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A *bcr*–v-*abl* Oncogene Induces Lymphomas in Transgenic Mice

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In chronic myeloid leukemia and some cases of acute lymphoblastic leukemia, a 9;22 chromosome translocation has fused most of the c-*abl* oncogene to a gene designated *bcr*. To explore in vivo the biological effects of the chimeric gene, we introduced a facsimile of the translocation product, a *bcr*–v-*abl* gene, into the mouse germ line under the control of the immunoglobulin heavy-chain enhancer or a retroviral long terminal repeat. Some transgenic mice bearing either construct developed clonal lymphoid tumors. T lymphomas predominated, but some pre-B lymphomas developed. The transgenes were expressed in the tumors but not detectably in the lymphoid tissues of nontumorous transgenic animals, implying that transcription is activated by a low-frequency somatic event. These results demonstrate that *bcr*–v-*abl* is tumorigenic in vivo and provide a new animal model for lymphomagenesis.

Chronic myeloid leukemia (CML) is a neoplasm of the pluripotent hematopoietic stem cell characterized by the accumulation of mature granulocytes and their precursors in the bone marrow and the blood. The disease eventually transforms into an acute leukemia in which the dominant cells are usually myeloblasts but can instead be pre-B lymphoblasts or, more rarely, T lymphoblasts (10). The lymphoid blast crisis of CML is very similar to de novo acute lymphoblastic leukemia (ALL).

The hallmark of CML is a foreshortened chromosome 22 (the Philadelphia chromosome), which is also found in some cases of de novo ALL. It is formed by a reciprocal translocation that fuses most of the *abl* oncogene from chromosome 9 to a gene denoted *bcr* (breakpoint cluster region) on chromosome 22 to form a hybrid *bcr-abl* gene (24, 48). In the resulting chimeric polypeptide, the normal N terminus of the *abl* polypeptide has been replaced by *bcr*-coded sequences. The *bcr-abl* polypeptide associated with CML (p210) includes either 902 or 927 *bcr* amino acid residues, whereas that sometimes found in de novo ALL has the same portion of c-*abl* fused to a *bcr*-coded N-terminal segment of only 436 amino acids (17, 30). The *bcr-abl* polypeptides have enhanced autophosphorylation activity in vitro (11) and hence resemble the oncoprotein of the Abelson murine leukemia virus (AMuLV), in which viral *gag* sequences replace the N terminus of c-*abl* (reviewed in reference 34).

Mice infected with AMuLV rapidly develop lymphoid tumors (34), but relatively little is known about the biological effects of *bcr-abl*. A *bcr-abl* retrovirus has been found to transform immature B lymphoid cells but only after prolonged culture (39, 55), which suggests that additional genetic changes are required. Introduction of *bcr-abl* into growth factor-dependent hematopoietic cell lines rendered the cells autonomous and tumorigenic (15, 27), as found also with AMuLV (13, 42, 44, 46). Unlike *gag*–v-*abl*, however, the *bcr-abl* gene did not transform NIH 3T3 fibroblasts (16).

Transgenic mice allow oncogenic potential to be assessed within the living animal (for reviews, see references 14 and 25). To explore the biological effects of the 9;22 translocation in vivo, we have introduced a synthetic *bcr-abl* gene into the mouse germ line. This gene, which mimics that found in CML, was constructed by fusing *bcr* and v-*abl* sequences (27). To encourage expression of the transgene in hematopoietic cells, we coupled the gene either to the immunoglobulin heavy-chain enhancer (Eμ), which is known to be very effective in transgenic animals (1, 2, 47, 51), or to part of the long terminal repeat (LTR) of the myeloproliferative sarcoma virus (MPSV) (18), which has not previously been tested in a transgene. With either construct, some of the mice developed lymphomas after a variable latent period. Since the tumors have proven to be clonal, tumorigenesis appears to require alterations within a rare cell.

MATERIALS AND METHODS

Transgene construction. Construction of the *bcr*–v-*abl* gene (Fig. 1A) has been described elsewhere (27). The $E\mu V_H$(*bcr*–v-*abl*) construct included the 1-kilobase-pair (kb) *Xba*I fragment spanning the *lgh* enhancer (3). Its orientation with respect to the transcription unit is opposite that in the *lgh* gene to prevent transcription of *bcr*–v-*abl* from the cryptic promoters within the enhancer (37). The V_H promoter fragment (21) extends from the *Hin*dII site at –600 (relative to the mRNA cap site) to the *Nde*I site at +2. The *bcr*–v-*abl* gene extends from the *Nae*I site 9 base pairs (bp) upstream of the translation initiation codon of human *bcr* to the *Dra*I site 110 bp 3′ to the termination codon of v-*abl*. The enhancer and promoter of the LTR(*bcr*–v-*abl*) transgene were contained in a fragment from the 3′ LTR of MPSV extending from the *Pvu*II site in the *env* gene to the *Sma*I site within the R region (30 bp 3′ to the transcription initiation site) (49). The *bcr*–v-*abl* gene in this construct included an additional 807 bp of 3′ untranslated sequence extending to the *Cla*I site located 917 bp 3′ to the stop codon.

