Increased Expression of Acetylcholinesterase T and R Transcripts during Hematopoietic Differentiation Is Accompanied by Parallel Elevations in the Levels of Their Respective Molecular Forms*

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Acetylcholinesterase (AChE) is responsible for inactivating acetylcholine at cholinergic synapses in both central and peripheral nervous systems. This enzyme displays a rich polymorphism because it exists as a variety of molecular forms that may be classified as either homomeric or heteromeric on the basis of their association with specialized structural subunits (1). Homomeric forms include the globular (G1) monomer and G2 dimer as well as a glycospholipid-linked dimer. Conversely, heteromers consist of the asymmetric forms A4, A8, or A12 in which one, two, or three soluble G4 tetramers attach to a collagenic structural subunit, respectively; and amphiphilic tetramers G4-linked to a 20-kDa hydrophobic anchor. Although the functional significance of this polymorphism is still elusive, it has been suggested that it allows the placement of catalytically active subunits in distinct cell types and subcellular locations to perform site-specific functions. In mammals, for example, the asymmetric forms of AChE are expressed exclusively in differentiated muscle and neuronal cells, whereas glycospholipid-linked dimers are found preferentially in tissues of hematopoietic origin. Such varied patterns of expression suggest that expression of AChE involves several levels of regulatory mechanisms ranging from tissue-specific transcriptional control to highly regulated post-translational events.

An interesting feature of AChE is that its expression is indeed not restricted to cholinergic tissues. In fact, its presence in non-cholinergic cells such as specific neuronal populations (2–4) and hematopoietic cells (5–7) has led to the proposal that AChE may participate in additional cellular functions distinct from its well known catalytic activity (for review, see Ref. 8). For example, converging lines of evidence indicate that the process of terminal differentiation in specific lineages of hematopoietic cells is accompanied by large increases in the levels of AChE activity (see Ref. 9). Moreover, it has been suggested that AChE may participate directly in the regulation of bone marrow cell development by reducing the proliferative and expansive capacity of pluripotent stem cells committed to erythropoiesis, megakaryopoiesis, and macrophage production and by promoting apoptosis in their progeny (10–12). Finally, deletion of the AChE locus at 7q22 has been shown to be associated with both myelodysplastic syndromes and acute myeloid leukemia, suggesting that AChE may in fact act as a tumor suppressor gene (14, 15). In light of these recent findings, it becomes important to elucidate the cellular and molecular mechanisms presiding over AChE expression in hematopoietic cells since it may provide additional insights into its physiological function in this cell type. Additionally, a thorough understanding of these mechanisms in skeletal muscle as well as in neuronal and hematopoietic cells will not only contribute to our knowledge of the regulatory mechanisms controlling tissue-specific expression of AChE, but it should also lead to the elucidation of the factors implicated in its highly controlled developmental regulation.

The goal of the present study was to examine the molecular mechanisms involved in regulating AChE expression during the differentiation of hematopoietic cells. We initiated our studies using murine erythroleukemia (MEL) cells because they can be induced easily to undergo terminal hematopoietic differentiation accompanied by hemoglobinization after exposure to inducing agents such as dimethyl sulfoxide (Me₂SO) (16).
This cell line represents an excellent model with which to study AChE expression during hematopoiesis because induced cells are known to express high levels of AChE compared with their uninduced counterparts (9, 17).

**EXPERIMENTAL PROCEDURES**

*Tissue Culture*—Friend MEL cells were grown in suspension in minimum essential medium supplemented with 5% fetal bovine serum and antibiotics (100 units/ml penicillin G and 100 μg/ml streptomycin sulfa-) at 37 °C in a water-saturated atmosphere containing 5% CO2. Differentiation of these cells was induced by adding 1.5% Me2SO to the medium. The number of cells was detected by using a hemacytometer. Cell viability was assessed using the trypan blue exclusion test.

**AChE Extraction and Analysis**—Extraction of intracellular AChE was performed on cells washed twice with phosphate-buffered saline and resuspended in 350–700 μl of a high salt detergent buffer containing antiproteolytic agents (10 mM Tris-HCl, pH 7.0; 10 mM EDTA; 1 mM NaCl; 1% Triton X-100; 1.0 mg/ml bacitracin; 2.5 mg/ml aprotinin). The cells were homogenized for 30 s with a Polytron and then centrifuged at 14,000 rpm for 15 min at 4 °C. The resulting supernatants were transferred to fresh tubes and stored at −80 °C for further analysis. To assay for secreted AChE, MEL cells were cultured in medium containing serum in which endogenous esterase activity was inhibited by treatment with diisopropyl fluorophosphate as described except for some modifications (18). Briefly, fetal bovine serum was incubated with 0.1 mM diisopropyl fluorophosphate overnight at room temperature on a rocking platform. The treated serum was then filter sterilized and left at 4 °C for 10 days at which point less than 1% of the original AChE activity remained. The treated serum displayed no inhibitory activity toward exogenously added AChE or toward cell growth and differentiation.

