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Transcription of three c-*myc* exons is enhanced in chicken bursal lymphoma cell lines

(*onc* gene/cellular transformation/enhancer sequence/viral long terminal repeat)

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ABSTRACT **The chicken c-*myc* gene, as defined by its homology to the v-*myc* gene of MC29 virus, is comprised of two exons. Using the techniques of runoff transcription, primer extension, and S1 nuclease protection, we demonstrate that there is a third c-*myc* exon of ≈345 base pairs (bp) located 0.7 kbp upstream of the 5′ end of the v-*myc* homology. This first exon is transcribed and present in *myc* mRNA in normal chicken cells. We also examined RNA from five cell lines derived from avian leukosis virus-induced bursal lymphomas. In all these lines, the level of transcription of the 2.2- to 2.5-kbp *myc* mRNA is increased 30- to 60-fold over normal cells. The *myc* mRNA in four of these lines also contains increased levels of the first noncoding exon, and evidence is presented that the long terminal repeat (LTR) in the vicinity of c-*myc* is functioning as an enhancer of c-*myc* transcription rather than as a promoter in several of these cell lines. In two cell lines in which the viral LTR has integrated between the first and second exons in the proper orientation for downstream promotion of *myc*, the LTR does not exhibit promoter function. The pattern of c-*myc* transcription observed by others in a vast majority of avian leukosis virus-induced neoplasms is not observed in any of the five cell lines examined.**

Activation of the c-*myc* locus is correlated with malignant transformation in a variety of diseases including avian leukosis virus (ALV)-induced bursal lymphomas of chickens (1–3), murine plasmacytomas, and Burkitt lymphomas in humans (4–11). In the murine and human cases, chromosomal translocations have occurred in the vicinity of the c-*myc* locus, whereas in the avian bursal lymphomas, activation appears to involve insertion of viral sequences, including the viral long terminal repeat (LTR) promoter region near c-*myc*. In a majority of the bursal tumors examined, insertion of the viral LTR leads to formation of RNA molecules containing both viral LTR U5 and *myc* sequences. The existence of such hybrid *myc* RNAs, which are present in higher amounts than c-*myc* RNA in normal tissue (1, 2, 12), has led to the hypothesis of promoter insertion and downstream promotion to explain the genesis of bursal lymphomas (1).

The c-*myc* oncogene has been defined by virtue of its homology with the v-*myc* transforming gene of the avian acute transforming virus MC29 (13–15, 16). The region of the chicken c-*myc* gene homologous to MC29 v-*myc* consists of two exons of 707 and 861 base pairs (bp), separated by an intron of 970 bp (17, 18). However, the size of the c-*myc* message (2.2–2.5 kbp) is ≈0.7 kbp larger than the MC29 defined coding sequence. In both humans and mice, evidence for a third, non-coding exon has been presented (10, 19). Additional transcribed sequences upstream of the two MC29-defined coding exons have also been reported in chickens (20). In most murine plasmacytomas, the rearrangement of the c-*myc* locus and the constant region of the immunoglobulin heavy chain has occurred such that the first upstream exon has been deleted from the region (4, 5). It has been suggested that, in mice, the non-coding exon might contain a control region that inhibits translation of the c-*myc* protein in normal cells (19). However, the correlation between the absence of the non-coding exon and transformation is not seen in many Burkitt lymphoma cells. Translocations in Burkitt lymphomas often do not remove part of the c-*myc* region (6–8, 10), and the c-*myc* RNAs in tumor and normal tissues are similar in size and appear to contain sequences from the upstream exon (9).

In the majority of ALV-induced bursal lymphomas thus far examined, the presence of hybrid v-*myc*–c-*myc* RNA molecules and the transcriptional orientation of the newly integrated LTRs are consistent with the promoter insertion model proposed by Hayward *et al.* (1). However, examples of primary tumors containing viral promoters in the opposite transcriptional orientation, or downstream of c-*myc*, have been reported (2). In addition, in some long term cell lines derived from bursal lymphomas, hybrid v-*myc* RNAs are not present (21). These cases suggest that the viral LTR can also activate or alter transcription of cellular sequences without initiating transcription at the viral promoter site—i.e., that the LTR can function as an enhancer (22, 23). The finding that major alterations in chromatin structure of the c-*myc* region are associated with viral integration into this region is also compatible with the view that these viral sequences serve functions other than transcriptional initiation (24).

