c-Myc Selectively Regulates the Latent Period and Erythroid-specific Genes in Murine Erythroleukemia Cell Differentiation

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During the latent period of murine erythroleukemia (MEL) cell differentiation, c-*myc* levels showed a significant change and the overexpression of the transferred c-*myc* gene inhibited the commitment and differentiation of MEL cells, suggesting that c-Myc may be a key molecule for the commitment. Since c-Myc may function as a DNA binding transcription factor, we examined whether c-Myc regulates the latent period genes (hsp and hsc70, *MER5*, Id and Spi-1 genes) and the erythroid-specific genes [β-globin, glycophorin, δ-aminolevulinic acid synthase (ALAS-E), GATA-1 and erythropoietin receptor (EpoR)] in the MEL cell transformant having transferred c-*myc* gene. The overexpression of c-*myc* gene affected the latent period genes in different ways: hsc and hsp 70 genes and Id gene were positively regulated, while expression of *MER5* gene was repressed. While c-*myc* is thought to be involved in DNA replication, its overexpression showed no effect on the expression of proliferating cell specific nuclear antigen or DNA polymerase α. The overexpression of c-*myc* repressed the expression of glycophorin, ALAS-E and β-globin genes, of the five erythroid-specific genes, but had no effect on expression of GATA-1 or EpoR gene. These results suggest that c-Myc differentially regulates the expression of the latent period and erythroid-specific genes.

Key words: c-Myc — Murine erythroleukemia — Differentiation — Transcription

When murine erythroleukemia (MEL) cells are induced with a variety of agents, expression of several erythroid-specific genes is apparent following a latent period.[1] During this latent period, which might require the reprogramming of the uncommitted cells to commit toward erythroid differentiation, changes in the expression of certain cellular oncogenes including c-*myc*[2] and heat shock protein hsc70[1] were observed. These latent period genes may have an important role in the commitment of MEL cells. The role of c-*myc* in the differentiation program was examined by introducing the c-*myc* gene into MEL cells.[3-5] The constitutively elevated expression of the transferred c-*myc* inhibited the differentiation of MEL cells without inhibiting commitment,[4] whereas the regulatable expression of the transferred c-*myc* inhibited both the commitment and differentiation of MEL cells in a dose-dependent manner.[5-7] By transferring human c-*myc* mutant genes, we have shown that almost the entire region of c-Myc is required for the inhibition of commitment, whereas only a limited region of c-Myc including the basic-helix-loop-helix-leucine repeat (b-HLH-LR) structures is required for the inhibition of terminal differentiation.[8] Other reports also showed the importance of these domains for the transforming activity of c-Myc.[9-11] The c-Myc protein has recently been shown to function as a sequence-specific DNA-binding protein that requires dimerization with a second related protein called Max.[12, 13] Max, like c-Myc,

contains adjacent b-HLH-LR structures that are also present in transcription factors such as TFE3, AP4, and upstream stimulating factor (USF).[14-16] Myc forms a heterodimer with Max and binds to a specific DNA sequence containing CACGTG.[11, 16-20] Presumably Myc/Max heterodimer binds to and trans-regulates a set of cellular target genes that participate in the commitment and differentiation processes of MEL cells. Thus, we examined the effect of overexpressed c-Myc on expression of the latent period genes and erythroid-specific genes.

MATERIALS AND METHODS

Culture and induction of MEL cell transformants One of the c-*myc* transfected cell lines (clone 38-2) used in this work was obtained by transferring rat c-*myc* gene under the control of human metallothionein gene promoter into B8/3 cells.[5] Cells were cultured in ES-medium (Nissui Seiyaku, Tokyo) supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) and maintained at 37°C in a humidified 5% CO_2 atmosphere. Erythroid differentiation of the MEL cells was induced by continuous exposure to 1.4% dimethylsulfoxide (DMSO). The overexpression of the transferred c-*myc* gene was induced by continuous exposure to 200 μM $ZnCl_2$ in the presence of DMSO as previously described.[7] **RNA isolation and Northern blot analysis of RNA** MEL cell samples were collected at the indicated times after exposure to DMSO in the presence or absence of Zn ions,

