Expression of *bcr* and *bcr-abl* Fusion Transcripts in Normal and Leukemic Cells

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The translocation of the *c-abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 in cases of Philadelphia chromosome-positive chronic myelogenous leukemia (CML) generates an aberrant *bcr-abl* fusion transcript which may be intimately related to the pathogenesis of CML. Because factors controlling normal *bcr* expression might also be involved in the expression of this aberrant *bcr-abl* transcript, we studied the patterns of expression of the normal *bcr* gene in different cell types. We found that the normal *bcr* gene was expressed in many different types of human cells. Moreover, the *bcr* gene was evolutionarily conserved, and homologous *bcr* genomic sequences and RNA transcripts were readily detected in chick tissue. The highest level of *bcr* expression in chick tissue was in brain tissue, the lowest level was in liver tissue, and a truncated *bcr* mRNA was noted in chick testes. Normal *bcr* transcripts, in addition to the aberrant *bcr-abl* hybrid transcripts, have been found in all Philadelphia chromosome-positive CML cells studied to date. Within a given CML sample, the relative amounts of normal *bcr* RNA and aberrant *bcr-abl* RNA were similar. In addition, the normal *bcr* and the aberrant *bcr-abl* hybrid transcripts demonstrated similarly prolonged half-lives compared with that of the normal *abl*-related transcripts in CML cells. These findings suggest that in CML cells, similar cellular mechanisms control the steady-state levels of both the normal *bcr* and the *bcr-abl* fusion RNAs.

The presence of the Philadelphia chromosome (22q⁻) (Ph¹) characterizes over 90% of cases of chronic myelogenous leukemia (CML). Formation of this chromosome in CML involves a translocation of the *c-abl* oncogene from its usual position on the distal end of the q arm of chromosome 9 to a relatively restricted region on the Ph¹ termed the breakpoint cluster region (*bcr*) (8, 10). This chromosome 22 breakpoint cluster region is part of a specific coding sequence locus which has been termed the *bcr* gene (11). As a result of this translocation, a hybrid *bcr-abl* transcript is generated, with the *bcr* sequences at the 5' end and the *c-abl* sequences at the 3' end of the transcript (2, 6, 22). This fusion transcript is abnormally large (8 kilobases [kb]) when compared with the normal-sized *c-abl* transcripts (7.4 and 6.6 kb), and this 8-kb transcript codes for the aberrantly large *abl*-related protein product (P 210) that has been demonstrated in CML cells (14, 15). These findings suggest that structural abnormalities in the *bcr* and *c-abl* genes and, in particular, expression of the aberrant 8-kb *bcr-abl* fusion transcript may be critical to the pathogenesis of CML.

Since *bcr* sequences form the 5' end of the aberrant *bcr-abl* transcript, it is probable that the normal *bcr* gene and the *bcr-abl* fusion gene share the same 5' regulatory sequences. This would suggest that factors regulating normal *bcr* gene expression may also be involved in regulating expression of the aberrant *bcr-abl* hybrid transcript. Thus, we have studied the patterns of expression of the normal *bcr* gene in an attempt to gain greater insight into the transcriptional control of the aberrant CML *bcr-abl* hybrid gene. Using a *bcr*-specific cDNA probe cloned from an HL-60 promyelocytic leukemia cDNA library, we found *bcr*-related transcripts in a wide variety of hematopoietic and nonhematopoietic cell types. Moreover, both the normal chromosome 22 *bcr* transcript and the aberrant Ph¹ *bcr-abl* hybrid tran-

script were expressed in CML cells. Within individual CML samples, the relative amounts of the normal *bcr* and aberrant *bcr-abl* transcripts were similar. In addition the half-lives of these transcripts were also similar and prolonged compared with that of the normal *c-abl* transcript. These results suggest that factors controlling the steady-state levels of normal *bcr* RNA also determine the levels of the aberrant *bcr-abl* fusion RNA.

