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Cellular RNA Homologous to the Abelson Murine Leukemia Virus Transforming Gene: Expression and Relationship to the Viral Sequence

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To examine the expression of the cellular homolog of the Abelson murine leukemia virus transforming gene (the v-*abl* sequence), a DNA probe representing the v-*abl* sequence was prepared. The probe detected two cytoplasmic polyadenylic acid-containing c-*abl* RNAs of about 6.5 and 5.5 kilobases in a variety of rodent cells, and slightly larger RNAs were detected in human cells. These two RNA species were found in all normal tissues or cell lines examined, but at differing concentrations: liver cells had the least, fibroblastic cell lines had the most. By using a probe able to detect the cellular but not the viral gene, the two RNAs were shown to be present in Abelson murine leukemia virus-transformed cells at levels found either in their untransformed counterparts or in similar cell types transformed by other means. The target cells of the virus have a somewhat elevated level of the two RNAs although expression of the c-*abl* gene is not restricted to these cells. The v-*abl* sequence lacks 0.35 and 0.85 kilobases of the c-*abl* RNA on the 5′ and 3′ ends, respectively. Thus, the Abelson murine leukemia virus transforming gene is an internal fragment of the transcript of a normal cellular gene.

Abelson murine leukemia virus (A-MuLV) has a hybrid genome composed of Moloney MuLV (M-MuLV) terminal sequences surrounding an internal sequence (v-*abl*) derived from a region of the normal mouse DNA (c-*abl*) (8, 17). The virus is similar in structure to many other transforming retroviruses (18). The v-*abl* sequence is linked in phase to the *gag* gene of M-MuLV, with terminal viral sequences providing the necessary signals for a high-level expression of the *gag*/v-*abl* fusion protein. The fusion protein is the only protein product of the A-MuLV genome and is responsible for the transforming activity of the virus (21).

The c-*abl* sequence homologous to the 4.3-kilobase (kb) v-*abl* is distributed along 30 kb of mouse DNA as what appears to be a series of short exons separated by long introns (8; J. Y. J. Wang, S. P. Goff, F. Ledley, Y. Groner, and D. Baltimore, manuscript in preparation). That the c-*abl* sequence represents exons is supported by the findings of polyadenylic acid [poly(A)]-containing RNA homologous to v-*abl* in various mammalian cells. Studies of the expression of c-*abl* RNA during murine embryogenesis (11) or in human tumors (6, 13) have been reported. We prepared a full-length v-*abl* probe and used it to examine c-*abl* RNA in mouse tissues and cell lines with an emphasis on lymphoid cells. The expression of c-*abl* RNA in A-MuLV-transformed cells was also examined. In addition, we have analyzed the structural relationship between the v-*abl* sequence and c-*abl* RNA.

MATERIALS AND METHODS

Construction of pABlsub9 probe. The plasmid pAB1 (Fig. 1) was digested with *Kpn*1 which cut the plasmid at a unique site in the long terminal repeated portion of the viral genome. The linearized molecule was treated with a predetermined amount of *Bal*31 nuclease and was then blunt-end ligated. *Escherichia coli* HB101 was transformed with the total ligated DNA, and the ampicillin-resistant colonies were hybridized with an M-MuLV DNA probe. Plasmid DNA was prepared from colonies that did not hybridize to the M-MuLV probe. From these plasmids, pAB1sub9 was chosen because of its restriction enzyme digestion pattern and because it hybridized well to A-MuLV DNA but poorly to M-MuLV DNA. The presence of a small amount of homology to M-MuLV ensured that this plasmid retained the complete 5′ end of the v-*abl* sequence. Its content of M-MuLV sequence was so short that it reacted with total cellular RNA and genomic DNA as a v-*abl*-specific probe.