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washed twice with phosphate-buffered saline (PBS, pH 7.4), and stored at $-80°C$ until use. Total cellular RNA was isolated by the guanidine extraction method. Briefly, cells were solubilized in a guanidinium thiocyanate-phenol-2-mercaptoethanol solution at $4°C$ and extracted with chloroform. RNA was precipitated with 50% isopropanol and washed with a 70% ethanol solution. Samples of RNA (30 μg) were denatured and separated on 1% formaldehyde-agarose gels. RNAs were transferred to nylon membrane (Schleicher & Schuell, Keene, N.H.) and then cross-linked to the membrane by UV exposure. Quality and equivalent loading of RNA were determined by ethidium bromide staining of ribosomal RNAs and detection of constitutively expressed actin mRNA on Northern blots. Messenger RNAs for the latent period genes and erythroid-specific genes were detected by hybridization with ^{32}P-labeled cDNA probes. Hsc70 cDNA (provided by Dr. K. Fujimoto of Mitsubishi Life Science Institute, Tokyo), *MER5* cDNA,[21] Spi-1 (PU.1) cDNA (provided by Dr. R. A. Maki of La Jolla Cancer Research Center, La Jolla), Id cDNA (provided by H. Weintraub of Fred Hutchinson Cancer Research Institute, Seattle), DNA polymerase α cDNA (provided by Dr. F. Hanaoka of Physical and Chemical Institute (RIKEN), Wako, Saitama), proliferating cell specific nuclear antigen (PCNA) cDNA (provided by Dr. A. Matsukage of Aichi Cancer Research Institute, Nagoya), β-globin gene, glycophorin cDNA,[22] and rat δ-aminolevulinic acid synthase (ALAS-E) cDNA (provided by Dr. M. Yamamoto of Tohoku University School of Medicine, Sendai), GATA-1 (cloned by the polymerase chain reaction method in our laboratory), erythropoietin receptor (EpoR) cDNA (provided by Dr. K. Todokoro of Tsukuba Life Science Center of RIKEN, Tsukuba) and β-actin cDNA were recovered from agarose gels after separation of the DNA fragments of the respective plasmid DNAs by restriction enzyme digestion and were labeled with ^{32}P-dCTP with a random oligonucleotide primer DNA-labeling kit (Boehringer, Mannheim, FRG). All hybridizations were performed overnight at $42°C$ in hybridization buffer (50% formamide, $5\times$SSC, 0.1% sodium dodecyl sulfate, 80 mM sodium phosphate (pH 6.5) containing 20 μg of salmon sperm DNA per ml, 50 μg of bovine serum albumin per ml, and 0.4 mg of yeast RNA (Sigma Chemical Co., St. Louis, Mo.) per ml. Hybridized blots were washed twice in $2\times$SSC solution containing 1% SDS for 1 h at $65°C$.

RESULTS

Effect of overexpression of c-*myc* on the latent period genes In the MEL cell transformant (clone 38-2) with a rat c-*myc* gene fused to the promoter of the human

metallothionein II gene, expression of the transferred c-*myc* could be manipulated artificially by the addition of Zn ions in a time-dependent and dose-dependent manner.[5-7] DMSO induced the differentiation of clone 38-2 to an extent similar to the parental cells in the absence of Zn ions, but the differentiation was strongly inhibited in the presence of Zn ions. Fig. 1 shows that the overexpression of the transferred c-*myc* gene was induced by 200 μM ZnCl$_2$, as previously reported.[5] Its overexpression reduced the proportion of committed cells to less than one-fifth that in the absence of Zn ions.[7]

Using this transformant, we examined the effect of overexpression of the transferred c-*myc* gene on expression of the latent period genes after induction with DMSO (Fig. 2). As latent period genes, we examined hsc70, *MER5*, Spi-1, and Id genes. Total RNAs were isolated from the transformants collected at the indicated times after exposure to DMSO in the presence or absence of Zn ions, and the level of mRNA for each gene was determined by Northern blot hybridization.
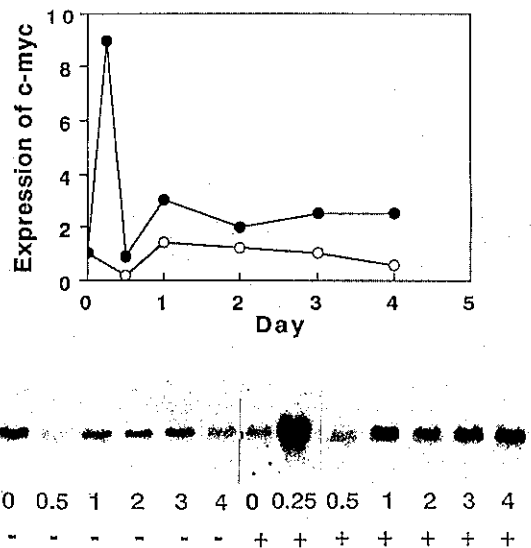

