bates the uncertainties in the total luminosity. Several investigators have suggested that since N_ν is expected to exceed 6, the observations are consistent only with very hard equations of state or with the formation of a black hole (*9*). These researchers have not included the uncertainties in the energy arising from small number statistics. Equation 3 shows that even if we assumed $N_\nu = 8$, the total energy emitted would still be consistent with the binding energy of a $1.4M_\odot$ neutron star (where $M_\odot$ is the solar mass) and a wide range of equations of state (*14*).

The success of this simplified model implies that it will be difficult to use the observed neutrino flux to confirm more detailed models. The supernova has confirmed the general picture of core collapse; however, it has not provided sufficient data to discriminate between equations of state or to validate specific detailed models. There is no need to evoke new particle physics or complicated astrophysical scenarios to explain the observed data. When a supernova is observed in our own galaxy, the detectors should record many hundreds of events and neutrino spectroscopy may then reveal surprises about stellar collapses and weak interaction physics.

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Differential Expression of c-*myb* mRNA in Murine B Lymphomas by a Block to Transcription Elongation

TIMOTHY P. BENDER, CRAIG B. THOMPSON, W. MICHAEL KUEHL

Expression of c-*myb* proto-oncogene messenger RNA (mRNA) and protein has been detected principally in tumors and in normal tissue of hematopoietic origin. In each hematopoietic lineage examined, expression of the c-*myb* gene is markedly downregulated during hematopoietic maturation. However, the mechanism by which differential expression of the c-*myb* gene is regulated is not known. In murine B-lymphoid tumor cell lines, the amount of steady-state c-*myb* mRNA is 10 to more than 100 times greater in pre-B cell lymphomas than in B cell lymphomas and plasmacytomas. The downregulation of c-*myb* mRNA correlates with events at the pre-B cell–B cell junction. Differential expression of c-*myb* mRNA levels detected between a pre-B cell lymphoma and a mature B cell lymphoma is now shown to be mediated by a block to transcription elongation in the first intron of the c-*myb* locus. In addition, this developmentally regulated difference in transcriptional activity is correlated with alterations in higher order chromatin structure as reflected by changes in the patterns of hypersensitivity to deoxyribonuclease I at the 5′ end of the c-*myb* transcription unit. Regulation of transcription elongation may provide a more sensitive mechanism for rapidly increasing and decreasing mRNA levels in response to external stimuli than regulation of the initiation of transcription.

THE c-*myb* PROTO-ONCOGENE, which encodes a nuclear DNA binding protein (*1*), is the normal cellular homolog of the transforming gene of avian myeloblastosis virus (*2*). Although its normal function is unknown, the c-*myb* gene is expressed predominantly at immature stages of development in the hematopoietic system (*3*). Levels of c-*myb* messenger RNA (mRNA) are markedly decreased during chemically induced differentiation of several hematopoietic tumor cell lines (*4*). Steady-state levels of c-*myb* mRNA in murine pre-B cell lymphomas are 10 to more than 100 times greater than those in B cell lymphomas and plasmacytomas, and the difference correlates with events at the pre-B cell–B cell junction (*5*). The steady-state level of c-*myb* mRNA in the pre-B cell lymphoma line 70Z/3B is 10 to 20 times greater than that expressed by the mature B cell lymphoma A20.2J (Fig. 1A) (*6*).

To examine the possibility that differences in steady-state levels of c-*myb* mRNA expression are due to differences in message stability, we measured the half-life of c-*myb* mRNA in these two cell lines after inhibition of RNA synthesis by actinomycin D. We found that the stability of c-*myb* mRNA does not differ significantly between these two cell lines (Fig. 1B). From this experiment, we estimate the half-life of c-*myb* mRNA to be approximately 3 hours in each cell line. For comparison, we determined the half-life of histone protein 2B mRNA to be approximately 30 minutes in each cell line, in good agreement with previously reported estimates (*7*). Thus, the 10- to 20-fold difference in c-*myb* mRNA expression between the 70Z/3B pre-B cell lymphoma and the A20.2J B cell lymphoma is not due to differences in c-*myb* mRNA stability.

