Journal of Cellular Biochemistry 67:13–23 (1997)

Downregulation of Telomerase Activity in HL60 Cells by Differentiating Agents Is Accompanied by Increased Expression of Telomerase-Associated Protein

Trevor W. Reichman,[1] Juan Albanell,[2] Xuening Wang,[1] Malcolm A.S. Moore,[2] and George P. Studzinski[1]*

[1]Department of Pathology, UMD, New Jersey Medical School, Newark, New Jersey 07103
[2]Laboratory of Developmental Hematopoiesis, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Abstract Telomerase activity provides a mechanism for the unlimited division potential of neoplastic cells. Induced differentiation of these cells was found to be associated with repression of telomerase activity irrespective of the inducing agent. We have employed a series of sublines of human promyelocytic leukemia line HL60 with differing degrees of resistance to differentiation to determine how tightly the expression of the differentiated phenotype is coupled to the downregulation of telomerase activity and to the expression of the recently identified telomerase-associated protein 1 (TP1). As expected, in the 1,25D_3-dihydroxyvitamin D_3 (1,25D_3)–resistant subclones (20A–100A cells), telomerase activity was not significantly downregulated by 1,25D_3 and, in most cases, by all-trans retinoic acid (atRA), to which these cells were cross-resistant, but telomerase activity was repressed by dimethylsulfoxide (DMSO) and phorbol-12-myristate-13-acetate (TPA), to which the sublines were in general sensitive. However, there were exceptions; in some instances telomerase activity was repressed in the absence of the expression of markers of differentiation. Also, there was an inverse relationship between telomerase activity and the cellular levels of TP1 transcripts. We conclude that in HL60 cells downregulation of telomerase is loosely associated with upregulation of differentiation markers and with other cellular changes which include an upregulation of TP1. J. Cell. Biochem. 67:13–23, 1997.

Key words: telomerase; TP1; HL60 cells; leukemia; differentiation therapy

Abrogation of the cancer cells' immortality is the goal of differentiation therapy. Unfortunately, in spite of temporary successes achieved in some instances, such as treatment of acute promyelocytic leukemia with all-trans retinoic acid (atRA) [Huang et al., 1988; Castalgne et al., 1990; Warrell et al., 1991], sustained remissions are difficult to achieve. Thus, even though telomerase activity, whose presence can help to explain the continuous cell replication of most malignant cells [Counter et al., 1992; Kim et al., 1994] has been shown to be repressed during induced differentiation of several human cell lines [Sharma et al., 1995; Albanell et al., 1996; Bestilny et al., 1996], telomerase activity again reappears during the recurrence of the disease and perhaps contributes to the renewed tumor growth. Further progress in differentiation therapy seems therefore to be dependent on a more complete understanding of the mechanisms of induced differentiation and of the basis for the reversibility of these processes, in which reactivation of telomerase may play an important part.

The recent development of a series of sublines of human promyelocytic leukemia HL60 designated 20AF–100AF, which have acquired the ability to proliferate in increasing concentrations of 1,25D_3-dihydroxyvitamin D_3 (1,25D_3), offers a system for studies of cellular events that result in the reversal and subsequent resistance to differentiation. These cells are the progeny of cells that were partially differentiated on first exposure to 1,25D_3 but, after prolonged cultivation in the presence of 1,25D_3, no longer

Contract grant sponsor: FIS BAE, contract grant number 96/5706; contract grant sponsor: CIRIT, contract grant number 1996BEAI200087.

Juan Albanell's current address is Oncology Service, Hospital Vall d'Hebron 119-129, Barcelona 08035, Spain.

*Correspondence to: George P. Studzinski, Department of Pathology, UMD, NJ Medical School, 185 S. Orange Avenue, Newark, NJ 07103.

Received 23 June 1997; accepted 27 June 1997

express markers of differentiation. The resistant cells are able to proliferate at rates more rapid than the parental cells, and some have near-tetraploid karyotype [Wajchman et al., 1996; Studzinski et al., 1996, 1997]. We show here that these sublines are cross-resistant to atRA, but only some sublines are also resistant to the differentiation-inducing effects of phorbol-12-myristate-13-acetate (TPA) or dimethylsulfoxide (DMSO), and therefore the series of these $1,25D_3$-resistant sublines provides a variety of differentiation-defective phenotypes. Since stringent downregulation of telomerase activity is potentially a way to ensure irreversibility of differentiation, we have used this system to investigate the strength of the reported link between telomerase repression and the differentiated phenotype and their relationship to differentiation-related regulation of the recently discovered telomerase-associated proteins. The human homolog, telomerase-associated protein 1 (TP1), was specifically shown to interact with mammalian telomerase RNA; however, no specific function of the protein was given [Harrington et al., 1997; Nakayamaa et al., 1997].

MATERIALS AND METHODS

Tissue Culture

The parental HL60-G clone and its $1,25D_3$-resistant sublines were obtained and cultured as described [Wajchman et al., 1996; Studzinski et al., 1996, 1997]. The numerical designation denotes the nanoMolar concentrations of $1,25D_3$ in which the cells were continuously propagated; the letter A indicates the first series derived from HL60-G cells, and the letter F indicates that cells used in the experiments were obtained from frozen stocks. All cells were cultivated in suspension in RPMI 1640 medium supplemented with glutamine to 2 mM and bovine calf serum (Hyclone, Logan, UT) to 10%. Antibiotics were not added to minimize the chance of inapparent mycoplasma infection, and the cultures were checked periodically to confirm the absence of mycoplasma [Studzinski et al., 1973]. Cell numbers were obtained using a Neubauer hemocytometer, and cellular viability was measured using the trypan blue exclusion technique.

For each experiment, cell flasks were seeded at 300K/ml and treated with the appropriate concentration of differentiating agent: 2×10^{-7} M $1,25D_3$ (Hoffmann-LaRoche, Nutley, NJ), 1 µM atRA (Hoffmann-LaRoche), 200 nM TPA (Sigma, St. Louis, MO), and 1.25% DMSO (Sigma). Fresh medium was added after 48 h and then as needed, which was determined by the growth rate. Cells were then harvested at the given time point, at which time the viability was again checked using the trypan blue exclusion technique.

