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Differential Promoter Utilization by the c-*myc* Gene in Mitogen- and Interleukin-2-Stimulated Human Lymphocytes

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Transcription of the c-*myc* gene is initiated from two principal promoters, P1 and P2. We demonstrate here that a shift in promoter utilization occurred with time in human peripheral blood mononuclear cells (PBMC) that had been stimulated to proliferate. The P1/P2 ratio reached a maximum of approximately 1.3 at 4 h after phytohemagglutinin stimulation and a minimum of 0.31 at 48 h. Actinomycin decay experiments demonstrated that both P1 and P2 transcripts had similar half-lives at early and late times after mitogen stimulation, indicating that the shift in promoter utilization was probably not posttranscriptionally regulated. Addition of interleukin-2 to previously activated PBMC increased c-*myc* mRNA, but unlike increases after mitogen stimulation, the P1/P2 ratio stayed less than 0.5. Our findings demonstrated that there was a difference between mitogen- and interleukin-2-stimulated increases in c-*myc* RNA in PBMC.

Abundant evidence implicates the c-*myc* gene in the neoplastic process. The c-*myc* locus in many types of tumors is altered by mechanisms which include gene amplification (8, 12), retroviral promoter insertion (9, 26), and chromosomal translocation (1, 17, 35, 38). In addition, numerous studies have indicated a role for the c-*myc* gene in normal cell proliferation (18, 20, 29). Hence, there is considerable interest in studying c-*myc* gene regulation and function.

The normal human c-*myc* gene has two principal promoter sites, and transcription initiates from within the untranslated first exon (2). The initiation sites are separated by 165 base pairs (bp) with TATA sequences 24 bp upstream from the first initiation site, termed promoter one (P1), and 35 bp upstream from the second initiation site, termed promoter two (P2) (40).

The high degree of evolutionary conservation is suggestive of an important regulatory role for the dual promoter structure of the c-*myc* gene (4, 5). Other evidence that the two promoters are involved in c-*myc* gene regulation derives from studies of c-*myc* expression in Burkitt's lymphoma, murine plasmacytomas, and lymphoblastic cell lines. A high percentage of Burkitt's lymphoma cell lines have translocations between the c-*myc* locus and immunoglobulin loci. Taub et al. (39) showed that, in some cases of Burkitt's lymphoma cell lines in which the c-*myc* gene promoter region remains intact after translocation, there is a significant shift in promoter utilization compared with lymphoblastic cell lines without a c-*myc* translocation, such that P1 is favored over P2. The P1 transcript is also increased relative to P2 in murine plasmacytomas (35, 41) with translocations which leave the c-*myc* promoter structure intact and in thymic lymphomas (33). Other reports concerning the increase or decrease of total c-*myc* mRNA in various cell culture systems and tissues have indicated that promoter utilization remains relatively unchanged, with the P2 transcript remaining predominant at all times (3, 20, 37). One exception is murine cerebellar tissue during development. Ruppert et al. (34) reported that the P1/P2 ratio decreases from 0.66 prenatally to <0.1 in mature animals.

Many reports have indicated that total c-*myc* mRNA increases in normal lymphocytes within minutes after mitogen stimulation (21, 29, 32) and in previously activated normal lymphocytes after exogenous interleukin-2 (IL-2) stimulation (15, 32). We decided to investigate whether there is a difference between the c-*myc* mRNA increase after mitogen compared with IL-2 stimulation by examining promoter utilization of the c-*myc* gene after stimulation with phytohemagglutinin P (PHA-P) and IL-2.

For lymphocytes, we used human peripheral blood mononuclear cells (PBMC) since they are normal, quiescent, nondividing cells when isolated fresh from whole blood (14). Stimulation of PBMC with mitogens causes the cells to increase expression of numerous genes besides c-*myc*, including the IL-2 and IL-2 receptor genes (7, 22). Interaction of IL-2 with the IL-2 receptor, which becomes maximal at about 20 to 24 h after mitogen stimulation, is a pivotal event required for the cells to progress to DNA synthesis at approximately 36 h and cell division at 40 to 48 h (28). Other events, including binding of transferrin to an induced receptor, must also occur for progression of the cells to DNA synthesis (25).