In this report, we present evidence that the c-*myc* locus in normal chicken cells is transcribed into an RNA containing ≈345 bases that are not present in the genome of MC29 virus. The cap site of the first exon has been mapped to a location 1048 bp upstream from the second exon. These nontranslated sequences are present in the poly(A)$^+$ RNA in four of five bursal lymphoma cell lines examined. In two of these lines, the viral LTR is integrated downstream from the location of the third exon. These data lend credence to the idea that the viral LTR can function solely as an enhancer of the c-*myc* locus.

MATERIALS AND METHODS

Cell Lines. The origin of the RP9, 1104HI(HI), BK25, and BK3A cell lines has been described (21, 24–26). The haploid *myc* line S13 was cloned from the diploid *myc* line 1104BI (25), which appears to be a mixture of cells of the HI and S13 phenotypes. MH2 P100$^+$ cells have been described (27, 28). MSB is a Marek virus immortalized chicken T-cell line (29).

c-*myc* Clones. An 8.2-kbp *Eco*RI/*Hin*dIII fragment obtained from a λ c-*myc* clone (24) was subcloned into PBR322. Specific fragments depicted in Fig. 2 were then subcloned into M13 phages mp8, -10, or -11 (30). The 5′ LTR (5′), and

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Abbreviations: LTR, long terminal repeat; ALV, avian leukosis virus; bp, base pair(s).

c-*myc* exon 1, 2, and 3 probes are described in ref. 22 and in Figs. 1, 2, and 4.

Primer Extension and S1 Nuclease Assays. The end-labeled 24-bp *Ava* II/*Bgl* I primer (bp 1230–1254 in ref. 20) was a gift of S. McKnight. Primer extension experiments were performed as described in the legend to Fig. 4 and in ref. 31. For S1 nuclease protection experiments an M13 clone containing the 1.0-kbp *Sma* I fragment (as shown in Fig. 1*A*) was uniformly labeled with [^{32}P]dCTP and [^{32}P]TTP, and the purified fragment was hybridized to poly(A)$^+$ RNA for 16 hr at 65°C in buffer containing 80% formamide, after heating to 80°C for 5 min. Reaction mixtures were then treated with S1 nuclease and analyzed using standard procedures (32).

Nuclear Runoff Transcription Assays. Transcription assays were done as described (33), except that single-stranded M13 clones containing the inserts detailed in Fig. 1*A* were used.

RNA Preparation and Analyses. RNA was extracted, poly(A)$^+$-containing RNA was selected, and RNA blot analysis was performed as described (21, 28). Thymic lymphocytes were prepared by scraping the thymic stroma with a scalpel, and thymic RNA was prepared from this lymphocytic cell-enriched population. For dot blot analyses, poly(A)$^+$ RNA was serially diluted (1:4 or 1:5), denatured in 7.4% formaldehyde, and then applied in 15× NaCl/Cit to a nitrocellulose filter previously wetted with H_2O and soaked in 15× NaCl/Cit, essentially as described (34) (1× NaCl/Cit = 0.15 M NaCl/0.015 M Na citrate).