MATERIALS AND METHODS

Cloning of *bcr*-specific genomic and cDNA probes. A human genomic Charon 4A library (obtained from T. Maniatis) was screened with a 1.2-kb *Hind*III-*Bgl*II genomic fragment (obtained from Oncogene Science, Mineola, N.Y.) containing noncoding sequences near the 3' end of the previously described chromosome 22 breakpoint cluster region (10). Several overlapping clones whose restriction maps closely resemble previously published genomic maps of the chromosome 22 *bcr* (10) were isolated, and various fragments from this region were subcloned and hybridized to Northern blots (RNA blots) of both CML and non-CML cells. A 1.4-kb *Bgl*II-*Sst*I fragment lying immediately 5' to the 5-kb *Bgl*II fragment within *bcr* (10, 11) identified RNA species corresponding to the normal 7- and 4.5-kb human *bcr* transcripts (22) and to the 8-kb *bcr-abl* fusion transcript in CML cells. This fragment was used to screen an HL-60 lambda gt 10 cDNA library constructed and provided by David Bentley. Ten positive clones were identified (8×10^5 total clones screened), and one of these clones contained a 3.0-kb cDNA insert with a restriction map matching a previously described *bcr* cDNA clone (11). Subclones from this *bcr* cDNA clone (Fig. 1) were used in the blot hybridization studies and in the isolation of a 0.7-kb chick *bcr* cDNA clone from a chick embryo cDNA library.

Cells. Peripheral blood mononuclear cells were isolated by buoyant density gradient centrifugation by using Isolymp

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FIG. 1. Restriction map of human *bcr* cDNA. This 3.0-kb *bcr* cDNA fragment was cloned from an HL-60 cDNA library as described in Materials and Methods. The arrow indicates the approximate region corresponding to the Ph¹ chromosome 22 breakpoint such that genomic sequences corresponding to cDNA sequences 5' to the arrow remain on chromosome 22, while those 3' to the arrow are translocated to chromosome 9 in the Ph¹ translocation (11, 22, 23). The 5' BCR subclone is a 600-bp *EcoRI*-*Pst* fragment which hybridizes to both normal *bcr* and *bcr-abl* hybrid transcripts. The BCR-22 subclone is a 700-bp *Pst* fragment which hybridizes only to normal *bcr* transcripts. H, *HindIII*; Bg, *BglII*; P, *Pst*; A, *AvaI*; R₁, *EcoRI*; The terminal *EcoRI* sites were introduced during the library construction.

(Gallard Schlesinger, Carle Place, N.Y.). The interface cells were washed several times in RPMI 1640 prior to RNA extraction. To isolate granulocytes, the erythrocyte (RBC)-granulocyte isolymp pellet was brought to 25 ml with plain RPMI 1640 and mixed with 25 ml of 3% dextran (T-500) in phosphate-buffered saline. The RBCs were allowed to sediment for at least 1 h, the supernatant was harvested, and the cells were pelleted. The contaminating RBCs were lysed several times in 0.2% NaCl. The pelleted cells were washed; they consisted of greater than 95% granulocytes. To isolate total leukocytes, peripheral blood was subjected to 3% dextran sedimentation followed by lysis of RBCs as described above.

Individual cell lines were cultured as described in the references cited and included the CML blast crisis cell lines K-562 (17), EM-3 (12), KCL-22 (16), the human promyelocytic leukemia cell line HL-60 (4), the human myeloid leukemia KG-1 (13), the human erythroleukemia cell line HEL (19), the T leukemia line Jurkat, and the B lymphoma line Daudi. Phytohemagglutinin-stimulated cells were obtained by culturing peripheral blood mononuclear cells at an initial concentration of 10⁶ cells per ml in RPMI 1640–5% fetal calf serum–1% phytohemagglutinin for 6 days.

RNA extraction. To extract total cell RNA, cells were washed in sterile isotonic medium and the pellet was vortexed vigorously in 6 ml of ice-cold 6 M guanidine hydrochloride–0.1 M potassium acetate (pH 5.0). The cells were then sonicated three times on ice with 20-s bursts. Nucleic acids were precipitated with the addition of 0.5 volume of 95% ethanol, and after vortexing, the samples were poured into a baked 15-ml Corex tube and precipitated overnight at –20°C. The samples were spun at 10,000 rpm (Sorvall SS34) for 20 min, and the pellet was dissolved in 1 ml of 6 M guanidine hydrochloride–0.1 M potassium acetate (pH of 2 M stock, 5.0)–25 mM EDTA (pH of 0.5 M stock, 8.0). The solution was drawn 10 times through a 22-gauge spinal needle and then precipitated with 0.5 volume of ethanol at –20°C for 1 h. The sample was again spun at 10,000 rpm for 20 min and again aspirated through the 22-gauge spinal needle. Following a third 10,000 rpm spin, the pellet was washed gently in 70% ethanol and then dissolved in 20 mM Tris (pH 8.0)–100 mM NaCl–10 mM EDTA–0.2% sodium dodecyl sulfate. Heating for 5 to 10 min at 60°C facilitated dissolving of the pellet. The RNA solution was then phenol-chloroform extracted, made 0.3 M sodium