To investigate the possibility that the difference in c-*myb* mRNA expression in these two lines is transcriptionally regulated, we used a nuclear run-on assay. When a plasmid containing a 2.4-kb murine c-*myb*

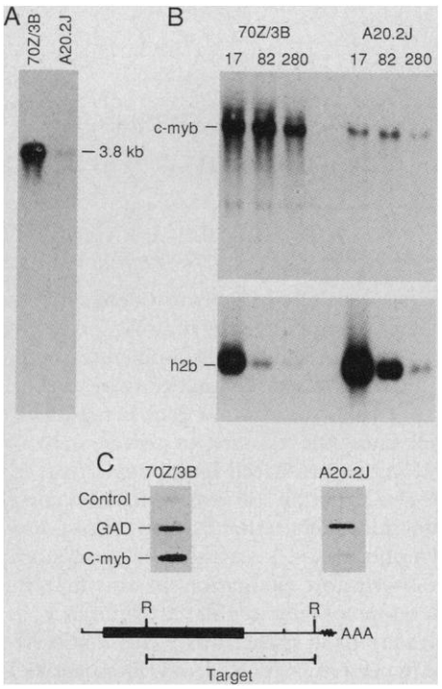
T. P. Bender and W. M. Kuehl, National Cancer Institute/Navy Medical Oncology Branch, Naval Hospital, Bethesda, MD 20814.
C. B. Thompson, Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109.

complementary DNA (cDNA) was used as a target, transcription of the c-*myb* locus was readily detected in 70Z/3B (Fig. 1C). However, c-*myb* transcription was not detected significantly above background in A20.2J. By contrast, transcription of the glyceraldehyde-3-phosphate dehydrogenase gene was equivalent in each cell line. The 10- to 20-fold difference in steady-state c-*myb* mRNA levels between 70Z/3B and A20.2J appears to be accounted for by differences in transcriptional activity at the c-*myb* locus.

For other genes that exhibit differential levels of transcription, changes in expression have been associated with alterations in higher order chromatin structure in the promoter region (*8*). Thus, the differences in c-*myb* transcription found between the pre-B cell lymphoma line 70Z/3B and the A20.2J B cell lymphoma line may be regulated by factors that also alter the higher order chromatin structure in the 5′ end of the gene. To examine this possibility we compared deoxyribonuclease I (DNase I) sensitivity of the genomic region that contains the first two exons of the c-*myb* locus (*6, 9*). A 7.6-kb genomic Eco RI fragment contains 1.1 kb of 5′ flanking/untranslated sequence plus the first and second exons of the c-*myb* locus (Fig. 2). Limited digestion of 70Z/3B and A20.2J nuclei with DNase I showed no significant difference in the overall sensitivity of this region between the two cell lines. However, of the six DNase I–hypersensitive sites identified in Fig. 2, these cell lines differ quantitatively at sites I, IV, and VI. Although differences at sites I and VI appear to be qualitative in Fig. 2, site I is detected in A20.2J and site VI is detected in 70Z/3B upon prolonged exposure. We confirmed the positions and the relative sensitivities of each hypersensitive site by the indirect labeling technique for which we used a 0.5-kb Eco RI–Hind III fragment derived from the 5′ end of this region and by using different restriction enzymes. Thus, the large quantitative differences in steady-state c-*myb* mRNA detected at different stages of B cell development are correlated with alterations in higher order chromatin structure in the region encompassing the first and second exons of the c-*myb* locus.

