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Rapid Induction of Polyadenylated H1 Histone mRNAs in Mouse Erythroleukemia Cells Is Regulated by c-*myc*

GENHONG CHENG AND ARTHUR I. SKOULTCHI*

Department of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

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Chemically induced differentiation of murine erythroleukemia cells is a multistep process involving a precommitment period in which exposure to inducer leads to cells that are irreversibly committed to terminal differentiation. Certain changes in the expression of cellular proto-oncogenes are an important feature of the precommitment phase. We have identified two H1 histone genes that are rapidly induced during this period. Unlike most histone genes, these two H1 genes encode polyadenylated mRNAs with long 3′ untranslated regions. To investigate the relationship between induction of the H1 mRNAs and changes in proto-oncogene expression, we studied two independent series of mouse erythroleukemia cell lines that are inhibited from differentiating because of deregulated expression of transfected copies of c-*myc* or c-*myb*. The results showed that induction of the H1 mRNAs was negatively regulated by c-*myc*. The two H1 histone genes are among the first examples of specific cellular genes that are regulated by c-*myc*. The timing of their induction suggests that they may play an important role in achieving commitment to terminal differentiation.

The murine erythroleukemia (MEL) cell system has provided a useful cell culture model for studying the regulation of erythroid development. These cells also offer the opportunity to investigate the molecular events occurring when tumor cells reinitiate a terminal differentiation program. MEL cells are transformed erythroid precursors that are blocked from completing the terminal stages of erythroid differentiation (16, 34). Treatment of these cells with a variety of low-molecular-weight compounds, notably dimethyl sulfoxide (DMSO) and hexamethylene bisacetamide (HMBA), causes them to reenter a differentiation program culminating after 4 to 5 days in terminal cell divisions and the accumulation of hemoglobin and other erythrocyte-specific proteins (34).

The continuous presence of an inducing agent is not required for terminal differentiation of MEL cells. After about 24 h of inducer treatment, a substantial fraction of the cells become irreversibly committed to terminal differentiation such that they can complete differentiation in the absence of inducer (15, 18). Whereas there are a number of specific markers characteristic of the terminally differentiated cells, only a few changes in cellular activities have been described to occur during the precommitment period. There are very rapid changes in the transport of ions and other small molecules (5, 14, 29, 33) and in phosphatidylinositol turnover (13) soon after addition of inducer. There also are rapid changes during the precommitment period in the expression of certain cellular proto-oncogenes (28, 41). For example, the amount of c-*myc* mRNA drops to a very low level within 1 h after addition of inducer. It is maintained at this low level during most of the precommitment period but is reexpressed to the original level just before cells begin to commit to terminal differentiation. The level of mRNA then declines again as cells undergo differentiation (28). The mRNA for c-*myb* exhibits very similar fluctuations (7, 41). Several groups of investigators have attempted to evaluate the significance of these changes in c-*myc* and c-*myb* gene expression by transfecting MEL cells with expression vectors containing these genes driven by heterologous promoters (7, 8, 10, 26, 40). The results have shown that these changes in expression are important for terminal differentiation of MEL cells. In particular, constitutive expression of exogenously introduced c-*myc* or c-*myb* causes a marked inhibition of terminal differentiation.

The mechanisms by which these two proto-oncogenes affect differentiation of MEL cells are not known, in large part because the cellular targets of their protein products remain to be elucidated. We have embarked on a program to isolate additional genes whose activities change during the precommitment period of MEL cell differentiation. We describe here the identification of two H1 histone genes that encode polyadenylated mRNAs that are induced during the precommitment period. Moreover, we found that the rapid induction of these two H1 mRNAs after inducer treatment was specifically regulated by c-*myc*. Studies of the effect of c-*myc* on expression of these two H1 histone genes should allow us to understand the mechanisms of action of this important proto-oncogene regulator of cell division.

MATERIALS AND METHODS

Cell culture. Clone DS19 MEL cells were grown in Dulbecco modified Eagle medium supplemented with 10% (vol/vol) fetal bovine serum. Differentiation was initiated by subculturing a logarithmic culture to 3×10^5 cells per ml in growth medium containing 5 mM HMBA. During growth in medium containing HMBA, the cells were subcultured to 3×10^5 to 4×10^5 cells per ml every 24 h as required to maintain the cell density at less than 10^6/ml. Under these conditions, accumulation of cells committed to differentiation was as described previously (26). After 18 h of HMBA treatment there was only a very slight increase in committed cells, by 24 h of treatment about 20% of the cells were committed, and at 48 h more than 95% were committed.

Deregulated c-*myc* MEL cell transfectants (clones 56 and 60) and the control cell line (clone 57) (10) were generously provided by W. Michael Kuehl. They were grown in RPMI

* Corresponding author.