RESULTS

Mapping of c-*myc* Transcripts in Bursal Lymphoma Cell Lines and Normal Chicken Cells. To determine the region of the c-*myc* transcriptional start site in chicken cells, runoff transcription experiments were performed. While the v-*myc* or MC29 homology begins about 100 bp 5′ of the *Pst* I site, as depicted in Fig. 1*A*, by analogy with mouse and human c-*myc* (10, 19), it was expected that there would be an additional upstream exon. Nuclear runoff transcription studies were performed using nuclei from normal chicken thymus cells as well as the bursal lymphoma-derived cell lines HI, S13, and BK25 (Fig. 1*B*), which contain only the LTR associated c-*myc* allele. As described previously (33), this technique measures the presence of elongating nascent RNAs along a specific gene. Isolated nuclei are incubated with [^{32}P]UTP for 5–20 min, and the resultant ^{32}P-labeled nascent RNA is hybridized to DNA bound to nitrocellulose. In these studies, single-stranded M13 clones extending ≈6 kbp upstream from the 5′ MC29 exon, in both orientations (Fig. 2*A*), were hybridized to [^{32}P]RNA synthesized in isolated nuclei. We found (Fig. 1*B*) that all the ^{32}P-labeled nascent RNAs hybridized well to the 3.2-kbp *Pst* I fragment containing the first 120 bases of the 5′ coding exon, or to the exon 2 *myc* probe. None of the RNA samples hybridized to any DNA sequences upstream of the 1.0-kbp *Sma* I fragment. However, transcription from this *Sma* I 1.0-kbp region was detected in all the cells tested, albeit weakly in the case of HI and thymus cells. In S13 and BK25 cells, we also analyzed transcription from a downstream 0.4-kbp *Sma* I fragment, which is probably part of an intron (20), and an upstream *Pst* I/*Sma* I 1.3-kbp fragment. While a significant signal was detected from the 0.4-kbp *Sma* I fragment (reflecting a lack of processing in the *in vitro* nuclear runoff assay), no transcription from the upstream *Pst* I/*Sma* I 1.3-kbp region was observed. Since this latter fragment is immediately 5′ to the *Sma* I 1.0-kbp fragment, we conclude that c-*myc* transcription is initiated within the *Sma* I 1.0-kbp region.

The finding that transcription in cell line BK25 initiated within the 1.0-kbp *Sma* I fragment was unexpected, because the LTR integration site near c-*myc* in this cell line is just upstream of the *Pst* I site (at ≈0.2 kbp in Fig. 1*A*) and in the same transcriptional orientation as c-*myc* (21, 24). Thus, the LTR would be expected to behave as a transcriptional promoter in this case, with transcription initiating within the LTR. To confirm the nature of the BK25 transcript, and the size and content of c-*myc* transcripts in the other cell lines, RNA blot and RNA dot blot analyses were performed (Fig. 2). For the upstream probe, a double-stranded probe for the *Sac* II 1.2-kbp fragment was used (Fig. 1*A*). The normal chicken c-*myc* 2.4-kbp mRNA hybridizes to the 1.2-kbp *Sac* II (first exon) probe as has been shown (20). In RP9 cells, a complex pattern of c-*myc* RNAs is seen; we have previously shown that only the 2.6-kbp mRNA encodes the *myc* protein (21). The 2.6-kbp RNA contains U5 LTR and exon 2 and 3 sequences (Fig. 2*A*), but it lacks exon 1 sequences (data not shown). In S13 cells, the LTR near c-*myc* is upstream and in the opposite transcriptional orientation (24). In this line, the major 2.4-kbp *myc* mRNA is identical in size to that of normal c-*myc* and contains sequences from all three exons but lacks LTR sequences (Fig. 2*A*; unpublished data). Lines BK25 and BK3A both contain LTRs slightly upstream of exon 2 in the same transcriptional orientation as c-*myc* (24). In BK25 cells, no normal c-*myc* allele is present; in BK3A cells, a normal c-*myc* allele is retained. However, transcription of all three c-*myc* exons is greatly increased in BK3A compared to that seen in normal cells (data not shown), and this increased transcription is presumed to be from the allele that contains a viral LTR. As shown in Fig. 2*A*, the c-*myc* mRNA in BK25 and BK3A cells is identical in size to that of

