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Premature termination of transcription from the P1 promoter of the mouse c-*myc* gene

(regulation/protooncogenes)

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ABSTRACT **Modulation of transcriptional elongation within the c-*myc* gene is thought to play a major role in determining levels of c-myc mRNA in both normal and tumor cells. A discrete site of blockage to transcriptional elongation has previously been localized at the 3′ end of exon 1 of the mouse and human c-*myc* genes. We here identify an additional site of transcriptional attenuation that is located between the P1 and P2 promoters of the c-*myc* gene and that mediates premature termination of transcripts initiating from the P1 promoter. A 95-nucleotide DNA fragment derived from this region prematurely terminated transcription when placed downstream from the promoter of the *H-2K*^{bm1} gene and assayed by expression in *Xenopus* oocytes. We also show that the previously identified attenuation signal in exon 1 of the mouse c-*myc* gene can mediate premature termination of P1-initiated transcripts. Premature termination of P1-initiated transcripts presumably increases transcription from the downstream P2 promoter; aberrant regulation of this termination may explain the increased use of the P1 promoter that is characteristic of certain tumors in which *myc* is overexpressed.**

The c-*myc* protein is thought to play a role in the control of cellular proliferation and differentiation. Aberrant expression of *myc* may be mediated by multiple mechanisms and is associated with the development of a wide variety of neoplasms.

The steady-state level of c-myc RNA in a cell may be modulated by regulating the rate of transcriptional initiation (1, 2), the rate of transcriptional elongation through a "block" near the end of the first exon (2–4), or the stability of the c-myc transcript (5, 6). Aberrant expression of the c-*myc* gene, as mediated by chromosomal translocation (7, 8), gene amplification (9, 10), or retroviral insertion (11), results from disruption of one or more of these regulatory mechanisms.

The site of "blockage" to transcriptional elongation within the mouse c-*myc* gene has been previously defined by run-off transcription analysis in isolated nuclei and is located near the end of the first exon of the gene (12–14). A 180-nucleotide (nt) DNA fragment derived from this region blocked transcriptional elongation when placed within a heterologous gene and assayed by transfection into eukaryotic cells (14). The expression of cloned c-*myc* genes in *Xenopus* oocytes has been used to define a site of premature transcriptional termination within this 180-nt region (13).

We here identify an additional site of transcriptional attenuation that is located between the P1 and P2 promoters of the c-*myc* gene and that mediates premature termination of P1-initiated transcripts. We suggest that this site may play a role in modulating the relative use of the two promoters of the c-*myc* gene. Premature termination of P1-initiated transcripts may account for the low representation (5–20%) of these transcripts in steady-state c-myc RNA of normal cells, and loss of the ability to regulate the premature termination of P1-initiated transcripts may explain reversal of the normal pattern of promoter use associated with overexpression of the c-*myc* gene in Burkitt lymphoma.

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MATERIALS AND METHODS

Cell Culture. MEL cells (F412-B2 line) (47) were grown in α-minimal Eagle's medium/10% fetal calf serum.

Microinjection of *Xenopus* Oocytes and RNA Isolation. Supercoiled plasmid DNA was injected into the germinal vesicle of *Xenopus* oocytes as described by Gurdon and Wickens (15). RNA was isolated as described by Bentley and Groudine (13).

Analysis of RNA by Hybridization to Uniformly Labeled RNA Probes. RNA was analyzed by hybridization to uniformly labeled RNA probes followed by digestion with RNase under described conditions (14). ^{32}P-labeled RNA probes were synthesized from Gem3 or Gem4 vectors (Promega) containing inserts indicated in the figure legends.

Run-Off Transcription. Isolation of nuclei and elongation of nascent transcripts *in vitro* were done exactly as described (14).

Plasmid Construction. Plasmids H2-B-CAT, P1-CAT-A, and P1-CAT-B (Figs. 3 and 4) were derived from the vector $pCAT_{Basic}$ (Promega) by insertion of the indicated restriction fragments into sites in the polylinker. The globin fragment in P1-CAT-A was derived from exon 2 of the human β-globin gene, and the *myc* Pst I fragment in P1-CAT-B was derived from exon 2 of the mouse c-*myc* gene; the purpose of these inserts was to provide different distances between the P1 promoter and the chloramphenicol acetyltransferase gene.

RESULTS

Run-Off Transcription Analysis of the Region Between the P1 and P2 Promoters of Murine c-*myc* Gene. Previous reports have described a block to transcriptional elongation near the 3′ end of exon 1 of the mouse c-*myc* gene (12–14). We noted that the nucleotide sequence at this site shares a region of homology with sequences located between the P1 and P2 promoters of the gene (see Fig. 2*a*) and were, therefore, interested in determining whether these latter sequences block the elongation of transcripts that initiate at the P1 promoter.