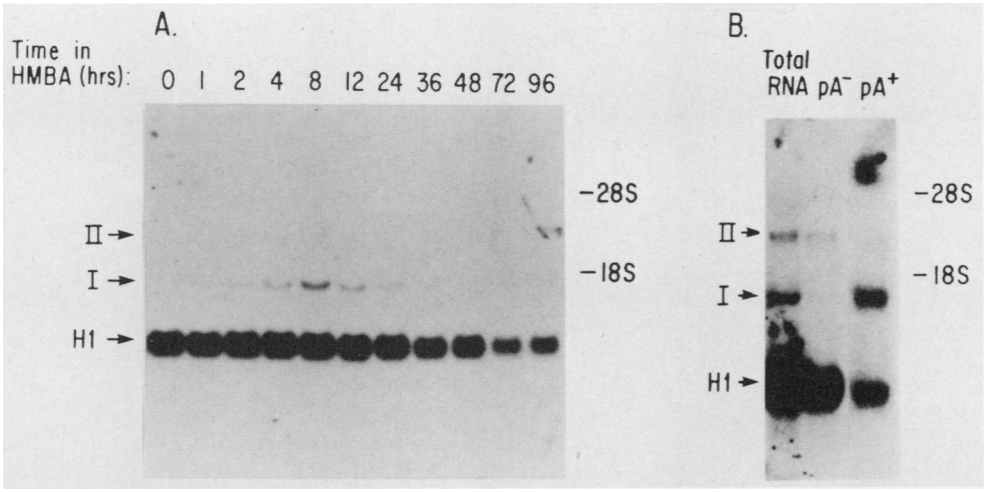

FIG. 1. Changes in H1 histone transcript levels during HMBA treatment detected with a human H1 probe. (A) MEL cells grown in the presence of 5 mM HMBA. At the times indicated, total cellular RNA was prepared and analyzed by blot hybridization, using an electroeluted *Eco*RI-*Sma*I DNA fragment isolated from a cloned human H1 histone gene, pHh8C (25). Hybridization was performed at 37°C in 30% formamide–5× SSC, and the blots were washed at 45°C in 0.1× SSC. The positions of the 28S and 18S rRNA markers, visualized by ethidium bromide staining of the gel, are indicated at the right. (B) MEL cells grown in the presence of 5 mM HMBA for 8 h. Total cellular RNA was prepared and fractionated by chromatography on oligo(dT)-cellulose. Bound (pA^+) and unbound (pA^-) RNA samples derived from an equal number of cells were analyzed by blot hybridization as described above.

1640 medium containing 10% fetal bovine serum. Transfectants SVmyc10 and SVmyc12 and the control cell line TK8 (8) were kindly provided by Michael D. Cole. They were grown in Ham F12 medium with 10% fetal bovine serum. Deregulated c-*myb* transfectants, clones 87C, 89A, and 91A (7), were a generous gift from Edward V. Prochownik. They were grown in Dulbecco modified Eagle medium with 10% fetal bovine serum. Differentiation of all transfected cell lines was induced as described above except that the growth medium contained 1.8% (vol/vol) DMSO. Benzidine-reactive cells were scored as described previously (4).

Isolation of H1 cDNA clones. H1 cDNA sequences were isolated from a cDNA library in bacteriophage lambda gt10. The library was constructed from polyadenylated, cytoplasmic RNA extracted from DS19 MEL cells that had been grown for 18 h in the presence of 5 mM HMBA. The first cDNA strand was synthesized with avian myeloblastosis virus DNA polymerase in the presence of actinomycin D. The second cDNA strand was synthesized with *Escherichia coli* DNA polymerase in the presence of RNase H. The double-stranded cDNA was incubated successively with *Eco*RI methylase, T4 DNA polymerase, and T4 DNA ligase in the presence of *Eco*RI linkers and *Eco*RI and then ligated to *Eco*RI-digested lambda gt10 bacteriophage DNA.

Recombinant phage containing H1 sequences were identified by hybridization with an *Eco*RI-*Sma*I fragment of a human H1 histone gene from pHh8c (25), generously provided by Nathaniel Heintz. Hybridization was performed in 30% formamide–5× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 37°C, followed by washes with 0.1× SSC–0.1% sodium dodecyl sulfate at 45°C. After four cycles of purification by plaque hybridization, the cDNA inserts were isolated by *Eco*RI digestion and categorized according to their hybridization properties with respect to the other cDNA inserts and uninduced and induced MEL cell RNA. The largest cDNA inserts in each category were isolated by *Eco*RI digestion and cloned into pGEM-3Z (Promega Biotec, Madison, Wis.). Dideoxy sequencing was performed on a nested set of deleted inserts prepared with exonuclease III according to the instructions of the manufacturer. The cDNA clone that hybridized to the 1.8-kilobase (kb) H1 mRNA, designated pcMH1-var.1, contained a 1.5-kb cDNA insert. Sequences representing primarily the coding region or the 3′ untranslated region were isolated by digesting pcMH1-var.1 with *Eco*RI and *Bst*NI, and the 0.6- and 0.46-kb fragments, respectively, were subcloned separately in pGEM-3Z. The cDNA clone that hybridized to the 2.2-kb H1 mRNA, designated $pcMH1^0$, contained a 1.8-kb cDNA insert. Sequences representing primarily coding or 3′ untranslated regions were isolated by digesting $pcMH1^0$ with *Eco*RI and *Bam*HI, and the 1.0- and 0.9-kb fragments, respectively, were subcloned in Bluescript plasmid pSKM13+ (Stratagene).

RNA analysis. Total cellular RNA was prepared by phenol extraction at 60°C (42). A 10-μg sample of RNA was dissolved in 50 mM boric acid–5 mM sodium borate–10 mM sodium sulfate–0.1 mM EDTA–50% formamide–6% formaldehyde, heated at 65°C for 5 min, and fractionated by electrophoresis in 1.2% agarose gels containing 3% formaldehyde. RNA was transferred to nitrocellulose papers by the method of Thomas (44). Unless otherwise indicated, the blots were hybridized with nick-translated DNA probes by incubation at 45°C in a solution containing 50% formamide, 5× SSC, 50 mM sodium phosphate (pH 6.8), 5× Denhardt solution, 0.1% sodium dodecyl sulfate, and 60 μg of denatured salmon sperm DNA per ml, followed by washing at 55°C with 0.1× SSC–0.1% sodium dodecyl sulfate.