Assessment of Differentiation

Phenotypic maturation was determined by flow cytometric measurement of the myeloid surface marker CD11b, as previously described in detail [Wajchman et al., 1996; Studzinski et al., 1996, 1997]

Cell Cycle Traverse

The presence and the extent of G1 to S phase block was assessed by the distribution of cells in each cell cycle compartment. This was obtained by propidium iodide staining of permeabilized cells, determination of the DNA content by flow cytometry, and an analysis of the data using the Multicycle Program (Phenix Flow Systems, San Diego, CA), as described [Wajchman et al., 1996].

Protein Extraction

Cells for telomerase activity were processed as described previously by Kim and others [Kim et al., 1994; Albanell et al., 1996; Wright et al., 1995]. Each sample was treated with ice-cold lysis buffer (10^6 cells/100 µl: 0.5% CHAPS, 10 mM Tris-HCL (pH 7.5), 1 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 5 mM β-mercaptoethanol, 10 ng/ml leupeptin) incubated on ice for 30 min and centrifuged at 12,000*g* for 30 min at 4°C; the supernatant stored at −80°C. Protein concentrations were measured using the BioRad (Hercules, CA) protein assay kit, and 1 µg/µl aliguots were stored at −80°C [Kim et al., 1994; Albanell et al., 1996].

Telomeric Repeat Amplification Protocol Assay

The telomerase assay was performed following a recently modified TRAP assay [Kim and Wu, 1997]. Two micrograms of protein extract were assayed in reaction tubes containing 50 µl of the TRAP reaction mixture. The TS primer (5′-AATCCGTGAGCAGAGTT-3′) was labeled using T4 polynucleotide kinase (PNK) (Promega, Madison, WI) and 25 µCi of 300 Ci/mmol [γ-^{32}P]ATP per 1 µg of TS. T4 PNK was used at 2.5 units per microgram of TS and incubated at 37°C for 20 min, followed by heat inactivation

at 95°C for 5 min. Each TRAP reaction consisted of 5 µl of 10x TRAP buffer, 50 µM dNTP's, 0.1 µg end-labeled TS, 0.1 µg ACX return primer, 0.1 µg of NT internal control primer, 0.01 amol of the TSNT internal control, 2 units of Taq polymerase (AmpliTaq; Perkin Elmer, Brachburg, NJ), and 2 µg protein extract. TSNT is a polymerase chain reaction (PCR) internal control amplified by the primers TS and NT that gives a 36 bp product. After 30 min incubation at room temperature for telomerase-mediated extension of the TS primer, the reaction mixture was immediately subjected to 30 PCR cycles of 94°C, 60°C, and 72°C for 30 s each. The PCR product was resolved by electrophoresis on a 10% polyacrylamide nondenaturing gel, and the gel was analyzed on a Phosphoimager (Molecular Dynamics, Sunnyvale, CA). In every gel a negative control (2 µg of CHAPS lysis buffer) and 0.1 amol of the quantification standard oligonucleotide, R8, were included. The telomerase activity was calculated as total product generated (TPG). The legend to Figure 2 provides further details of quantitation. All protein extracts were analyzed in two independent TRAP assays, and the average telomerase activity was calculated. Relative telomerase activity was calculated in inducer-treated cells, attributing 100% activity to the control (medium alone) extract of each cell line.

RT-PCR Measurement of TP1 Transcripts

Total cellular RNA was extracted using TRIzol reagent (Life Technologies, Inc., Gaithersburg, MD) according to the supplier's directions. RNA was quantitated using a spectrophotometer (260/280). One microgram was used per RT-PCR measurement. RT-PCR was carried out using a RT-PCR kit (Perkin Elmer) according to the manufacturer's directions. The primers used for this experiment were derived from the sequence obtained from Genbank (NCBI, NIH, Bethesda, MD), accession #486136. The two primers were 5′-GTGTACTGCGTTCGACTAAA-3′ (antisense) and 5′-TACAAGCTGAGTTTCAGCCA-3′ (sense). These primers produced a fragment 440 base pairs in length corresponding to the 61–500 bp 5′-end region of TP1 mRNA. For the reverse transcription of the mRNA to cDNA, RNA was transcribed at 42°C for 15 min, denatured at 99°C for 5 min, and then cooled to 5°C for 10 min. Amplification was then carried out by first heating the reaction mix to 95°C for 105 s, followed by 36 two-step cycles (denature at 95°C for 15 s and anneal-extend at 50°C for 30 s) and a final step at 72°C for 7 min. The final volume for each reaction was 50 µl. From this reaction a 20 µl aliquot was taken and separated on a 1.8% agarose gel run in Tris-Bonite-EDTA buffer and stained with ethidium bromide. The bands were quantitated using a fluorimager (Molecular Dynamics) and expressed as the ratio to the optical density of the internal control β-actin cDNA. Commercially available human β-actin primers (Stratagene, La Jolla, CA) were used to measure mRNA levels for β-actin and produced a fragment 661 bp in length. The TP1 band was sequenced to verify that it was the correct fragment.

RESULTS

Cross-Resistance of 1,25D_3-Resistant Sublines to Other Differentiation-Inducing Agents

The A series sublines are able to proliferate in the presence of 1,25D_3 in the 20–100 nM range and do not express markers of differentiation such as CD11b [Wajchman et al., 1996; Studzinski et al., 1996]. To determine if this confers resistance to other differentiation inducers, we have tested the effects of treatment for 96 h with atRA, 9-cisRA, TPA, and DMSO. Preliminary experiments showed that the actions of atRA and 9-cisRA were identical, so in subsequent experiments only atRA was used. All the sublines were highly resistant to induction of CD11b by RA, and most of the resistant sublines responded to TPA and DMSO (Fig. 1A). However, when 30A cells were treated with DMSO and 40AF cells with TPA, neither cell line expressed the CD11b surface marker. Figure 1A also shows that there did not appear to be any sequential change in the development of either resistance or sensitivity to TPA or DMSO as the sublines evolved from growth in 20 nM ambient 1,25D_3 (20AF cells) in successive stages to 100 nM 1,25D_3 (100AF cells).