Total AChE activity was determined using a modified version of the spectrophotometric method of Ellman (19) in the presence of 10−5 M tetraisopropylpyrophosphoramide, which inhibits nonspecific cholinesterase as described previously (20). Specific AChE activity was monitored by comparing substrate hydrolysis in the absence or presence of the AChE-specific inhibitor 1,5-bis(4-allyldimethylammoniumphenyl)pentane-3-one dibromide. Protein concentration was determined using a bicinchoninic (BCA) assay kit.

The content of AChE molecular forms in induced versus uninduced cells and in the growth medium was determined by velocity sedimentation as described in detail elsewhere (18, 20). Briefly, 100-μl samples were layered onto 5–20% linear sucrose gradients containing either Tris–HCl or Tris–HCl, pH 8.3. Gradients were centrifuged in a Beckman SW41 rotor at 40,000 rpm for 16 h at 4 °C. Approximately 45 fractions were collected from the bottom of the tubes and assayed for AChE activity. Peaks of activity corresponding to distinct molecular forms were identified according to the nomenclature of Bon et al. (21) on the basis of their apparent sedimentation coefficients.

**RNA Extraction, Quantitative RT-PCR, and mRNA Stability Assay**—Cell pellets were centrifuged at 1,500 rpm for 5 min. The cell pellets were then dissolved in 1.0 ml of Trizol (Life Technologies, Inc.). After phenol/chloroform extraction and ethanol washes, RNA pellets were redissolved in an appropriate volume of RNase-free water. 100–1,000 ng of total RNA was subsequently reverse transcribed to generate cDNAs. The reverse transcription mixture contained 5 mM MgCl2, 1 μM PCR primer II (50 mM KCl; 10 mM Tris-HCl, pH 8.3), 1.0 μM dNTPs, 20 units of RNase inhibitor, 50 units of Moloney murine leukemia virus reverse transcriptase, and 2.5 μM random hexamer primers. Negative controls consisted of reverse transcription mixtures in which RNA was replaced by RNase-free water.

To amplify AChE cDNAs, synthetic primers required for selective amplification of the T, H, and R transcripts were used (22). PCR assays were performed as described previously in detail (23, 24). Briefly, 5 μl of the reverse transcribed sample was added to 20 μl of a PCR mixture containing 0.3125 unit of AmpliTaq DNA polymerase, a 1.5 μM concentration of each of the appropriate 5′- and 3′-primers, and MgCl2 and PCR buffer II at final concentrations of 2 mM and 1 ×, respectively. The PCR conditions involved denaturation at 94 °C for 1 min and primer annealing and extension at 72 °C for 30 s for 30–50 cycles. PCR products were fractionated by electrophoresis, using either 1 or 2% agarose gels containing ethidium bromide and quantitated using 1.5% agarose gels containing VistraGreen (Amersham). The relative amounts of PCR products were quantitated using a Storm PhosphorImager (Molecular Dynamics; Sunnyvale, CA). For quantitation of the relative abundance of the AChE transcripts, PCR analyses of reverse transcribed samples from MEL cell RNA were performed in parallel with serial dilutions of an external standard control plasmid designated as C5 which contains an engineered AChE cDNA with a deletion (kindly provided by Dr. Claire Legacy; for details, see Ref. 22). The amount of AChE transcripts was determined by comparing the intensity of the PCR product from MEL cell samples against a standard curve generated from the quantitative analyses of serial dilutions of the control plasmid. We previously used a similar approach to quantitate the amount of AChE mRNA at the avian neuromuscular junction (see Ref. 23).

To assess the stability of the various AChE transcripts, actinomycin D (5 μM) or 5,6-dichlorobenzimidazole riboside (20 μM) was used to inhibit transcription in day 2 uninduced and induced MEL cell cultures. Total RNA was extracted from these samples at different time intervals thereafter and analyzed by RT-PCR.