In both constructs, an intervening sequence and polyadenylation site were provided by the *Apa*I-*Bam*HI fragment of pSV2gpt (40). Since procaryotic vector sequences can interfere with tissue-specific expression in transgenic animals (43, 52), both constructs are bounded by *Not*I sites. These occur very infrequently in eucaryotic DNA, and the constructs were readily freed of the pUC19 plasmid sequences by *Not*I digestion and agarose gel electrophoresis. The DNA was subsequently purified by treatment with perchlorate and adsorption to glass fiber filters (9).

Generation of transgenic mice. The genes shown in Fig. 1 were microinjected (at 6 μg/ml) into the fertilized eggs of

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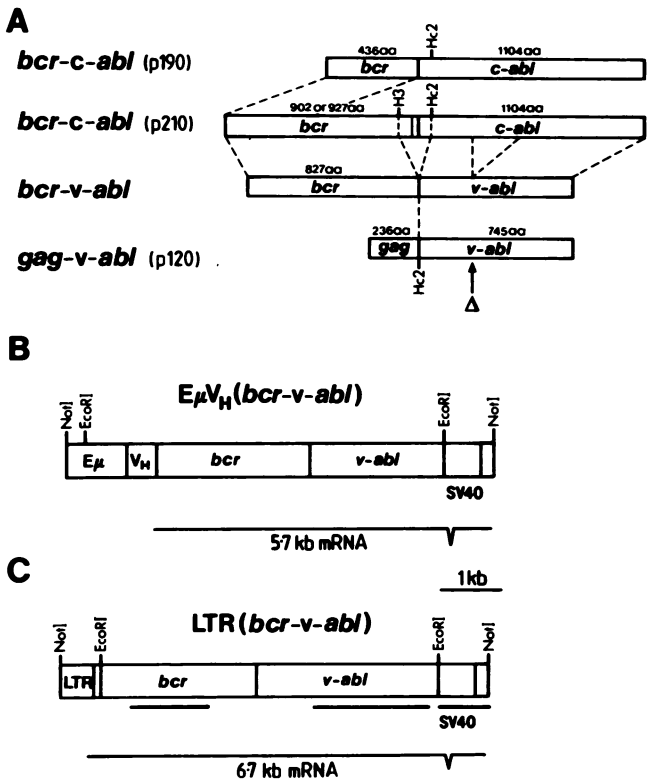

FIG. 1. Transgene constructs. (A) Structure of the hybrid *bcr*–v-*abl* gene. The region 5′ to the *Hin*dIII (H3) site of human *bcr* was fused to the v-*abl* (p120) sequences 3′ to the *Hin*cII (Hc2) site (27). Δ, Additional 263 amino acids found in the p160 form of the v-*abl* polypeptide and in murine c-*abl*. (B and C) The *bcr*–v-*abl* gene coupled either to the murine *lgh* enhancer (Eμ) and promoter (V_H) or to the MPSV LTR. A small intron and polyadenylation site from the early region of SV40 were included. DNA was injected as a gel-purified *Not*I fragment. The *bcr* probe (C) was the middle *Bam*HI fragment from *bcr-29* (26), the v-*abl* probe was a *Sma*I-*Hin*dIII fragment from pAb3sub3 (20), and the SV40 probe was the *Apa*I-to-*Bam*HI fragment from pSV2gpt (40).

(C57BL/6JWehi × SJL/JWehi)F_1 hybrid mice as described by Brinster et al. (9). Primary transgenic mice and offspring bearing the transgene were identified by dot hybridization to DNA from the tails of weanling mice (9), using a simian virus 40 (SV40) probe. Breeding lines were produced by mating transgenic animals with normal (C57BL/6 × SJL)F_1 hybrids.

Cell phenotyping. Flow cytometric analysis of immunofluorescence and cell size was performed as described by Langdon et al. (36). Additional monoclonal antibodies used were fluorescein-conjugated anti-Ly2 (CD8) and phycoerythrin-conjugated anti-L3T4 (CD4), obtained from Becton-Dickinson and Co. (Mountain View, Calif.).