A representative autoradiograph showing temporal expression of the two principal c-*myc* mRNA transcripts in PBMC after stimulation with PHA-P is shown in Fig. 1B. Human PBMC were isolated and cultured with PHA-P as described previously (32). At various times after stimulation, total cellular RNA was isolated (32), and this RNA was subjected to S1 nuclease analysis with a uniformly labeled (23, 27) 610-bp DNA probe (Fig. 1A) spanning the two major c-*myc* transcription initiation sites and most of the 5′ noncoding exon (a gift of K. Nishikura, Wistar Institute of Anatomy and Biology, Philadelphia, Pa). The relative levels of the c-*myc* transcripts were measured by densitometry with compensation for the number of labeled nucleotides in each protected probe fragment. The ratios of the P1 to P2 mRNA levels for the time points in Fig. 1B are listed below each lane, and the mean ± the standard error of the mean for multiple experiments is shown graphically in Fig. 1C.

The P1/P2 ratio averaged 0.90 before stimulation with PHA-P (Fig. 1C). After stimulation, the average P1/P2 ratio increased, reaching a maximum of 1.3 by 4 h. The P1

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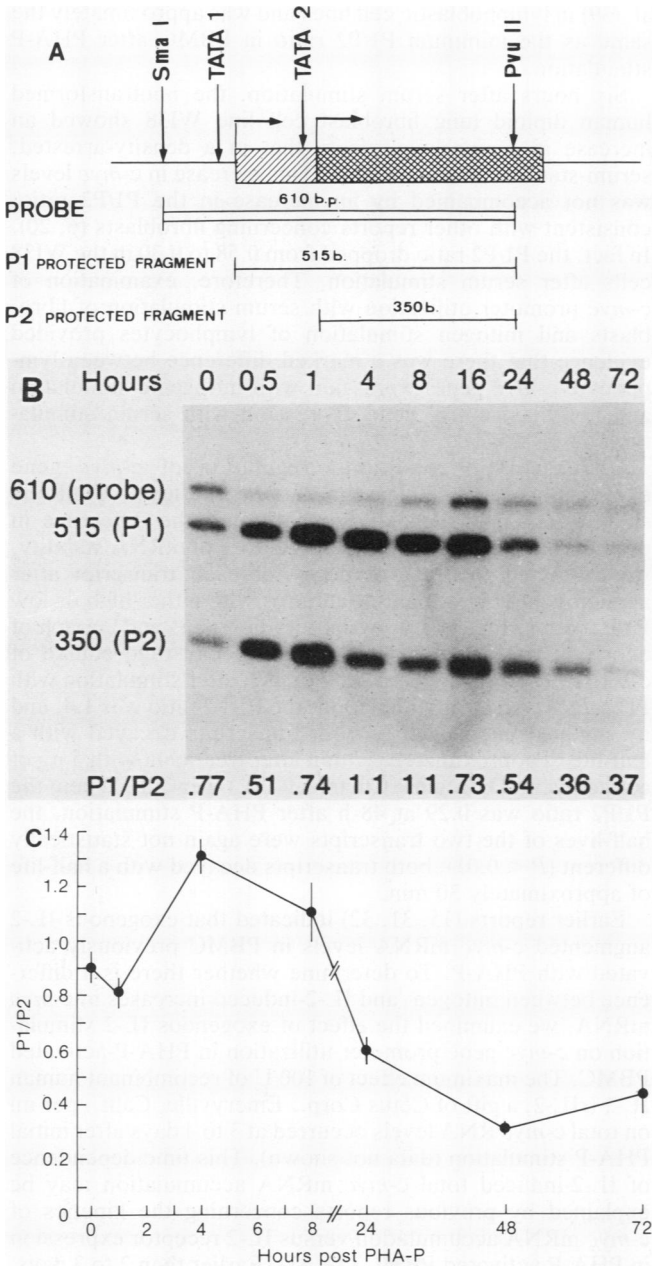

FIG. 1. Relative use of the two principal c-*myc* promoters with time in PBMC stimulated with PHA-P. PBMC were cultured at 1×10^6 to 2×10^6/ml in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum and 1 μg of PHA-P per ml as described previously (32). At the times listed, PBMC were harvested, and total cellular RNA was isolated and analyzed (20 μg per lane) for relative levels of transcripts from the two principal c-*myc* promoters by S1 nuclease analysis. (A) Diagram of the probe used in the S1 analyses; the probe was a 610-bp *Sma*I-*Pvu*II double-stranded DNA fragment with the strand complementary to the RNA uniformly labeled as described by Ley et al. (23). The probe spanned the two c-*myc* transcription initiation sites indicated by the right-angle-bent arrows adjacent to the two TATA sequences in the diagram. The expected S1 nuclease-protected DNA products are illustrated in the diagram. These protected fragments, with lengths of 515 and 350 nucleotides correspond to the distance from the *Pvu*II site in the diagram to the two transcription initiation sites as described previously (27). The hatched box in the diagram indicates exon 1 of c-*myc*. (B) Autoradiograph of the S1-resistant fragments at each time point. Densitometry tracings were performed for each lane, and the relative density of the 515-base (b.) transcript (P1 initiated) to the 350-base transcript (P2 initiated) is expressed as the ratio P1/P2 below each respective lane. In all cases, corrections were made for the amount of radioactivity incorporated as a function of the size of the protected fragment. (C) Graph of the mean (± the standard error of the mean) P1/P2 ratios from replicate experiments, as determined by scanning densitometry.