RESULTS

Isolation of cDNA clones of inducible, polyadenylated H1 histone mRNAs. To evaluate the changes in H1 histone mRNA levels during MEL cell differentiation and to determine whether any distinct H1 histone mRNAs were induced during differentiation, we used a DNA probe derived from a human H1 histone gene (25). The probe contained primarily H1 histone-coding sequences, and hybridization was performed under nonstringent conditions so as to maximize the

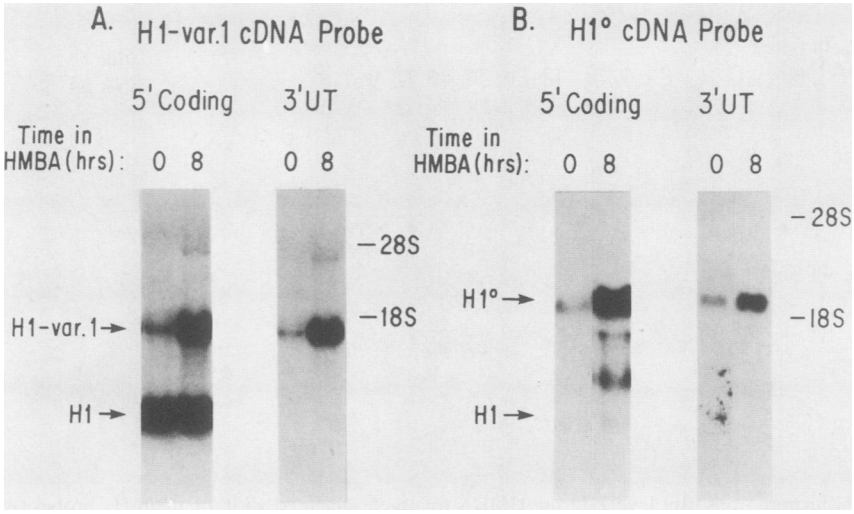

FIG. 2. Analysis of H1 transcripts with coding and noncoding segments of H1 cDNA clones. (A) Uninduced MEL cells (0 h) and cells grown in the presence of 5 mM HMBA for 8 h. Total cellular RNA was prepared, and duplicate RNA blots were analyzed by hybridization with electroeluted DNA fragments derived from either the coding region or the 3′ untranslated (3′UT) region of pcMH1-var.1. (B) Duplicate RNA blots prepared as described above and analyzed by hybridization with electroeluted DNA fragments from either the coding region or the 3′ untranslated region of pcMH1^0. Positions of RNA species are as described in the legend to Fig. 1.

possibility of detecting cross-reacting H1 mRNA species. The probe was hybridized to RNA blots prepared from total cellular RNA isolated from MEL cells treated with HMBA for various lengths of time (Fig. 1A). The human H1 histone probe hybridized quite strongly with the majority of mouse H1 histone mRNAs, which were about 700 to 800 bases long. These mRNAs were primarily nonadenylated (Fig. 1B). Their levels were relatively constant in MEL cells treated for up to 24 h with HMBA. Starting at about 24 h of HMBA treatment, the levels of nonadenylated H1 histone mRNAs began to decline and were substantially reduced as differentiation was completed (Fig. 1A).

The human H1 histone probe used in these experiments also detected two additional RNA species (Fig. 1). One (marked II in Fig. 1) was about 3 kb long and was also nonadenylated. This RNA species has not been characterized further. However, another RNA species (marked I in Fig. 1), about 1.8 kb long, was found to be retained on oligo(dT)-cellulose (Fig. 1B). Furthermore, this polyadenylated RNA was observed to be induced in MEL cells during the first 24 h of HMBA treatment.

Since the human H1 histone probe would be expected to hybridize with many of the mouse H1 histone family members present in a mouse genomic library, we used an alternate strategy to clone sequences for HMBA-inducible, polyadenylated H1 mRNAs. A cDNA library was prepared in lambda gt10, using polyadenylated mRNA from HMBA-induced MEL cells as a template for cDNA synthesis. The library was screened by hybridization with the human H1 histone probe under nonstringent conditions. Two categories of cDNA clones were characterized.

One category hybridized strongly under stringent conditions with the 1.8-kb inducible mRNA detected with the human H1 probe (Fig. 2A). Results of dideoxy sequencing on the largest cDNA inserts in this category showed that the 1.8-kb mRNA encoded an H1 histone. Overall, 28% of the residues were lysine, whereas in the carboxy-terminal half, 39% of the residues were lysine. There are a number of electrophoretically separable H1 histone proteins in the mouse (31), but the amino acid sequences of these H1 variants are not known. Therefore, we have designated the sequence encoded by the cDNA that hybridized with the 1.8-kb mRNA as H1-var.1. A computer search of the PIR protein data base of the National Biomedical Research Foundation (release 18.0; September 1988) showed that H1-var.1 is 76% homologous with rabbit histone H1.3. There is also extensive amino acid sequence homology with other vertebrate H1 histones. The amino acid-coding sequence in the cDNA clone is followed by a long 3′ untranslated region that contains a polyadenylation signal, AATAAA, about 15 nucleotides upstream from the apparent site of polyadenylation. Segments of the cDNA clone representing the coding sequences and the 3′ untranslated region were subcloned separately in plasmid vectors. A probe containing the coding sequences hybridized to the 1.8-kb inducible mRNA as well as to the smaller, nonadenylated H1 mRNAs (Fig. 2A). A probe derived from the 3′ untranslated region hybridized specifically with the 1.8-kb H1-var.1 mRNA, which indicated that the cDNA clone was homologous to the polyadenylated, inducible 1.8-kb H1 mRNA.