RESULTS

Production of *bcr*–v-*abl* transgenic mice. The structure of the *bcr*–v-*abl* gene (27) is compared with those of the two *bcr*–c-*abl* forms and the AMuLV transforming gene in Fig. 1A. Its *bcr* moiety encompasses most of the segment present within the *bcr*–c-*abl* gene of CML and far more than that within the ALL form. The v-*abl* moiety used bears a few amino acid substitutions and an internal deletion with respect to the homologous portion of c-*abl* (45), but these alterations are unlikely to contribute greatly to the activation of c-*abl* as a transforming gene, since a fusion gene linking *gag* to the homologous region of c-*abl* transforms fibroblasts and a factor-dependent lymphoid cell line (5). Moreover, the effects of *bcr*–v-*abl* on a fibroblast and a myeloid cell line have proved to be very similar to those of *bcr*–c-*abl* (27). Hence *bcr*–v-*abl* appears to be a reasonable facsimile of *bcr*–c-*abl*, although it does lack a 5′ region that recent work suggests may restrain the oncogenic potential of c-*abl* (19, 32).

TABLE 1. Primary transgenic mice

Primary mouse[a]	No. of transgenic progeny[b]	No. of transgenic progeny with tumors
$E\mu V_H$(*bcr*–v-*abl*)		
EBvA1 (tumor 17d)	NB	
EBvA3	10	0
EBvA4	19	0
EBvA5 (tumor 52d)	NB	
EBvA6	0[c]	
EBvA11	0[c]	
EBvA16	3	0
EBvA21	49	1
EBvA22	11	0
EBvA25	22	0
EBvA26	27	0
EBvA32 (tumor 79d)	69	8
LTR(*bcr*–v-*abl*)		
LBvA11 (tumor 55d)	NB	
LBvA23	33	1
LBvA30	17	8

[a] Observed for 12 months.
[b] Observed for at least 6 months. NB, Not bred.
[c] The absence of transgenic animals among 29 progeny of EBvA6 and 35 progeny of EBvA11 suggests that these two founders were transgene mosaics.

Two constructs were used as transgenes. The $E\mu V_H$ construct (Fig. 1B) employs the enhancer and promoter from a murine *lgh* gene. Because the enhancer functions in both B and T lymphocytes (22) and in some myeloid cell lines (33), we reasoned that it might also function in a primitive hematopoietic cell that engenders all of these lineages. The other construct (Fig. 1C) uses the promoter and enhancer of the MPSV LTR, chosen because, unlike other LTRs, it is active in embryonal carcinoma cells (18) and hence might escape the inactivation that often occurs when retroviral sequences pass through the germ line (53). Moreover, the MPSV LTR drives retroviral expression very effectively in diverse hematopoietic cells, both in vitro and in vivo (6, 7). Both constructs include an SV40 early-region intron and polyadenylation site.

With the Eμ–*bcr*–v-*abl* construct, 12 of 42 pups that developed from microinjected eggs carried the transgene. With the LTR (*bcr*–v-*abl*) construct, only 3 of 71 pups were transgenic, raising the possibility that expression of the transgene early in embryonic development is lethal.

Tumor incidence in *bcr*–v-*abl* mice. The *bcr*–v-*abl* transgenes have both proven tumorigenic in vivo. Of the primary transgenic mice, 3 of 12 bearing the $E\mu V_H$ construct became ill with tumors between 2 and 11 weeks of age, and 1 of the 3 carrying the LTR construct died after 55 days (Table 1). The other primary transgenic mice are now more than 12 months of age and have still not developed tumors.

Because of the early deaths, a breeding line (EBvA32) could be established from only one primary mouse that developed a tumor. To date, tumors have developed in 8 of its 69 descendants born more than 6 months ago; curiously,

TABLE 2. Characteristics of transgenic tumors

Mouse[a]	Sex[b]	Age[c] (days)	Tumor site[d]	Transplant ability[e]	Surface marker(s)[f]	DNA status[g]			Diagnosis
						Igh	TCRγ	TCRβ	
Eμ(*bcr*–v-*abl*)									
EBvA1	M	17	Cervical LN	–	$B220^+$ Ig^-	ND[h]	ND	ND	Pre-B lymphoma
EBvA5	F	52	Thymus, LNs	+	$Thy\text{-}1^+$	G[i]	R	R[j]	T lymphoma
EBvA32	M	79	Thymus	+	$Thy\text{-}1^+$	G	R	R	T lymphoma
EBvA32-A26	F	224	Thymus	+	$Thy\text{-}1^+$	G	R	R	T lymphoma
EBvA32-A31	F	72	Thymus	+	$Thy\text{-}1^+$	G[i]	R	R	T lymphoma
EBvA32-B112	F	22	Cervical LN	–	$B220^+$ Ig^-	R	G	ND	Pre-B lymphoma
EBvA32-B116	M	54	Brachial LN	–	$B220^+$ Ig^-	R	R	ND	Pre-B lymphoma
EBvA32-B119	M	66	Thymus	+	$Thy\text{-}1^+$	G	R	R	T lymphoma
EBvA21-A10	M	118	Thymus	–	$Thy\text{-}1^+$	R	R	R	T lymphoma
LTR(*bcr*–v-*abl*)									
LBvA11	F	55	Thymus	+	$Thy\text{-}1^+$	G[i]	R	R	T lymphoma
LBvA30-A6	M	165	Thymus	ND	ND	ND	ND	ND	? T lymphoma
LBvA30-A21	F	149	Thymus	+	$Thy\text{-}1^+$	G[i]	R	R	T lymphoma
LBvA30-A56	M	94	Thymus	ND	ND	ND	ND	ND	? T lymphoma
LBvA30-A36	F	150	Thymus	–	$Thy\text{-}1^+$	ND	ND	R	T lymphoma