Fig. 1. Overexpression of the transferred c-*myc* gene induced by the addition of Zn ions. MEL cells (c-*myc* transformant clone 38-2) were induced to differentiate with 1.4% DMSO in the presence or absence of Zn ions and sampled on the indicated days. Lower figure; Northern blot analysis of total RNA with c-*myc* gene probe. Quality and equivalent loading of RNA were determined by ethidium bromide staining of ribosomal RNAs and detection of constitutively expressed β-actin mRNA (see Fig. 3, lower lane). Upper figure; Relative expression of c-*myc* gene. Relative expression of c-*myc* gene to β-actin gene in the presence (●) or absence (○) of Zn ions was determined by laser densitometric analysis of the autoradiographs from Northern blot analysis.
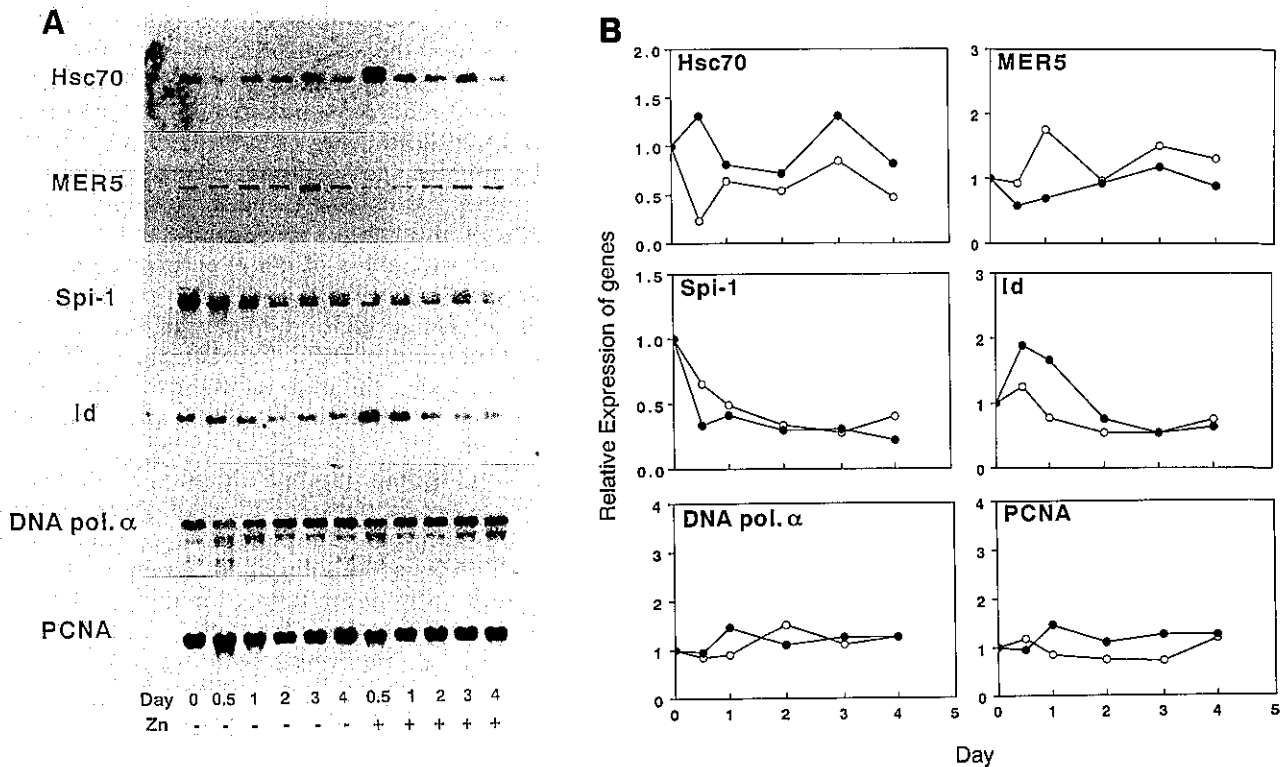
Fig. 2. Effect of the overexpression of c-myc on the latent period genes. MEL cells (c-myc transformant clone 38-2) were induced to differentiate with 1.4% DMSO in the presence or absence of Zn ions and sampled on the indicated days. (A) Northern blot analysis of total RNA with 6 latent period gene cDNA probes. (B) Relative expression of the latent period genes. Relative expression of each latent period gene with respect to β-actin gene in the presence (●) or absence (○) of Zn ions was determined as described in the legend to Fig. 1.

Hensold and Housman[1] reported that a decrease in the rate of synthesis of the constitutively expressed isoform of the 70-kilodalton (kDa) heat shock protein, hsc70, occurs prior to the onset of differentiation of inducer-exposed MEL cells. We measured the levels of hsc70 mRNA after induction of 38-2 cells with DMSO in the presence or absence of Zn ions. In the absence of Zn ions, a rapid decrease in hsc70 mRNA was observed within 0.5 day after induction; it recovered slightly at 1 day, but thereafter remained low as reported earlier.[1] Surprisingly, a new mRNA band with high molecular weight was observed in addition to hsc70 mRNA at 0.5 day, when the mRNA levels were measured in the transformant exposed to DMSO in the presence of Zn ions. The higher-molecular mRNA is believed to be hsp70 mRNA from its size. This dramatic increase was not observed in the parental cell line (B8/3) exposed to DMSO and Zn ions (Fig. 3). This is consistent with the previous report that MEL cells fail to transcriptionally activate and accumulate mRNA for the gene of hsp70, the heat-inducible form of the 70-kDa heat shock protein

in response to stress.[23] A slight increase in hsc70 mRNA, rather than a decrease, was apparent at 0.5 day (Fig. 2 A and B; Fig. 2B shows the levels of the low-molecular-weight mRNA for hsc70). Thus, the overexpression of c-Myc induces the expression of hsc70 and hsp70 genes in MEL cells.