We first determined by run-off transcription whether all transcripts that initiate at the P1 promoter extend to the 3′ end of the gene. Nuclei were isolated from MEL cells, and nascent transcripts were elongated in the presence of [^{32}P]UTP. Labeled run-off transcripts were hybridized to short single-stranded DNA probes that detected transcription

Abbreviation: nt, nucleotide(s).

across regions within and 5′ to the mouse c-*myc* gene (Fig. 1). The amount of transcription across different regions at the 5′ end of the gene (probes A, B, and C) was measured relative to that at the 3′ end (probe D). Probes A, B, and C were used in separate hybridizations, so that they would not compete for hybridization to the same run-off transcript. After correction for the uridine content of the transcripts, transcription between the P1 and P2 promoters (detected by probe B) was determined to be ≈8-fold greater than that found 5′ to the gene (detected by probe A), implying that most transcripts within region B initiated at the P1 promoter. Probes C and D detected total transcription initiating from the P1 and P2 promoters. The ratio of P1- to P2-initiated c-*myc* transcripts in steady-state RNA in MEL cells is ≈1:8 (data not shown), implying that P1-initiated transcripts represented approximately one-ninth of the signal detected by probe D. The amount of P1-initiated transcription detected by probe B, however, was similar to the total c-*myc* transcription across region D, indicating that most transcripts that initiate at the P1 promoter do not reach the 3′ end of the gene. The presence of P2-initiated transcripts made it impossible to determine by run-off transcription analysis whether the blockage to elongation of P1-initiated transcripts occurs within the previously identified site at the end of exon 1 or at an upstream site.

Premature Termination of P1-Initiated Transcripts upon Expression of a Genomic c-*myc* Clone in *Xenopus* Oocytes. Prematurely terminated transcripts are stable in *Xenopus* oocytes, whereas in mammalian nuclei they are undetectable; the oocyte system thus provides a means to accurately map the presumed sites of transcriptional termination.

A mouse c-*myc* genomic clone was injected into *Xenopus* oocytes to determine whether transcripts that initiate at the P1 promoter terminate prematurely within DNA sequences between the P1 and P2 promoters. Plasmid DNA was injected at a concentration at which RNA polymerase III does not use the c-*myc* promoters (16). RNA from injected oocytes was analyzed by hybridization to a uniformly labeled RNA probe that spanned the region between the P1 and P2 promoters (Fig. 2*b*, probe A, experiment 1). This probe detected major hybridizing fragments of 221, 60, and 155 nt. The 221- and 60-nt fragments represented transcripts that initiated at the P1 and P2 promoters, respectively, and continued past the 3′ end of the probe. The fragment of 155 nt could represent transcripts that initiate at the P1 promoter and terminate prematurely upstream of P2, or, alternatively, this fragment could indicate an additional site of transcription initiation located between the P1 and P2 promoters.

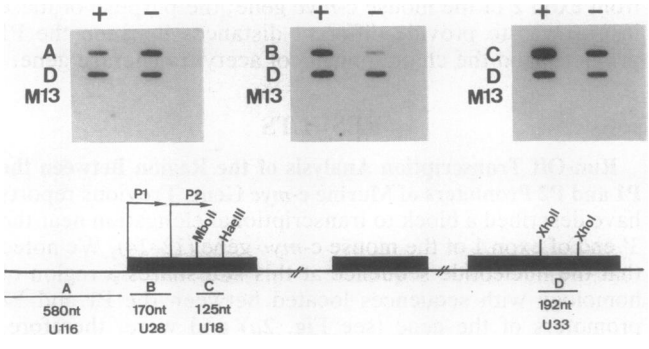

FIG. 1. Run-off transcription analysis of the c-*myc* gene in MEL cells. Labeled run-off transcripts were synthesized in nuclei isolated from MEL cells and hybridized to immobilized single-stranded DNA probes that detected transcription in the sense (+) or antisense (−) direction across regions A–D of the mouse c-*myc* gene as indicated. Independent hybridizations were used to detect transcription across each of regions A, B, and C, and transcription in each of these regions was measured relative to that across probe D. Each hybridization contained 10^8 cpm. Filters also contained single-stranded M13mp18 DNA as a control for nonspecific hybridization. Sizes of probes are given in nucleotides, and the uridine content of the region in the corresponding transcript is indicated.