[a] A and B animals represent, respectively, the first- and second-generation descendants of founder animals.
[b] M, Male; F, female.
[c] Age at sacrifice, when ill, except for LBvA30-A6 and -A56, which were found dead with tumors.
[d] Major tumor mass; LN, lymph node.
[e] Cells (10^6) were injected intraperitoneally and subcutaneously into $(C57BL/6 \times SJL)F_1$ mice, which were monitored for at least 3 months.
[f] Determined by flow cytometry. Markers B220, Thy-1, and Ig were analyzed routinely.
[g] Analysis of immunoglobulin heavy-chain and TCR γ and β genes. G, Germ line; R, rearranged.
[h] ND, Not done.
[i] Low levels of two to three rearranged J_H fragments were also present.
[j] Tumors in thymus and lymph node had different TCRβ rearrangements (see Fig. 4).

all three transgenic offspring in one litter developed tumors by 9 weeks of age. Breeding lines were also initiated from 12 other primary mice. Tumors have arisen in three of these lines, albeit only a single case each in the EBvA21 and LBvA23 lines. The third line, LBvA30, is the most tumor prone, as lymphomas have developed in 6 of 17 descendants born more than 6 months ago and in 2 animals of a younger group.

Tumors are pre-B or T lymphomas. All of the tumors have been lymphomas, and they have proven to be of two types. The thymus was the predominant site of growth in all 10 tumors bearing the LTR-driven transgene and 9 of 12 bearing the Eμ-driven gene. They were classified as T lymphomas because all those examined bore the Thy-1 surface antigen and not the B-lineage marker Ly-5(B220) or immunoglobulin (Ig) (Table 2). Five of those tested for additional T-cell markers proved to be mixtures of 60 to 80% $CD4^+$ $CD8^+$ and 40 to 20% $CD4^-$ $CD8^+$ cells. The remaining three tumors of Eμ(*bcr*–v-*abl*) mice expressed B220 but not immunoglobulin or Thy-1 and hence were classified as pre-B lymphomas. Whereas pre-B lymphomas of Eμ-*myc* transgenic mice are usually disseminated (28, 29), all three of these tumors were most prominent in a single cervical or brachial lymph node, with little tumor growth in other lymph nodes or the spleen (Table 2).

To evaluate the autonomy of the tumors, we tested their transplantability into histocompatible recipients and attempted to derive cultured cell lines from them. Seven of eight T lymphomas were transplantable (Table 2), but none yielded a cell line under conditions that were regularly successful with other lymphomas, even with medium supplemented with sources of interleukin-2 and interleukin-4. In marked contrast to the pre-B lymphomas from Eμ-*myc* mice (29), none of the three pre-B lymphomas of $E\mu V_H$(*bcr*–v-*abl*) mice was transplantable, and none could be grown as a continuous cell line in vitro. These results suggest that the pre-B lymphomas are not fully autonomous despite their inexorable growth in the primary mice.

The tumors are clonal. To determine whether the tumors represented a monoclonal or polyclonal lymphoproliferation, we monitored rearrangements of the *lgh* and T-cell receptor (TCR) loci by Southern blot analysis. The results (Table 2) suggested that the tumors were monoclonal. Every T lymphoma examined displayed a unique and simple pattern of TCRβ rearrangement (Fig. 2) and had also undergone rearrangement at the γ locus (Fig. 3B). Both pre-B lymphomas examined (32-B112 and 32-B116) exhibited two rearranged J_H fragments in disproportionate yield, suggestive of ongoing rearrangement; B112 also retained an unrearranged allele (Fig. 3A). One of the T lymphomas (EBvA21-A10) displayed a rearranged J_H allele, and one of the pre-B tumors (EBvA32-B116) had undergone rearrangement at the TCRγ locus (Table 2). It is noteworthy that the thymic tumor in mouse EBvA5 had a pattern of TCRβ rearrangement different from that of tumor tissue in its mesenteric lymph node (Fig. 2). Although these tumors may have arisen independently, it is also possible that the rearrangement in the lymph node tumor arose from that in the thymus.