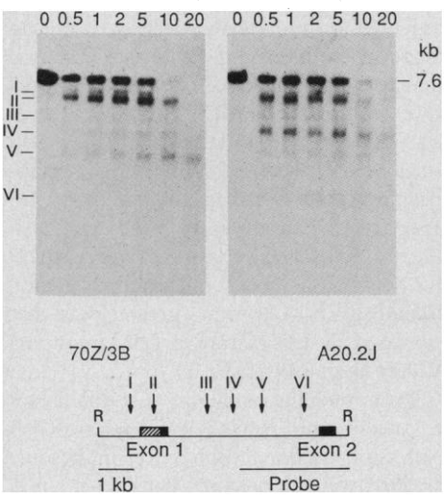
Using the nuclear run-on transcription assay, we examined transcription across the 7.6-kb Eco RI genomic region that contains the first two exons of the murine c-*myb* locus. The 7.6-kb Eco RI fragment was divided into four regions (fragments A to D) (Fig. 3A). Transcription was detected in the 70Z/3B pre-B cell lymphoma line in the regions containing the first exon, first intron, and the second exon (fragments B to D) but not in fragment A, which is located on the 5′ side of the first exon. Hybridization to fragment A could not be detected even after prolonged exposure of the film. In addition, when the 7.6-kb Eco RI fragment was examined by Bam HI digestion, which generates a 1.1-kb fragment on the 5′ side of the first exon, transcription was not detected in this region. Thus, if transcription occurs on the 5′ side of the first exon, it is below the limits of detection in the 70Z/3B cell line. When nuclear run-on probes were generated from the A20.2J B cell lymphoma line, transcription detected with fragment B (which contains exon 1) was equivalent to that seen in 70Z/3B. However, in the the A20.2J cell line, transcription detected with fragment C was one-fifth to one-third of

Fig. 1. (**A**) Expression of steady-state c-*myb* mRNA levels in the 70Z/3B and A20.2J tumor cell lines. Total cellular RNA was prepared from exponentially growing cells by the guanidinium isothiocyanate method (*16*), fractionated on a 1.0% agarose gel containing 0.22*M* formaldehyde, and transferred to a nitrocellulose filter (*17*). Each lane contains 10 μg of total cellular RNA. The filter was hybridized to a [^{32}P]αdCTP nick-translated (*18*) 2.4-kb Eco RI murine c-*myb* cDNA fragment (*9*). The filter was washed twice in 0.1× SSC and 0.1% SDS at 56°C for 30 minutes, dried, and exposed to Kodak XAR-5 film with an enhancer screen for 12 hours. (**B**) Half-life of c-*myb* mRNA after treatment with actinomycin D. Exponentially growing 70Z/3B and A20.2J cells were treated with actinomycin D at a final concentration of 8 μg/ml. Total cellular RNA was prepared 17, 82, and 280 minutes after addition of actinomycin D. RNA blots, for which 15 μg of 70Z/3B and 45 μg of A20.2J total RNA were used, were prepared and washed as described above. The c-*myb* probe was as described above. The h2b probe was a 1.1-kb Xho I–Sac II fragment containing the avian h2b gene (*19*). In two independent experiments the half-lives for c-*myb* mRNA were 165 and 190 minutes for 70Z/3B, and 155 and 190 minutes for A20.2J. (**C**) The transcription rate of c-*myb* and *gpd* in the 70Z/3B and A20.2J cell lines. Nuclei were prepared from exponentially growing cells and frozen at −80°C as previously described (*20*). Nuclei from each cell line were thawed simultaneously and assayed by nuclear run-on transcription as described by Groudine *et al.* (*20*) and modified elsewhere (*21*). Equal numbers of counts per minute of [^{32}P]αUTP-labeled RNA probes prepared from each cell line were hybridized to nitrocellulose filters containing 5 μg of each plasmid. Slot-blotted filters were prepared, hybridized for 36 hours at 65°C, and washed as



previously described (*21*). The plasmids used are as follows: pBR322 is a plasmid control; p10.10.1 is pBR322 containing a 2.4-kb Eco RI murine c-*myb* cDNA fragment (*9*); and pGAD-28 is pBR322 containing a 1.26-kb cDNA insert with the avian glyceraldehyde-3-phosphate dehydrogenase coding region (*22*). Nitrocellulose filters were exposed for 4 days to Kodak XAR-5 film with an enhancer screen at −80°C.