Preparation of cellular RNA. Total cellular RNA was prepared from an 8 M guanidine-hydrochloride lysate of cells by repeated precipitation with 33% ethanol, as described by Deeley et al. (5). Cytoplasmic RNA was isolated by lysing the cells in buffer containing 10 mM NaCl, 10 mM Tris-hydrochloride (pH 7.4), 10 mM

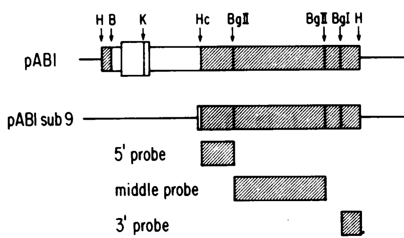


FIG. 1. v-*abl* probes used for the detection of homologous cellular RNA. Plasmid pAB1 is a permuted clone of the A-MuLV genome (8). Plasmid pAB1sub9 was derived from pAB1 as described in the text. The 5′ probe, middle probe, and 3′ probe were fragments released by restriction enzyme digestion of pAB1sub9 and were prepared by preparative gel electrophoresis. The clear area represents sequences derived from M-MuLV, the cross-hatched area represents the A-MuLV specific sequences, and the thin line represents pBR322 sequences. Restriction enzymes: H, *Hin*dIII; B, *Bam*HI; K, *Kpn*I; Hc, *Hin*cII; BgI, *Bgl*I; BgII, *Bgl*II.

$MgCl_2$, 0.5% Nonidet P-40, 0.5% deoxycholate, 500 μg of heparin per ml, and 500 μg of spermidine per ml. After 10 strokes with a Dounce homogenizer, nuclei were removed by centrifugation, and RNA was precipitated with 66% ethanol. The pellet was then dissolved in the 8 M guanidine-hydrochloride buffer, and repeated precipitation with 33% ethanol was carried out.

After the final 33% ethanol precipitation step, the RNA pellet was dissolved in 20 mM EDTA (pH 7.4) and extracted three times with chloroform-isobutanol (4:1). The extracted RNA was dissolved in 10 mM Tris-hydrochloride (pH 7.4)–0.5% sodium dodecyl sulfate–1 mM EDTA plus 400 mM NaCl at a concentration so that the absorbance at 260 nm of the solution was 10. Poly(A)-containing RNA was then selected by chromatography on oligodeoxythymidylic acid-cellulose. Those RNAs that were bound to oligodeoxythymidylic acid-cellulose at 400 mM NaCl and eluted with the same buffer minus NaCl were used in the hybridization experiments.

RNA hybridizations. Poly(A)-containing RNA was denatured in 10 mM methylmercury and applied to a 1% agarose gel in 5 mM methylmercury-containing E buffer (50 mM boric acid, 5 mM $Na_2B_4O_7 \cdot 10H_2O$, 10 mM sodium sulfate, 1 mM EDTA [pH 8.2]). After electrophoresis (100 V, 6 h), the RNA was transferred onto diazobenzyloxymethyl-paper (DBM-paper) as described (2).

The DBM-paper was hybridized with ^{32}P-labeled probes (2×10^8 to 6×10^8 cpm/μg) by using dextran sulfate according to the method of Wahl et al. (19). To reuse the blot, labeled probe was washed off in 99% formamide–0.1% sodium dodecyl sulfate at 65°C.

Quantitative dot-blot hybridization. A given quantity of poly(A)-containing RNA in 1 μl was mixed with 1 μl of 100 mM NaOH at room temperature for 2 min and then neutralized with 1 μl of 100 mM HCl. The sample was diluted to 10 μl with 0.2 M sodium acetate (pH 4.5) and spotted in 2-μl aliquots onto freshly activated DBM-paper. Hybridization with ^{32}P-labeled probes was as described above. As a standard for comparison, poly(A)-containing RNA from ANN-1 cells (an A-MuLV-transformed fibroblastic line) or 2M3 cells (an A-MuLV-transformed pre-B cell line) was applied to the DBM-paper in 10 μl containing 0.1, 0.05, 0.02, 0.01, and 0.005 μg. As a negative control, 1 μg of poliovirus RNA was used. The other RNA samples were spotted on the same sheet of paper at given amounts. The intensity of hybridization was compared with the standards, and the level of the c-*abl* RNA as a percentage of the A-MuLV viral RNA was determined.