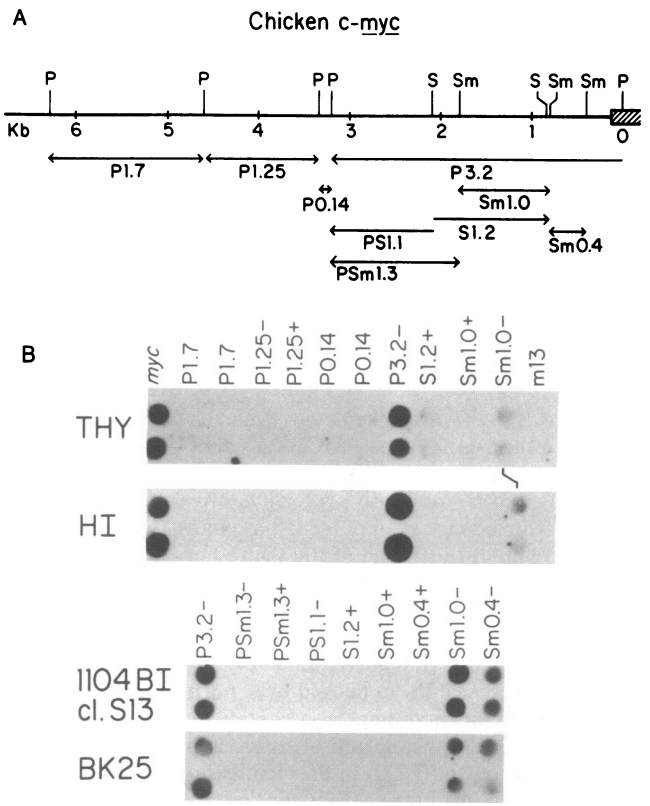

FIG. 1. Hybridization of nuclear runoff transcripts to M13 c-*myc* clones. (*A*) Summary of clones used. Arrow indicates orientation of *myc* insert; hatched box denotes extent of v-*myc* homology. In two cases, P1.7 and P0.14, the orientation was not determined. It has not been possible to clone the P3.2 fragment or any large fragment from this region in the opposite orientation because of its instability in a variety of M13 vectors. (*B*) Hybridization of runoff products to clones depicted in *A*; 1-μg duplicates of indicated clones were dotted onto nitrocellulose filters. In all cases, hybridizations were expected to be in DNA excess. The *myc* probe was a 118-base *Sst* I/*Hin*cII fragment from the 5′ coding exon described in Fig. 3. (−) indicates clones that are complementary to *myc* mRNA.

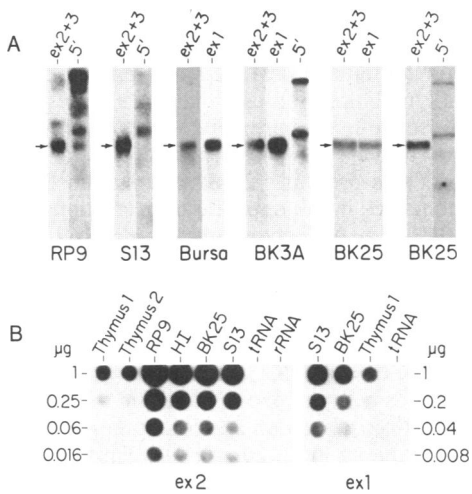

FIG. 2. RNA blot and RNA dot blot analysis of c-*myc* transcripts. (*A*) Poly(A)$^+$ RNA (2 μg) from indicated cell lines and 10 μg from bursal lymphocytes were analyzed by RNA blot technique as described (21). The same blots were hybridized to the 3.2-kbp *Sst* I probe containing c-*myc* coding exons 2 and 3 (ex2+3), the 1.2-kbp *Sac* II probe (Fig. 1*A*) containing c-*myc* non-coding exon 1 (ex1), or the 5′ LTR probes. In the case of BK25, two separate blots were used as indicated. The U5 LTR probe hybridizes to full-length viral RNA (8.2 kbp) and *env* mRNA (3.6 kbp) as well as to other uncharacterized mRNAs. Arrow indicates location of the major c-*myc* mRNA. Given the differences in specific activities of the probes and times of exposure of the blots to film, no quantitative conclusions can be drawn from this figure. However, analysis of the original films shows that the content of exon 1 and exon 2 and 3 sequences is greater in all the cell lines as compared to normal cells. (*B*) Dot blot analysis was performed as described in *Materials and Methods* and ref. 33, using poly(A)$^+$ RNAs.