Fig. 2. DNase I–hypersensitive sites within the 5′ end of the c-*myb* gene. Nuclei were isolated from exponentially growing 70Z/3B and A20.2J cell lines as described in the legend to Fig. 1. The nuclei were resuspended at a concentration of approximately 10^8 per milliliter, and aliquots were exposed to increasing concentrations of DNase I (0.1 to 20 μg/ml) for 15 minutes at 37°C. The digestions were stopped by adding an equal volume of a solution containing 1% SDS, 600 m*M* NaCl, 20 m*M* tris (*p*H 7.4), and 5 m*M* EDTA. DNA was then isolated as described previously (*23*), digested with Eco RI, size-fractionated on 1.0% agarose gels, and transferred to a nitrocellulose filter (*24*). The filter was hybridized with a 3.3-kb Sma I–Eco RI fragment labeled with ^{32}P by nick-translation (*18*). The filter was washed twice in 0.1× SSC and 0.1% SDS at 56°C for 30 minutes, dried, and exposed to Kodak XAR-5 film with an enhancer screen for 24 hours. The resultant audioradiograms are shown, and the positions of the observed hypersensitive sites are indicated by arrows.



that relative to 70Z/3B, and no transcription was detected with fragment D (which contains the second exon). After prolonged exposure, hybridization of nuclear run-on probes (derived from A20.2J nuclei) to fragment D could not be detected above background levels of hybridization to pBR322. Although a small amount of steady-state c-*myb* mRNA is detected by RNA blot analysis in A20.2J (Fig. 1A), low transcription levels of a moderately stable mRNA are difficult to detect by the nuclear run-on assay and would account for the lack of hybridization to fragment D in this case (*10*). As shown in Fig. 3A, all of the transcription detected with the 7.6-kb Eco RI fragment was sensitive to α-amanitin at levels shown to inhibit transcription by RNA polymerase II (*11*). Under these same conditions, transcription by RNA polymerase I was not affected as measured by hybridization of nuclear run-on probes to a target specific for the 28*S* ribosomal RNA (Fig. 3A). In addition, hybridization did not detect reiterated sequences in the 7.6-kb Eco RI fragment, since it hybridized only to a single band when used as a probe on genomic DNA and RNA blot analysis. Southern blot analysis has shown that the c-*myb* locus is not amplified in either the 70Z/3B or the A20.2J cell lines. Thus, the difference in the amount of steady-state c-*myb* mRNA between the 70Z/3B pre-B cell lymphoma line and the A20.2J B cell lymphoma line appears to be the result of a block to transcription elongation rather than a difference in transcription initiation.

Hybridization of 70Z/3B nuclear run-on probes to fragment B was consistently two to three times higher, relative to hybridization to fragment D, than would be predicted solely on the basis of target size. One possible explanation for this observation would be that antisense transcription takes place in this region as has been recently reported in the c-*myb* gene (*12, 13*). To examine this possibility, we used single-stranded M13 clones that contain a 1.05-kb insert covering the first exon region (Fig. 3B) (*9*). As shown in Fig. 3B, hybridization to both sense and antisense strands was detected with nuclear run-on probes from both the 70Z/3B and A20.2J cell lines. Since transcription is approximately equivalent on both strands, antisense transcription appears to account for the discrepancy in hybridization to fragment B relative to fragment D. We do not detect antisense transcription in the region containing the second exon when we use single-stranded M13 clones. Thus, all of the hybridization to fragment D in Fig. 3 represents sense transcription. In addition, when differences in target size are considered, the level of sense transcription detected by hybridization to the 1.05-kb Bam HI target is approximately equivalent to that detected by hybridization to the 2.4-kb cDNA target. Although these findings do not rule out the possibility that some block to transcription elongation occurs in 70Z/3B, it suggests that most of the sense transcription that begins in exon 1 continues through to the second exon in this cell line.