Primer extension. Restriction fragments of the v-*abl* region were prepared and labeled at their 5′-ends with [γ-^{32}P]ATP and polynucleotide kinase. The labeled DNA fragment plus 20 μg of poly(A)-containing RNA in 10 μl of water was boiled for 2 min and chilled immediately on dry ice. Hybridization was carried out in 80% (vol/vol) formamide (deionized and recrystallized)–0.4 M NaCl–0.01 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)-hydrochloride] (pH 6.4)–2 mM EDTA at 42°C for 22 h. The hybridization mixture was diluted with 200 μl of water and ethanol precipitated to remove formamide. The precipitate was dissolved in 100 μl of 50 mM Tris-hydrochloride (pH 8.3), 50 mM KCl, 10 mM dithiothreitol, 10 mM $MgCl_2$, 40 μg of actinomycin D per ml, 600 μM each of dATP, dGTP, dCTP, and dTTP, and 6 U of reverse transcriptase. After elongation of the primer for 1 h at 37°C, NaOH was added to 0.1 M and the RNA was hydrolyzed at 68°C for 10 min. The extended DNA was precipitated in ethanol, extracted with phenol, dissolved in 1.5 M glyoxal, and analyzed by electrophoresis through a 1.6% agarose gel.

RESULTS

c-*abl* RNA in mammalian cells. To examine cells for c-*abl* RNA, total poly(A)-containing RNA was prepared, size-fractionated by electrophoresis, transferred to derivatized paper, and hybridized to one of the variety of v-*abl* probes described in Fig. 1. When a probe for the whole v-*abl* region was used (pAB1sub9), two RNA species were detected in several rodent and human cell lines (Fig. 2). The RNAs were about 6.5 and 5.5 kb in rodent cells and 7.0 and 6.0 kb in human (HeLa) cells. Both RNAs were present in L-cell cytoplasmic RNA preparation (Fig. 2, lane B), showing that the larger is not a nuclear precursor of the smaller. All of the probes described in Fig. 1 hybridized to both RNAs, and the relative intensity of the two bands was constant (data not shown). These results demonstrate that the two RNAs are closely related, and they suggest that v-*abl* is derived from a single gene that gives rise to two different sizes of mRNA.

Distribution of c-*abl* RNA in various mouse cells. Twelve different types of cells have been examined for the expression of c-*abl* RNA. They include normal tissues and mouse cell lines. In all cases examined, c-*abl* RNA of 6.5 and 5.5 kb could be detected in approximately constant ratios, although the absolute amount of total c-

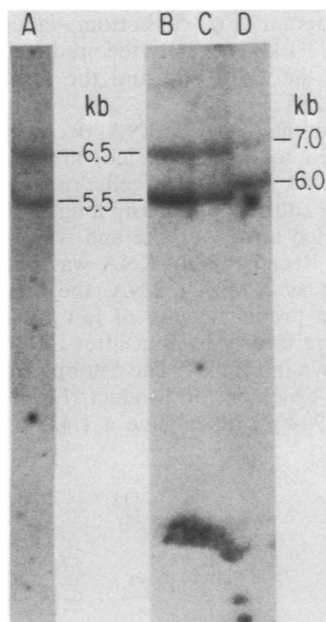


FIG. 2. c-*abl* RNA in mammalian cells. Poly(A)-containing RNA was prepared and analyzed by hybridization to ^{32}P-labeled pAB1sub9 as described in the text. Lanes: (A) 10 μg of total poly(A)-containing RNA from rat hepatoma Fao cell line; (B) 10 μg of cytoplasmic poly(A)-containing RNA from mouse L cells; (C) 10 μg of total poly(A)-containing RNA from mouse L cells; (D) 20 μg of total poly(A)-containing RNA from human HeLa cells.

abl RNA varied. The c-*abl* RNA content was quantitated by dot-blot hybridization by using varying amounts of RNA from A-MuLV-transformed cells as a standard for comparison (Table 1). The highest expression of c-*abl* RNA was found in established untransformed fibroblastic cell lines; its level there was almost 1/10 that of the v-*abl* RNA in transformed fibroblasts. A primary fibroblast culture from BALB/c mouse embryos was also found to contain this high level of c-*abl* RNA. The cell density of the cultures had no apparent effect on the level of c-*abl* RNA. Among lymphoid cells, normal thymocytes and a pre-B lymphoma line (70Z/3) were the highest producers of c-*abl* RNA, and a radiation-induced thymoma cell line, L691, had half the amount of c-*abl* RNA of normal thymocytes. Normal splenocytes from 5-week-old mice had sixfold less c-*abl* RNA than thymocytes from the same animals. In neonatal spleen, however, the level of c-*abl* RNA was comparable to that of thymocytes (11). It is not known how the c-*abl* RNA is distributed among the different cells making up thymocyte and splenocyte populations. Normal liver had the least amount of c-*abl* RNA, although a rat hepatoma cell line contained about fourfold more (Table 1).