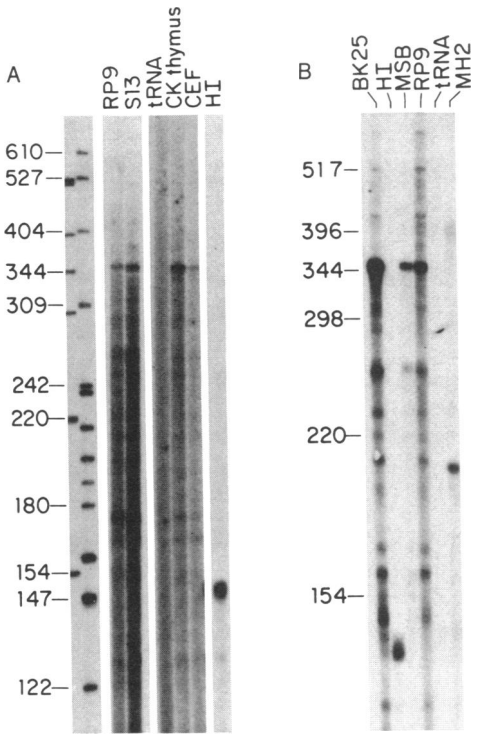

FIG. 3. S1 nuclease protection of 1.0-kbp *Sma* I fragment. Poly(A)$^+$ RNA from the indicated types was hybridized to uniformly ^{32}P-labeled DNA, and hybrids were treated with S1 nuclease and analyzed on 6% polyacrylamide denaturing gels. (*A*) Amounts of RNA used are as follows: RP9, S13, HI, and thymus, 10–11 μg; fibroblasts, 23 μg; *Escherichia coli* tRNA, 30 μg. Exposure of tRNA, thymus, and fibroblast in *A* is 3 times that of RP9, S13, and HI. (*B*) Amounts of RNA used are as follows: BK25, HI, MSB, RP9, and MH2, 5 μg; calf liver tRNA, 35 μg.

normal cells, and like normal c-*myc*, contains sequences of exons 1, 2, and 3, but no U5 sequences. Thus, the RNA blot data confirm the runoff transcription data for line BK25.

Dot blot analysis (Fig. 2*B*) confirms that BK25 cells have increased transcription of both exons 1 and 2, relative to that observed for normal thymus. This, and the fact that only a single BK25 c-*myc* RNA band is detected on gels, strongly suggests that all the BK25 c-*myc* RNA contains exon 1.

Mapping of the First Chicken c-*myc* Exon. To delineate the extent of the first c-*myc* exon, S1 nuclease protection experiments were done using a uniformly labeled 1.0-kbp *Sma* I fragment in which c-*myc* transcription initiates (Fig. 1). Fig. 3 shows the results of this analysis. In cells with unrearranged *myc* loci, normal chicken thymus cells or embryo fibroblasts (Fig. 3*A*) or MSB cells (Fig. 3*B*), a major fragment of ≈345 bases is seen. The same major protected fragment is also detected in RP9 and S13 cells. It is surprising that RP9 mRNA protects the same number of bases of exon 1 as does normal cell RNA, which suggests that there are transcripts initiating at the normal c-*myc* promoter. These transcripts could arise from either the normal c-*myc* allele in RP9 cells or from LTR-mediated enhancement of the rearranged allele. The large amount of bona fide exon 1 makes it most likely that the LTR is functioning as both an enhancer and a promoter of the same allele in these cells. Poly(A)$^+$ RNA from line BK25 (Fig. 3*B*) protects the identical 345-base fragment as normal cells, indicating that identical exon 1 sequences are transcribed. In all these cases (most evident in the MSB lane in Fig. 3*B*) a second fragment of ≈260 bases is seen that could represent transcription from a secondary downstream promoter. In HI cells, the 345-base fragment is not protected; instead a 145-base fragment is seen. This is consistent with integration of a LTR within the first exon, a situation deduced from restriction mapping (21, 24). Furthermore, the lack of the 345-base fragment in HI cells indicates that protection of this fragment in other tumor cells and in normal cells is not an artefact caused by a G+C-rich region. MH2 virus-infected quail cells were also examined. The MH2 genome contains the c-*myc* exons 2 and 3, but not exon 1 (35). Thus the 210-base fragment protected in these cells is due to hybridization of the chicken exon 1 sequences to homologous sequences in Japanese quail cells, and it indicates conservation of this region between those two species. It is likely that the entire first exon is contained within the 1.0-kbp *Sma* I fragment, because we have found no sequences protected in the immediate 3′ 0.4-kbp *Sma* I fragment as shown in Fig. 1*A* (unpublished data). This is consistent with the hybridization data of Shih *et al.* (20).