Although A series sublines are highly resistant to induction of differentiation markers even when challenged by addition of concentrations of 1,25D_3 higher than the concentration present in their ambient medium, they do show a partial G1 to S phase block, which is not, however, progressive, and the cells continue to proliferate in the presence of raised concentrations of 1,25D_3 [Wajchman et al., 1996; Studzinski et al., 1996]. A similar situation was seen when these sublines were exposed to atRA; in spite of

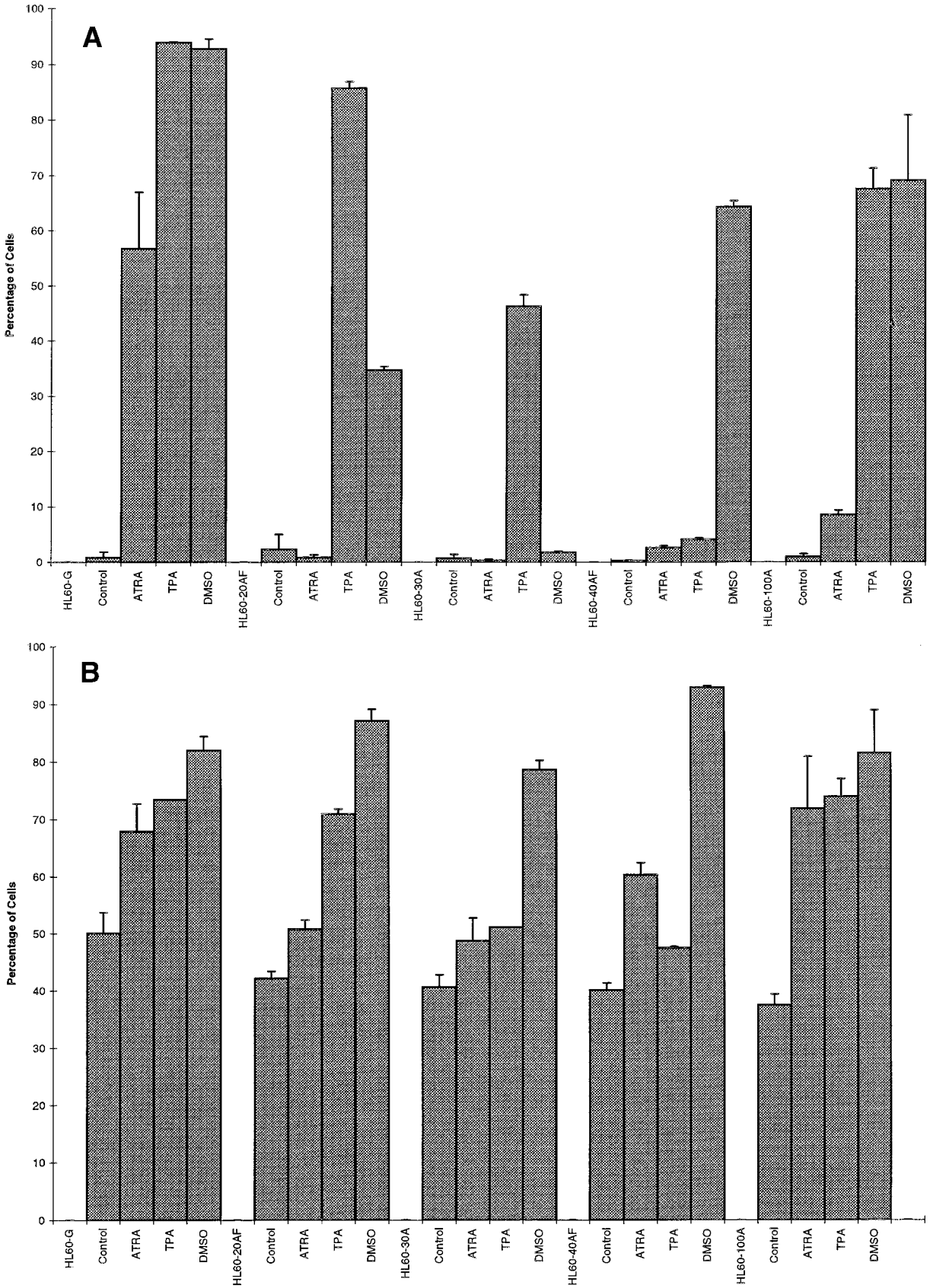

Fig. 1. The resistance of $1,25D_3$-resistant cell lines to other differentiating agents was determined by several parameters. Following treatment, cells were analyzed for (**A**) percentage of cells positive for CD11b, (**B**) percentage of cells in G1 phase, (**C**) percentage of cells in S phase, and (**D**) cell proliferation.