We then examined another latent period gene, MER5,[21] whose pattern of expression is similar to that of c-myc. In 38-2 transformant, a transient increase in MER5 mRNA 1 day after induction with DMSO was observed in the absence of Zn ions, whereas such an increase was not observed in the presence of Zn ions, indicating that the overexpression of c-Myc affects expression of the MER5 gene.

Expression of mRNA for Id, a negative regulator of differentiation,[24] was measured during induction of the transformant exposed to DMSO in the absence of Zn ions. Reduction in Id mRNA after a transient increase was observed, as reported.[24] The overexpression of c-myc gene clearly affected the expression of Id gene during the latent period.

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1   2

Hsp70 3.3kb ►
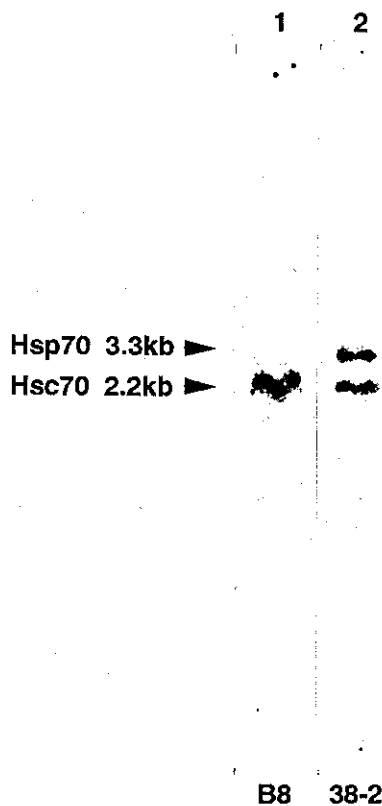
Hsc70 2.2kb ►

B8   38-2

Fig. 3.   Expression of hsp70 and hsc70 genes in the parental (B8/3) and the c-*myc* transformant (38-2). Both cells were induced with 1.4% DMSO in the presence of 200 μM ZnCl$_2$ for 12 h and the total RNAs were subjected to Northern blot analysis. Both 3.8 kb hsp70 and 2.2 kb hsc70 mRNAs were detected in the transformant (38-2), while only 2.2 kb hsc70 mRNA was detected in the parental cells (B8/3).

Spi-1 is an oncogene activated during leukemogenesis induced by Friend virus.[25, 26] Spi-1 is identified as PU.1, which is a transcription factor and one of the Ets family of oncoproteins. The parental MEL cell line, B8/3, was shown to have the activated form of Spi-1. The importance of Spi-1 activation during Friend virus-induced leukemogenesis has been suggested, but it is not yet known how this gene is involved in the transformation and differentiation of erythroid cells. We examined the expression of Spi-1 gene during induction of MEL cells and found that the gene was rapidly down-regulated in these cells after induction with DMSO (Fig. 2). However, the overexpression of c-*myc* gene did not affect expression of the Spi-1 gene.

Effect of overexpression of c-*myc* on DNA polymerase α and PCNA   The strong correlation between proliferation and expression of c-*myc* raised the possibility of the

involvement of c-Myc in DNA replication.[15] We examined whether the overexpression of c-*myc* affects expression of DNA polymerase α[26] and PCNA[27] genes, whose protein products are involved in DNA replication. While DNA replication ceases in the later stage of MEL cell differentiation, no significant change in expression of either gene was observed, and the overexpression of c-*myc* did not affect their expression.

Effect of overexpression of c-*myc* on expression of the erythroid-specific genes   In addition to its involvement in the latent period genes, c-Myc may act as a transcriptional regulator of the differentiation-specific genes. Thus, we examined the effect of the overexpression of c-*myc* on several erythroid-specific genes using 38-2 transformant.

Genes for β-globin, glycophorin A,[22] and ALAS-E, an erythroid-specific type of δ-aminolevulinic acid synthase,[28] are specifically expressed in erythroid cells and were shown to be induced during induction of MEL cell differentiation. Messenger RNAs for these three genes were induced after addition of DMSO in the absence of Zn ions, but the overexpression of c-*myc* strongly inhibited their induction (Fig. 4).

Genes for GATA-1,[29, 30] a hematopoietic cell lineage-specific transcription factor, and EpoR,[31, 32] a membrane receptor for erythropoietin, an erythroid-lineage specific cytokine, are co-expressed in the erythroid progenitors and they may have important functions in the growth and differentiation of erythroid cells.[33] In contrast to the above three erythroid-specific genes, expression of GATA-1 and EpoR genes was observed at constant levels throughout the induction of MEL cells, as shown in Fig. 4. The levels of their expression were not affected by the overexpression of c-*myc*.