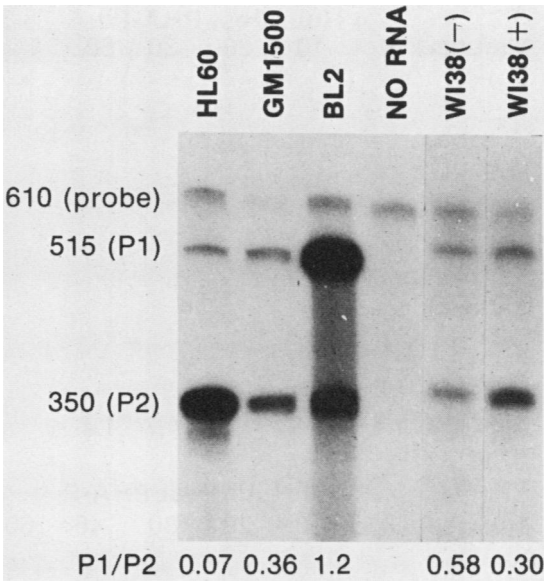

FIG. 2. S1 nuclease analyses of control RNA samples for c-*myc* promoter utilization. All S1 analyses were as described in the legend to Fig. 1. S1 nuclease analyses of total cellular RNA from the human promyelocytic leukemia cell line HL60 (12), the Epstein-Barr Virus-transformed human myeloma cell line GM-1500 (10), and the Epstein-Barr virus-negative Burkitt's lymphoma cell line BL2 (27) are shown in the appropriately labeled lanes. S1 nuclease analysis of total cellular RNA from the human diploid lung fibroblast cell line WI-38 are shown in a density-arrested, serum-starved state [labeled WI-38(−)] and after 6 h of 15% fetal calf serum stimulation [labeled WI-38(+)]. The lane labeled NO RNA is an S1 nuclease analysis of probe DNA with no test cellular RNA. The relative ratios of the two principal transcripts were determined as described in the legend to Fig. 1 and are listed under each lane in the row labeled P1/P2. The numbers on the left indicate size in nucleotides.

predominance lasted until 8 to 16 h. The P1/P2 ratio then decreased to a mean low of 0.31 at 48 h. Consistent with previous reports (20, 32), the total c-*myc* mRNA levels had decreased almost to prestimulation levels by 72 h.

Figure 2 shows controls which verify that our S1 nuclease analysis produced results consistent with previous reports. The control lane using HL60 RNA showed a large predominance of the P2 transcript as reported previously (3, 27). Also consistent with previous reports were the P1/P2 ratios measured in RNAs from the Epstein-Barr virus-negative Burkitt's lymphoma cell line BL2 (11, 27) and the Epstein-Barr virus-transformed immunoglobulin G-producing human myeloma cell line GM-1500 (10). The Burkitt's lymphoma cell line BL2 had a P1/P2 ratio of 1.2, which corresponded to the 0.9 to 1.3 P1/P2 ratios identified in similar Burkitt's lymphoma cell lines by Taub et al. (39), and this ratio was approximately the same as the maximum P1/P2 ratio in PBMC after PHA-P stimulation. In contrast, the GM1500 cell line had a P1/P2 ratio of 0.36, which corresponded approximately to the 0.21 to 0.26 ratios reported by Taub et