acetate, and precipitated with 2 volumes of ethanol at –70°C for 10 to 20 min. The precipitate was spun in an Eppendorf Microfuge, and the pellet was washed in 70% ethanol and dissolved in H₂O. The RNA sample concentrations were estimated by the optical density at 260 nm, and samples (2 to 4 µg) were routinely run on a formaldehyde minigel with ethidium staining to identify the 28S and 18S ribosomal bands to determine whether any significant RNA degradation was present. This total RNA was poly(A)⁺ selected on an oligo(dT) cellulose column as previously described (1).

DNA extractions. DNA extractions were performed by digesting nuclei with protease K, followed by ethanol precipitation as previously described (5).

Northern, Southern, and slot blot hybridizations. Poly(A)⁺ RNA was electrophoresed through formaldehyde denaturing gels, followed by Northern blotting by standard procedures (18). Southern blots on restriction endonuclease-digested genomic DNA were performed as previously described (5). For slot blot hybridization, quantities of poly(A)⁺ RNA were serially diluted in 100 µl of water containing 6× SSC (standard citrate; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 2.2 M formaldehyde and then heated for 15 min at 60°C. The samples were cooled on ice and applied with a slot blotter (Minifold II; Schleicher & Schuell, Keene, N.H.) to a nitrocellulose filter previously soaked in 20× SSC. The filter was then air dried, baked, and hybridized. Southern, Northern, and slot blots were all hybridized to nick-translated probes and subsequently washed under exactly the same conditions described previously (5).

Molecular probes. In addition to the *bcr* probes described in Fig. 1, we used as a *v-abl* probe a 1.5-kb *BglII* fragment isolated from the *v-abl* insert of the pAB sub 9 plasmid (25); as an actin probe, we used a 2.0-kb chick pA 1β actin cDNA *Pst* fragment (3), and as a *c-myc* probe, we used a 1.8-kb human *c-myc* cDNA fragment (provided by David Bentley).

RESULTS

Cloning of *bcr* sequences. As detailed in Materials and Methods, we isolated a 3.0-kb *bcr*-specific cDNA clone from an HL-60 cDNA library (Fig. 1). We isolated multiple *bcr*-specific clones from the HL-60 cDNA library and could readily identify *bcr*-specific transcripts in HL-60 cell RNA by Northern blot analysis. As depicted in Fig. 1, two subclones from this 3.0-kb *bcr* cDNA were obtained, representing sequences at the 5' and 3' ends of the *bcr* transcript (designated 5' BCR and BCR-22, respectively).

***bcr* expression.** Using this *bcr*-specific human cDNA clone as a probe, we performed Northern blot analysis on poly(A)⁺ RNA isolated from a wide variety of different human cells and cell lines. In all cell samples tested, we found *bcr*-specific transcripts approximately 7 and 4.5 kb in size (Fig. 2). These cells, in addition to HL-60, included an erythroleukemia cell line (HEL), the myeloid cell line KG-1, B and T leukemic cell lines (Daudi and Jurkat), Epstein-Barr virus-transformed B lymphoblast cell lines, and normal skin and bone marrow fibroblasts. We also noted a minor approximately 3-kb *bcr*-related transcript in some of these samples (Fig. 2). Although there was some variation in the total amount of *bcr* transcripts from sample to sample, we observed no clear-cut pattern relating quantitative *bcr* expression with a particular differentiative lineage. Moreover, similar amounts of transcripts were noted in resting and actively proliferating cells. These included resting peripheral blood mononuclear cells and the same cells stimulated to divide with phytohemagglutinin (Fig. 2, lanes 8 and 9), as

well as actively proliferating HL-60 cells and the same cells induced to terminally differentiate to granulocytes with 10^{-6} M retinoic acid and to macrophagelike cells with 10^{-8} M tetradecanoyl-phorbol acetate (TPA) (Fig. 2, lanes 6, 7, and 11).