Retroviral RNA is usually present as 1% of the total polyadenylated RNA (7). Because the level of c-*abl* RNA in fibroblasts, thymocytes, and a pre-B lymphoma was about 4 to 7% that of the viral RNA in an A-MuLV transformant (Table 1), the level of c-*abl* RNA in these cells is approximately 0.05% of the total poly(A)-containing RNA. The level of c-*abl* expression as a percentage of the total poly(A)-containing RNA in 13 samples is summarized in Table 1.

Expression of c-*abl* RNA in A-MuLV-transformed cells. A-MuLV transforms fibroblasts and pre-B lymphocytes in vitro (15, 16). Because both fibroblasts and a pre-B lymphoma line had relatively high levels of c-*abl* RNA, we examined whether c-*abl* RNA could be detected in A-MuLV-transformed fibroblasts and pre-B cells. A-MuLV RNA is of similar size as, and it is at least 10-fold more abundant than, the endogenous RNA, so that the v-*abl* probes used in earlier experiments could not be used to detect c-*abl* RNA in A-MuLV-transformed cells. A solution to this problem became available when the genome of another A-MuLV variant, A-MuLV(P160), was molecularly cloned (10). The A-MuLV(P160) genome contains 800 base pairs (bp) more sequence than the A-MuLV(P120) genome, which was used to prepare the

TABLE 1. Levels of c-*abl* RNA in mouse cells

Cell line	Cell type	c-*abl* as % of v-*abl*[a]	c-*abl* as % total poly(A)-RNA
NIH 3T3	Fibroblasts	7.0	0.07
BALB/3T3	Fibroblasts	7.0	0.07
L cells	Fibroblasts	4.0	0.04
	Normal thymocytes (5-week-old mice)	5.0	0.05
L691	Radiation-induced thymoma	2.0	0.02
70Z/3	Chemically induced pre-B lymphoma	4.0	0.04
MOPC-11	Myeloma	2.0	0.02
MOPC-104E	Myeloma	1.0	0.01
WEHI231	Chemically induced B-cell lymphoma	1.4	0.014
745PC4	Friend virus-induced erythroblastoid	2.0	0.02
	Normal splenocytes (5-week-old mice)	0.8	0.008
	Liver	0.4	0.004
Fao	Hepatoma (rat)	1.5	0.015

[a] Comparison of hybridization intensity between RNA samples containing c-*abl* RNA and those containing v-*abl* RNA on a dot-blot is described in the text. For example, 1.5 μg of poly(A)-containing RNA from NIH 3T3 fibroblasts gave the same intensity as 0.1 μg of poly(A)-containing RNA from ANN-1 or 2M3 cells. Thus, the c-*abl* RNA is 1/15 of the v-*abl* RNA, about 7%.

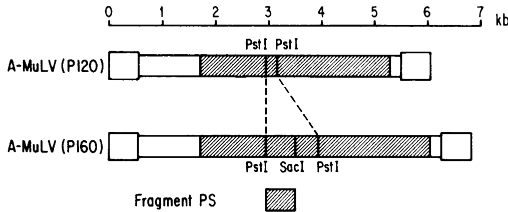


FIG. 3. DNA probe used for the detection of c-*abl* RNA in A-MuLV-transformed cells. The genome of A-MuLV(P160) is larger than that of A-MuLV(P120) by 800 bp located within the *Pst*I restriction fragment indicated in the Figure. Within the 800-bp sequence is a single *Sac*I site. The indicated restriction fragment PS of 550 bp was chosen because it did not hybridize to A-MuLV(P120) DNA but did hybridize to c-*abl* RNA.