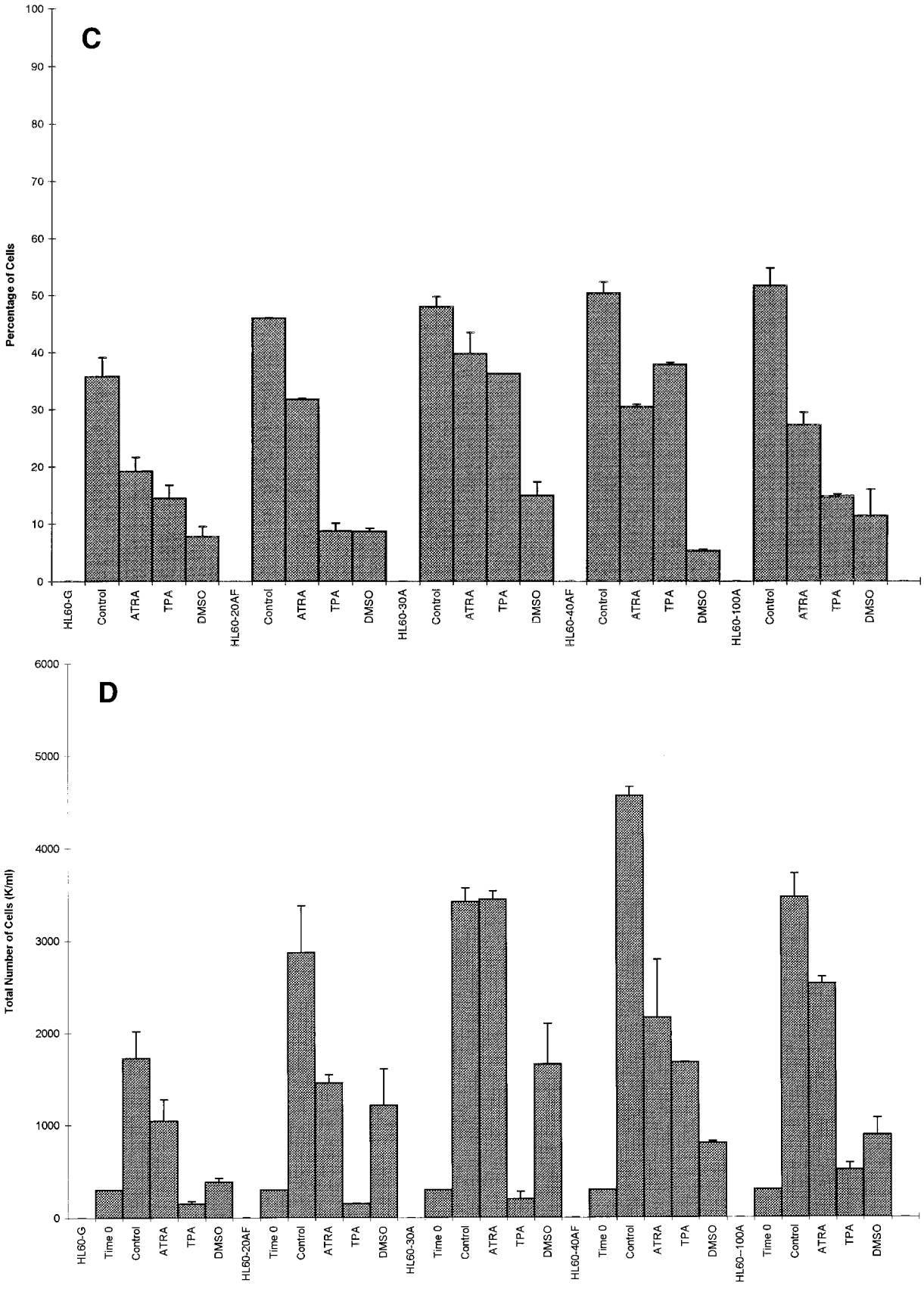

Figure 1. (Continued)

essentially complete resistance to induction of a differentiation marker, CD11b, by atRA, there was an increase in the number of cells in the G1 compartment of the cell cycle (Fig. 1B), with a corresponding decrease in the S phase compartment (Fig. 1C). However, the rate of cell proliferation was reduced only to an extent similar to the reduction of cell proliferation in the parental HL60-G cells (Fig. 1D). Treatment with TPA or with DMSO produced more pronounced G1/S blocks and more pronounced inhibition of cell proliferation than treatment with atRA, generally consistent with the induction of differentiation markers by these agents. An exception was the absence of DMSO-induced differentiation in 30A cells in the presence of a marked G1 to S phase block and a reduction in the rate of cell proliferation (Fig. 1A–D). Thus, the resistance to growth inhibitory effects of the inducing agents is less marked than the resistance to the induction of phenotypic differentiation in these sublines.

Downregulation of Telomerase Activity Is Not Tightly Coupled to Differentiation

Determination of telomerase activity by the TRAP procedure that includes a novel internal control and a quantitation standard (R8) showed that in the parental cells the activity was downregulated by all differentiation-inducing compounds tested, but only DMSO downregulated telomerase in every resistant subline examined (Fig. 2A–C). TPA was also a relatively potent downregulator of telomerase activity in several of the sublines tested (e.g., G, 20AF, and 30A) but was less potent in the sublines that have a hypotetraploid karyotype [Wajchman et al., 1996], the 30AF, 40AF, and 100AF cells (Fig. 2C,D and data not shown). The resistance of the sublines to $1,25D_3$ and the strong cross-resistance to atRA correlated with little or no repression of telomerase activity by these agents (Fig. 2B,D), except that atRA repressed telomerase in 20AF cells.

A correlation between the expression of CD11b and the downregulation of telomerase activity was noted when the time course of the effects of TPA on the sublines was studied. Figure 3 shows that a 24 h exposure to TPA had little effect on either the expression of CD11b or the telomerase activity, but by 48 h there was an approximately parallel change in both of these parameters. After 96 h, there was an even further increase in telomerase inhibition which was tightly associated with an identical increase in the expression of CD11b in HL60-G and 20AF cells. Both cell lines were also shown to have similar telomerase activity (data not shown). However, in 30A cells after 96 h, the correlation between a decrease in telomerase activity and the expression of CD11b was not apparent. Telomerase activity in 30A cells is almost twofold greater than in the parental HL60-G cells (Fig. 2D), possibly indicating that a constitutive increase in telomerase activity might be associated with a partial disassociation of differentiation (as measured by CD11b) from the downregulation of telomerase activity.