DISCUSSION

The functions of c-Myc oncoprotein appear to be linked to proliferation, and the protein may play a major role in cell differentiation.[15] The various motifs found in the Myc oncoproteins suggest that they participate in the regulation of gene expression.[10, 11, 13, 17, 34, 35] It is not known, however, which genes are responsible for c-Myc's transcriptional regulation, though c-Myc/Max heterodimer can bind to a core nucleotide sequence (CAC(G/A)TG).[17] c-Myc was concluded to regulate erythroleukemic cell differentiation, since its overexpression inhibits commitment and differentiation of MEL cells.[5] In the present work, we examined the effect of the overexpression of c-Myc on expression of the latent period genes involved in the commitment, as well as its effect on expression of the erythroid-specific genes using the MEL transformant with the transferred c-*myc* gene under the inducible gene promoter.
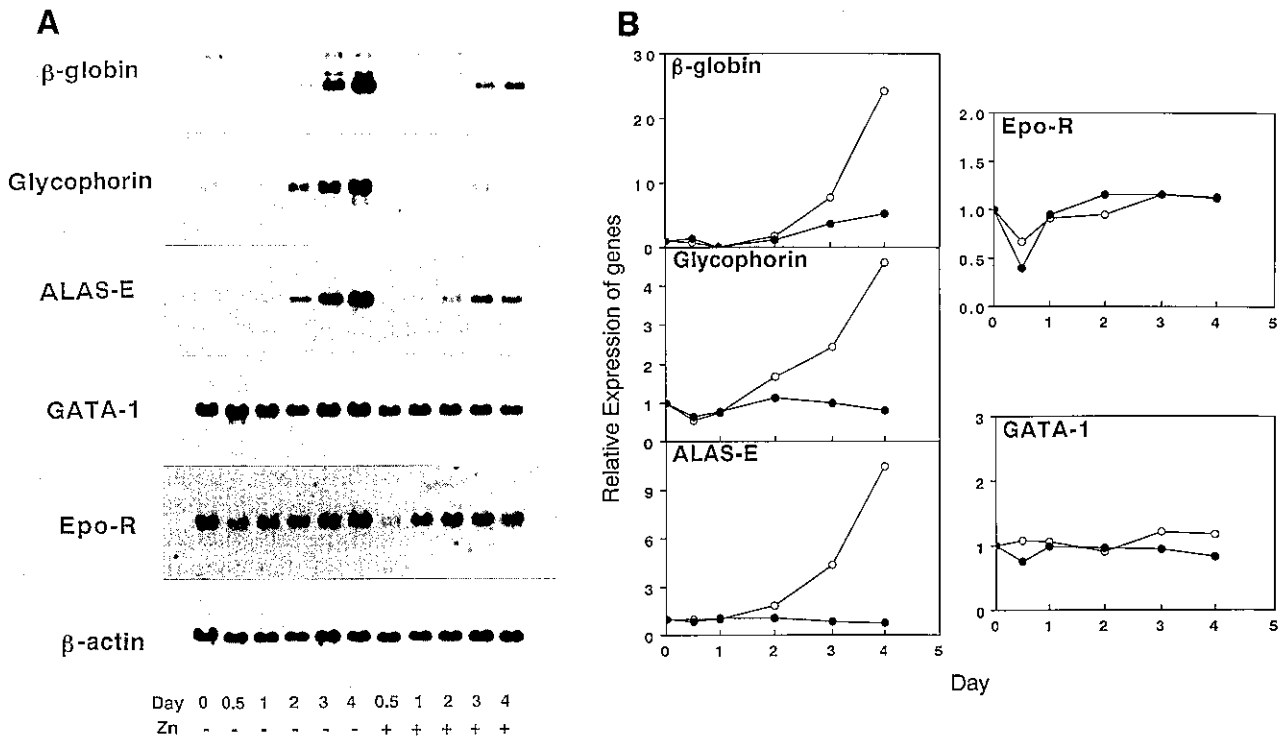
Fig. 4. Effect of the overexpression of c-myc on the erythroid-specific genes. RNA samples were the same as in Fig. 1. (A) Northern blot analysis of total RNA with 5 erythroid-specific genes and β-actin gene cDNA probes. (B) Relative expression of the erythroid-specific genes. Relative expression of the erythroid-specific genes with respect to β-actin gene in the presence (●) or absence (○) of Zn ions was determined as described in Fig. 1.

Among the effects of c-Myc on the latent period genes, its effect on heat shock genes is interesting. Despite the high degree of conservation of heat shock response, MEL cells are known to lack this response. Activation of mammalian heat shock factor by heat requires at least two separate steps: an alteration of binding activity followed by further modification. Hensold et al.[23] showed that DNA binding of heat shock factor (HSF) to the heat shock element of hsp70 gene is insufficient for transcriptional activation in MEL cells. We demonstrated that c-Myc induced expression of hsp70 gene in the latent period in MEL cell differentiation without heat shock (Fig. 2). The induction of hsp70 gene in MEL cells is not due to the presence of Zn ions, since the parental cell line showed no response to the same condition (Fig. 3). Our results favor the possibility that c-Myc can affect transcription of the hsp70 gene, as previously shown by Kaddurah-Daouk et al.,[34] rather than that c-Myc induces a modification of the HSF. However, the observed effect of c-Myc on heat shock genes may be due to combined action with other factor(s).