A second category of cDNA clones isolated with the human H1 histone probe hybridized strongly with a 2.2-kb mRNA (Fig. 2B) that was not detected in RNA blots with the human H1 probe. This H1 mRNA species was also polyadenylated (data not shown) and was induced during MEL cell differentiation. DNA sequence analysis indicated that these cDNA clones encode an H1^0 histone. The coding DNA sequence is identical to a recently published sequence of a mouse H1^0 histone cDNA clone (3) (unpublished results). Like the H1-var.1 mRNA, the mouse H1^0 mRNA contains a long 3′ untranslated region. Probes derived from the coding sequences of mouse H1^0 reacted weakly with the major, nonadenylated H1 mRNAs and with another RNA band about 1.0 kb in length whose structure is being investigated (Fig. 2B). Sequences from the 3′ untranslated region hybridized only with the 2.2-kb H1^0 mRNA. The complete DNA sequences of the mouse H1-var.1 and H1^0 genes will be published separately.

Polyadenylated H1 mRNAs are induced during the precommitment period of MEL cell differentiation. Beginning at

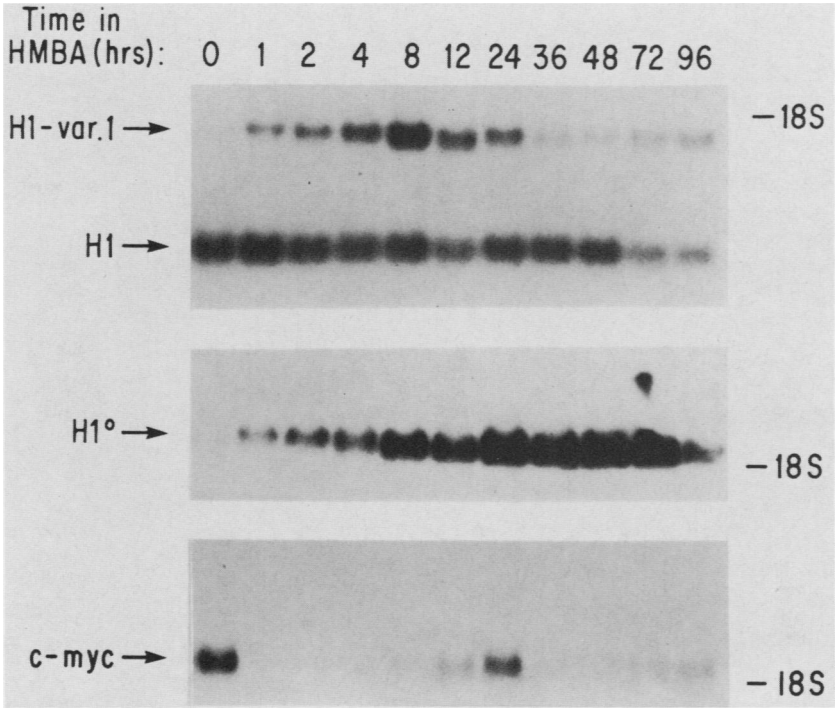

FIG. 3. Induction of polyadenylated H1 mRNAs during HMBA treatment. Total cellular RNA was prepared from MEL cells grown in the presence of 5 mM HMBA for the times indicated. Triplicate RNA blots were prepared and hybridized with three DNA probes: pcMH1-var.1 (A), pcMH1^0 (B), and pcmyc54 (43) (C).

about 24 h of inducer treatment, MEL cells undergo an irreversible commitment to differentiation (15, 18). Most cells are committed by 48 h of inducer treatment, and globin mRNAs, which begin to accumulate shortly after 24 h, continue to accumulate until about 96 h (17). If the inducer is removed during the precommitment period, before 24 h nearly all cells retain the unlimited proliferative capacity exhibited by untreated cells.

Levels of the major nonadenylated H1 histone mRNAs were quite constant in MEL cells during the first 24 h of HMBA treatment (Fig. 1A). In contrast, the polyadenylated mRNAs for H1-var.1 and H1^0 were very strongly induced during this precommitment period (Fig. 3). Interestingly, the kinetics of accumulation and decay of the two types of polyadenylated H1 mRNAs were different. Both mRNAs were detected at very low levels in cultures of untreated cells. Their presence in untreated cells may have been due to a low spontaneous rate of differentiation exhibited by MEL cell lines. The mRNAs for H1-var.1 and H1^0 were rapidly induced within 1 h of HMBA treatment. The amount of H1-var.1 mRNA continued to rise until about 8 h of HMBA treatment, whereafter it declined rapidly and was then maintained at a low level throughout the differentiation period. The level of H1^0 mRNA rose more gradually, reaching a maximum at 24 h and gradually declining thereafter as the cells underwent differentiation.

We have described previously the biphasic change in c-*myc* mRNA levels that occurs during the precommitment period when MEL cells are treated with inducing agents (27, 28). The amount of c-*myc* mRNA was greatly reduced within 1 h of HMBA treatment (Fig. 3C) and was maintained at a low level until 18 to 24 h, when it accumulated to the level present in untreated cells. Thereafter, it declined again as cells became committed to differentiation. It is apparent from these results that induction of the two polyadenylated H1 mRNAs occurred primarily during the period when c-*myc* mRNA levels were very low. Moreover, the down-regulation of H1-var.1 mRNA appeared to coincide with the reexpression of c-*myc* mRNA. These observations suggested to us the possibility that the changes in levels of the polyadenylated H1 mRNAs were related to changes in c-*myc* gene expression.