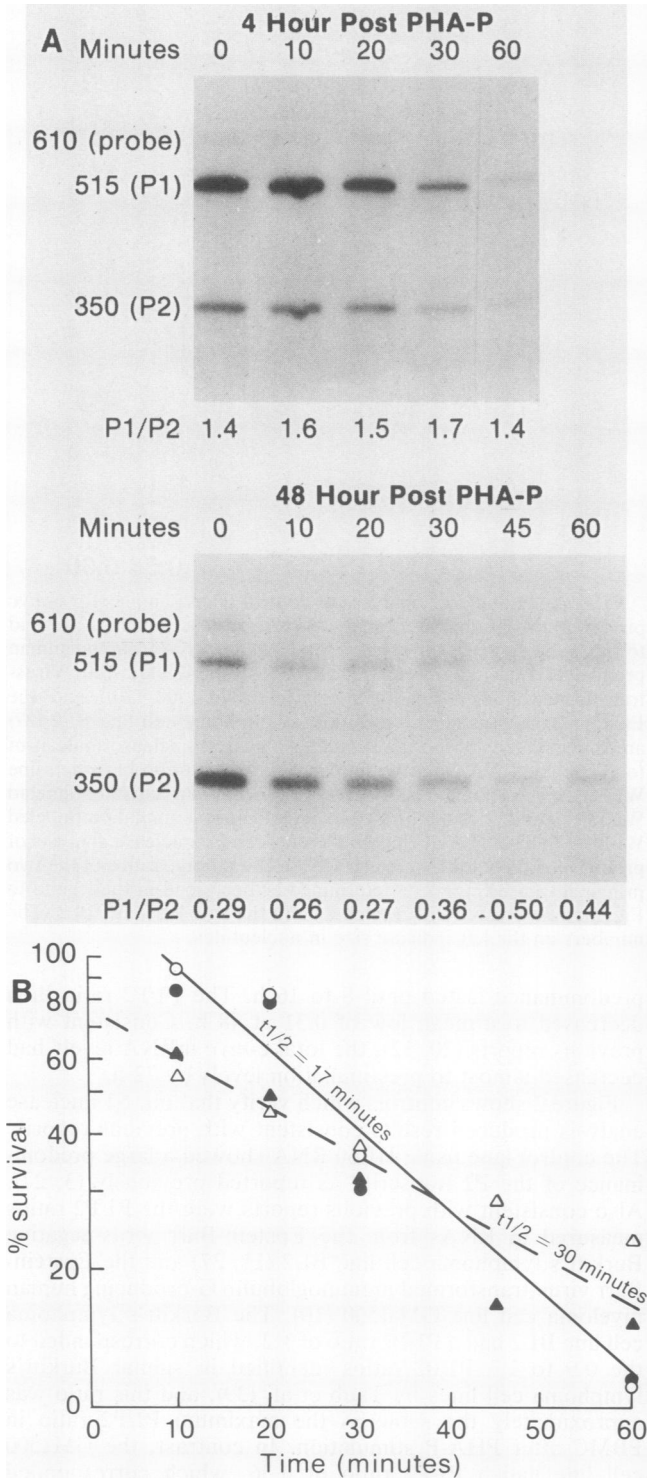

FIG. 3. Relative survival of the two principal c-*myc* transcripts following actinomycin D treatment of cells. (A) PBMC were cultured for 4 or 48 h, as indicated, with PHA-P, 5 μg of actinomycin D per ml was added to the cultures, and total cellular RNA was isolated at 0 to 60 min after addition of actinomycin D. These RNA samples were analyzed for c-*myc* promoter utilization, and the results were labeled with the number of minutes after the addition of actinomycin D. The decay of the two principal c-*myc* transcripts was quantified by densitometry. The relative ratios of the two principal transcripts are listed below the lanes, in the rows labeled P1/P2. The numbers on the left indicate size in nucleotides. (B) Densitometric values were normalized to 100% at 0 min for each transcript, and the data were plotted on a semilog graph. The lines of best fit were determined by ordinary least-squares regression, omitting the 0-min time point to account for the fact that actinomycin D does not act instantaneously upon addition. Simultaneous tests for slope and intercept indicated that the regression lines were significantly different ($P < 0.01$). Symbols: ○, P1 transcript at 4 h post-PHA-P; ●, P2 transcript at 4 h post-PHA-P; △, P1 transcript at 48 h post-PHA-P; ▲, P2 transcript at 48 h post-PHA-P.

al. (39) in lymphoblastic cell lines and was approximately the same as the minimum P1/P2 ratio in PBMC after PHA-P stimulation.

Six hours after serum stimulation, the nontransformed human diploid lung fibroblast cell line WI38 showed an increase in c-*myc* levels from that in a density-arrested, serum-starved, quiescent state. This increase in c-*myc* levels was not accompanied by an increase in the P1/P2 ratio, consistent with other reports concerning fibroblasts (6, 20). In fact, the P1/P2 ratio dropped from 0.58 to 0.30 in the WI38 cells after serum stimulation. Therefore, examination of c-*myc* promoter utilization with serum stimulation of fibroblasts and mitogen stimulation of lymphocytes provided evidence that there was a marked difference between lymphocyte c-*myc* gene expression with mitogenic stimulation and fibroblast c-*myc* gene expression with serum stimulation.