From the sequence of the c-*myc* region and the tentative location of the first exon (Fig. 1; ref. 20), a primer that is located about 30 bp downstream from a likely "TATA" box was obtained by restriction enzyme digestion. This primer was used for primer extension studies using RNA from the chicken T-cell line MSB in which the c-*myc* region is not rearranged (24), and also from *Xenopus laevis* oocytes microinjected with the 1.0-kbp *Sma* I fragment containing this TATA sequence. The results are shown in Fig. 4. A cluster of four start sites is seen that is identical in both *Xenopus* oocytes and chicken cells. This is not likely to represent a strong stop for reverse transcriptase, because RNA from RP9 cells extended the primer past these sites (data not shown). The sequence of this region of c-*myc* is shown in Fig. 4*B* and its location relative to the rest of chicken c-*myc* is shown in Fig. 4*C*. It is interesting to note that the hexanucleotide C-C-G-C-C-C, which is necessary for RNA transcription in *Xenopus* oocytes (16), is present three times upstream of the T-T-T-A-A-A promoter. This sequence is also

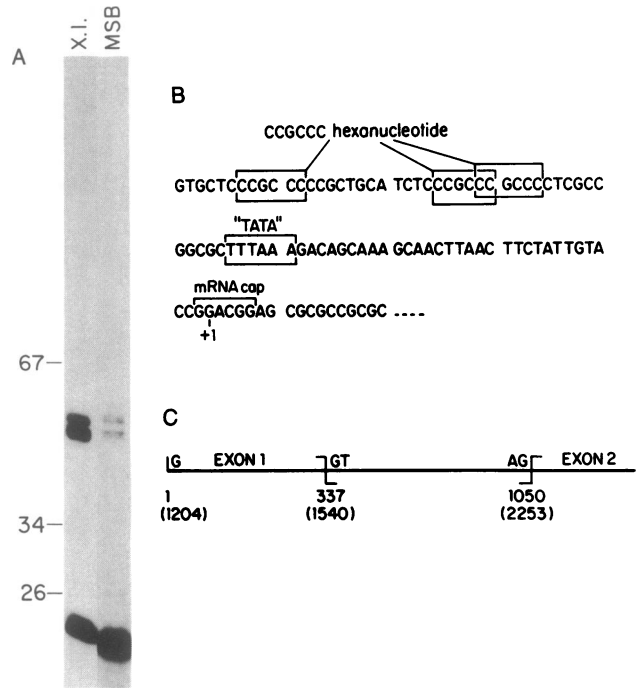

FIG. 4. Analysis of c-*myc* transcriptional start site using primer extension. (*A*) A single-stranded primer from an *Ava* II/*Bgl* I restriction digestion of the *Sma* I 1.0-kbp fragment (Fig. 1*A*) cloned in pUC-9 (pUCSml*myc*) and labeled at the 5′ end with [^{32}P]TTP and [^{32}P]dCTP was hybridized to 10 μg of poly(A)$^+$ RNA extracted from MSB cells or 5 μg of RNA extracted from *Xenopus laevis* oocytes microinjected with pUCSml*myc*. Microinjection, RNA extraction, hybridization, and analysis of the product are described in refs. 30 and 36. (*B*) Location of the cap site for c-*myc* mRNA synthesis, using the sequence from ref. 20. (*C*) Location of the first chicken c-*myc* exon. Numbers in parentheses refer to numbering of sequence in ref. 20.

present in the 21-bp repeat region of the simian virus 40 early promoter and has been shown to be important in the binding of a transcription factor (37).