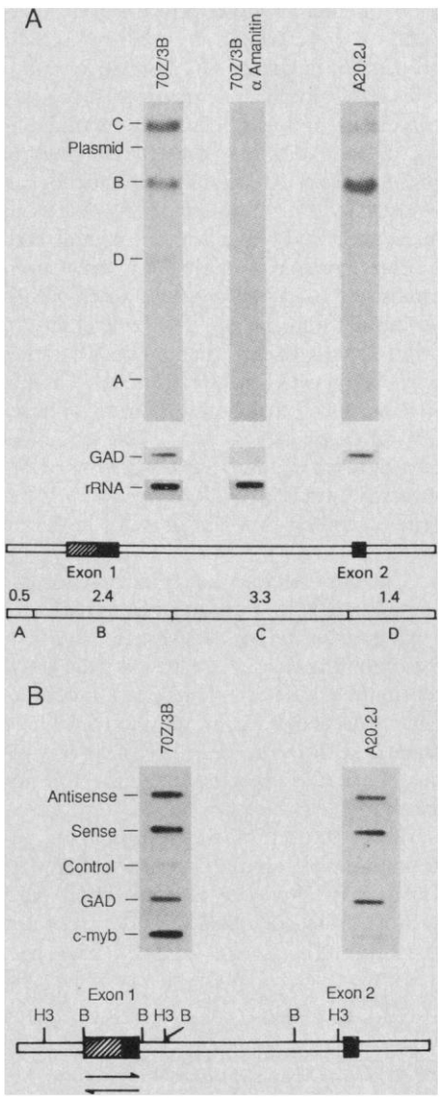


Fig. 3. (**A**) Transcription of the c-*myb* locus in the exon 1 and exon 2 genomic regions assayed by nuclear run-on transcription. Nuclei from exponentially growing 70Z/3B and A20.2J cell lines as well as nuclear run-on probes were prepared as described in the legend to Fig. 1. The plasmid p*myb*13 contains a 7.6-kb Eco RI fragment of murine genomic DNA which encompasses the c-*myb* exon 1 and exon 2 regions (*6, 9*). The restriction map and the locations of exon 1 and exon 2 are as shown. The hatched region in exon 1 represents the region of heterogeneity defined by S1 nuclease protection at the 5′ end of c-*myb* mRNA (*9*). Ten micrograms of p*myb*13 were digested with Eco RI and Hind III, fractionated on a 1.0% agarose gel, and transferred to nitrocellulose filters (*24*). Control filters are slot-blotted with 5 μg each of pGAD-28 and pB, which contains a portion of the human 18*S* ribosomal RNA gene (*25*). The filters were hybridized to nuclear run-on probes and washed as described in the legend to Fig. 1. After being washed, filters were dried and exposed to Kodak XAR-5 film with an enhancer screen at −80°C. (**B**) Detection of both sense and antisense transcription in exon 1 of the murine c-*myb* locus. Nuclei were prepared and run-on transcription assays were performed as described in the legend to Fig. 1. [^{32}P]αUTP-labeled RNA from 70Z/3B or A20.2J was hybridized to filters containing 5 μg of each of the following plasmids or M13 clones: sense and antisense transcription are detected by M13mp10 clones with the 1.05-kb Bam HI exon 1 region (*9*) in appropriate orientations; M13mp10 single-stranded DNA control; and p10.10.1 (c-*myb*) and pGAD-28 as described in Fig. 1. Filters were dried and exposed to Kodak XAR-5 film with an enhancer screen for 4 days. B, Bam HI; H3, Hind III.

The region in which transcription elongation is

Our data indicate that the transcriptional downregulation of c-*myb* mRNA in the A20.2J B cell lymphoma line results primarily from a block to transcription elongation rather than to differences in the intrinsic rate at which transcription is initiated. Although transcription elongation is blocked at this point, these experiments do not distinguish whether this is the result of premature chain termination or a pausing of RNA polymerase prior to further chain elongation. Regardless, the functional block to transcription elongation within the region of intron 1 is a general property of murine B lymphoid tumor cell lines beyond the pre-B cell stages of development. There is little or no block to transcription elongation in a second pre-B cell lymphoma, 1881.B4, but such a block does account for the downregulation of c-*myb* mRNA in the immature B cell lymphoma cell line WEHI-231 and the plasmacytoma cell line MPC-11. The rate of transcription initiation in these cell lines does not differ significantly from the rate reported here for the 70Z/3B and A20.2J cell lines.

