and G. Kobs.** 1986. H4 histone messenger RNA decay in cell-free extracts initiates at or near the 3′ terminus and proceeds 3′ to 5′. J. Mol. Biol. **188**:579–593.
41. **Rutherford, T., J. B. Clegg, D. R. Higgs, R. W. Jones, J. Thompson, and D. J. Weatherall.** 1981. Embryonic erythroid differentiation in the human leukemic cell line K562. Proc. Natl. Acad. Sci. USA **78**:348–352.
42. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., p. 7.66–7.70. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Sariban, E., K. Imamura, R. Luebbers, and D. Kufe.** 1988. Transcriptional and posttranscriptional regulation of tumor necrosis factor gene expression in human monocytes. J. Clin. Invest. **81**:1506–1510.
44. **Schule, R., P. Rangarajan, S. Kliewer, L. Ransone, J. Bolado, N. Yang, I. M. Verma, and R. M. Evans.** 1990. Functional antagonism between oncoprotein c-*jun* and the glucocorticoid receptor. Cell **62**:1217–1226.
45. **Sen, R., and D. Baltimore.** 1986. Inducibility of κ immunoglobulin enhancer binding protein NF-κB by a post-translational mechanism. Cell **47**:921–928.
46. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46**:659–667.
47. **Smithies, O., R. G. Gregg, S. S. Boggs, M. A. Karalewski, and R. S. Kucherlapati.** 1985. Insertion of DNA sequences into the human chromosome β-globin locus by homologous recombination. Nature (London) **317**:230–234.
48. **Stolle, C. A., and E. J. Benz, Jr.** 1988. Cellular factors affecting the stability of β-globin mRNA. Gene **62**:65–74.
49. **Sugden, B., K. Marsh, and J. Yates.** 1985. A vector that replicates as a plasmid and can be efficiently selected in β-lymphoblasts transformed by Epstein-Barr virus. Mol. Cell. Biol. **5**:410–413.
50. **Tetteroo, P. A. T., F. Massaro, A. Mulder, R. Schrender-van Gelder, and A. E. G. von dem Borne.** 1984. Megakaryoblastic differentiation of proerythroblastic K562 cell-line cells. Leukocyte Res. **8**:197–206.
51. **Tuan, D. Y., W. B. Solomon, I. M. London, and D. P. Lee.** 1989. An erythroid-specific, developmental-stage-independent enhancer far upstream of the human "β-like globin" genes. Proc. Natl. Acad. Sci. USA **86**:2554–2558.
52. **Volloch, V., and D. Housman.** 1981. Stability of globin mRNA in terminally differentiating murine erythroleukemia cells. Cell **23**:509–514.
53. **Wager, R. E., and R. K. Assoian.** 1990. A phorbol ester-regulated ribonuclease system controlling transforming growth factor β1 gene expression in hematopoietic cells. Mol. Cell. Biol. **10**:5983–5990.
54. **Weinberger, C., C. C. Thompson, E. S. Ong, R. Lebo, D. J. Gruoe, and R. M. Evans.** 1986. The c-*erbA* gene encodes a thyroid hormone receptor. Nature (London) **324**:641–646.
55. **Wilson, T., and R. Treisman.** 1988. Removal of poly(A) and consequent degradation of c-*fos* mRNA facilitated by 3′ AU-rich sequences. Nature (London) **336**:396–399.
56. **Zenke, M., A. Munoz, J. Sap, B. Vennstrom, and B. Beug.** 1990. v-*erbA* oncogene activation entails the loss of hormone-dependent regulator activity of c-*erbA*. Cell **61**:1035–1049.