Comparison of Figure 1A with Figure 2D also shows that in general the downregulation of telomerase activity was accompanied by an expression of the CD11b differentiation marker, but there were exceptions. Most notably, 30A cells treated with DMSO showed downregulated telomerase activity but no expression of CD11b (Figs. 1A, 2D). Thus, a variety of altered phenotypes were exhibited by the $1,25D_3$-resis-

Fig. 2. Changes in telomerase activity in $1,25D_3$-resistant cell lines treated with $1,25D_3$, atRA, TPA, and DMSO for 96 h (**A–D**). Telomerase activity was assayed by a recently modified TRAP assay [Kim and Wu, 1997]. Representative TRAP gels of control and inducer-treated HL60 cell lines are shown (**A–C**). Panel **A** displays the R8 quantitation standard **(lane 1)**, the negative control **(lane 2)**, and the TRAP products of the untreated cell lines **(lanes 3–7)**. The R8 quantification standard oligonucleotide produces a characteristic banding pattern of six bands corresponding to the first to sixth TRAP products. The assay incorporates an internal PCR control of 36 bp product (designated TSNT), running 14 bp below the smallest TRAP band. This is used to monitor the PCR efficiency during the PCR step of this assay. The amount of telomerase activity from a given reaction was calculated using the formula

$$\mathrm{TPG} = \frac{(\mathrm{T} - \mathrm{B})/(\mathrm{CT})}{(\mathrm{R8} - \mathrm{B})/(\mathrm{CR8})} \times 100$$

where T is the radioactive counts from telomerase bands from the protein extract, B is the counts from negative control (background), R8 is the counts from R8 (0.1 amol), and CT is the count from the internal control TSNT (0.01 amol) of the R8 (0.1 amol). The final quantitation was expressed as TPG (total product generated). One unit of TPG was defined as 0.001 amol, or 600 molecules, of TS primers extended by telomerase present in the extract with at least three telomeric repeats. A telomerase activity level of 1 TPG corresponds approximately to telomerase activity from one immortal cell [Kim and Wu, submitted]. **B:** Telomerase activity following a 96 h exposure to $1,25\ D_3$ (2×10^{-7} M) **(lanes 1–5)** or atRA (1 μM) **(lanes 6–10)**. **C:** Telomerase activity following exposure to TPA (200 nM) **(lanes 1–5)** or DMSO (1.25%) **(lanes 6–10)**. **D:** Telomerase activity (as measured by TPG) following 96 h of inducer treatment in control and inducer-treated HL60 cell lines.

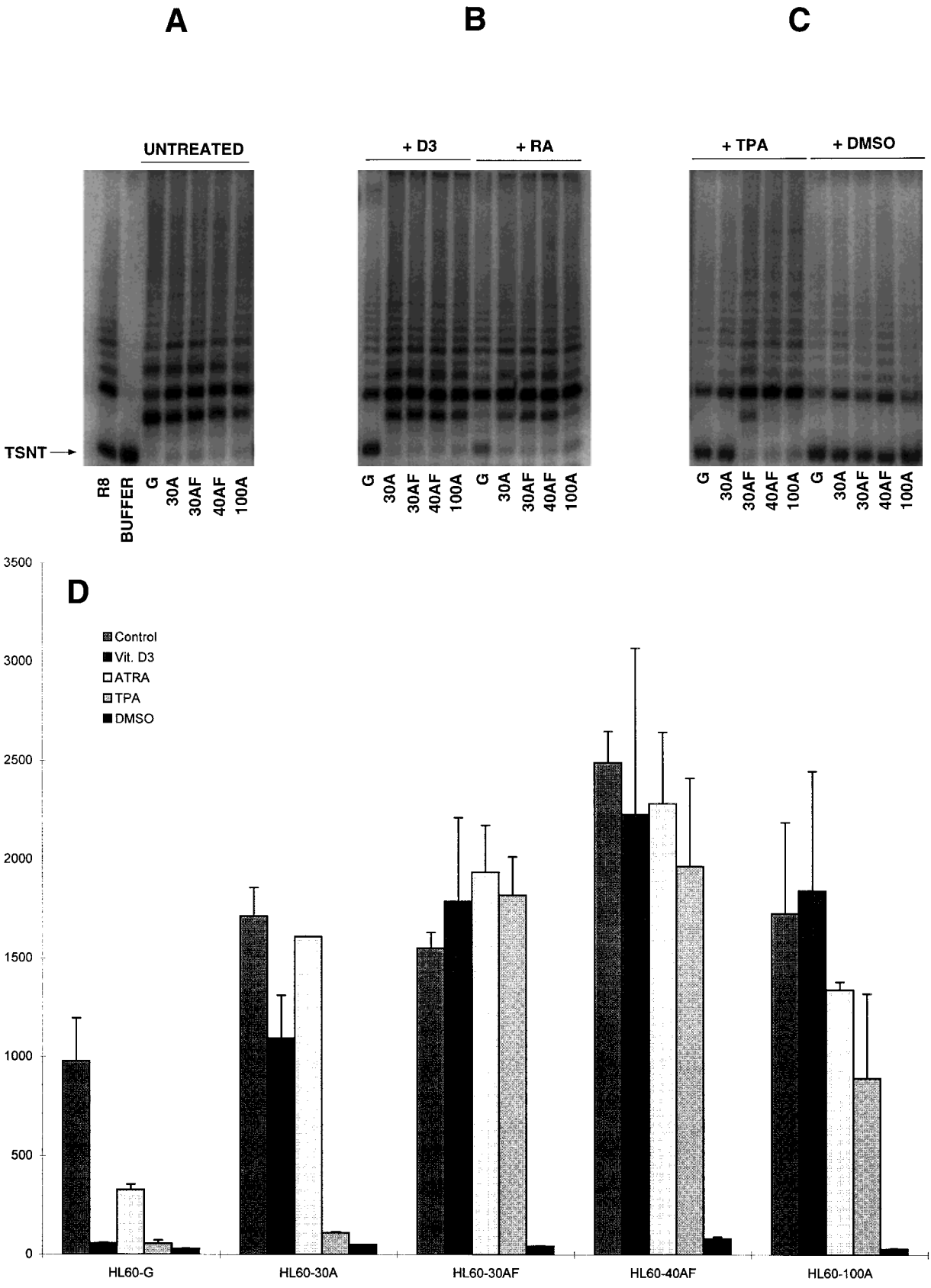
A
B
C
UNTREATED
+ D3
+ RA
+ TPA
+ DMSO
TSNT
R8
BUFFER
G
30A
30AF
40AF
100A
D
Control
Vit. D3
ATRA
TPA
DMSO
3500
3000
2500
2000
1500
1000
500
0
HL60-G
HL60-30A
HL60-30AF
HL60-40AF
HL60-100A

Figure 2.