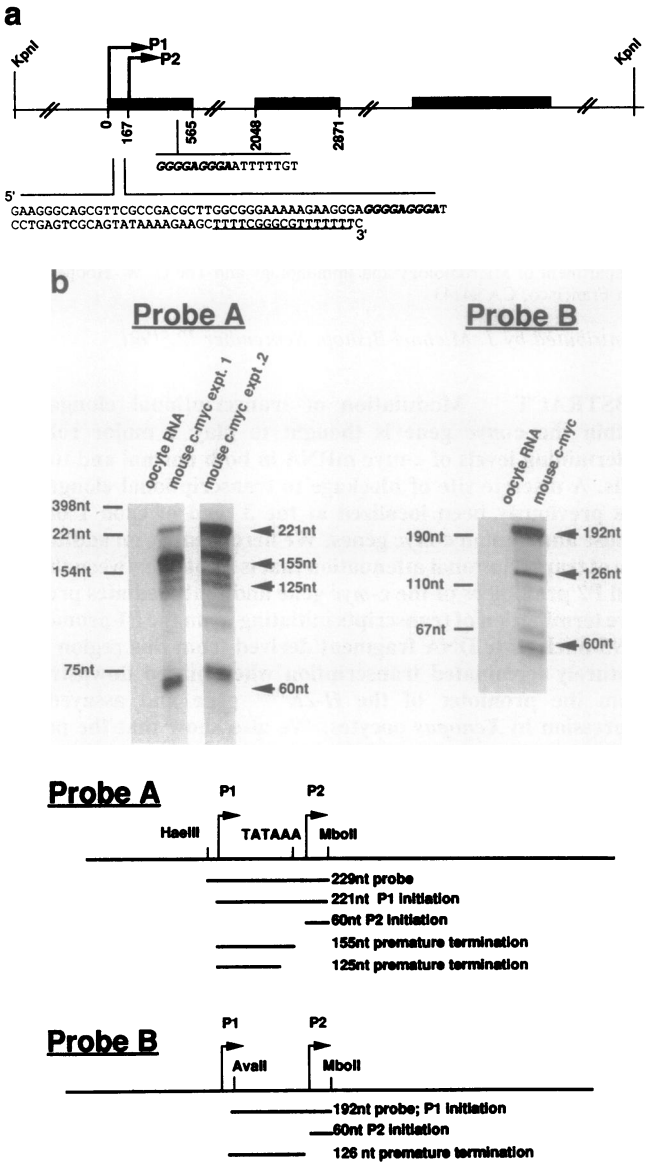

FIG. 2. Expression of an injected genomic c-*myc* gene in *Xenopus* oocytes. (*a*) Structure of the mouse c-*myc* genomic clone. The construct used for microinjection into *Xenopus* oocytes contained a 10-kilobase *Kpn* I restriction fragment inserted into the vector pML. The region between the P1 and P2 promoters that was cloned into vectors indicated in Fig. 3 is shown below; nucleotide sequences homologous to the attenuator in exon 1 of the gene are shown in boldface type. The region of premature transcriptional termination is underlined. (*b*) RNase protection analysis of c-*myc* transcripts in injected oocytes. RNA was isolated from injected oocytes and hybridized to probes A and B. Lanes correspond to RNA isolated from uninjected oocytes (oocyte RNA) or oocytes injected with the mouse c-*myc* clone (mouse c-*myc*). Sizes of P1- and P2-initiated transcripts and of prematurely terminated transcripts are indicated. expt., Experiment.

Hybridization of the oocyte RNA to a uniformly labeled RNA probe spanning the region between the *Ava* II and the *Mbo II* sites of the c-*myc* gene yielded protected fragments of 60, 192, and 126 nt, the last of these confirming that transcripts initiating from the P1 promoter terminated at sites upstream from P2 (Fig. 2*b*, probe B). Prematurely terminated transcripts represented 30–80% of total P1-initiated transcripts. A variable amount of premature transcriptional ter-

mination was also seen at a region located immediately upstream from the TATAAA box of the P2 promoter (Fig. 2*b*, probe A, experiment 2, 125-nt fragments); this accounted for 5–50% of the total amount of prematurely terminated transcripts.

Hybridization of the injected oocyte RNA to a single-stranded 3′ end-labeled DNA probe, followed by digestion with S1 nuclease, showed that the 3′ ends of the prematurely terminated transcripts mapped in two clusters (data not shown). The major site is located within the runs of thymine residues found just upstream of the P2 promoter, and the minor one is located just upstream of the TATAAA box of the P2 promoter; the latter site was used to a variable extent.