**Nuclear Run-on Assays**—Two × 106 MEL cells were washed in cold phosphate-buffered saline, resuspended, and lysed in 10 ml of cold lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl, 5 mM MgCl2; 0.2% Nonidet P-40) by vortexing for 10 s and incubating them on ice for 10 min. Nuclei were then sedimented by centrifugation at 850 × g for 5 min at 4 °C and subsequently washed twice in lysis buffer. Nuclei pellets thus obtained were resuspended in 400 μl of freezing buffer (50 mM Tris-HCl, pH 8.3; 40% glycerol; 5 mM MgCl2; 0.1 mM EDTA) and used immediately or stored in liquid nitrogen.

Run-on transcription assays were performed as described in Chan et al. (25). [α-32P]UTP (200 μCi) was used to label nascent transcripts for 30 min at 27 °C. After DNA purification and protein denaturation, radiolabeled RNA was extracted using Trizol and hybridized to filters containing 10 μg of immobilized cDNAs encoding AChE, β-globin, and glyceraldehyde-3-phosphate dehydrogenase. The intensity of the radioactive signals was quantitated using a Storm PhosphorImager. All values were standardized according to glyceraldehyde-3-phosphate dehydrogenase, used as an internal standard, because transcriptional rates of this gene have been shown previously to remain unchanged during differentiation of MEL cells (25).

**RESULTS**

**Differential of MEL Cells Results in Large Increases in AChE Activity**—After a 3-day exposure to Me2SO, differentiation of MEL cells could be confirmed easily by examining their striking red color caused by the accumulation of hemoglobin (data not shown). Compared with uninduced control cells, expression of AChE was increased dramatically in induced MEL cells (Fig. 1). In fact, intracellular and secreted AChE activity increased by approximately 5- and 10-fold, respectively, in induced cells (Fig. 1). As shown in Fig. 1, it also appears that in both uninduced and induced MEL cells, more than 80% of the total enzyme activity was actually in the growth medium, indicating that these cells secrete large amounts of AChE. Unlike differentiating neuronal cells (18), the proportion of enzyme secreted remained unchanged during hematoepoietic differentiation of MEL cells.

To determine whether differentiation was accompanied by selective increases in the amount of specific molecular forms, we separated by velocity sedimentation using sucrose gradients aliquots of the cell extracts and growth media obtained from both uninduced and induced MEL cells and measured the AChE enzyme activity in each fraction. As shown in Fig. 2, A and C, uninduced MEL cell extract and media contained only a small amount of the monomeric G1 species of AChE. By contrast, we detected in induced cell extracts high levels of G1 together with moderate amounts of G4 (Fig. 2B). In these experiments, we also observed a high level of G1 in the medium of induced MEL cells (Fig. 2D). Together, these results indicate that the large increases in cell-associated and secreted total AChE enzyme activity are accounted for by selective increases in the levels of the globular forms of AChE.

In separate experiments, we also determined the amphiphilic character of the G1 monomers expressed by these cells using sucrose gradients containing Brij-96. Although essentially all monomers present in the media appeared non-amphiphilic (data not shown), we observed routinely that approximately half of the cell-associated G1 molecular form in either uninduced or induced cells displayed a reduced sedimentation coef-
Increased Stability of AChE mRNA during Hematopoiesis

It is well established that AChE is expressed in hematopoietic cells and that significant changes in total enzyme activity accompany the process of terminal hematopoietic differentiation. However, little is known about the cellular and molecular mechanisms underlying the expression of AChE in hematopoietic cells as well as its induction during differentiation. We employed MEL cells as a model system to begin addressing these issues. In the present study we focused on the pattern of

DISCUSSION

To compare the relative abundance of the T and R transcripts in uninduced and induced MEL cells, we determined the absolute amount of each mRNA in day 3 samples by quantitative RT-PCR using the external C5 control plasmid (see Ref. 22). Using 1 μg of total RNA (analysis of 1 μg of total RNA was necessary to visualize the PCR products corresponding to the T transcript in ethidium bromide-stained gels), we observed that in uninduced MEL cells there was approximately $1.1 \times 10^5$ copies of AChE T mRNA (Fig. 4). Similar experiments revealed that 0.1 μg of total RNA from uninduced cells contained $1.8 \times 10^5$ copies of AChE R transcripts, indicating that as observed previously by RNase protection assays (27, 28), the R transcript is slightly more abundant than the T mRNA in uninduced MEL cells when values are corrected for total RNA. As shown in this figure, differentiation increased the expression of both transcripts by approximately 3-fold without affecting the ratio of T versus R transcripts significantly. In agreement with previous findings obtained with muscle fibers (see Ref. 23), these experiments also indicated that both AChE mRNAs are rare to moderately abundant transcripts in MEL cells.