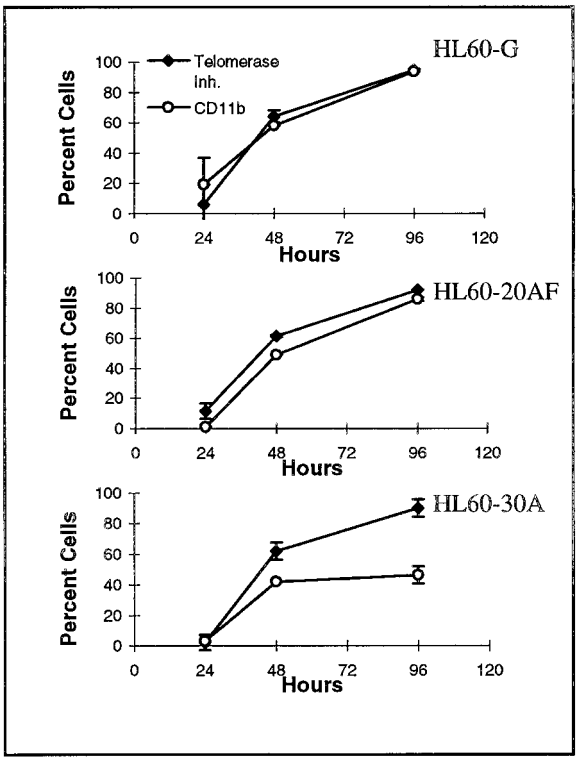

Fig. 3. HL60-G, HL60-20AF, and HL60-30A cells were treated with TPA (200 nM) for 24, 48, or 96 h. TRAP assays were performed on cell lysates following each exposure. Telomerase activity from the inducer-treated cells was expressed as relative telomerase activity, attributing a 100% activity to the control (medium alone) extract of each cell line. The percent inhibition was then calculated by subtracting each number from 100%. These values were then plotted as a function of time. CD11b surface antigen was also measured by flow cytometry (as described in Materials and Methods) following the timed exposure to TPA. The percentages of cells staining positive for CD11b were then plotted as a function of time.

tant cells exposed to a panel of differentiation inducers, and repression of telomerase activity was only loosely coupled to the expression of CD11b. The expression of the CD14 cell marker or of nonspecific esterase, a cytoplasmic marker of differentiation, did not correlate with repression of telomerase activity in these sublines (data not shown).

Expression of Telomerase-Associated Protein 1 In HL60 Sublines Treated With Inducers of Differentiation

Changes in the expression of TP1 gene in this experimental system were followed by the RT-PCR of RNA extracted from these cells. Addition of 2×10^{-7} M $1,25D_3$ to HL60-G cells resulted in increased levels of TP1 mRNA within 24 h, and levels continued to increase during the period of observation (Fig. 4A). When HL60-G cells were treated with other inducers of differentiation for 96 h, TP1 mRNA levels were also increased, with the greatest increases resulting from treatment with DMSO, to which most sublines were most sensitive (Fig. 1), and $1,25D_3$ followed by TPA and atRA (Fig. 4B). The relative increases in mRNA expression were tightly linked to a similar downregulation in telomerase activity. Similar results were also obtained when the $1,25D_3$-resistant subline, 40AF, was treated with the same inducing agents. The largest increase was noted in the DMSO-treated group, to which the cells were fully sensitive. However, only slight increases in TP1 mRNA were seen in cells treated with $1,25D_3$, atRA, and TPA. Again, relative increases in TP1 mRNA correlated with similar relative decreases in telomerase activity (Fig. 4B).

DISCUSSION

This study allowed several new generalizations to be made and led to the novel and unexpected finding that TP1 expression is upregulated when telomerase activity declines during induced differentiation of HL60 cells.

The sublines derived by resistance to $1,25D_3$ showed strong cross-resistance to atRA, but all were differentiation-competent since differentiation could be induced by either TPA, DMSO, or both. The resistance did not appear to be related to the lineage of differentiation, since $1,25D_3$ is an inducer of monocytic differentiation, while atRA is an inducer of granulocytic differentiation, showing that these pathways of induced differentiation diverge only after a step controlled by both $1,25D_3$ and RA. It is also apparent that the differentiation signals provided by TPA and DMSO are transduced by other pathways than those utilized by $1,25D_3$ and RA and are independent from each other (e.g., 30A cells are sensitive only to TPA, while 40AF cells are sensitive only to DMSO).

Telomerase repression appears in this context to be another independent marker of differentiation. It does not appear to be tightly linked to the inhibition of cell proliferation or to the G1/S block, which was apparent to varying degrees after treatment of parental or resistant sublines with any of the agents, since the G1/S phase block was not always accompanied by reduced telomerase activity. Similarly, the expression of differentiation markers CD14 or the nonspecific esterase showed little correlation