DNA Sequences Located Between the P1 and P2 Promoters of the Mouse c-*myc* Gene Block Transcriptional Elongation When Placed Downstream from a Heterologous Promoter. It has previously been shown that a 180-nt DNA fragment derived from the first exon of the mouse c-*myc* gene will block transcriptional elongation when placed downstream from heterologous promoters. We, therefore, determined whether a similar transportable element was present at the site of premature termination of P1-initiated transcripts.

The previously defined 180-nt attenuator region was deleted from exon 1 of a mouse c-*myc* genomic clone and replaced with a candidate 95-nt fragment that derived from the region between the P1 and P2 promoters and contained the site of premature termination of P1-initiated transcripts (Figs. 2*a* and 3*a*, construct A). Expression of this construct in *Xenopus* oocytes was analyzed by hybridization of RNA to a uniformly labeled RNA probe that spanned the transferred 95-nt fragment (Fig. 3*b*, probe A). After digestion with RNase, protected fragments of 95, 180, 140, and 110 nt were obtained. The fragments of 140 and 110 nt corresponded to premature termination of transcription within the 95-nt region at sites similar or identical to those used when this fragment is found in its natural position downstream from the P1 promoter. The hybridizing band of 180 nt represented transcripts that read through the entire length of the probe; the band of 95 nt represented hybridization to transcripts that initiate from the natural P1 promoter, which was also present in the injected *myc* gene constructs. The 95-nt fragment, therefore, functioned as a transcriptional terminator in *Xenopus* oocytes when positioned in place of the previously identified terminator within exon 1 of the mouse c-*myc* gene.

To determine whether the 95-nt fragment would cause transcriptional termination when placed downstream from a heterologous promoter, this fragment was placed 220 nt downstream from the promoter of the *H-2K*^{bm1} gene in the construct H2-B-CAT (Fig. 3*a*, construct B). Expression of this construct was analyzed after microinjection into *Xenopus* oocytes by hybridization of RNA to an RNA probe that spanned the transferred 95-nt region (Fig. 3*b*, probe B). The protected fragments of 140 and 110 nt represented transcripts that terminated prematurely within the 95-nt fragment derived from the region between P1 and P2, thus indicating that this element will also function as a transcriptional terminator when placed downstream from a heterologous promoter.

Exon 1 Sequences of the Mouse c-*myc* Gene Will Mediate Attenuation of P1-Initiated Transcripts When the P2 Promoter and Upstream Attenuator Sequences Are Absent. Overexpression of the c-*myc* gene in Burkitt lymphoma is characterized both by a loss of the block to transcriptional elongation at the end of exon 1 (17, 18) and by an alteration in relative use of the two promoters of the c-*myc* gene, with the normally less used and more 5′ promoter P1 now predominating (19, 20). It has, therefore, been suggested that loss of attenuation results from the switch in promoter use—there being an intrinsic inability of P1-initiated transcripts to terminate prematurely within exon 1 sequences. However, after showing that P1-initiated transcripts may terminate prematurely at a site located between the P1 and P2 promoters, we examined the ability of exon 1 sequences to mediate termination when placed downstream from the P1 promoter in constructs in which the P2 promoter and upstream attenuator were absent.