The transgene is expressed in the tumors. As expected, the *bcr*–v-*abl* transgene was expressed in every tumor tested. Northern (RNA) blot analysis (Fig. 4) with an SV40 probe confirmed that the tumors from LTR(*bcr*–v-*abl*) mice expressed the predicted 6.7-kb mRNA, while those from $E\mu V_H$(*bcr*–v-*abl*) mice yielded the expected 5.7-kb mRNA. The latter also consistently yielded 3.5- and 2.8-kb transcripts, which also hybridized to a 3′ v-*abl* probe and an SV40 probe that detects only the sense orientation but not to the *bcr* probe indicated in Fig. 1C (data not shown). Hence, they may initiate from cryptic promoters within the transgene or be generated by an aberrant splice that deletes much of the coding region. While the shorter transcripts might encode altered polypeptides, they are unlikely to account for

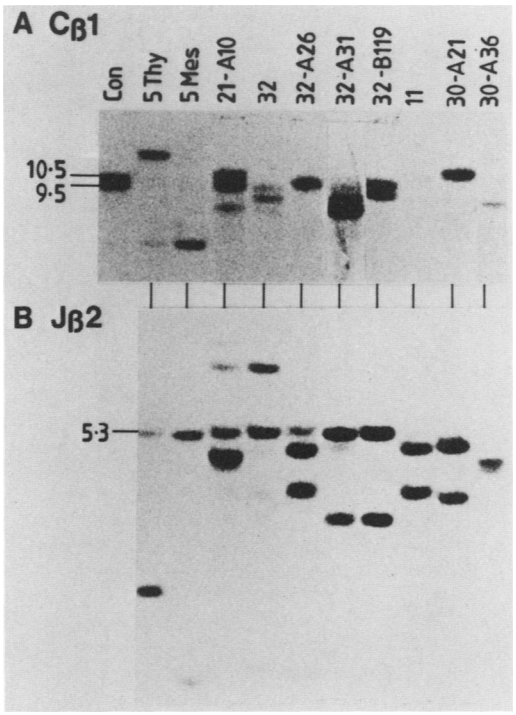

FIG. 2. TCRβ gene rearrangements in transgenic T-lymphoid tumors. (A) Southern blot analysis of *Hin*dIII-digested DNA from (C57BL/6 × SJL)F_1 liver (Con) and transgenic tumors using a $C_{\beta 1}$ probe, the 2.2-kb *Eco*RI fragment encompassing $J_{\beta 1}$ and most of $C_{\beta 1}$ (38). The germ line SJL $C_{\beta 1}$ band is 10.5 kb, and that from C57BL/6 is 9.5 kb; since the transgenic mice derive from a pool of F_2 hybrids, they may have either or both alleles. The probe also detects the 3-kb $C_{\beta 2}$ fragment (not shown). Deletion of the $C_{\beta 1}$ region (as in LBvA11) can result from a D-J joining event involving $J_{\beta 2}$ and a D segment 5′ to $C_{\beta 1}$. (B) The same filter hybridized with a 2-kb *Eco*RI fragment encompassing the $J_{\beta 2}$ region. The position of the 5.3-kb germ line *Hin*dIII fragment is indicated. The higher-molecular-weight bands in EBvA21-A10 and EBv 32 probably represent partial digests.

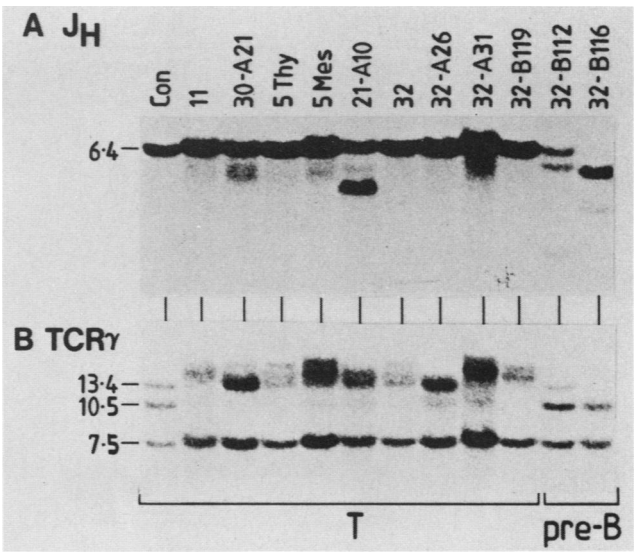

FIG. 3. *Igh* and TCR γ gene structures in transgenic tumors. (A) Southern blot analysis of *Eco*RI-digested DNA from SJL liver (Con) and transgenic tumors using a J_H probe (a 670-bp *Pst*I-*Nae*I fragment immediately 3′ of J_{H3} and spanning J_{H4}). The germ line fragment is 6.4 kb. (B) The same filter hybridized with a TCRγ probe, a 1-kb *Ava*I-*Bam*HI C_γ fragment (31). Positions of the germ line bands (13.4, 10.5, and 7.5 kb) are indicated. In EBvA32-B116, a rearranged band of 20 kb (faint in this autoradiograph), which resulted from loss of one copy of the 13.4-kb fragment, was reproducibly observed

the oncogenic action of the transgene, since neither was present in significant amounts in the LTR(*bcr*–v-*abl*) tumors.