Under certain conditions, regulation of c-*myc* gene expression occurs, at least in part, at the level of mRNA stability (13, 30). To determine whether the difference in promoter utilization occurred at the level of mRNA stability, we examined the rate of decay of each transcript after actinomycin D treatment of cultures with either high or low P1/P2 ratios. Figure 3 shows autoradiographs and a graph of densitometry measurements for the decay time course of c-*myc* transcripts in PBMC at 4 or 48 h after stimulation with PHA-P. At 4 h after stimulation, the P1/P2 ratio was 1.4, and it remained unchanged as both transcripts decayed with a half-life of approximately 17 min after treatment with 5 μg of actinomycin D (Sigma Chemical Co.) per ml. When the P1/P2 ratio was 0.29 at 48 h after PHA-P stimulation, the half-lives of the two transcripts were again not statistically different ($P < 0.01$); both transcripts decayed with a half-life of approximately 30 min.

Earlier reports (15, 31, 32) indicated that exogenous IL-2 augmented c-*myc* mRNA levels in PBMC previously activated with PHA-P. To determine whether there is a difference between mitogen- and IL-2-induced increases in c-*myc* mRNA, we examined the effect of exogenous IL-2 stimulation on c-*myc* gene promoter utilization in PHA-P-activated PBMC. The maximum effect of 100 U of recombinant human IL-2 (rIL-2; a gift of Cetus Corp., Emeryville, Calif.) per ml on total c-*myc* RNA levels occurred at 3 to 4 days after initial PHA-P stimulation (data not shown). This time dependence of IL-2-induced total c-*myc* mRNA accumulation may be explained by previous reports concerning the kinetics of c-*myc* mRNA accumulation versus IL-2 receptor expression in PHA-P-activated PBMC (22, 31). Earlier than 2 to 3 days, the c-*myc* RNA levels present from the initial PHA-P stimulation overshadowed the effect of exogenous rIL-2, and at times later than 4 days, the effect of exogenous IL-2 diminished, probably because of the decrease in IL-2 receptors.

Figure 4 shows the differential effect of PHA-P and exogenous rIL-2 on c-*myc* promoter utilization in freshly isolated PBMC and PBMC that were in culture for 4 days after PHA stimulation. Stimulation of freshly isolated PBMC

with rIL-2 for 4 h without PHA-P prestimulation showed no appreciable increase in c-*myc* mRNA from either principal initiation site, consistent with the absence of IL-2 receptors on these cells. PHA-P stimulation for 4 h showed the usual dramatic increase in c-*myc* mRNA with a predominance of the P1 over the P2 transcript. In contrast, rIL-2 stimulation of PBMC 4 days after initial PHA-P stimulation (to induce IL-2 receptors) resulted in an appreciable increase in total c-*myc* mRNA. PHA-P restimulation of the PBMC 4 days after initial PHA-P stimulation caused an even greater increase in total c-*myc* mRNA than that caused by exogenous rIL-2. Both PHA-P- and rIL-2-induced increases in c-*myc* total RNA reached maximal levels 2 to 4 h after stimulation (data not shown). However, whereas the P1/P2 ratio was 0.71 after 4 h of PHA-P restimulation, it was 0.23 after stimulation of previously activated PBMC with exogenous rIL-2. This ratio difference indicated that the maximum IL-2-stimulated increase in c-*myc* RNA is not associated with as high a P1/P2 ratio as the maximum PHA-P-stimulated increase. By 24 h after PHA-P restimulation, the P1/P2 ratio had fallen to 0.39, a 45% drop, which is similar to that observed between 4 and 24 h after initial PHA-P stimulation of the PBMC. Therefore, the shift in promoter utilization after mitogen stimulation occurred in previously activated, as well as freshly isolated, PBMC.

Maintaining PBMC in culture after mitogen stimulation results in expansion of T cells (36). Freshly isolated PBMC were 70 to 90% OKT_{11} positive, as determined by fluorescence-activated cell sorter analysis of the cells after immunofluorescence staining with anti-OKT_{11} (Ortho Diagnostic Systems, Raritan, N.J.). After 4 days in culture, the cells were over 95% OKT_{11} positive. Since mitogen stimulation of both freshly isolated and 4-day-cultured lymphoblasts showed similar shifts in promoter utilization, the shift probably occurred in the T lymphocytes. We also tested a T-cell-enriched population of freshly isolated PBMC and found a similar shift in promoter utilization after PHA-P stimulation. Neuraminidase-treated sheep erythrocyte rosetting of PBMC followed by Ficoll-Hypaque separation by the method of Moretta et al. (24) enriched the OKT_{11}-positive cells to 90%. Figure 5 shows the results of S1 nuclease analysis for c-*myc* promoter utilization at 0, 4, and 24 h after PHA-P stimulation of T-cell-enriched PBMC by sheep erythrocyte rosetting. There was a 64% drop in the P1/P2 ratio from 1.2 at 4 h to 0.43 at 24 h. Therefore, populations of PBMC enriched for T cells by 4 days of culturing or by sheep erythrocyte rosetting still demonstrated a shift in c-*myc* promoter utilization after mitogen stimulation.