The MER5 gene was cloned as a latent period gene.[21] Overexpression of transferred antisense MER5 cDNA inhibited the differentiation of MEL cells without inhibiting commitment. Thus, the MER5 gene is expected to have a positive function in the erythroid differentiation.[36] The Id gene was cloned from MEL cell cDNA library as a gene encoding an HLH protein that represses the activity of several b-HLH proteins involved in cell-type specific transcription and cell lineage commitment.[24] Id is expected to be a negative regulator of differentiation of hematopoietic cells. Although the functional mechanisms of the MER5 gene and Id gene are different, c-Myc acts as a positive regulator for Id gene and a negative regulator for the MER5 gene, and leads to a blocking of the differentiation of MEL cells. Though the effect of overexpression of c-myc was clearly observed in expression of hsp70, hsc70, Id and the MER5 genes, no effect was apparent on Spi-1 oncogene, which encodes a sequence-specific DNA binding factor, PU.1.

Several experimental results are consistent with the notion that c-Myc might be involved in replication, but leave it unclear where c-Myc acts.[15] DNA polymerase α and PCNA were induced after a transient increase in immediate early response genes, such as c-fos and c-myc, during serum-induced cell growth of BALB 3T3 cells

following serum deprivation.[35] We speculated that these mRNAs might decrease after the cells differentiate because DNA synrthesis ceases; however, both mRNAs persisted even after DNA synthesis had stopped. Although overexpression of c-*myc* blocked differentiation of MEL cells and their DNA synthesis, the levels of the two mRNAs did not increase. Thus, c-Myc may not regulate expression of these genes. A recent report that c-Myc interacts with retinoblastoma gene product (Rb)[37] suggests the possibility of an indirect effect of c-Myc on replication.

c-Myc may regulate the differentiation-specific genes[38-40] as a transcription factor. We have demonstrated that a limited domain of c-Myc shows the ability to inhibit the terminal differentiation without affecting commitment of MEL cells.[8] It is likely that c-Myc affects the erythroid-specific genes directly rather than through the indirect effect of inhibition of the commitment process. The five erythroid-specific genes examined here contain binding motifs for GATA-1, a hematopoietic cell lineage-specific transcription factor,[29, 30] within their *cis*-acting elements. GATA-1 and EpoR genes are expressed in early erythroid progenitor cells, whereas glycophorin, globin and ALAS-E genes[28, 41] are expressed after the proerythroblast stage. Differential expression of these five erythroid-specific genes were observed in the MEL cell differentiation process: expression of glycophorin, ALAS-A and β-globin genes was induced only after induction with DMSO, while GATA-1 and EpoR genes were expressed before induction (Fig. 4). Erythroid-specific expression of these genes may be regulated by combinatorial action, as shown in the human β-globin gene.[42-45] We found that c-Myc interferes with the expression of the three inducible genes but not with that of GATA-1 and EpoR genes. If c-Myc interferes with common factors among the five genes, expression of

GATA-1 and EpoR genes should be repressed. However, a more intriguing idea arising from our results is that c-Myc interferes only with the factor(s) involved in the inducible genes but not in GATA-1 and EpoR genes. Alternatively c-Myc may interfere with the activation process of erythroid-specific genes rather than simply interfering with the function of *trans*-acting factors. We have reported that c-Myc interferes with the appearance of the specific DNase I hypersensitive site of β-globin gene chromatin.[7] The active conformation of chromatin might be formed by the competitive binding of specific *trans*-acting factors and nucleosomes along the *cis*-acting elements of specific genes through combinatorial interaction of these factors. The appearance of DNase I hypersensitive sites of β-globin gene chromatin is assumed to be tightly coupled with the commitment of MEL cells. c-Myc may regulate the activation of chromatin conformation of the erythroid-specific genes by interacting with the transcription factor(s), but once the active conformation is formed, c-Myc has no effect on its active structure. This is consistent with the role of c-Myc in the commitment of MEL cells.

The present work suggests that c-Myc may selectively regulate a set of genes and that this selective regulation may be caused by multiple binding capacity of c-Myc with other *trans*-acting factors. Although direct interaction has been observed only with Max and Rb,[33] there might be other factors interacting with c-Myc which function in the selective gene regulation.

ACKNOWLEDGMENTS

REFERENCES

1) Hensold, J. and Housman, D. Decreased expression of the stress protein HSP70 is an early event in murine erythroleukemia cell differentiation. *Mol. Cell. Biol.*, **8**, 2219–2223 (1988).

2) Lachman, H. M. and Skoultchi, A. I. Expression of c-myc changes during differentiation of mouse erythroleukemia cells. *Nature*, **310**, 592–594 (1984).