Evolutionary conservation and expression of *bcr* sequences. The universal expression of *bcr* sequences in all human cells examined to date suggest that the *bcr* gene plays an essential, albeit unknown, role in normal cell physiology. If this is true, then one might predict that this gene would be evolutionarily conserved and be readily demonstrable in other species. We hybridized Southern blots of restriction endonuclease-digested genomic mouse, chick, and human DNA to the human *bcr* cDNA probe. Under stringent hybridization and wash conditions, this probe identified faint but distinct bands in chick genomic DNA, in addition to the expected relatively intense bands observed in human DNA (Fig. 3A). In addition, using similar hybridization conditions, we readily detected homologous *bcr* fragments in mouse genomic DNA (blot not shown). To analyze patterns of *bcr* transcription in chick cells, we first screened a chick cDNA library with the human *bcr*-specific cDNA probe and isolated a single 0.7-kb chick cDNA fragment. This chick cDNA clone, when used as a probe on Southern blots, identified the same chick and human fragments as the human *bcr* probe did, but as predicted, the chick bands were considerably more intense than the human bands (Fig. 3B). We used this chick *bcr* cDNA clone as a probe on Northern blots of various chick tissue RNA extracted from 2-week-old chicks

FIG. 2. Patterns of expression of the normal *bcr* gene. Equal amounts (4 μ g) of poly(A)⁺ RNA were electrophoresed through formaldehyde gels, transferred to nitrocellulose, and successively hybridized to a nick-translated 3.0-kb *bcr* cDNA probe (Fig. 1) and to an actin probe. Lanes: 1, Daudi B-cell lymphoma; 2, Jurkat T-cell lymphoma; 3, HEL erythroleukemia cell line; 4, bone marrow fibroblasts; 5, skin fibroblasts; 6, HL-60 uninduced; 7, HL-60 induced with 10^{-6} M retinoic acid for 3 days; 8, resting mononuclear peripheral blood cells; 9, mononuclear peripheral blood cells stimulated with phytohemagglutinin; 10, KG-1 cell line; 11, HL-60 induced with 10^{-8} M TPA for 2 days; 12 to 14, individual Epstein-Barr virus-transformed B lymphoblast cell lines. Approximately sizes of the transcripts are shown in kilobases.

FIG. 3. Evolutionary conservation of *bcr* sequences. (A) Genomic DNA (10 μ g) from chick embryo fibroblasts (lanes 1 to 3) and from the human HL-60 cell line (lanes 4 to 6) were digested with restriction endonucleases, Southern blotted, and hybridized to the 3.0-kb human *bcr* cDNA probe. Hybridization and washes were under stringent conditions exactly as previously described (5). Approximate sizes of the chick fragments in kilobases are noted. Lanes: 1 and 4, *Eco*RI digests; 2 and 5, *Hind*III digests; 3 and 6, *Bam*HI digests. Lanes 1 to 3 were exposed for 2 days, while lanes 4 to 6 were exposed for 16 h. (B) The same blot as in panel A was rehybridized to a 700-bp chick cDNA clone homologous to the human 3.0-kb *bcr* cDNA clone. Hybridization and washes were performed under the same stringent conditions described previously (5). Exposure time was 20 h.

and noted predominant transcripts of approximately 6 and 4 kb in most chick tissue (Fig. 4A). The highest levels of these transcripts were noted in chick brain, while low, barely detectable levels were noted in chick liver. Chick testes were unusual in exhibiting a truncated approximately 1-kb *bcr*-related message (Fig. 4A, lane C). Although the fact that an actin probe hybridized to this same blot suggests that less testes RNA is loaded in this lane (Fig. 4B), the actin probe did not indicate any testes RNA degradation, and the low-molecular-weight testes-specific *bcr* transcript is probably not a degradation product.

Expression of *bcr*-related transcripts in Ph¹ positive CML cells. When Northern blots of RNA from Ph¹-positive CML cells are hybridized to an *abl*-specific probe, an aberrantly large 8-kb message representing a *bcr-abl* fusion transcript is noted (2, 6, 22, 23). We have found that the amount of this abnormal transcript varies in different CML samples, with blast-phase CML cells generally expressing higher levels of this transcript than chronic phase cells do (F. Andrews and S. Collins, manuscript in preparation). This variation in the amount of the 8-kb transcript as revealed by an *abl*-specific probe is demonstrated by the Northern blot in Fig. 5A. When these same Ph¹-positive CML samples were hybridized to the 5' BCR human cDNA probe (Fig. 5B), the following observations were made. (i) The *bcr* probe identified an 8-kb transcript that comigrated with the aberrant transcript identified by the *abl* probe. This confirms the previous observation that this aberrant RNA is indeed a *bcr-abl* hybrid transcript which is transcribed from the Ph¹ (22, 23). (ii) All these CML samples exhibited the normal 7- and 4.5-kb *bcr* transcripts. These transcripts most likely originate from the *bcr* gene on the normal intact chromosome 22 in these CML cells. Thus, there appears to be no allelic exclusion of *bcr* transcription in these CML samples. (iii) With the exception of K-562 cells (see below), the relative amounts of Ph¹ *bcr-abl* hybrid transcripts and normal chromosome 22 *bcr* transcripts were similar within individual CML samples. For example, samples in Fig. 5B, lanes 1, 4, 9, and 10 expressing relatively large amounts of the *bcr-abl* hybrid Ph¹ transcript also expressed relatively