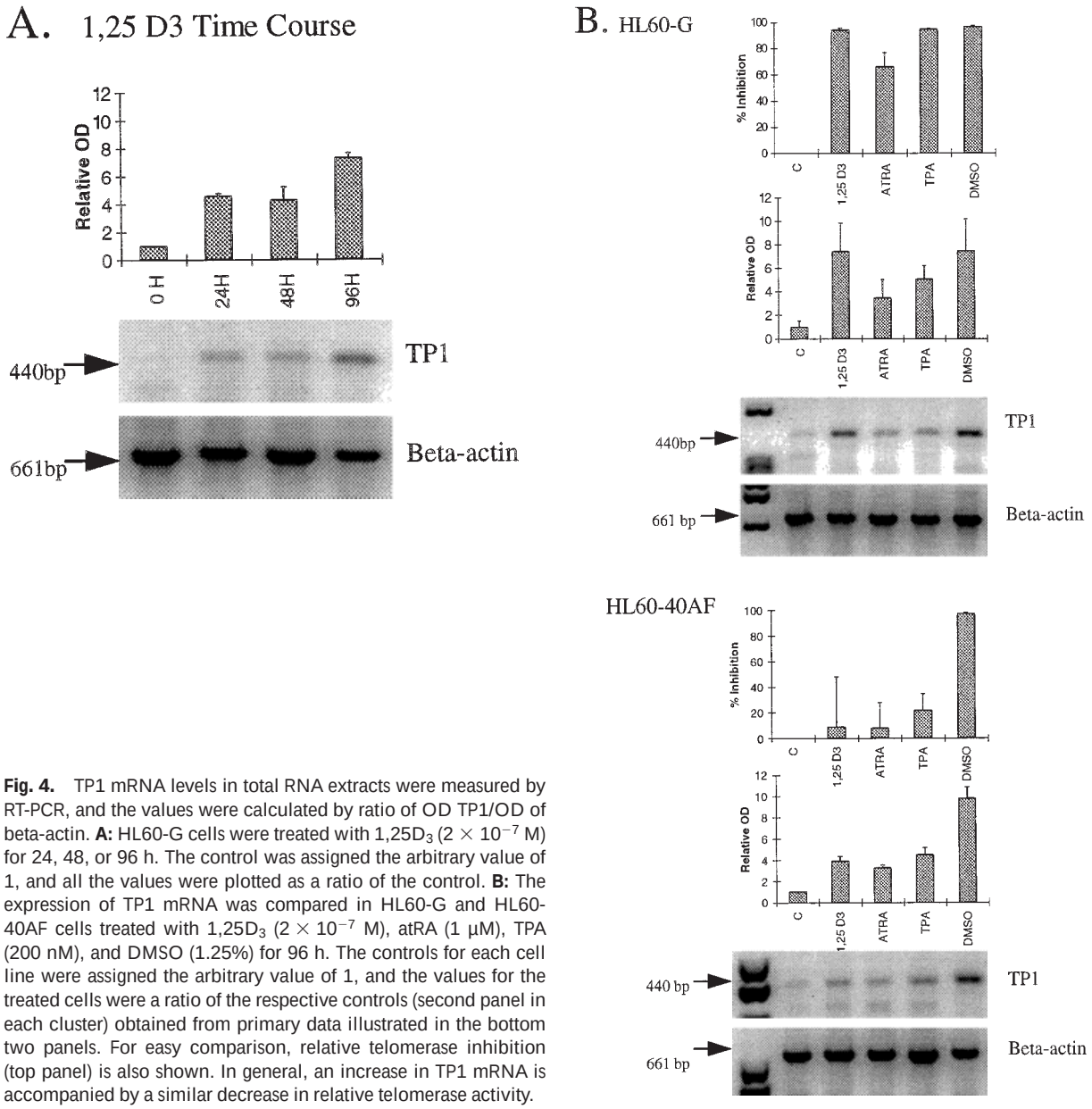

Fig. 4. TP1 mRNA levels in total RNA extracts were measured by RT-PCR, and the values were calculated by ratio of OD TP1/OD of beta-actin. **A:** HL60-G cells were treated with $1,25D_3$ (2×10^{-7} M) for 24, 48, or 96 h. The control was assigned the arbitrary value of 1, and all the values were plotted as a ratio of the control. **B:** The expression of TP1 mRNA was compared in HL60-G and HL60-40AF cells treated with $1,25D_3$ (2×10^{-7} M), atRA (1 μM), TPA (200 nM), and DMSO (1.25%) for 96 h. The controls for each cell line were assigned the arbitrary value of 1, and the values for the treated cells were a ratio of the respective controls (second panel in each cluster) obtained from primary data illustrated in the bottom two panels. For easy comparison, relative telomerase inhibition (top panel) is also shown. In general, an increase in TP1 mRNA is accompanied by a similar decrease in relative telomerase activity.

with telomerase activity (data not shown). The best correlation was between the expression of the CD11b adhesion molecule and the repression of telomerase activity, but even here the association was not invariable. Thus, further studies are necessary to elucidate what events precede and mediate the signal for the down-regulation of telomerase activity.

The sublines resistant to concentrations of $1,25D_3$ higher then 20 nM exhibited considerably higher telomerase activity than the parental, fully differentiation-sensitive, cells. This cannot be due to an acquisition of higher ploidy levels by the resistant cells, since the increased telomerase activity was also apparent in 30A cells. While no definitive explanation for this observation is available, it could perhaps be related to the increased proliferation rate of the cells in untreated resistant cultures [Wajchman et al., 1996; Studzinski et al., 1996].

The relationship of telomerase activity to the cell cycle traverse is not well defined. When quiescent cells are stimulated to reenter the cell cycle, telomerase activity is upregulated when cells exit G0 and enter G1 but then remains active in all phases of the cell cycle in proliferating cells [Mantell and Greider, 1994; Holt et al., 1996], though a transient increase

in telomerase activity driving the early S phase has also been reported [Kruk et al., 1997]. Conversely, telomerase activity becomes repressed when cell cycle progress is blocked by differentiating agents [Sharma et al., 1995; Albanell et al., 1996; Bestilny et al., 1996], though apparently not when cells are treated with direct inhibitors of DNA synthesis such as hydroxyurea [Buchkovich and Greider, 1996], but exceptions to these generalizations have been reported [Zhu et al., 1996]. In our experiments, no strict relationship between the inhibition of cell cycle progression and telomerase activity was discerned; for instance, 24 h treatment with TPA produced a marked G1 block in several sublines (data not shown), but there was no significant repression of telomerase activity at this time, though such repression was noted at later times (Fig. 3). In general, downregulation of telomerase appeared to follow the onset of the G1 to S phase block, suggesting that the G1 block may trigger molecular events that result in repression of telomerase activity. However, it is unlikely that repression of telomerase activity contributes to the initiation of the G1 block.

Recently, two groups reported cloning of the mammalian homologs of the Tetrahymena telomerase protein p80: the human homolog TP1 which is 75% identical to the mouse homolog at the amino acid level [Harrington et al., 1997] and the rat homolog TLP1, which encodes two large proteins, p240 and p230, the latter being thought to be associated with the active enzyme [Nakayamaa et al., 1997]. Even more recently, the cloning of the catalytic subunit responsible for telomerase extension in E. aediculatus and in yeast was reported. The catalytic protein was shown to have reverse transcriptase motifs, and single amino acid mutations in the sequence resulted in shortened telomeres and cell senescence in yeast, indicating the importance of reverse transcriptase in normal chromosomal telomere replication [Lingner et al., 1997]. The relevance of this protein to human cells is not known at this time, unlike TP1, which is a component of human cells [Harrington et al., 1997].