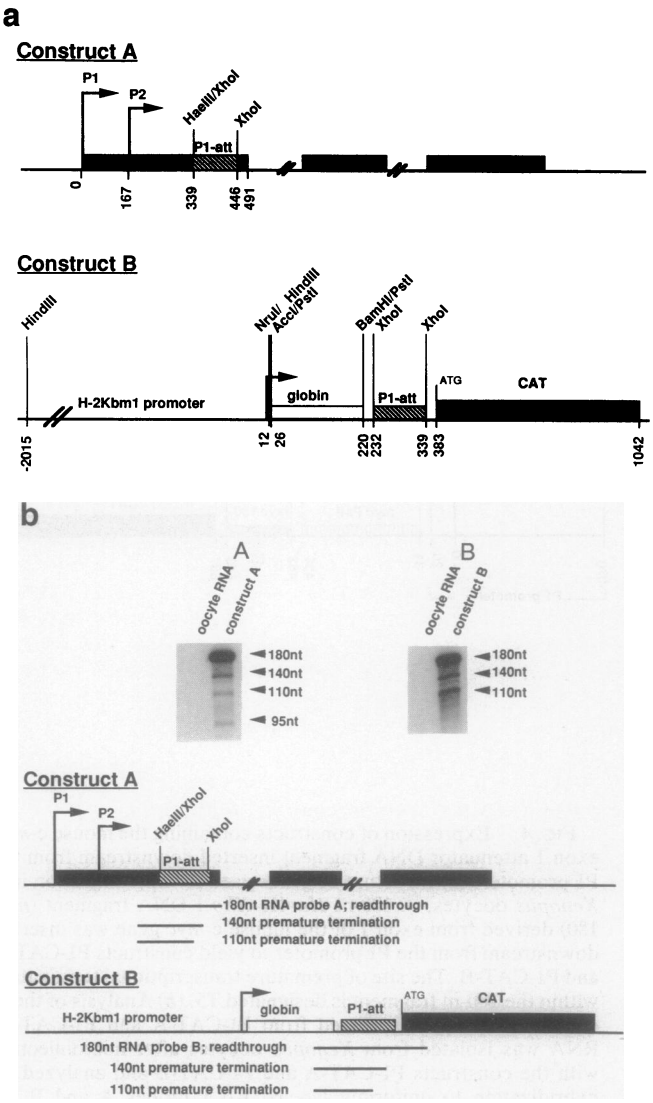

FIG. 3. Expression of genes containing a transplanted candidate P1-attenuator fragment in *Xenopus* oocytes. (*a*) Structure of genes used for microinjection into *Xenopus* oocytes. Construct A: the 180-nt *Hae*III–*Xho* I fragment containing the site of premature transcriptional termination within exon 1 of mouse c-*myc* gene was deleted and replaced with a 95-nt fragment (P1-att; hatched boxes), which derived from the region between the P1 and P2 promoters shown in Fig. 2*a*. Construct B: the 95-nt fragment described above (see Fig. 2*a*) was inserted into the unique *Xho* I site of vector H2-B-CAT. (*b*) RNase protection analysis of transcripts derived from constructs containing a transplanted P1-attenuator fragment. RNA was isolated from *Xenopus* oocytes after microinjection with constructs A and B. Samples analyzed by hybridization to the uniformly labeled RNA probes and the expected sizes of protected fragments representing transcriptional readthrough and premature termination within the candidate P1-attenuator fragment are indicated. Lanes correspond to RNA isolated from uninjected oocytes or oocytes injected with construct A or B. CAT, chloramphenicol acetyltransferase.

The 180-nt exon 1 attenuator fragment was positioned at distances 240 and 463 nt downstream from the P1 promoter, corresponding to its approximate natural distance from the P2 and P1 promoters, respectively (Fig. 4*a*). Transcription of these constructs in *Xenopus* oocytes initiated at the P1 cap