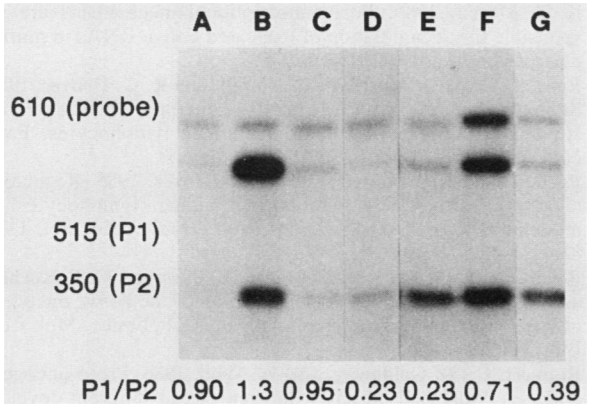

FIG. 4. Relative use of the two principal c-*myc* promoters in PBMC stimulated with IL-2 and PHA-P. S1 nuclease analysis for c-*myc* promoter utilization was performed on RNA samples from the following cells (lanes): A, PBMC; B, PBMC after 4 h of PHA-P stimulation; C, PBMC after 4 h of stimulation with rIL2. For lanes D through G, PBMC were cultured in PHA-P for 72 h to induce IL-2 responsiveness and then replated in medium free of mitogens and IL-2 for 18 h. S1 nuclease analyses for c-*myc* promoter utilization was then performed on RNAs isolated from the cells with the following additional treatments (lanes): D, no addition; E, rIL-2 for 4 h; F, PHA-P for 4 h; G, PHA-P for 24 h. The relative ratios of the two transcripts are listed under each lane in the row labeled P1/P2. The numbers on the left indicate size in nucleotides.

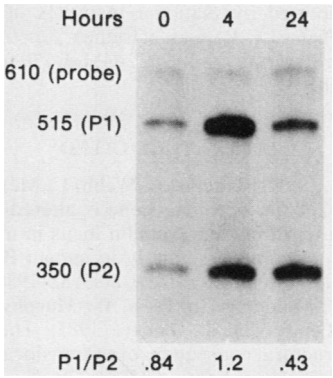

FIG. 5. Relative use of the two c-*myc* promoters in T-cell-enriched PBMC after PHA-P stimulation. PBMC were enriched for OKT_{11}-positive cells by rosetting with neuraminidase-treated sheep erythrocytes followed by Ficoll-Hypaque separation. These enriched cells were stimulated with PHA-P, and total cellular RNA was analyzed for c-*myc* promoter utilization as described in the legend to Fig. 1. The lanes labeled 0, 4, and 24 h refer to duration of PHA-P stimulation. The relative ratios of the two principal transcripts were determined as described in the legend to Fig. 1 and listed under each lane in the row labeled P1/P2. The numbers on the left indicate size in nucleotides.

Our data suggest a possible role for IL-2 in mediating the shift in promoter utilization in mitogen-stimulated PBMC. There are two lines of correlative evidence implicating IL-2 in this role. (i) The decrease in the P1/P2 ratio after mitogen stimulation of PBMC begins by approximately 8 to 16 h, with a nadir at 48 to 72 h. This time course corresponds with the time course of endogenous IL-2 interacting with its induced receptor (16, 19, 22). (ii) Unlike the PHA-P-stimulated increase in c-*myc* RNA, the P1/P2 ratio stays less than 0.5 after exogenous IL-2 stimulation of preactivated PBMC. This P1/P2 ratio corresponds with the ratio present in PBMC at the time of maximum interaction between endogenous IL-2 and its induced receptor.

The observed differences in c-*myc* promoter utilization after mitogen stimulation compared with IL-2 stimulation of lymphocytes indicate that these stimuli may activate c-*myc* transcription through different mechanisms. These mechanisms could involve differential activation of the two c-*myc* promoters.