Polyadenylated H1 mRNAs are not induced in deregulated c-*myc* MEL cell transfectants. To investigate the relationship between induction of the polyadenylated H1 mRNAs and the early down-regulation of c-*myc*, we studied gene expression changes in several MEL cell lines transfected with c-*myc* genes driven by viral promoters. These lines have been described previously (8, 10). Clones 56 and 60 contain copies of a human c-*myc* gene driven by the Moloney leukemia virus long-terminal-repeat promoter region (10). Clones SVmyc10 and SVmyc12 contain copies of the mouse c-*myc* coding exons under control of the simian virus 40 early promoter (8). Both pairs of lines exhibited high levels of expression of the exogenous c-*myc* transcripts after induction with DMSO (Fig. 4h and 4i), whereas endogenous c-*myc* transcripts undergo a biphasic change in expression similar to that shown in Fig. 3 (8, 10). Deregulated expression of the exogenous c-*myc* gene in these lines causes them to produce much lower numbers of differentiated cells after treatment with DMSO (8, 10). As controls, we also studied MEL cell transfectants that did not express or did not contain the exogenous c-*myc* gene (clone 57 or TK 8, respectively [8, 10]) but did express the cotransfected selection marker. These lines differentiated normally and exhibited a biphasic change in c-*myc* mRNA levels characteristic of differentiating MEL cells (Fig. 4g).

Figure 4a shows the pattern of induction of the H1-var.1 mRNA in the control transfectant clone 57 (not expressing exogenous c-*myc*) treated with DMSO. The H1-var.1 mRNA

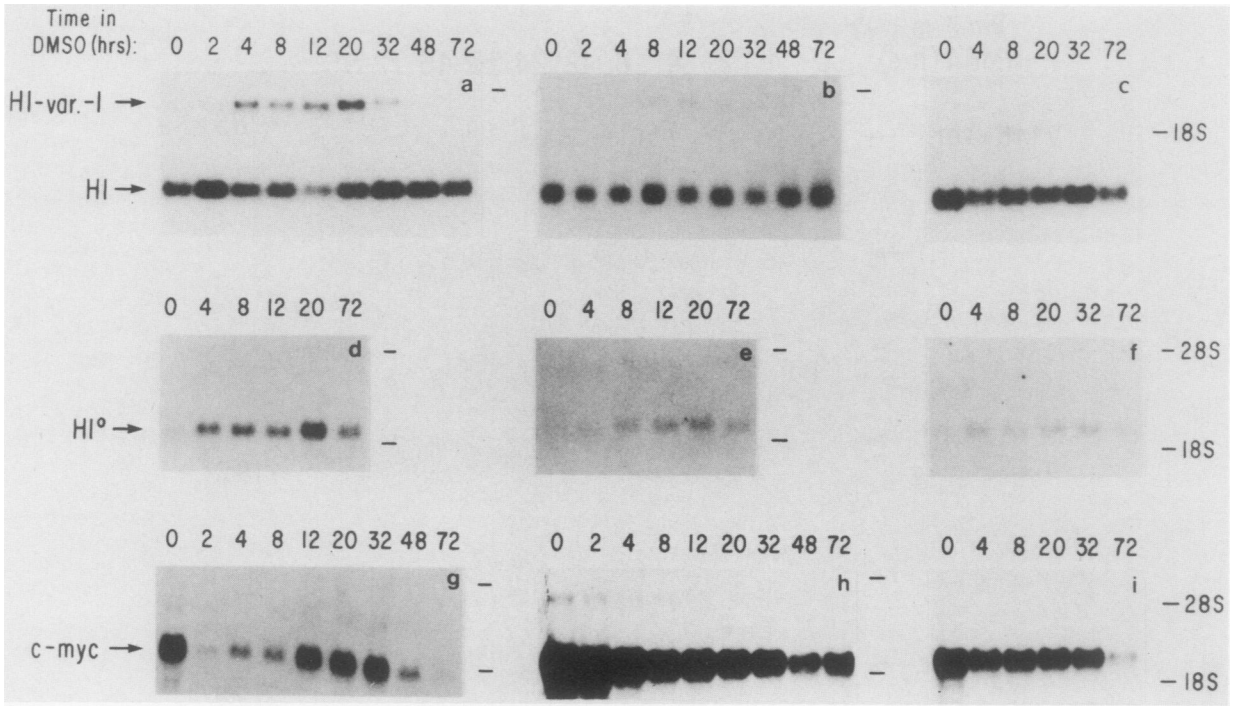

FIG. 4. Polyadenylated H1 mRNA levels during DMSO treatment of deregulated c-*myc* transfectants. Three MEL cell transfectants, clones 57 (panels a, d, and g), 56 (panels b, e, and h), and 60 (panels c, f, and i) (10), were grown in the presence of 1.8% DMSO. At the times indicated, total cellular RNA was prepared and analyzed by blot hybridization with the following electroeluted DNA fragments: an *Eco*RI insert from pcMH1-var.1 (panels a through c), an *Eco*RI insert from pcMH1^0 (panels d through f), and a 1.4-kb *Xho* fragment from pcmyc54 (43) (panels g through i). After 5 days of DMSO treatment, clone 57 produced 85% benzidine-positive cells, whereas clones 56 and 60 produced less than 1% positive cells.