The TRAP procedure used here, although sensitive, allows measurement of telomerase activity only slightly more precise than its description as "semi quantitative" [Wright et al., 1995]. Thus, there is a possibility that some of the exceptions we have noted in the correlations between telomerase activity and manifestations of differentiation may have been due to the lack of sufficient precision of the method. Additionally, telomerase activity measured in vitro may not represent its true activity in the cell, as has been reviewed already [Lundblad and Wright, 1996]. For instance, the abundance of primers supplied in the assay may not reflect the situation in the cell, where nucleotide 3′ overhangs and other structural features at telomerase may also be limiting for the enzyme activity [Greider and Blackburn, 1987; Lingner et al., 1995]. Additionally, positively and negatively regulatory proteins may determine telomerase activity, and TP1 is potentially one of such factors in the system studied here. However, the reservations discussed above do not allow a clear statement regarding the physiological role of TP1 in this system.

ACKNOWLEDGMENTS

We thank Dr. Milan R. Uskokovic for his generous gifts of $1,25D_3$ and retinoic acid (all-trans and 9-cis) and Richard Schweitzer for help with the demonstration of the cross-resistance of the sublines. This work was supported by grant RO1-CA 44722 (G.P.S.). J.A. was supported by FIS BAE and CIRIT.

REFERENCES

Albanell J, Han W, Mellado B, Gunawardane R, Scher HI, Dmitrovsky E, Moore MAS (1996): Telomerase activity is repressed during differentiation of maturation-sensitive but not resistant human tumor cell lines. Cancer Res 56:1503–1508.

Bestilny LJ, Brown CB, Miura Y, Robertson LD, Riabowol KT (1996): Selective inhibition of telomerase activity during terminal differentiation of immortal cell lines. Cancer Res 56:3796–3802.

Buchkovich KJ, Greider CW (1996): Telomerase regulation during entry into the cell cycle in normal human T cells. Mol Biol Cell 7:1443–1454.

Castalgne S, Chomienne C, Daniel MT, Berger R, Miclea JM, Ballerini P, Degos L (1990): Retinoic acids in the treatment of acute promyelocytic leukemia. Nouv Rev Fr Hematol 32:36–38.

Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, Bacchetti S (1992): Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 11:1921–1929.

Greider CW, Blackburn EH (1987): The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 51:887–898.

Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Program AEST, Bass MB, Arruda I, Robinson MO (1997): A mammalian telomerase associated protein. Science 275: 973–977.

Holt SE, Wright WE, Shay JW (1996): Regulation of telomerase activity in immortal cell lines. Mol Cell Biol 16: 2932–2939.

Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhoa L, Gu LJ, Wang ZY (1988): Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood, 72: 567–572.

Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994): Specific association of human telomerase activity with immortal cells and cancer. Science 266:2011–2015.

Kim NW, Wu F (1997): Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). Nucleic Acids Research 25:2595–2597.

Kruk PA, Orren DK, Bohr VA (1997): Telomerase activity is elevated in early S phase in hamster cells. Biochem Biophys Res Commun 233:717–722.

Lingner J, Cooper JP, Cech TR (1995): Telomerase and DNA end replication: No longer a lagging strand problem? Science 269:1533–1534.

Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech T (1997): Reverse transcriptase motifs in the catalytic subunit of telomerase. Science 276:561–567.

Lundblad V, Wright WE (1996): Telomeres and telomerase: A simple picture becomes complex. Cell 87:369–375.

Mantell LL, Greider CW (1994): Telomerase activity in germline and embryonic cell of Xenopus. EMBO J 13: 3211–3217.

Nakayama J, Saito M, Nakamura H, Matsuura A, Ishikawa F (1997): TLP1: A gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. Cell 88:875–884.

Sharma HW, Sokoloski JA, Perez JR, Maltese JY, Sartorelli AC, Stein CA, Nichols G, Khaled Z, Telang NT, Narayanan R (1995): Differentiation of immortal cells inhibits telomerase activity. Proc Natl Acad Sci USA 92:12343–12346.

Studzinski GP, Gierthy JF, Cholon JJ (1973): An autoradiographic screening test for mycoplasmal contamination of mammalian cell cultures. In Vitro 8:466–472.

Studzinski GP, Rathod B, Rao J, Kheir A, Wajchman HJ, Zhang F, Finan FB, Nowell PC (1996): Transition to tetraploidy in 1,25-$(OH)_2$-vitamin D_3 resistant HL60 cells is preceded by reduced growth factor dependence and constitutive up-regulation of Sp1 and AP-1 transcription factors. Cancer Res 56:5513–5521.

Studzinski GP, Rathod B, Wang QM, Rao J, Zhang F (1997): Uncoupling of cell cycle arrest from the expression of monocytic differentiation markers in HL60 cell variants. Exp Cell Res 232:376–387.

Wajchman HJ, Rathod B, Song S, Xu H, Wang X, Uskokovic MR, Studzinski GP (1996): Loss of deoxycytidine kinase expression and tetraploidization of HL60 cells following long-term culture in 1,25-dihydroxyvitamin D_3. Exp Cell Res 224:312–322.

Warrell RP, Frankel SR, Miller WH Jr, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreeff M, Tafuri A, Jakubowski A, Gabrilove J, Gordon MS, Dmitrovsky E (1991): Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans retinoic acid). N Engl J Med 324:1385–1393.

Wright WE, Shay JW, Piatyszek MW (1995): Modifications of a telomeric repeat amplification protocol (TRAP) result in increased reliability, linearity and sensitivity. Nucleic Acids Res 23:3794–3795.

Zhu X, Kumar R, Mandal M, Sharma N, Sharma HW, Dhingra U, Sokoloski JA, Hsiao R, Narayanan R (1996): Cell cycle–dependent modulation of telomerase activity in tumor cells. Proc Natl Acad Sci U S A 93:6091–6095.