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There is currently little information concerning the transcriptional regulation of the AChE gene in hematopoietic cells. However, the presence of a consensus binding site for GATA-1, a transcription factor important for erythroid and megakaryocytic development, has been identified in the upstream regulatory regions of both rat (29) and human (30) AChE genes. In separate experiments we examined whether the increase in AChE mRNAs which occurs in differentiating MEL cells was caused by a concomitant increase in the transcriptional activity of the AChE gene. Nuclear run-on assays performed with nuclei isolated from MEL cells at day 3 showed, as expected (see Ref. 25), a large 10-fold increase in the transcriptional activity of the β-globin gene in induced versus uninduced cells (Fig. 5). By contrast, these experiments demonstrated that transcription of the AChE gene remained largely unaffected in differentiated MEL cells. Consistent with previous observations (see Ref. 31), approximately 75% of the AChE signal was attributed to nascent transcripts bound to antisense cDNAs. Control experiments using α-amanitin inhibited transcription in both directions, indicating that AChE mRNA synthesis resulted primarily from the activity of the RNA polymerase II (data not shown). Together these findings show that the increased AChE mRNA levels seen in induced cells do not result from an enhancement in the transcriptional activity of the AChE gene.

**Differentiation Increases the Stability of AChE Transcripts**

Increases in the levels of AChE mRNA in induced MEL cells may have also occurred via post-transcriptional regulatory mechanisms. We therefore examined whether differentiation of MEL cells was accompanied by an increase in the stability of AChE transcripts. For these experiments we inhibited the activity of the RNA polymerase II by adding actinomycin D to the media of uninduced and 2-day induced cells and measured the levels of T and R transcripts, the predominant species of AChE mRNA in MEL cells. Total RNA was extracted at different time intervals thereafter, and the levels of these two transcripts were determined by RT-PCR. As shown in Fig. 6, the half-lives of the R and T transcripts were only $4.1 \pm 0.9 h$ ($n = 3$) and $4.5 \pm 1.5 h$ ($n = 3$), respectively. As expected on the basis of our nuclear run-on assays, the stability of both AChE transcripts increased markedly in induced MEL cells. In separate experiments, we observed a similar effect on the stabilization of the R and T transcripts using 5,6-dichlorobenzimidazole riboside (data not shown).

**Increased Stability of AChE mRNA during Hematopoiesis**

It is well established that AChE is expressed in hematopoietic cells and that significant changes in total enzyme activity accompany the process of terminal hematopoietic differentiation. However, little is known about the cellular and molecular mechanisms underlying the expression of AChE in hematopoietic cells as well as its induction during differentiation. We employed MEL cells as a model system to begin addressing these issues. In the present study we focused on the pattern of
AChE molecular forms and the different transcripts expressed during hematopoiesis as well as on the relative contribution of transcriptional versus post-transcriptional regulatory mechanisms in the overall induction of AChE activity.

Exposure of MEL cells to Me₂SO, an inducer of differentiation, resulted in large increases in cell-associated and secreted AChE activity as described previously (9, 17). Interestingly, these changes in AChE expression were accounted for mostly by dramatic increases in the levels of G₁ rather than by changes in the amount of membrane-bound, glycophospholipid-linked G₂ dimer, which is highly expressed in mature erythrocytes (1). Because uninduced and induced MEL cells are equivalent to erythroblasts and normoblasts, respectively, which are early erythroid precursors residing in the bone marrow (32), our data suggest that expression of monomeric AChE is probably a characteristic feature in less mature hematopoietic cells. In support of this hypothesis, histochemical staining of normoblasts has revealed previously the presence of AChE activity inside the cells rather than on the cell surface (33). Furthermore, recent studies of immature human megakaryocytes showed that AChE is located intracellularly (34).