pAB1sub9 probe. [A-MuLV(P120) is actually a deletion mutant of A-MuLV(P160).] As shown in Fig. 3, this 800-bp extra segment is located in a *Pst*I fragment in the v-*abl* region. A *Pst*I to *Sac*I fragment (PS) derived from this region was found to be specific to the A-MuLV(P160) genome in that it did not hybridize to A-MuLV(P120) DNA. Therefore, fragment PS could be used to detect c-*abl* RNA in cells transformed by the A-MuLV(P120) variant. When the pAB1sub9 and PS probes were hybridized to RNA from NIH 3T3 or 70Z/3 cells, both probes detected the same c-*abl* RNAs (Fig. 4, lanes 1 and 2). When RNA from A-MuLV(P120)-transformed lymphoid cells was hybridized with these probes, pAB1sub9 gave rise to the 5.5-kb viral RNA band (Fig. 4A, lanes 3, 4 and 6), but fragment PS detected the two c-*abl* RNAs (Fig. 4B, lanes 3, 4, and 6). An RNA sample from line 22D6, an A-MuLV(P160)-transformed line, was also included in the experiment to show that both probes hybridized to the 6.3-kb viral RNA of A-MuLV(P160) (Fig. 4, lanes 5). In fibroblasts transformed by A-MuLV(P120), the two c-*abl* RNAs were also detected. Thus, expression of the A-MuLV genome does not repress the synthesis of c-*abl* RNA.

Relationship between c-*abl* RNA and v-*abl* sequence. Because c-*abl* RNA is larger than the v-*abl* sequence, we examined whether the ends of the c-*abl* sequence were deleted during the formation of the A-MuLV genome by primer extension experiments. Three different restriction fragments from the 5′ portion of the v-*abl* sequence were used as primers for reverse transcriptase (Fig. 5). As a positive control, the primer labeled at the *Bgl*II site was hybridized to poly(A)-containing RNA from the A-MuLV-transformed cell line, ANN-1. The longest reverse-transcription product was 2.1 kb, as evidenced by two different exposures of the autoradiogram (Fig. 5, bottom, lanes 1). The length of 2.1 kb is exactly the predicted distance between the *Bgl*II site and the 5′ end of A-MuLV RNA.

To examine the c-*abl* RNA, two primers were hybridized to, and extended on, poly(A)-containing RNA of mouse L cells: one was labeled at a *Sma*I site and was 550 bp long, and the other was labeled at a *Sac*I site and was 90 bp long (Fig. 5). Because c-*abl* RNA was only 1/10 as abundant as A-MuLV RNA, the bands of the extension products were of low intensity and were more clearly evident after the long exposure shown in Fig. 5B. The 550-bp primer yielded a 900-bp extension product (Fig. 5, lane 2), and the 90-bp primer gave a 1,425-bp product