DISCUSSION

We have analyzed the transcriptional products in normal chicken cells and in five bursal lymphoma lines in which viral LTR sequences have integrated in the proximity of the c-*myc* locus. In three of these lines, there are no viral LTR sequences associated with the c-*myc* mRNA (ref. 21; Fig. 1). In all five of these lines, there is a 30- to 60-fold increase in the amount of poly(A)$^+$ *myc* RNA compared to the amount of c-*myc* poly(A)$^+$ RNA in normal tissues. We have also shown that in normal cells, c-*myc* RNA contains at least 345 bases not present in the sequence of the MC29 v-*myc* gene (17). The start of c-*myc* transcription has been located 1048 bases upstream from the end of the MC29 homology (Fig. 4). This additional exon does not encode a portion of the myc protein, as it contains no open reading frame (20). A hypothesis has been advanced that the first exon in mammalian cell c-*myc* might prevent efficient translation at the AUG codon 4–600 bases downstream, either by the large distance between the transcriptional start site and the cap site, or by imposition of secondary structure (19, 38). Although the potential for secondary structure exists in chicken c-*myc* (20), the fact that the RNA in many bursal lymphoma cell lines contains all three exons argues against this model for chicken cells.

The promoter insertion model of oncogenesis was originally proposed by Hayward *et al.* (1) based on results obtained from analysis of primary bursal lymphomas. Hayward *et al.* (1) found, as did Payne *et al.* (2), that a vast majority of bursal tumors induced by ALV synthesized increased levels of myc RNAs containing viral LTR sequences. Our data with the bursal lymphoma cell lines that have been maintained in culture for several years are not consistent with this finding. A summary of our results is presented in Fig. 5. In line S13, the viral LTR is present ≈2.7 kbp upstream from the *myc* coding sequences in the opposite transcriptional orientation (24). S13 RNA, as expected, contains no viral promoter sequences, but it does contain the upstream non-coding sequences. Since the level of c-*myc* transcription is elevated in these cells relative to normal chicken cells, the LTR appears to be acting as an enhancer of transcription. More surprising, perhaps, is the case of BK25 and BK3A cells. In these lines, the viral LTR is between exons 1 and 2 in the same transcriptional orientation, a position and orientation seen in a majority of bursal tumors (1, 2, 20, 40, 41). Thus, the LTR could behave as a transcriptional promoter. However, we have shown that no viral LTR U5 sequences are present in BK25 or BK3A RNAs (Fig. 2). Furthermore, these cells contain increased amounts of the first exon (Figs. 1, 2*B*, and 3), which is located upstream of the site of viral LTR integration. Thus, in these cases as well, the viral LTR appears to be acting as an enhancer. Since the original tumor tissues are not available for comparison, we do not know whether this represents a switch in LTR function after establishment of the cell line, or whether the LTR acted as an enhancer in the original tumor. In RP9 cells that encode a complex array of LTR myc RNAs, our data suggest that the viral LTR is acting as a promoter, with the viral LTR sequences spliced to