3) Dmitrovsky, E. Kuehl, W. M., Hollis, G. F., Kirsch, I. R., Bendoer, T. P. and Segal, S. Expression of a transfected human c-myc oncogene inhibits differentiation of a mouse erythroleukemia cell line. *Nature*, **322**, 748–750 (1986).

4) Coppola, J. A. and Cole, M. D. Constitutive c-myc oncogene expression blocks mouse erythroleukemia cell differentiation but not commitment. *Nature*, **320**, 761–763

(1986).

5) Kume, T. U., Takada, S. and Obinata, M. Probability that the commitment of murine erythroleukemia cell differentiation is determined by the c-myc level. *J. Mol. Biol.*, **202**, 779–786 (1988).

6) Yamamoto, T., Masuko, K., Kume, U.-T., Takada, S. and Obinata, M. A balance between self-renewal and commitment in the murine erythroleukemia cells with the transferred c-myc gene: an *in vitro* stochastic model. *Cell Differ. Dev.*, **28**, 129–134 (1989).

7) Takada, S. and Obinata, M. Overexpression of c-Myc inhibits the appearance of a specific DNase I hypersensitive site in the β-globin chromatin in murine erythroleukemia cells. *Jpn. J. Cancer Res.*, **82**, 376–379

(1991).

8) Ohmori, Y., Tanabe, J., Takada, S., Lee, W. M. F. and Obinata, M. Functional domains of c-Myc involved in the commitment and differentiation of murine erythroleukemia cells. *Oncogene,* **8**, 379–386 (1993).

9) Stones, J. T., deLange, G., Ramsay, E., Jakobovits, E., Bishop, J. M., Varmus, H. E. and Lee, W. Definition of regions in human c-myc that are involved in transformation and nuclear localization. *Mol. Cell. Biol.,* **7**, 1697–1709 (1987).

10) Dang, C. V. and Lee, W. M. F. Identification of the human c-Myc protein nuclear translocation signal. *Mol. Cell. Biol.,* **8**, 4048–4054 (1988).

11) Dang, C. V., McGuire, M., Buckmire, M. and Lee, W. M. F. Involvement of the 'leucine zipper' region in the oligomerization and transforming activity of human c-myc protein. *Nature,* **337**, 664–666 (1989).

12) Blackwell, T. K., Kretzner, L., Blackwood, E. M., Eisenman, R. N. and Weintraub, H. Sequence-specific DNA binding by the c-Myc protein. *Science,* **250**, 1149–1151 (1989).

13) Blackwood, E. and Eisenman, R. N. Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA binding complex with Myc. *Science,* **251**, 1211–1217 (1991).

14) Beckmann, H., Su, L.-K. and Kadesch, T. TFE3: A helix-loop-helix protein that activates transcription through the immunoglobin enhancer μE3 motif. *Gene Dev.,* **4**, 167–179 (1990).

15) Luscher, B. and Eisenman, R. N. New light on Myc and Myb. Part 1. Myc. *Genes Dev.,* **4**, 2025–2035 (1990).

16) Blackwood, E. M., Luscher, B. and Eisenman, R. N. Myc and Max associate *in vivo. Genes Dev.,* **6**, 71–80 (1992).

17) Kato, G. J., Barrett, J., Villa-Garcia, M. and Dang, C. V. An amino-terminal c-Myc domain required for neoplastic transformation activates transcription. *Mol. Cell. Biol.,* **10**, 5914–5920 (1990).

18) Prendergast, G. C., Laew, D. and Ziff, E. B. Association of Myn, the murine homolog and Ras cotransformation. *Cell,* **65**, 395–407 (1991).

19) Prendergast, G. C. and Ziff, E. B. Methylation-sensitive sequence specific DNA binding by the c-Myc basic region. *Science,* **251**, 186–189 (1991).

20) Wenzel, A., Cziepluch, C., Hamann, U., Schurmann, J. and Schwab, M. The N-Myc oncoprotein is associated *in vivo* with the phosphoprotein Max(p20/22) in human neuroblastoma cells. *EMBO J.,* **10**, 3703–3712 (1991).

21) Yamamoto, T. Matsui, Y. Natori, S. and Obinata, M. Cloning of a housekeeping gene (MER5) preferentially expressed in murine erythroleukemia cells. *Gene,* **80**, 337–343 (1989).

22) Matsui, Y. Natori, Y. and Obinata, M. Isolation of the cDNA clone for mouse glycophorin, erythroid-specific membrane protein. *Gene,* **77**, 325–332 (1989).

23) Hensold, J. O., Hunt, C. R., Calderwood, S. K., Housman, D. E. and Kingston, R. T. DNA binding of heat shock

factor to the heat shock element is insufficient for transcriptional activation in murine erythroleukemia cells. *Mol. Cell. Biol.,* **10**, 1600–1608 (1990).