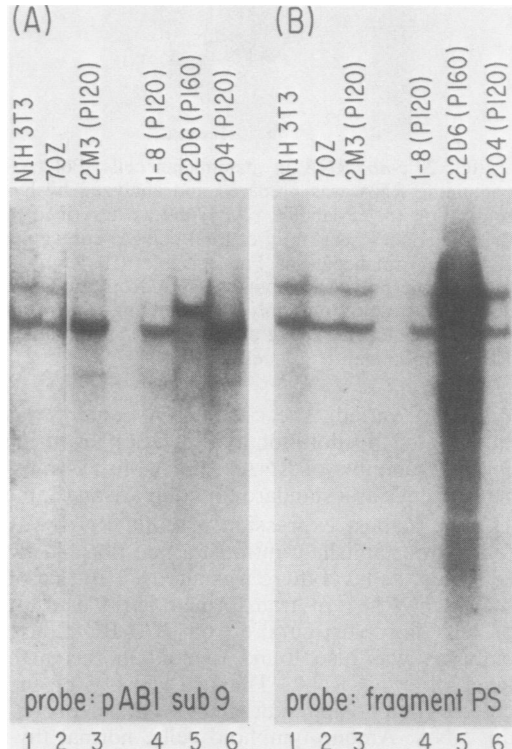
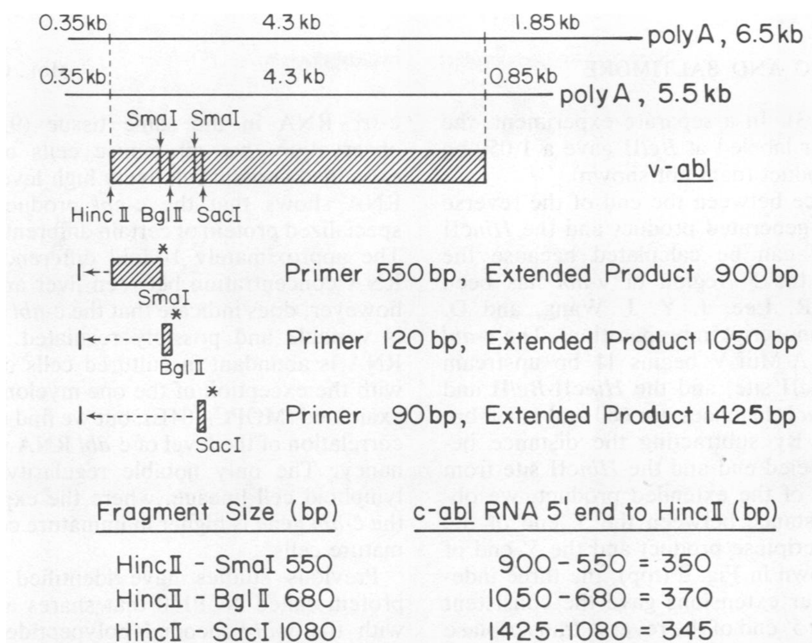


FIG. 4. A-MuLV-transformed cells contain c-*abl* RNA. Ten micrograms of total poly(A)-containing RNA from the following cell lines was analyzed by hybridization to ^{32}P-labeled probes: (1) NIH 3T3, (2) 70Z/3, (3) 2M3, (4) 1-8, (5) 22D6, (6) 204. Cell lines 2M3, 1-8, and 204 were transformed by A-MuLV(P120), whereas cell line 22D6 was derived by transformation with A-MuLV(P160). The DBM-paper was first hybridized to ^{32}P-labeled pAB1sub9 (A). After exposure of the paper to X-ray film, the labeled probe was washed off and a second hybridization with ^{32}P-labeled fragment PS was performed (B). In (A), lanes 1 and 2 are from a longer exposure (2 days) and lanes 3 to 6 are from a shorter exposure (16 h) of the same paper.