During MEL cell differentiation, the increases in AChE activity were accompanied by significant increases in the levels of AChE transcripts as revealed by our RT-PCR analysis. Although AChE is transcribed by a single gene, alternative exon usage achieved by differential splicing gives rise to three distinct transcripts designated T, H, and R. Previous studies have shown that the T transcript is the only splice variant expressed in adult muscle (22) and arises from splicing of exon 4 to 6. Alternatively, the H transcript results from splicing of exon 4 to 5 and appears exclusively in cells of hematopoietic origin, whereas the R transcript results from reading through the splice donor site of exon 4 into the subsequent genomic sequence (see Refs. 1 and 26). In agreement with previous findings (27, 28), we observed in uninduced MEL cells that approximately 60% of the total AChE mRNA corresponded to the R transcript, and the remaining portion consisted mostly of the T mRNA. Because levels of these transcripts are controlled by their rate of synthesis and degradation, our data showing a similar half-life for both of these transcripts in uninduced MEL cells suggest that the synthesis of the R transcript is likely occurring at a slightly faster rate. It is therefore possible that although transcription of the AChE gene provides precursor mRNA molecules for the formation of all AChE transcripts, splicing from exon 4 to 6 needed for T transcript maturation is a less preferred splicing pathway in uninduced MEL cells. Because the H transcript is barely detectable in these cells (see Refs. 27 and 28 and this study), the splicing from exon 4 to 5 necessary for its maturation appears as the least used option for alternative splicing of immature AChE mRNA. This particular mode of splicing likely persists during MEL cell differentiation as the ratio of R to T transcripts remained essentially unchanged in induced MEL cells despite the increases in the levels of both species of AChE transcripts. Interestingly, this splicing pattern is possibly not limited to early erythroid precursors such as uninduced and induced MEL cells. It may in
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AChE transcripts between uninduced and induced samples.

Above PCR products) to demonstrate the differences in the levels of amplified DNA were removed at different cycle numbers (numbers above PCR products) to show the differences in the levels of AChE transcripts in 3-day uninduced (CTL) and induced MEL cells. Aliquots of amplified DNA were removed at different cycle numbers (numbers above PCR products) to demonstrate the differences in the levels of AChE transcripts between uninduced and induced samples.

The fact extend to most early hematopoietic precursor cells because RNAse protection assays showed previously that the splicing pattern of AChE mRNA in MEL cells was similar to that in the bone marrow (27), which contains a pool of immature hematopoietic cells.

Because the H transcript encodes the glycoprophospholipid-linked dimer of AChE which is expressed abundantly in mature erythrocytes (1), the low level of this transcript in both uninduced and induced MEL cells suggests that the splicing event necessary for synthesis of this transcript is likely activated at a later stage of hematopoietic differentiation. Accordingly, this hypothesis raises the possibilities that (i) differential splicing of AChE mRNA is regulated developmentally in erythropoietic tissues as observed previously in skeletal muscle (22) and (ii) specific molecular forms of AChE which are destined to distinct subcellular locations are required at different stages of hematopoietic differentiation. It will thus be interesting to examine whether the H transcript is more prevalent than the other AChE transcripts in more mature erythroid elements such as reticulocytes.

In both uninduced and induced MEL cells, more than 80% of the total AChE enzyme activity corresponded to non-amphiphilic monomers secreted in the medium. Interestingly, the R transcript that encodes this monomeric species was only slightly more abundant than the T mRNA. Therefore, these results suggest that the R transcript may be translated more efficiently than the T mRNA. Alternatively, it is also possible that the secreted AChE, unlike intracellular AChE, is not subjected to degradation by the normal protein turnover machinery operating inside the cells.

To date, the molecular mechanisms regulating AChE gene expression have been studied mostly in cultured muscle (31, 35) and neuronal cells (18). In the C2 myogenic cell line, it has been demonstrated that the increase in the levels of AChE mRNA accompanying the fusion of myoblasts into multinucleated myotubes results primarily from an increase in the stability of existing transcripts as opposed to a transcriptional activation of the AChE gene (31). Similarly, post-transcriptional regulatory mechanisms have also been suggested to account for the large induction in the levels of AChE mRNA in P19 embryonic carcinoma cells induced to differentiate into neuronal and glial cells (18). Using MEL cells as a model system to study the molecular mechanisms underlying the increase in AChE expression during hematopoiesis, we show in the present study that the observed increase in AChE mRNA levels also results primarily from an increase in the stability of existing transcripts. Thus, post-transcriptional mechanisms, operating at the level of transcript stability, appear as a major regulatory step dictating levels of AChE mRNA in all cell types.