Differential expression of c-*myc* mRNA is regulated partially by a block to transcription elongation in the first intron of the c-*myc* gene (*12, 13, 14*). As both the c-*myc* and c-*myb* genes are associated with cell growth and proliferation, regulation of transcription elongation may provide a more sensitive mechanism for the rapid increase or decrease of mRNA levels in response to external stimuli than regulation of the initiation of transcription. Thus, it is of interest that expression of both c-*myc* and c-*myb* mRNA increases markedly during progression from the G_0 to the G_1 stage of the cell cycle (*15*). By keeping these genes in a transcriptionally active state, simple removal or alteration of the block would provide a rapid means to increase steady-state mRNA levels. The region in which transcription elongation is

blocked maps to an approximately 3.3-kb Hind III fragment (Fig. 3A). It is interesting that DNase I hypersensitive site IV maps to this same region of the first intron. Site IV is the location of the major quantitative difference in DNase hypersensitivity between the 70Z/3B and the A20.2J cell lines, and may be functionally associated with the block to transcription elongation. Thus, it will be of interest to determine whether the DNase I hypersensitivity in this region reflects binding of a trans-acting factor that can mediate a block to transcription elongation.

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Expression of an Exogenous Growth Hormone Gene by Transplantable Human Epidermal Cells

JEFFREY R. MORGAN, YANN BARRANDON, HOWARD GREEN, RICHARD C. MULLIGAN

Retrovirus-mediated gene transfer was used to introduce a recombinant human growth hormone gene into cultured human keratinocytes. The transduced keratinocytes secreted biologically active growth hormone into the culture medium. When grafted as an epithelial sheet onto athymic mice, these cultured keratinocytes reconstituted an epidermis that was similar in appearance to that resulting from normal cells, but from which human growth hormone could be extracted. Transduced epidermal cells may prove to be a general vehicle for the delivery of gene products by means of grafting.

THE EPIDERMIS IS A STRATIFIED EPITHELIUM whose principal cell type, the keratinocyte, can be serially propagated in culture (*1*). Human keratinocytes grow rapidly under appropriate culture conditions, and it is possible to expand a 1-cm^2 biopsy to 1 m^2 of epithelium within about 3 weeks. The cultured epithelium can be detached from the surface of a dish as an intact sheet and grafted onto a suitable bed. Epithelia prepared in this way have been extensively used to regenerate an epidermis on burned humans (*2*). The cultivability of keratinocytes makes them suitable target cells for genetic manipulation. As a first step, we have used highly transmissible retroviral vectors to transfer DNA sequences into cultured keratinocytes. We now report successful transfer and expression of the gene encoding human growth hormone (hGH).

Gene transfer into keratinocytes was accomplished with transmissible retroviruses generated from the ψAM cell line (*3*). Recombinant viruses produced by the ψAM cell are free of detectable replication-competent virus and have an amphotropic host range and therefore can infect cells of various mammalian species, including the human.

The structures of the recombinant genomes that we used are shown in Fig. 1A. The vectors ZipneoSV(X) and DOL have been described (*4*). Cell lines derived from ψAM that produce ZipneoSV(X) virus (termed ψAM2275) and DOL-hGH virus (ψAM DOL-hGH) both yielded titers of 10^5 G418-resistant colonies per milliliter when assayed on NIH 3T3 cells. To infect human keratinocytes, we cocultivated them with lethally irradiated producer ψAM cells, since direct contact of virus-producing cells with recipient cells is an efficient means of transmitting the virus (*5*). The producer ψAM cells also provided fibroblast support necessary for optimal growth of the human keratinocytes (*1*). Three strains of keratinocytes derived from the foreskins of newborns (AY, YF17, and YF19) were cocultivated with lethally irradiated ψAM2275 or ψAM DOL-hGH cells for 3 to 4 days. The ψAM cells were selectively removed by a brief EDTA treatment, and the adherent keratinocytes were then detached with trypsin and inoculated onto a feeder layer of G418-resistant 3T3-J2 cells in medium containing G418 at 0.6 mg/ml. By 6 days, all drug-sensitive cells were eliminated, and the cultures were then grown in nonselective medium for 2 to 3 days before transfer.

The effectiveness of the gene transfer was determined by plating freshly infected keratinocytes at clonal density in medium with and without G418. The number of resistant colonies was found to be 0.1 to 0.5% of the total number of colonies, for both of the viral constructs and for the three strains of keratinocytes.

The G418-resistant keratinocyte colonies were indistinguishable from the uninfected colonies by their doubling time and morphological appearance, but their life span in culture was variably shorter.

The ZipneoSV(X) and DOL-hGH

J. R. Morgan and R. C. Mulligan, Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Y. Barrandon and H. Green, Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115.