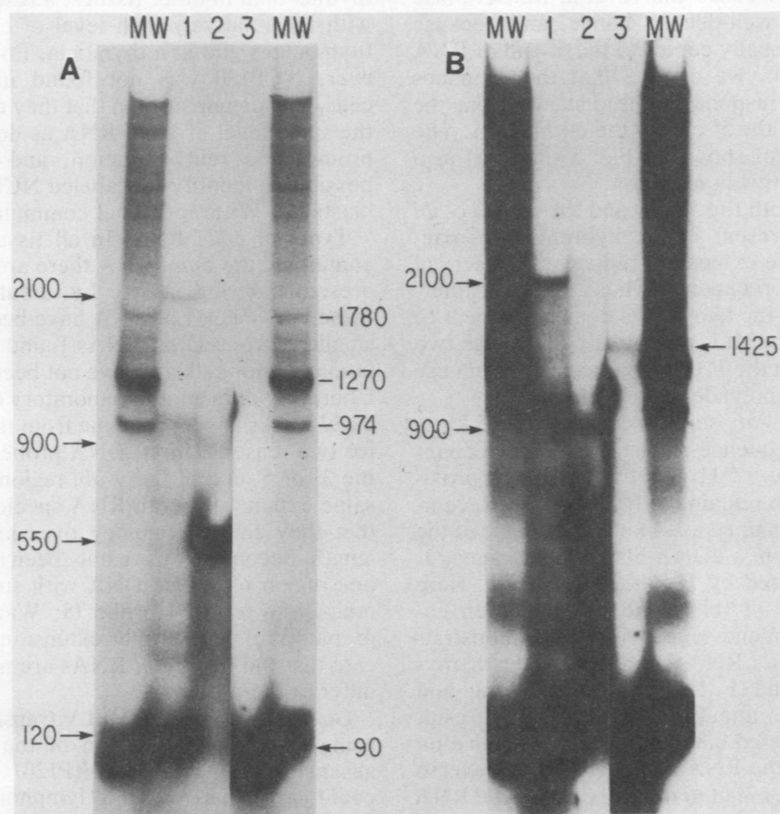


FIG. 5. Primer extension of v-*abl* sequences on A-MuLV and c-*abl* RNA. The length and positions of the three v-*abl* restriction fragments are as indicated (top). They were labeled at the 5′ end with ^{32}P, hybridized to RNA, and extended with reverse transcriptase as described in the text. The molecular weight markers (MW) are the in vitro run-off transcripts of adenovirus promoters (1,780, 1,270, and 974 bp) provided by Andrew Fire (M.I.T). Two different exposures of a gel are presented: exposure (A) was for 24 h, exposure (B) was for 4 days (bottom). (Lane 1) The *Sma*I-*Bgl*II fragment hybridized to 10 μg of poly(A)-containing RNA from A-MuLV-transformed fibroblasts, ANN-1, and extended with reverse transcriptase. (Lane 2) The *Hinc*II-*Sma*I fragment hybridized to 20 μg of poly(A)-containing RNA from mouse L cells and extended. (Lane 3) The *Sma*I-*Sac*I fragment hybridized to 20 μg of poly(A)-containing RNA from mouse L cells and extended.

(Fig. 5, lane 3). In a separate experiment, the 120-bp primer labeled at *Bgl*II gave a 1,050-bp extension product (data not shown).

The distance between the end of the reverse transcriptase-generated product and the *Hin*cII site in v-*abl* can be calculated because the sequence of the 5′ region of v-*abl* has been determined (R. Lee, J. Y. J. Wang, and D. Baltimore, manuscript in preparation). The v-*abl* sequence of A-MuLV begins 11 bp upstream from the *Hin*cII site, and the *Hin*cII-*Bgl*II and the *Hin*cII-*Sac*I distances are 680 and 1,080 bp, respectively. By subtracting the distance between the labeled end and the *Hin*cII site from the final size of the extended product, we obtained the distance between the 5′ end of the reverse transcriptase product and the 5′ end of v-*abl*. As shown in Fig. 5 (top), the three independent primer extensions gave the consistent result that the 5′ end of the reverse transcriptase product was about 355 bp upstream from the *Hin*cII site. Because the reverse transcriptase products are well-defined bands, and because the enzyme usually copies to the 5′ end of RNA molecules (14), we assume that the distances measured correspond to the distance from the *Hin*cII site to the 5′ end of the c-*abl* RNA. The positive control shown in Fig. 5 (lanes 1) also substantiates this assumption.

Although both the 5.5-kb and the 6.5-kb c-*abl* RNA were present in the hybridization reactions, only one extended product was observed with all three fragments. Thus, the 1-kb difference between the two RNAs does not appear to be at the 5′ end. It is most likely that these two RNAs differ at the 3′ end and they arise from use of alternative polyadenylation sites.

The 4.3-kb v-*abl* sequence plus the 0.35-kb c-*abl*-specific sequence at the 5′ end can account for 85% of the 5.5-kb c-*abl* RNA, but approximately 0.85 kb remains to be localized. Recently, we have obtained a 1.4-kb cDNA clone of the c-*abl* RNA from a cDNA library from mouse L cells constructed by H. Okayama and P. Berg (12). Analysis of this clone by restriction enzyme digestion and sequencing has demonstrated that the c-*abl* RNA is 0.85 kb longer than v-*abl* at the 3′ end (F. Ledley, J. Y. J. Wang, and D. Baltimore, unpublished data). This result places the deleted 0.85 kb of c-*abl* sequence on the 3′ end of the RNA and verifies that reverse transcriptase copied to the end of the c-*abl* RNA in the primer extension experiment.