24) Benezra, R. Davis, R. L., Lockshon, D., Turner, D. L. and Weintraub, H. The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. *Cell,* **61**, 49–59 (1990).

25) Moreau-Gachelin, F., Tavitian, A. and Tambourin, P. Spi-1 is a putative oncogene in virally induced murine erythroleukemias. *Nature,* **331**, 277–280 (1988).

26) Ben-David, Y., Giddens, E. B., Letwin, K. and Bernstein, A. Erythroleukemia induction by Friend murine leukemia virus: insertional activation of a new member of the ets gene family, Fli-1, closely linked to c-ets-1. *Genes Dev.,* **5**, 908–918 (1991).

27) Jaskulski, D., Gatti, C., Travali, S., Cababretta, B. and Beserga, R. Regulation of the proliferating cell nuclear antigen cyclin and thymidine kinase mRNA levels by growth factors. *J. Biol. Chem.,* **263**, 10175–10179 (1988).

28) Fujita, H., Yamamoto, M., Yamagami, T., Hayashi, N. and Sassa, S. Erythroleukemia differentiation. Distinctive responses of the erythroid-specific and the nonspecific δ-aminolevulinate synthase gene. *J. Biol. Chem.,* **266**, 17494–17502 (1991).

29) Tsai, S. F., Martin, D. I. K., Zon, L. I., D'Andrea, A. D., Wong, G. G. and Orkin, S. H. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. *Nature,* **339**, 446–451 (1989).

30) Romeo, P.-H., Prandini, M. H., Joulin, V., Mignotte, V., Prenant, M., Vainchenker, W., Marguerie, G. and Uzan, G. Megakaryocytic and erythrocytic lineages share specific transcription factors. *Nature,* **344**, 447–449 (1990).

31) D'Andrea, A. D., Lodish, H. F. and Wong, G. G. Expression cloning of the murine erythropoietin receptor. *Cell,* **57**, 277–285 (1989).

32) Youssoufian, H., Zon, L., Orkin, S. H., D'Andrea, A. D. and Lodish, H. F. Structure and transcription of the mouse erythropoietin receptor gene. *Mol. Cell. Biol.,* **10**, 3675–3682 (1990).

33) Chiba, T., Ikawa, Y. and Todokoro, K. GATA-1 transactivates erythropoietin receptor gene, and erythropoietin receptor-mediated signals enhance GATA-1 gene expression. *Nucleic Acids Res.,* **19**, 3843–3848 (1991).

34) Kaddurah-Daouk,R., Greene, J. M., Baldwin, A. S. and Kingston, R. E. Activation and repression of mammalian gene expression by the c-myc protein. *Gene Dev.,* **1**, 347–357 (1987).

35) Greenberg, M. E. and Ziff, E. B. Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene. *Nature,* **311**, 433–438 (1984).

36) Nemoto, Y., Yamamoto, T., Takada, S., Matsui, Y. and Obinata, M. Antisense RNA of the latent period gene (MER5) inhibits the differentiation of murine erythroleukemia cells. *Gene,* **91**, 261–265 (1990).

37) Rustgi, A. K., Dyson, N. and Bernards, R. Amino-

terminal domains of c-myc and N-myc proteins mediate binding to the retinoblastoma gene product. *Nature,* **352,** 541–544 (1991).

38) Bernards, R., Dessain, S. K. and Weinberg, R. A. N-myc amplification causes down-modulation of MHC class I antigen expression in neuroblastoma. *Cell,* **47,** 667–674 (1986).

39) Versteg, R., Noordermeer, I. A., Kruse-Wolters, M., Ruiter, D. J. and Shier, P. I. c-myc down-regulates class I HLA expression in human melanomas. *EMBO J.,* **7,** 1023–1029 (1988).

40) Akeson, R. and Bernards, R. N-myc down regulates neural cell adhesion molecule expression in rat neuroblastoma. *Mol. Cell. Biol.,* **10,** 2012–2016 (1988).

41) Cox, T. C., Baldwin, M. J., Martin, A. and May, K. Human erythroid 5-aminolevulinate synthase: promoter analysis and identification of an iron-responsive element in the mRNA. *EMBO J.,* **10,** 1891–1902 (1991).

42) Grosveld, F., van Assendelft, G. B., Greaves, D. R. and Kollias, B. Position-independent, high-level expression of the human beta-globin gene in transgenic mice. *Cell,* **51,** 975–985 (1987).

43) Orkin, S. H. Globin gene regulation and switching: circa 1990. *Cell,* **63,** 665–672 (1990).

44) Ney, P. A., Sorrentino, B. P., McDonagh, K. T. and Hienhuis, A. W. Tandem AP-1-binding sites within the human β-globin dominant control region function as an inducible enhancer in erythroid cells. *Genes Dev.,* **4,** 993–1006 (1990).

45) Antoniou, M. and Grosveld, F. β-Globin dominant control region interacts differently with distal and proximal promoter elements. *Genes Dev.,* **4,** 1007–1013 (1990).