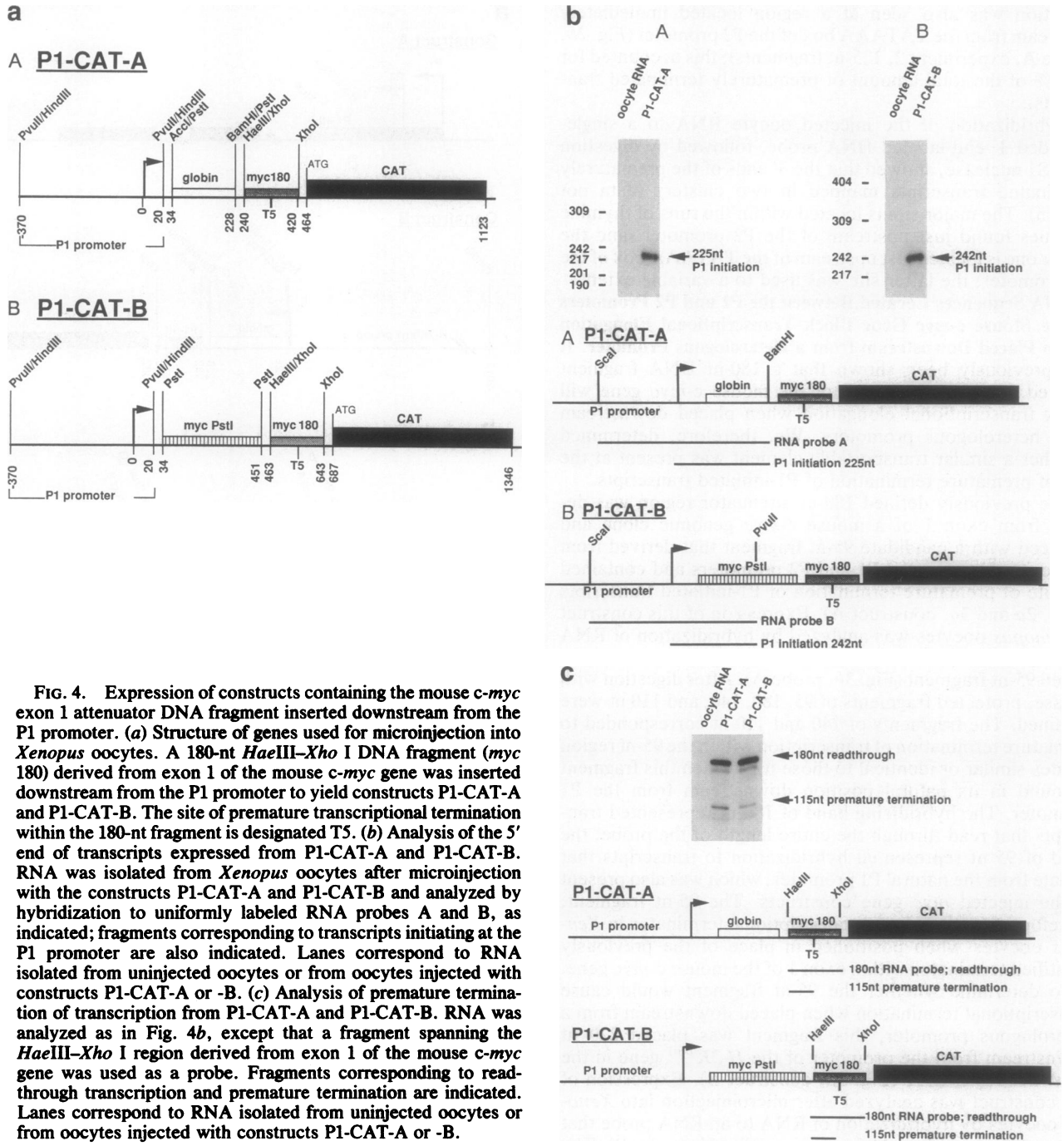

FIG. 4. Expression of constructs containing the mouse c-*myc* exon 1 attenuator DNA fragment inserted downstream from the P1 promoter. (*a*) Structure of genes used for microinjection into *Xenopus* oocytes. A 180-nt *Hae*III–*Xho* I DNA fragment (*myc* 180) derived from exon 1 of the mouse c-*myc* gene was inserted downstream from the P1 promoter to yield constructs P1-CAT-A and P1-CAT-B. The site of premature transcriptional termination within the 180-nt fragment is designated T5. (*b*) Analysis of the 5′ end of transcripts expressed from P1-CAT-A and P1-CAT-B. RNA was isolated from *Xenopus* oocytes after microinjection with the constructs P1-CAT-A and P1-CAT-B and analyzed by hybridization to uniformly labeled RNA probes A and B, as indicated; fragments corresponding to transcripts initiating at the P1 promoter are also indicated. Lanes correspond to RNA isolated from uninjected oocytes or from oocytes injected with constructs P1-CAT-A or -B. (*c*) Analysis of premature termination of transcription from P1-CAT-A and P1-CAT-B. RNA was analyzed as in Fig. 4*b*, except that a fragment spanning the *Hae*III–*Xho* I region derived from exon 1 of the mouse c-*myc* gene was used as a probe. Fragments corresponding to readthrough transcription and premature termination are indicated. Lanes correspond to RNA isolated from uninjected oocytes or from oocytes injected with constructs P1-CAT-A or -B.

site, as shown by hybridization of the oocyte RNA to uniformly labeled RNA probes that spanned the 5′ end of the gene (Fig. 4*b*). Analysis of the RNA by hybridization to probes that spanned the 180-nt exon 1 sequences showed that transcripts terminated within these sequences (Fig. 4*c*). The efficiency of premature termination was the same when the 180-nt fragment was placed either 240 or 463 nt downstream from the P1 promoter. This result indicated that, in contrast to previous suggestions, transcripts that initiate at the P1 promoter can terminate within exon 1 sequences of the c-*myc* gene.

DISCUSSION

The expression of eukaryotic genes is modulated by multiple mechanisms. Blocks to transcriptional elongation have now been described in several eukaryotic genes in addition to *myc* (2–4, 21–29), and it has been suggested that modulation of the degree of blockage could regulate genes in response to different physiological signals. The mechanisms used to modulate transcriptional elongation and termination within these genes remain to be elucidated.

The DNA sequences that mediate the block to transcriptional elongation within exon 1 of the human and mouse c-*myc* genes have been defined by nuclear run-off analysis of cloned genes after transfection into eukaryotic cells (12–14) and by localizing sites of premature transcriptional termination upon expression of genes injected into *Xenopus* oocytes (13). The regulatory regions include stretches of thymidine residues on the nontranscribed DNA strand that are preceded by sequences capable of forming secondary structures in the corresponding RNA. The role of these structural features, which are reminiscent of prokaryotic transcriptional termination signals (30), is not known.