Nontumorous mice display no abnormalities or evidence of transgene expression. To assess whether lymphoid development is perturbed before tumors arise, as it is in Eμ-*myc* mice (36), lymphoid organs from several healthy EBvA32, EBvA21, and LBvA30 mice were compared with those of normal littermates. None were enlarged. Flow cytometric analysis of bone marrow, thymus, and mesenteric lymph node for lineage-specific surface markers (bone marrow cells were examined for the markers Mac-1, B220, and Ig; lymph node cells were examined for Ig and Thy-1) provided no evidence of hematopoietic abnormality. There was no deficiency or excess of granulocyte-macrophage, B-lymphoid, or T-lymphoid cells, nor was there any increase in cell size to indicate abnormal proliferative activity. The four subsets of thymic lymphocytes ($CD4^+$ $CD8^+$, $CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$, and $CD4^-$ $CD8^-$) remained in normal proportions.

To assess whether the transgene was expressed in lymphoid tissues, poly(A)$^+$ RNA from healthy transgenic mice of both the EBvA32 and LBvA30 lines was analyzed on Northern blots. No *bcr*–v-*abl* RNA was detected in any of the tissues analyzed, although transcripts were readily detected in equivalent or even much smaller amounts of mRNA from tumors (Fig. 5). This result suggests that the transgene is transcriptionally silent in most lymphoid cells of the transgenic mice, although the possibility remains that a small proportion of cell express significant levels.

A sensitive test for *bcr*–v-*abl* expression in a minority of cells might be the ability of this gene to collaborate with another oncogene. We performed such a test with the c-*myc* gene by mating an EBVA32 mouse with Eμ-*myc* mice, which express *myc* constitutively within B-lineage cells (2,

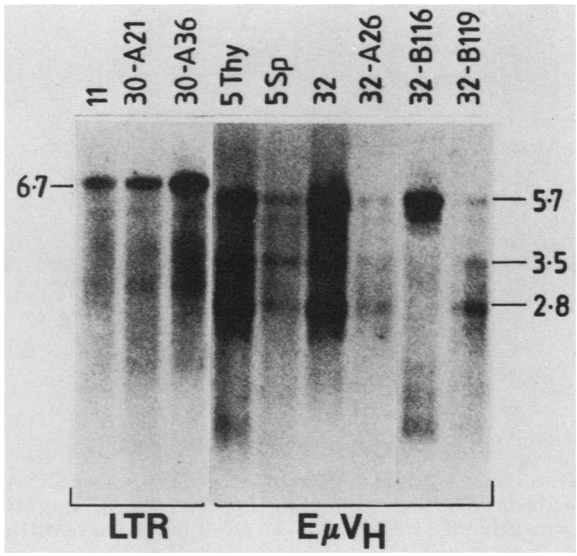

FIG. 4. Northern blot analysis of *bcr*–v-*abl* RNA in transgenic tumors. Poly(A$^+$ RNA (4 μg) from the indicated tumors (Table 2) was hybridized to the v-*abl* probe indicated in Fig. 1C.

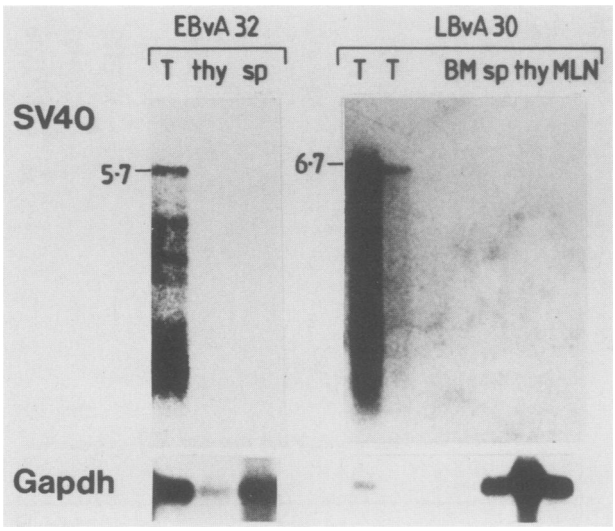

FIG. 5. Northern blot analysis of transgene RNA in tumors and lymphoid tissue. Poly(A)$^+$ RNAs from hematopoietic tissues of healthy transgenic mice of lines EBvA32 and LBvA30 were hybridized with the SV40 probe and then with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh). For EBvA32, all RNAs obtained from two thymuses (thy) and two spleens (sp) were compared with 0.1 μg obtained from a thymic tumor. For LBvA30, 0.3 and 0.1 μg from a tumor was compared with 4 μg prepared from the spleens, thymuses, and mesenteric lymph nodes (MLN) of 5 mice and all marrow (BM) isolated from 10 femurs.