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LITERATURE CITED

1. **Adams, J. M., S. Gerondakis, E. Webb, L. M. Corcoran, and S. Cory.** 1983. Cellular myc oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt's lymphomas. Proc. Natl. Acad. Sci. USA **80**:1982–1986.
2. **Battey, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenir, and P. Leder.** 1983. The human c-myc oncogene: structural consequences of translocation into the IgH locus in Burkitt's lymphoma. Cell **34**:779–787.
3. **Bentley, D. L., and M. Groudine.** 1986. A block to elongation is largely responsible for decreased transcription of c-myc in differentiated HL60 cells. Nature (London) **321**:702–706.
4. **Bernard, O., S. Cory, S. Gerondakis, E. Webb, and J. M. Adams.** 1983. Sequence of the murine and human cellular *myc* oncogenes and two modes of *myc* transcription resulting from chromosome translocation in B lymphoid tumors. EMBO J. **2**:2375–2383.
5. **Bishop, J. M.** 1983. Cellular oncogenes and retroviruses. Annu. Rev. Biochem. **52**:301–354.
6. **Campisi, J., H. E. Gray, A. B. Pardee, M. Dean, and G. E. Sonenshein.** 1984. Cell cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. Cell **36**: 241–247.
7. **Cantrell, D. A., and K. A. Smith.** 1983. Transient expression of interleukin 2 receptors: consequences for T cell growth. J. Exp. Med. **158**:1895–1911.
8. **Collins, S., and M. Groudine.** 1982. Amplification of endogenous myc-related DNA sequences in a human myeloid leukemia cell line. Nature (London) **298**:679–681.
9. **Corcoran, L. M., J. M. Adams, A. R. Dunn, and S. Cory.** 1984. Murine T lymphomas in which the cellular myc oncogene has been activated by retroviral insertion. Cell **37**:113–122.
10. **Croce, C. M., J. Erikson, A. Ar-Rushdi, D. Aden, and K. Nishikura.** 1984. Translocated c-*myc* oncogene of Burkitt's lymphoma is transcribed in plasma cells and repressed in lymphoblastoid cells. Proc. Natl. Acad. Sci. USA **81**:3170–3174.
11. **Croce, C. M., W. Thierfelder, J. Erikson, K. Nishikura, J. Finin, G. M. Lenoir, and P. C. Nowell.** 1983. Transcriptional activation of an unrearranged and untranslocated c-myc oncogene by translocation of a C locus in Burkitt's lymphoma cells. Proc. Natl. Acad. Sci. USA **80**:6922–6926.
12. **Dalla Favera, R., F. Wong-Staal, and R. C. Gallo.** 1982. Onc gene amplification in promyelocytic leukemia cell line HL-60 and primary leukemic cells of the same patient. Nature (London) **299**:61–63.
13. **Dani, C., J. M. Blanchard, M. Piechaczyk, S. El Sabouty, L. Marty, and P. Jeauteur.** 1984. Extreme instability of *myc* mRNA in normal and transformed cells. Proc. Natl. Acad. Sci. USA **82**:4896–4899.
14. **Darzykiewicz, Z., and F. Traganos.** 1982. RNA content and chromatin structure in cycling and noncycling populations studied by flow cytometry, p. 103–128. *In* G. M. Padilla and R. S. McCanty (ed.), Genetic expression in the cell cycle. Academic Press, Inc., New York.
15. **Depper, J. M., W. J. Leonard, C. Drogula, M. Krönke, T. A. Waldmann, and W. C. Greene.** 1985. Interleukin 2 (IL-2) augments transcription of the IL-2 receptor gene. Proc. Natl. Acad. Sci. USA **82**:4230–4234.
16. **Gillis, S., M. M. Ferm, W. Ou, and K. A. Smith.** 1978. T cell growth factor: parameters of production and a quantitative microassay for activity. J. Immunol. **6**:2027–2032.
17. **Hamlyn, P. H., and T. H. Rabbitts.** 1983. Translocation joins c-myc and immunoglobulin gamma-1 in a Burkitt's lymphoma revealing a third exon in the c-myc oncogene. Nature (London) **304**:135–139.
18. **Kaczmarek, L., J. K. Hyland, R. Watt, M. Rosenberg, and R. Baserga.** 1985. Microinjected c-myc as a competence factor. Science **228**:1313–1315.
19. **Kaepfer, R., and S. Efrat.** 1986. Regulation of human interleukin-2 gene expression, p. 57–68. *In* J. J. Oppenheim and D. M. Jacobs (ed.), Progress in leukocyte biology, vol. 5. Alan R. Liss, Inc., New York.
20. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
21. **Krönke, M., W. J. Leonard, J. M. Depper, and W. C. Greene.** 1985. Sequential expression of genes involved in human T lymphocyte growth and differentiation. J. Exp. Med. **161**:1593–1598.