We have now shown that transcripts initiating from the P1 promoter of the mouse c-*myc* gene terminate prematurely

within a thymidine-rich region just upstream from the P2 promoter. The amount of premature termination of P1-initiated transcripts we observed indicated that this process may, in part, account for the low ratio of P1- to P2-initiated transcripts in steady-state c-*myc* RNA in normal cells because transcriptional interference might normally be expected to occlude the more downstream P2 promoter (31, 32).

We also tested the ability of the previously defined attenuation sequences within exon 1 of the mouse c-*myc* gene to mediate attenuation of P1-initiated transcripts in constructs in which both the P2 promoter and upstream attenuator sequences were absent. The 180-nt exon 1 attenuator fragment mediated premature termination of P1-initiated transcripts when placed at distances from P1 that approximated its natural distance from either the P1 or the P2 promoter of the c-*myc* gene. These data indicated that there is no intrinsic inability of P1-initiated transcripts to terminate within exon 1 sequences of the mouse c-*myc* gene, and the inability to detect this in previous reports was presumably due to the normal premature termination of most P1-initiated transcripts at sites upstream from P2. The work reported here thus raises the possibility that the loss of transcriptional attenuation within exon 1 of the c-*myc* gene in Burkitt lymphoma is not solely a reflection of increased use of the P1 promoter. The increased amount of P1-initiated transcripts in Burkitt lymphoma cells could reflect a decrease in the amount of premature termination of P1-initiated transcripts or an increase in transcriptional initiation at the P1 promoter.

Transcriptional termination within promoter sequences has also been reported for the *Xenopus laevis* ribosomal RNA genes, which are transcribed by RNA polymerase I. Transcripts originating from the intergenic spacer terminate within the promoter of the adjacent gene, thus protecting the latter promoter from occlusion (33). In this case, a specific DNA sequence serves to both terminate transcription and to stimulate transcriptional initiation from the adjacent promoter. It is possible that the same sequences that mediate premature termination of P1-initiated transcripts also stimulate transcriptional initiation from the adjacent P2 promoter.

Premature termination of P1-initiated transcripts in normal cells presumably allows for more efficient use of the P2 promoter. No differences in properties of P1- and P2-initiated c-*myc* transcripts have been reported, although they could conceivably differ in the relative efficiencies at which they are translated into the two forms of c-*myc* protein, the larger of which is suppressed in many Burkitt lymphomas (34).

Diverse mechanisms for transcriptional termination have been defined in both prokaryotes and eukaryotes. Termination and antitermination may involve recognition of sequences at the site of termination itself (35–37), sequences within the promoter (38–40), or regions located between (41, 42). These recognitions may involve features in either the DNA (35–37) or transcribed RNA (43–46). The definition of sites of transcriptional elongation blockage within eukaryotic genes may reveal features held in common between the diverse genes that are regulated by this mechanism and also give hints as to how this process may confer specific regulation on different genes in response to varying physiological signals.