36). Three litters yielded 14 mice bearing both transgenes. They developed the typical disseminated B-lineage tumors of the Eμ-*myc* strain, with no evidence of accelerated onset. The tumors expressed the Eμ-*myc* transgene but no *bcr*–v-*abl* (Fig. 6). Hence, that transgene may be silent in B-lineage cells.

The transgene is altered in some tumors. To investigate whether the transgene has been activated in tumors by DNA rearrangement, DNAs from several *bcr*–v-*abl* tumors were compared by Southern blot hybridization with kidney DNA from the same animals. Amplification was evident in two tumors (e.g., the thymic tumor EBvA32 shown in Fig. 7), but no changes were observed in the other six examined [e.g., EBvA5 in Fig. 7]. Hence, amplification or gross rearrangement of the transgene can occur but is not a prerequisite for tumorigenesis. Of course, this analysis cannot reveal small alterations such as point mutations.

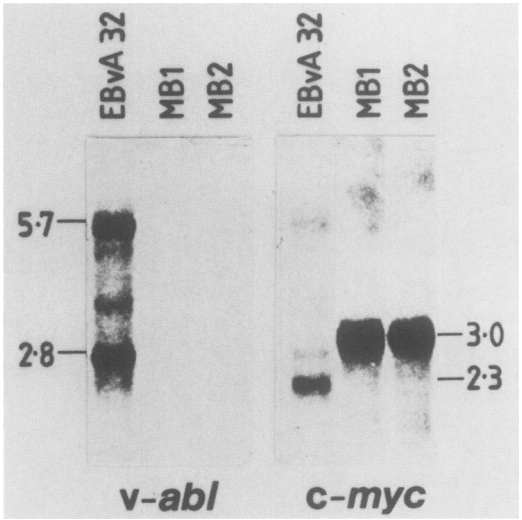

FIG. 6. Northern blot analysis of *myc* and *bcr*–v-*abl* RNA in tumors of mice bearing both genes. Poly(A)$^+$ RNAs (4 μg) from thymoma EBvA32 (Table 2) and two pre-B lymphomas (MB1 and MB2) arising in double-transgenic offspring of an Eμ-*myc* × EμV_H (*bcr-abl*) cross were hybridized to a v-*abl* probe and then to a fragment of a murine c-*myc* cDNA. The endogenous c-*myc* transcript is 2.3 kb; that from the Eμ-*myc* transgene is 3.0 kb.

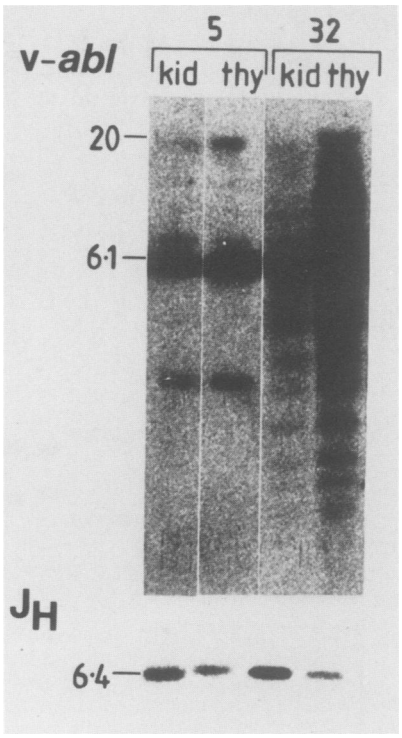

FIG. 7. Southern blot analysis of transgene DNA in two mice bearing tumors. DNA from kidneys and tumors of two transgenic mice, EBvA5 and EBvA32, was digested with *Eco*RI. The v-*abl* probe was a *Sma*I-*Hin*dIII fragment from the 3′ part of v-*abl*. The J_H probe was used to compare levels of DNA loaded; neither of these tumors has *lgh* rearrangements. The origin of the multiple small fragments from tumor 32 is not known.

DISCUSSION

To explore the effects of *bcr-abl* in vivo, we introduced into the mouse germ line a *bcr*–v-*abl* gene coupled to regulatory sequences from either the *lgh* gene or the MPSV LTR. Both constructs were oncogenic (Table 1). Three of twelve primary mice bearing the EμV_H(*bcr*–v-*abl*) transgene developed lymphomas, as did eight descendants of one of them (EBvA32) and one from another (EBvA21). One of the three primary mice harboring the LTR-driven gene also died with a lymphoma, and the two others engendered lines in which thus far nine descendants have succumbed.