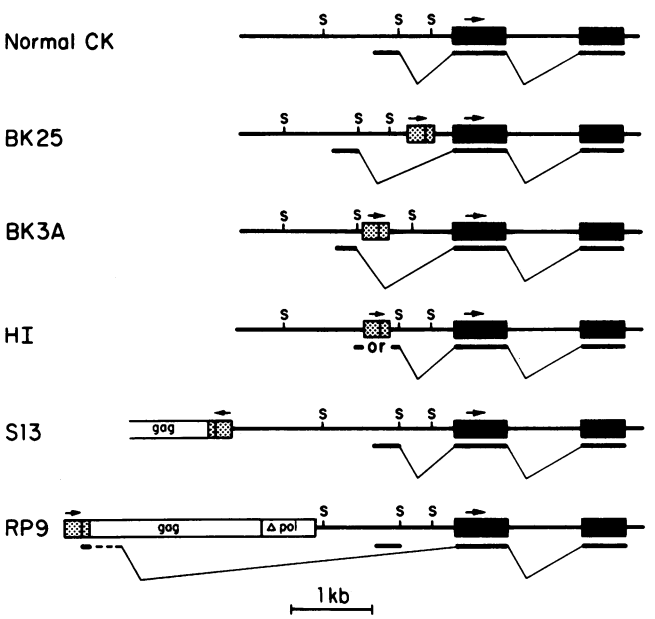

FIG. 5. Summary of transcriptional mapping data. *Sma* I sites of interest are denoted by S on the map of c-*myc* DNA. Black boxes indicate the MC29 defined exons that encode the *myc* protein; stippled boxes indicate ALV LTRs as previously mapped (21, 22); white boxes denote the rest of the proviral genome present in S13 and RP9 lines; broken line in RP9 denotes the possible use of the splice donor site in p19 of the *gag* gene (39). The 3′ terminus of the S13 provirus is not known, although there appear to be at least some envelope gene sequences present (unpublished data). RNA transcripts are depicted under the DNA map; solid lines represent exons in the c-*myc* mRNA and thin lines represent sequences that are spliced out in the major *myc* mRNA. In the case of the RP9 cells, only the 2.5-kbp mRNA is considered; the large 5′ *gag–myc* mRNAs previously seen are not responsible for encoding the *myc* protein (21). The two alternative transcripts in HI cells are shown. Arrows indicate the direction of transcription of *myc* and viral sequences.

the two c-*myc* coding exons with the elimination of the three introns, as well as the first exon, from the 2.6-kbp RNA. In HI cells, the LTR integration has occurred within the first *myc* exon, and the LTR could be acting as a promoter for a portion of the first exon. However, we have not been able to unequivocally demonstrate U5 sequences on the HI *myc* mRNA (ref. 21; data not shown). Thus, primer extension and/or sequence data will be required to determine the RNA structure in this cell line.

Our data indicate that maintenance of the LTR promoter function is not required for maintenance of the transformed phenotype in tissue culture. However, since increased levels of c-*myc* RNA (Fig. 2*B*) and protein (ref. 16; S. Hann and R. Eisenman, personal communication) are maintained in cell lines, the enhancer function of the LTR is retained. These results could indicate that the selective advantage conferred by LTR insertion near c-*myc* is the enhancement of c-*myc* transcription, regardless of which promoter (LTR or c-*myc*) is used. In this context, the enhancement of c-*myc* transcription via the LTR promoter in most primary tumors and some cell lines would be the reflection of the proximity of this functional promoter to the LTR enhancer. The loss of downstream promoter function and the enhancement of transcription from the normal c-*myc* promoter in the cell lines examined in our analysis could be due to alterations in the LTR promoter resulting from continued passage of these cells. Thus, under these circumstances, the normal c-*myc* promoter would be the most proximal functional promoter to the LTR enhancer, and the enhanced transcription from this promoter would continue to confer selective advantage to these cells. Alternatively, use of the viral promoter might be incompatible with sustained growth *in vitro* either because of the possible toxicity associated with increased levels of LTR promoted c-*myc* transcripts or the lack of normal cellular regulation of the LTR compared to the c-*myc* promoter. Thus, tissue culture might select for cells in which the LTR promoter has mutated. To distinguish between these alternatives, it will be important to directly compare c-*myc* transcription and LTR sequences in primary ALV-induced tumors and in cell lines derived from these tumors.

Note Added in Proof. Recent analysis of the BK25 c-*myc* locus using a plasmid clone of this region (obtained from W. Schubach) reveals that ≈4 kb of non-viral DNA has been inserted between c-*myc* exons 1 and 2 in both the DNA clone and BK25 cellular DNA. Thus, in the cell line, the viral enhancer is acting on the c-*myc* promoter over a distance of ≈5 kb. Therefore, in the BK25 line of Fig. 5, the first c-*myc* exon should be displaced ≈5 kb to the left of the second exon.

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