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1. Einat, M., Resnitzky, D. & Kimchi, A. (1985) *Nature (London)* **313**, 597–600.
2. Nepveu, A., Levine, R. A., Campisi, J., Greenberg, M. E., Ziff, E. & Marcu, K. B. (1987) *Oncogene* **1**, 243–250.
3. Bentley, D. L. & Groudine, M. (1986) *Nature (London)* **321**, 702–706.
4. Nepveu, A., Marcu, K. B., Skoultchi, A. I. & Lachman, H. M. (1987) *Genes Dev.* **1**, 938–945.
5. Dani, C., Blanchard, J. M., Piechaczyk, M., El Sabouty, S., Marty, L. & Jeanteur, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7046–7050.
6. Dani, C., Mechti, N., Piechaczyk, M., Lebleu, B., Jeanteur, P. & Blanchard, J. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4896–4899.
7. Cole, M. D. (1986) *Annu. Rev. Genet.* **20**, 361–384.
8. Cory, S. (1986) *Adv. Cancer Res.* **47**, 188–234.
9. Alitalo, K. & Schwab, M. (1986) *Adv. Cancer Res.* **47**, 235–282.
10. Schwab, M. & Amler, L. C. (1990) *Genes Chromosomes Cancer* **1**, 181–194.
11. Varmus, H. E. (1984) *Annu. Rev. Genet.* **18**, 553–612.
12. Miller, H., Asselin, C., Dufort, D., Yand, J.-Q., Gupta, K., Marcu, K. B. & Nepveu, A. (1989) *Mol. Cell. Biol.* **9**, 5340–5349.
13. Bentley, D. L. & Groudine, M. (1988) *Cell* **53**, 245–256.
14. Wright, S. & Bishop, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 505–509.
15. Gurdon, J. B. & Wickens, M. P. (1983) *Methods Enzymol.* **101**, 370–386.
16. Bentley, D. L., Brown, W. L. & Groudine, M. (1989) *Genes Dev.* **3**, 1179–1189.
17. Cesarman, E., Dalla-Favera, R., Bentley, D. & Groudine, M. (1987) *Science* **238**, 1272–1275.
18. Spencer, C. A., Lestrange, R. C., Novak, U., Hayward, W. S. & Groudine, M. (1990) *Genes Dev.* **4**, 75–88.
19. Taub, R., Moulding, C., Battey, J., Murphy, W., Vasicek, T., Lenoir, G. M. & Leder, P. (1984) *Cell* **36**, 339–348.
20. Taub, R., Kelly, K., Battey, J., Latt, S., Lenoir, G. M., Tantravahi, U., Tu, Z. & Leder, P. (1984) *Cell* **37**, 511–520.
21. Nepveu, A. & Marcu, K. B. (1986) *EMBO J.* **5**, 2859–2865.
22. Eick, D. & Bornkamm, G. W. (1986) *Nucleic Acids Res.* **14**, 8331–8346.
23. Eick, D., Berger, R., Polack, A. & Bornkamm, G. W. (1987) *Oncogene* **2**, 61–65.
24. Bender, T. P., Thompson, C. B. & Kuehl, W. M. (1987) *Science* **237**, 1473–1476.
25. Watson, R. J. (1988) *Oncogene* **2**, 267–272.
26. Fort, P., Rech, J., Vie, A., Piechaczyk, M., Bonnieu, A., Jeanteur, P. & Blanchard, J.-M. (1987) *Nucleic Acids Res.* **15**, 5657–5667.
27. Middleton, K. M. & Morgan, G. T. (1990) *Mol. Cell. Biol.* **10**, 727–735.
28. Chen, Z., Harless, M. L., Wright, D. A. & Kellems, R. E. (1990) *Mol. Cell. Biol.* **10**, 4555–4564.
29. Ramamurthy, V., Maa, M.-C., Harless, M. L., Wright, D. A. & Kellems, R. E. (1990) *Mol. Cell. Biol.* **10**, 1484–1491.
30. Platt, T. (1986) *Annu. Rev. Biol.* **55**, 339–372.
31. Cullen, B. R., Lemedico, P. T. & Ju, G. (1984) *Nature (London)* **307**, 241–245.
32. Proudfoot, N. J. (1986) *Nature (London)* **322**, 562–565.
33. McStay, B. & Reeder, R. H. (1990) *Genes Dev.* **4**, 1240–1251.
34. Hann, S. R., King, M. W., Bentley, D. L., Anderson, C. W. & Eisenman, R. N. (1988) *Cell* **52**, 185–195.
35. Kuhn, A., Bartsch, I. & Grummt, I. (1990) *Nature (London)* **344**, 559–562.
36. Pfleiderer, C., Smid, A., Bartsch, I. & Grummt, I. (1990) *Nucleic Acids Res.* **18**, 4727–4736.
37. McStay, B. & Reeder, R. H. (1990) *Mol. Cell. Biol.* **10**, 2793–2800.
38. Neuman de Vegvar, H. E., Lund, E. & Dahlberg, J. E. (1986) *Cell* **47**, 259–266.
39. Hernandez, N. & Lucito, R. (1988) *EMBO J.* **7**, 3125–3134.
40. Grayhack, E. J., Yang, X., Lau, L. F. & Roberts, J. W. (1985) *Cell* **42**, 259–269.
41. Barik, S., Ghosh, B., Whalen, W., Lazinski, D. & Das, A. (1987) *Cell* **50**, 885–899.
42. Horwitz, R. J., Li, J. & Greenblatt, J. (1987) *Cell* **51**, 631–641.
43. Gottlieb, E. & Steitz, J. A. (1989) *EMBO J.* **8**, 851–861.
44. Gottlieb, E. & Steitz, J. A. (1989) *EMBO J.* **8**, 841–850.
45. Lazinski, D., Grzadzielska, E. & Das, A. (1989) *Cell* **59**, 207–218.
46. Faus, I. & Richardson, J. P. (1990) *J. Mol. Biol.* **212**, 53–66.
47. Spandidos, D. A. & Paul, J. (1982) *EMBO J.* **1**, 15–20.