was strongly induced during the first 24 h of DMSO treatment. Induction of H1-var.1 mRNA was nearly completely inhibited in the two transfectant cell lines (clones 56 and 60) expressing high levels of exogenous human c-*myc* (Fig. 4b and c). The presence of the cross-hybridizing, nonadenylated H1 mRNA band provided an internal control for the amount of RNA loaded and the efficiency of transfer and hybridization. Similar results were obtained with lines expressing exogenous simian virus 40-promoted mouse c-*myc* (data not shown). The induction of H1^0 mRNA also was sensitive to inhibition by an exogenous c-*myc* gene (Fig. 4d through f). H1^0 mRNA was strongly induced in clone 57, which differentiated normally, whereas its induction was drastically reduced in the transfectant lines expressing exogenous c-*myc* genes. These results indicate that the induction of both polyadenylated H1 mRNAs, which normally occurs during the precommitment period, does not take place when an exogenous c-*myc* gene is expressed at high levels during this early period.

Specificity of the effect of c-*myc* on polyadenylated H1 mRNA induction. The deregulated c-*myc* MEL cell transfectants used in this study do not differentiate, as judged by their failure to accumulate hemoglobin and withdraw from the cell cycle (8). Although these events occur late in the MEL cell differentiation program, whereas the induction of the polyadenylated H1 mRNAs occurs much earlier, it is possible that the effect of deregulated c-*myc* expression on H1 mRNA induction is due to a general effect on all aspects exhibited by differentiating MEL cells.

Relatively few early changes in gene expression after inducer treatment have been described. However, in addition to the early biphasic change in c-*myc* mRNA and the induction of polyadenylated H1 mRNAs described here, there are changes in the mRNA levels for several other nuclear proto-oncogenes during the first 24 h of MEL cell differentiation. Dmitrovsky et al. (10) have studied the early decline and reexpression of endogenous c-*myb* and c-*myc* transcripts in the deregulated c-*myc* transfectants (clones 56 and 60) used in our study. These authors found that the early changes in the two endogenous proto-oncogene mRNAs were not affected by the deregulated expression of the transfected human c-*myc* gene. Thus, two types of early changes in mRNA levels characteristic of inducer-treated MEL cells are unaffected by deregulated c-*myc* expression, whereas induction of the polyadenylated H1 mRNAs is markedly inhibited.

DMSO induction of MEL cell differentiation also can be inhibited by deregulated expression of a c-*myb* gene (7). To determine whether the effect of c-*myc* on polyadenylated H1 mRNA induction is specific, we studied expression of the mRNAs in several MEL cell lines that had been transfected with a full-length human c-*myb* cDNA driven by simian virus 40 transcription signals. These lines produced high levels of human c-*myb* transcripts after DMSO treatment, whereas endogenous mouse c-*myb* expression undergoes an early biphasic change similar to that seen in untransfected MEL cells (7). Deregulated expression of the human c-*myb* cDNA led to a marked inhibition in the production of hemoglobin-accumulating cells after DMSO treatment. Results of expression studies of the polyadenylated H1 mRNAs in these c-*myb*-inhibited lines showed that despite the failure of these lines to undergo differentiation, the pattern of induction of

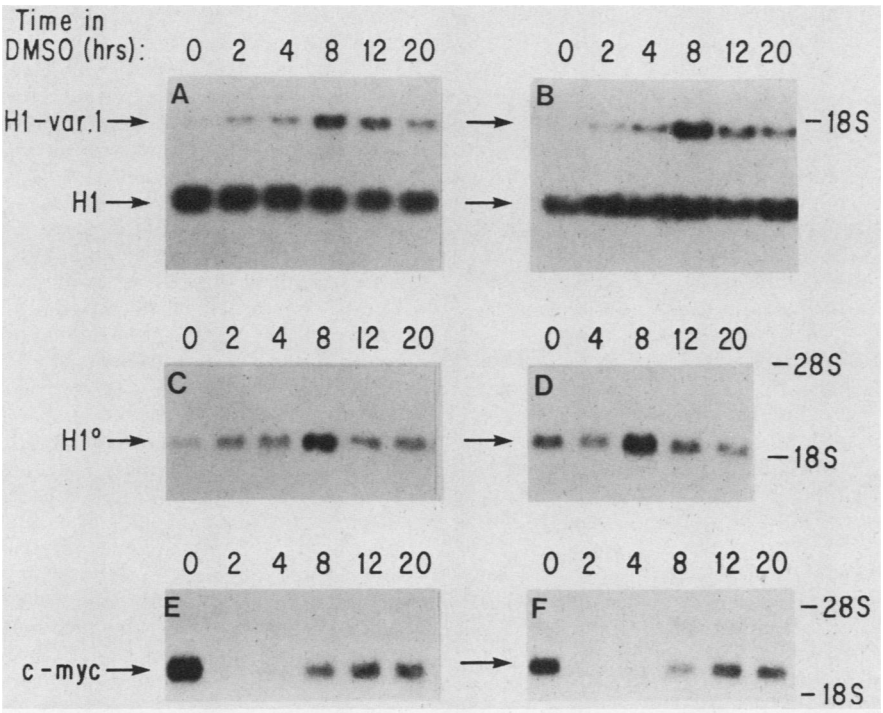

FIG. 5. Induction of polyadenylated H1 mRNAs during DMSO treatment of deregulated c-*myb* transfectants. Two MEL cell transfectants, clones 87C (panels A, C, and E) and 91A (panels B, D, and F) (7), were grown in the presence of 1.8% DMSO. At the times indicated, total cellular RNA was prepared and analyzed by blot hybridization with the following electroeluted DNA fragments: an *Eco*RI insert from pcMH1-var.1 (panels A and B), an *Eco*RI insert from pcMH1^0 (panels C and D), and a 1.4 kb *Xho* fragment from pcmyc54 (43) (panels E and F). After 5 days of DMSO treatment, both clones produced about 5% benzidine-positive cells.