DISCUSSION

Levels of c-*abl* RNA. Compared to other c-*onc* RNAs, the levels of c-*abl* transcripts are, in general, higher. Even at its lowest, i.e., in the liver, the level of c-*abl* RNA is an order of magnitude higher than that of c-*myb*, c-*myc*, or c-*src* RNA in the same tissue (9, 11). The observation that all mouse cells and tissues examined contain relatively high levels of c-*abl* RNA shows that the c-*abl* product is not a specialized protein of certain differentiated cells. The approximately 10-fold difference of c-*abl* RNA concentration between liver and thymus, however, does indicate that the c-*abl* expression is variable and possibly regulated. The c-*abl* RNA is abundant in cultured cells of all types with the exception of the one myeloma cell line examined (MOPC-104E), but we find no obvious correlation of the level of c-*abl* RNA with malignancy. The only notable regularity is in the lymphoid cell lineage, where the expression of the c-*abl* gene is higher in immature cells than in mature cells.

Previous studies have identified a cellular protein called NCP150 that shares antigenicity with the v-*abl*-encoded polypeptide sequence (20). That protein was much more abundant in thymus than in other tissues, a result consistent with the relatively high level of c-*abl* RNA in thymocytes and in a thymoma. Formerly, however, NCP150 was not found in fibroblastic cells. The demonstration that they contain about the same level of c-*abl* RNA as do thymocytes prompted a reinvestigation, and it has been possible to identify ^{32}P-labeled NCP150 in fibroblasts (O. Witte, personal communication).

Types of c-*abl* RNA. In all tissues and cells containing the c-*abl* RNA there are two species present differing in size by about 1 kb. Only these two species of RNA have been observed; smaller c-*abl*-related RNAs found by others in human tumor cells (6) have not been seen in this laboratory or in another laboratory (13). The two c-*abl* RNAs appear to come from the same gene for two reasons. First, DNA probes specific for the 3′ or 5′ end of the v-*abl* region react to the same extent with both RNA species, indicating that they share homology over much of their length. Second, we have only been able to detect one region of mouse DNA with substantial homology to a v-*abl* probe (8; Wang et al., in preparation). The primer extension results indicate that the two c-*abl* RNAs are most likely to differ at their 3′ ends.

c-*abl* expression in A-MuLV transformants. By using as a probe a region of the v-*abl* DNA absent from the A-MuLV(P120) genome, we could show that A-MuLV lymphoid transformants contain about as much c-*abl* RNA as a cell line of similar phenotype transformed by other means (70Z/3 cells). A-MuLV fibroblastic transformants also have about the same level of c-*abl* RNA as their untransformed counterparts. Thus, expression of the A-MuLV(P120) genome neither enhances nor represses the expression of the c-*abl* gene.

Assuming that the level of c-*abl* RNA in the A-MuLV transformant is characteristic of its level in the target cell (pre-B lymphocytes), the A-MuLV target cell would appear to have a relatively high expression of the c-*abl* gene. The A-MuLV transformant is probably a good model of its normal counterpart because of their similar surface antigens (3) and their immunoglobulin gene configuration (1). Other cells that have relatively high levels of c-*abl* RNA (thymocytes and fibroblasts) are also targets for A-MuLV transformation under certain circumstances (4, 16). It is possible that cells with higher expression of the c-*abl* gene are more susceptible to A-MuLV transformation. This observed correlation between c-*abl* expression and A-MuLV transformation, however, does not necessarily explain the target specificity.

A-MuLV contains a fragment of the c-*abl* RNA. Our results show that the v-*abl* region of the A-MuLV(P160) genome is an internal fragment of the c-*abl* RNA and that about 0.35 kb on the 5′ end and 0.85 kb (or 1.85 kb) on the 3′ end of the cellular RNA were eliminated during the formation of the A-MuLV hybrid genome. Some of the c-*abl* RNA coding sequence on the 5′ end must be deleted because there is no ATG codon at the extreme 5′ end of v-*abl*. How much, if any, of the 3′ coding sequence is present in the 0.85-kb deletion is unknown; the size of the c-*abl*-encoded protein (M_r = 150,000) compared to that encoded by v-*abl* (M_r = 130,000) suggests that some c-*abl* coding sequence is absent from A-MuLV. It is possible that the loss of c-*abl* coding sequence contributes to the oncogenicity of A-MuLV.

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