22. **Leonard, W. J., M. Krönke, N. J. Peffer, J. M. Depper, and W. C. Greene.** 1985. Interleukin 2 receptor gene expression in normal human T lymphocytes. Proc. Natl. Acad. Sci. USA **82**:6281–6285.
23. **Ley, T. J., N. P. Anagnou, G. Pepe, and A. W. Nienhius.** 1982. RNA processing errors in patients with beta-thalassemia. Proc. Natl. Acad. Sci. USA **79**:4775–4779.
24. **Moretta, L., M. Ferrarini, M. L. Duranti, and M. C. Minigari.** 1975. Expression of a receptor for IgM by human T cells *in vitro*. Eur. J. Immunol. **5**:565–569.
25. **Neckers, L. M., and J. Cossman.** 1983. Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. Proc. Natl. Acad. Sci. USA **80**:3494–3498.
26. **Neil, J. C., D. Hughes, R. McFarlane, N. M. Wilkie, D. E. Onions, G. Lees, and O. Jarrett.** 1984. Transduction and rearrangement of the myc gene by feline leukemia virus in naturally occurring T cell leukemias. Nature (London) **308**:814–820.
27. **Nishikura, K., S. Goldflam, and G. A. Vuocolo.** 1985. Accurate and efficient transcription of human c-*myc* genes injected into *Xenopus laevis* oocytes. Mol. Cell. Biol. **5**:1434–1441.
28. **Nowell, P. C., R. P. Daniele, and L. A. Winger.** 1975. Kinetics of human lymphocyte proliferation: proportion of cells responsive to phytohemagglutinin and correlation with E rosette formation. J. Reticuloendothel. Soc. **17**:47–52.
29. **Persson, H., L. Hennighausen, R. Taub, W. DuGrode, and P. Leder.** 1984. Antibodies to human c-myc oncogene product: evidence of an evolutionarily conserved protein induced during cell proliferation. Science **225**:687–693.
30. **Piechaczyk, M., J. Q. Yang, J. M. Blanchard, P. Jeanteur, and K. B. Marcu.** 1985. Post-transcriptional mechanisms are responsible for accumulation of truncated c-*myc* RNAs in murine plasma cell tumors. Cell **42**:589–597.
31. **Reed, J. C., J. D. Alpers, P. C. Nowell, and R. G. Hoover.** 1986. Sequential expression of proto-oncogenes during lectin-stimulated mitogenesis of normal human lymphocytes. Proc. Natl. Acad. Sci. USA **83**:3982–3986.
32. **Reed, J. C., P. C. Nowell, and R. G. Hoover.** 1985. Regulation of c-myc mRNA levels in normal human lymphocytes by modulators of cell proliferation. Proc. Natl. Acad. Sci. USA **82**:4221–4224.
33. **Reicin, A., J.-Q. Yang, K. B. Marcu, E. Fleissner, C. F. Koehne, and P. V. O'Donnell.** 1986. Deregulation of the c-*myc* oncogene in virus-induced thymic lymphomas of AKR/J mice. Mol. Cell. Biol. **6**:4088–4092.
34. **Ruppert, C., D. Goldowitz, and W. Willi.** 1986. Proto-oncogene c-*myc* is expressed in cerebellar neurons at different developmental stages. EMBO J. *5*:1897–1901.
35. **Stanton, L. W., R. Watt, and K. B. Marcu.** 1983. Translocation, breakage, and truncated transcripts of c-myc oncogene in murine plasmacytomas. Nature (London) **303**:401–406.
36. **Stern, J. B., and K. A. Smith.** 1986. Interleukin-2 induction of T-cell G_1 progression and c-*myb* expression. Science **233**:203–206.
37. **Stewart, T. A., A. R. Bellve, and P. Leder.** 1984. Transcription and promoter usage of the myc gene in normal somatic and spermatogenic cells. Science **226**:707–710.
38. **Taub, R., I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, and P. Leder.** 1982. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human

Burkitt's lymphoma and murine plasmacytoma cells. Proc. Natl. Acad. Sci. USA **79**:7837–7841.

39. **Taub, R., C. Moulding, J. Battey, W. Murphy, T. Vasicek, G. M. Lenoir, and P. Leder.** 1984. Activation and somatic mutation of the translocated c-myc gene in Burkitt's lymphoma cells. Cell **36**:339–348.
40. **Watt, R., K. Nishikura, J. Sorrentino, A. Ar-Rushdi, C. M. Croce, and G. Rovera.** 1983. The structure and nucleotide sequence of the 5′ end of the human c-myc oncogene. Proc. Natl. Acad. Sci. USA **80**:6307–6311.
41. **Yang, J., S. R. Bauer, J. F. Mushinski, and K. B. Marcu.** 1985. Chromosome translocations clustered 5′ of the murine c-myc gene quantitatively affect promoter usage: implications for the site of normal c-myc regulation. EMBO J **4**:1441–1447.