**Figure 3. Expression of AChE mRNA in MEL cells.** Panel A shows ethidium bromide-stained gels of PCR products corresponding to the T (670 base pairs) and R (668 base pairs) transcripts in MEL cells grown in the absence (control, CTL) or presence (INDUCED) of MeSO over 4 days. Note that the highest mRNA levels were observed at day 3 in both control and induced cells. The numbers refer to the days in culture. Left panels show the 100-base pair DNA molecular mass marker. Panel B shows ethidium bromide-stained gels of PCR products for the T and R transcripts in 3-day uninduced (CTL) and induced MEL cells. Aliquots of amplified DNA were removed at different cycle numbers (numbers above PCR products) to demonstrate the differences in the levels of AChE transcripts between uninduced and induced samples.

**Figure 4. Quantitation of AChE T and R transcript levels by RT-PCR.** Serial dilutions of the control plasmid (C5) that contained an engineered AChE cDNA were amplified in parallel with reverse-transcribed cDNAs from uninduced and induced MEL cells for 35 and 34 cycles for the quantitation of the T (panel A) and R (panel B) transcripts, respectively. Note that 1 μg of total RNA from MEL cell samples was used for quantitation of the R transcripts, whereas 0.1 μg was used for the T. Shown are ethidium bromide-stained gels of PCR products amplified from the C5 plasmid (numbers denote the number of copies used) and from uninduced (U) and induced (I) MEL cell samples. For quantitation, aliquots of the same samples were electrophoresed in agarose gels containing VistraGreen and scanned using a Storm PhosphorImager. Data obtained from the serial dilutions were plotted as standard curves (amount of C5 plasmid versus PCR signal); arrows indicate the levels of the transcripts in uninduced and induced MEL cells. Note that the level of both transcripts increased by approximately 3-fold. These results are representative examples obtained from four independent experiments.
known to express significant amounts of this enzyme. However, despite the apparent role of these post-transcriptional mechanisms in the control of AChE levels in various cell types, it is still of considerable interest to examine the relative contribution of specific AChE promoter elements to the transcriptional regulation of the AChE gene, particularly as it pertains to tissue-specific expression.

In recent years, there has been increasing evidence indicating that the stability of mRNA plays a crucial role in the basic mechanisms underlying gene expression (for review, see Refs. 36 and 37). Such regulatory mechanisms involve not only distinct cytoplasmic factors but also the contribution of sequence determinants lying both within and outside the coding regions of mature transcripts. On the basis of our data it appears that the predominant increase in AChE transcripts becomes stabilized during hematopoiesis, thereby raising the possibility of a general decrease in the activity of an endonuclease in induced MEL cells. This mechanism can be ruled out, however, because our previous studies have shown that the stability of the housekeeping actin mRNA is not affected following differentiation of MEL cells (25); thus, increased AChE mRNA stability appears to be a specific event accompanying hematopoietic differentiation. In muscle cells undergoing differentiation, the influx of Ca²⁺ via L-type channels has been linked to the increased stability of AChE transcripts (35). Although L-type Ca²⁺ channels have not been found in MEL cells, a surge of intracellular Ca²⁺ occurs in these cells after exposure to Me₂SO (38). Furthermore, treatment with the Ca²⁺ ionophore A23187 has been shown to induce an early and transient decrease in the levels of c-myb mRNA whose regulation has been implicated in the control of hematopoietic growth and differentiation (13). Therefore, it remains to be established whether Ca²⁺ does play a direct role in controlling AChE mRNA stability in hematopoietic cells. In differentiating C2 cells, an increase in AChE mRNA levels after protein synthesis inhibition has led to the suggestion that some labile proteins are involved in the destabilization of AChE mRNAs (31). Therefore, uninduced MEL cells may provide an interesting model in the search of destabilizing proteins specific for AChE mRNAs.

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**FIG. 5.** Differentiation of MEL cells does not affect transcriptional activity of the AChE gene. **Panel A** shows an autoradiogram of the nuclear run-on assay for control and differentiated (INDUCED) MEL cells. **Panel B** represents the quantitation of newly synthesized β-globin and AChE transcripts in MEL cells. Note that in contrast to the marked increase in the transcriptional activity of the β-globin gene in induced MEL cells, the transcriptional rate of the AChE gene remained largely unaffected. Values for these two transcripts were normalized to the internal standard glyceraldehyde-3-phosphate dehydrogenase and are expressed as arbitrary units.

**FIG. 6.** Stabilization of AChE mRNA in differentiated MEL cells. Both T (panel A) and R (panel B) transcripts displayed increased stability in induced compared with uninduced (CTL) cells. Representative examples obtained from three independent experiments are shown.
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