the H1-var.1 mRNA was retained (Fig. 5). The mRNA was strongly induced after 8 h of DMSO treatment (Fig. 5A and 5B), similar to the induction seen in wild-type MEL cells treated with HMBA (Fig. 3). The early induction of H1^0 mRNA also was observed in these c-*myb*-transfected lines (Fig. 5C and D). However, the pattern observed in these transfected lines after the initial induction was different from that observed in untransfected MEL cells. Whereas in MEL cells H1^0 mRNA was maintained at high levels until the final stages of differentiation (Fig. 3), the H1^0 mRNA induced in the deregulated c-*myb* transfectants declined after 8 h. Thus, although deregulated c-*myb* expression did not affect the early induction of H1^0 mRNA, it did appear to have an effect on the maintenance of H1^0 mRNA levels at later times. As reported previously (7) and shown in Fig. 5E and F, the early biphasic change in c-*myc* mRNA levels was not altered in these transfected cells.

To make a direct, quantitative comparison of the expression patterns of the polyadenylated H1 mRNAs in the two types of transfectants, we prepared single RNA blots with RNAs from both deregulated c-*myc* and deregulated c-*myb* transfectants as well as from a control transfectant line not expressing an exogenous oncogene. After hybridization with either the H1-var.1 or the H1^0 probe, the resulting bands in the autoradiogram were quantitated by densitometry. In comparison with the control transfectant line and the c-*myb* transfectant, the deregulated c-*myc* transfectant exhibited a 10- to 20-fold reduction in expression of the H1-var.1 mRNA and a 5- to 10-fold reduction in expression of the H1^0 mRNA (Fig. 6). These results indicate that the inhibition of polyadenylated H1 mRNA induction observed in deregulated c-*myc* transfectants is specific. This inhibition did not occur when expression of another nuclear proto-oncogene, c-*myb*,

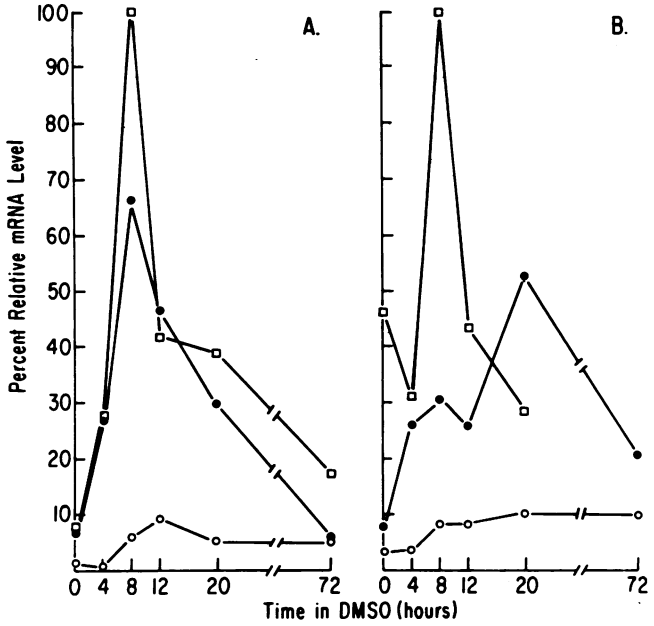

FIG. 6. Quantitative comparison of H1 mRNA induction in transfectants. RNA was prepared from a MEL cell control transfectant (clone 57; ●), a deregulated c-*myc* transfectant (clone 56; ○), and a deregulated c-*myb* transfectant (clone 91A; □) at various times during growth in 1.8% DMSO. Single RNA blots containing the RNAs from the three cell lines were prepared and hybridized with the *Eco*RI insert from either pcMH1-var.1 (A) or pcMH1^0 (B). The relative intensity of each band in the autoradiogram was determined by scanning with a Quantimet analyzer. Relative mRNA levels were normalized to the most intense band obtained with each probe, which was arbitrarily assigned a value of 100%.

was deregulated, even though such deregulation inhibited the ability of MEL cells to undergo terminal differentiation.

DISCUSSION

The H1 histone proteins of mammals can be resolved into multiple subtypes that differ in primary structure. At least seven unphosphorylated subtypes can be identified by two-dimensional gel electrophoresis, and further extensive heterogeneity is achieved by phosphorylations (30–32). The amounts, relative rates of synthesis, and metabolic stabilities of the various primary forms differ in different tissues. To a significant extent, these differences appear to correlate with the rate of cell division in the tissues. For example, subtypes designated H1a and H1b are markedly decreased in the chromatin of nondividing mammalian cells (30). Conversely, $H1^0$ accumulates in cultured cells when growth has been arrested (9, 37, 39) and in adult tissues containing a high proportion of nondividing cells (36). It also is present in the chromatin of certain terminally differentiated cells, including MEL cells (12, 22, 35). Very little is known about the significance of these changes in H1 subtypes for the functional state of the cell. H1 histones are involved in the formation of higher-order chromatin structures. Since changes in chromatin structure and transcriptional activity occur extensively during development and cell differentiation, specific changes in the H1 subtypes in chromatin could play an important role in these processes. However, direct proof for such a role is lacking.