The tumors found to date consist of 19 T lymphomas and 3 pre-B lymphomas. Since the *lgh* enhancer is active not only in B-lymphoid but also in some T-lymphoid and myeloid cell lines (33), and a rearranged *lgh* transgene is expressed in both B and T lymphocytes (22), it is not surprising that the EμV_H construct can target effects to both the B and T lineages. It is, however, unexpected that mice harboring the LTR-driven construct have so far developed

only T lymphomas, because the MPSV LTR is active in diverse cell types (6, 7). To date, no myeloid leukemia has developed with either construct. Since the *bcr*-v-*abl* gene efficiently transforms a murine myeloid cell line in vitro (27), the absence of myeloid leukemias in the transgenic mice may indicate that the transgene cannot be expressed in the myeloid cells susceptible to transformation by *bcr*-v-*abl*. Thus, these animals develop a disease analogous to 9;22 translocation-bearing ALL rather than to CML. Their tumors are also reminiscent of those inducible by AMuLV, with its *gag*-v-*abl* fusion gene; infection of mice by conventional routes induces pre-B-cell tumors (54), whereas intrathymic injection elicits T lymphomas (12).

The transgenic tumors are clonal, as each displays unique rearrangements of either the *lgh* or TCR gene (Fig. 2 and 3). Since the tumors arose in a small proportion of the mice and with variable latencies, it is likely that the transgene confers a predisposition to tumorigenesis but that rare genetic alterations are required to generate a tumor.

Activation of the transgene accompanies tumorigenesis. The transgene was expressed in every tumor examined (Fig. 4) but not in the normal lymphoid tissues (Fig. 5). Consistent with this finding, the hematopoietic populations in healthy transgenic mice were unperturbed, and pre-B lymphomas harboring both the *myc* and *bcr*-v-*abl* transgenes expressed only *myc* (Fig. 6). A plausible explanation is that the *bcr*-v-*abl* transgene is transcriptionally silent in normal lymphoid tissue and becomes activated by a stochastic somatic event, whereupon it initiates the tumorigeneic process. An alternative explanation is that the transgene is always expressed in a minor lymphoid subpopulation (probably less than 5%). Expression would then be detectable only when this small subpopulation became dominant, i.e., a tumor. A third possibility is that the transgene is expressed in a small proportion of the mice. Since the transgenic mice are an F_2 pool of C57BL/6 and SJL strains, a particular combination of alleles might facilitate expression. The occurrence of lymphomas in all three transgenic members of one litter might indicate that the transgene can occasionally be activated in the germ line, a possibility being investigated by further breeding experiments.

Why are the transgenes silent and how are they activated? With the LTR construct, the transcriptional silence in normal tissues may be related to the inactivation of retroviral genomes passed through the germ line (53), although our construct lacks an element just 3′ to the LTR that contributes to inactivation (4). A transgene construct using the Moloney murine leukemia virus LTR appears to be expressed at low levels and only in macrophages (35). The inactivity of the EμV_H construct is perhaps more surprising, because closely related transgene constructs using the SV40 promoter have been effective (28, 51). Moreover, the V_H promoter used here derives from a μ gene expressed very efficiently in transgenic mice (22). A V_H promoter, however, may be strongly influenced by unknown elements within the μ gene (21), perhaps involving an intron (41).

Recent evidence suggests that a promoter and enhancer may not be sufficient for consistent transgene expression. The absence of introns, for example, can markedly diminish transcriptional efficiency, and normal activity may not be restored by a heterologous intron [8]. Efficient transgene expression has also been linked to the presence of the DNase hypersensitivity regions thought to delineate large chromosomal domains (23). Thus, the rules governing transgene expression remain unclear.

Transcriptional activation of *bcr*-v-*abl* may result from changes within the transgene which increase its accessibility to transcription factors. In a few of the tumors, DNA amplification is implicated (Fig. 7). Amplification is thought to be initiated by illegitimate DNA replication and to persist if it confers a growth advantage (50), as might be expected in the *bcr*-v-*abl* tumors. As DNA rearrangement often accompanies amplification, subtle alteration of the transgene rather than amplification per se may have activated its transcription. Activation in the tumors lacking amplification may result from less obvious changes such as point mutation or epigenetic events such as demethylation.

Our strains of transgenic mice have demonstrated the tumorigenic potential of a *bcr*-v-*abl* fusion gene. Since they appear to yield exclusively lymphoid tumors, we are continuing efforts with modified *bcr-abl* constructs to generate a mouse model for CML.

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