The results reported here suggest that the MEL cell system may provide a useful model for investigating the role of H1 subtypes in a specific differentiation process. The MEL cell differentiation program is quite well defined at the temporal level. It consists of a precommitment period lasting about 24 h. During this period, the cells undergo certain changes which cause them to become irreversibly committed to terminal differentiation. Once a cell is committed, it no longer requires the presence of inducer to execute the differentiation process. Differentiation, occurring between 24 and 96 h, involves the accumulation of globin mRNA, the synthesis of hemoglobin and other erythrocyte-specific proteins, and the cessation of cell division. It appears that induction of the polyadenylated H1 mRNAs is not simply a consequence of the differentiation process (Fig. 3). Instead, the kinetics of induction suggests that these mRNAs may be induced as part of the events leading to cell commitment. Induction of both mRNAs was detected as early as 1 h after inducer was added. Moreover, induction did not depend on the differentiation process, since the early induction of the two mRNAs also was observed in deregulated c-*myb* transfectants that failed to differentiate. It seems, then, that induction of the two H1 mRNAs is a result of changes taking place very early during the precommitment period. In the case of H1-var.1, maximal accumulation of the mRNA was observed after 8 h of inducer treatment, and this mRNA began to decline before a substantial fraction of the cells underwent commitment. Only low levels of the H1-var.1 mRNA were present during the differentiation process occurring after 24 h. Thus, the activity of this gene appears to be restricted primarily to the precommitment period. Although $H1^0$ mRNA was also detected soon after inducer treatment, its level continued to rise throughout the precommitment period, reaching a maximum at 24 h, when committed cells begin to accumulate. Furthermore, $H1^0$ mRNA was maintained at a relatively high level throughout most of the differentiation period. These results suggest that $H1^0$ may play a role both in the commitment of cells to differentiate and in the differentiation process itself. The observed differences in the accumulation and decay of these two H1 mRNAs support the view that changes in the chromatin content of H1 subtypes may play specific roles in the modulation of cellular activities.

Recent progress in understanding some of the events required for MEL cell differentiation has come with the recognition that expression of certain cellular proto-oncogenes undergoes complex changes both before and after commitment of the cells to terminal differentiation (28, 41). Transfection studies using vectors that direct expression of c-*myc* and c-*myb* have shown that constitutive expression of either of these two proto-oncogenes blocks MEL cell differentiation (7, 8, 10, 26, 40). However differentiation is a complex, multistep process, and it is not known whether these two proto-oncogenes affect differentiation by interacting with the same or different cellular targets. The time of their action in the differentiation program also is not known. After treatment of MEL cells with an inducer, the mRNA levels from both proto-oncogenes exhibit a rapid decline, but they are reexpressed at intermediate times and then decline again as the cells differentiate (7, 28). A recent study suggests that for c-*myc*, the later but not the earlier decline is required for differentiation (24). Our results suggest that both the targets and the timing of the effect of the two proto-oncogenes in MEL cells may be different. Inhibition of the early induction of the polyadenylated H1 mRNAs was observed in deregulated c-*myc* but not c-*myb* transfectants. Thus, with induction of the H1 mRNAs used as a more specific measure, it seems that the effects of the two proto-oncogenes are different. Moreover, it is clear from our observations that deregulated expression of c-*myc* does affect early events in the MEL cell differentiation program. We also found an effect of deregulated c-*myb* expression on the maintenance of $H1^0$ mRNA levels after the initial induction. On the basis of this observation, we would tentatively suggest that the timing of the c-*myb* effect on differentiation may be later than that of c-*myc*.

Determining the mechanism by which the c-*myc* proto-oncogene exerts an effect on cell division is of fundamental importance to understanding the role of c-*myc* in control of normal and abnormal growth. A crucial step in achieving this goal is the identification of specific cellular genes that are regulated by c-*myc* and the demonstration that such genes participate in the control of cell division. The c-*myc* protein is localized in the nucleus and appears to have DNA-binding activity (1, 2, 11, 19, 38), although specific binding has yet to be demonstrated. These observations have led to the suggestion that the c-*myc* protein may be a regulator of transcription. Support for this idea has come from experiments demonstrating that c-*myc* can stimulate expression of *Drosophila* and human heat shock protein genes in cotransfection assays (20, 23). These studies also showed that c-*myc* can inhibit expression from the mouse metallothionein I gene, which indicates that it also can exert negative control on gene expression. It seems likely that the heat shock protein and metallothionein genes are involved in cellular responses to stress conditions rather than in control of cell division. The synthesis of $H1^0$, on the other hand, is correlated with growth arrest (9, 37, 39). Furthermore, we have found that both H1-var.1 and $H1^0$ transcripts are induced in mouse fibroblasts arrested by serum starvation and that these mRNAs disappear rapidly from the cells when growth is stimulated by restoration of serum levels (unpublished observations). Previous work has shown that c-*myc* mRNA

levels are low in serum-starved fibroblasts and that they rise during serum stimulation (6, 21). We also found that the levels of both polyadenylated H1 transcripts are inversely correlated with c-*myc* mRNA levels in a variety of fetal and adult mouse tissues (unpublished observations). Thus, the inverse relationship between c-*myc* expression and expression of the two H1 histone genes appears to be quite general. These studies with c-*myc* transfectants have allowed us to demonstrate directly that c-*myc* is involved in controlling expression of both H1 genes. These two H1 histone genes are promising candidates for studies of the mechanisms of specific gene regulation by c-*myc*.

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