FIG. 4. Expression of the chick *bcr* gene. Equal quantities (10 μ g) of RNA extracted from various chick tissues were electrophoresed in formaldehyde gels, transferred to nitrocellulose, and hybridized to the 700-bp chick *bcr* probe and to an actin probe. Exposure times were 8 days for the *bcr* probe and 1 day for the actin probe. Lanes: A, thymus; B, spleen; C, testes; D, pancreas; E, liver; F, brain; G, gut; H, bursa; I, kidney. Approximate sizes of the transcripts are shown in kilobases.

large amounts of the chromosome 22 normal *bcr* transcripts. Conversely, samples in Fig. 5B, lanes 3 and 11 to 13 expressing small amounts of the aberrant hybrid transcript also expressed relatively small amounts of the normal *bcr* transcripts. Probing the same blot with an actin probe (Fig. 5C) indicates that these differences in hybridization intensity did not result from different amounts of RNA inadvertently loaded in each lane.

To better quantitate this latter observation, we performed a slot blot analysis of equal amounts of poly(A)⁺ RNA from these CML samples hybridized to both a *v-abl* probe to detect the Ph¹ *bcr-abl* hybrid transcript and a *bcr* probe specific for the normal chromosome 22 *bcr* transcripts. This latter probe (designated BCR-22) is a 700-base-pair (bp) *Pst* *bcr* cDNA fragment corresponding to genomic *bcr* coding regions that are located 3' to the chromosome 22 breakpoint (Fig. 1), and which therefore hybridize only to normal chromosome 22 *bcr* transcripts. Again, the CML samples demonstrating enhanced hybridization to the *v-abl* probe also showed enhanced hybridization to the BCR-22 probe (Fig. 6). Hybridization of the same samples to an actin probe indicate that these differences were not due to the inadvertent loading of different amounts of poly(A)⁺ RNA on the blot. A notable exception to the above observation is demonstrated by the CML blast crisis cell line K-562. In these

cells, the amount of *bcr-abl* hybrid transcript was relatively large, while the amount of normal chromosome 22 *bcr* transcripts was relatively small (Fig. 5 and 6, lanes 5). As we have previously demonstrated, K-562 is exceptional among these CML samples in demonstrating an approximately four- to eight-fold amplification of the translocated *c-abl* oncogene (5).

Relative stability of *bcr*, *bcr-abl*, and *abl* transcripts. To compare the stabilities of normal *bcr*, normal *abl*, and the aberrant *bcr-abl* hybrid transcripts, we incubated the KCL-22 CML blast crisis cell line with actinomycin D to block transcription. After various times of incubation, we isolated poly(A)⁺ RNA from these cells and then performed Northern blot analysis to compare the half-lives of these various RNA transcripts. We chose these particular CML cells because they expressed the aberrant *bcr-abl* hybrid transcript as well as readily detectable levels of the normal chromosome 22 *bcr* and chromosome 9 *abl* transcripts. We observed that all four *bcr*-related transcripts in this cell line (the normal 7-, 4.5-, and 3-kb transcripts as well as the aberrant 8-kb *bcr-abl* fusion transcript) had similar, relatively prolonged half-lives, with little degradation of any of these four transcripts occurring until 4 to 8 h after actinomycin D treatment (Fig. 7). In contrast, *c-myc* transcripts in

FIG. 5. Expression of normal *bcr* and *bcr-abl* fusion transcripts in Ph¹-positive CML samples. Equal quantities (4 μ g) of poly(A)⁺ RNA extracted from Ph¹-positive CML cells were electrophoresed in formaldehyde gels, transferred to nitrocellulose, and hybridized to the indicated probes. Lanes: 5, K-562; 8, KCL-22; 9, EM-3. All other lanes contain RNA from fresh CML samples at different stages of the disease. Approximate sizes of the transcripts are shown in kilobases.

this particular cell line were more unstable, with significant degradation of the 2.5-kb *c-myc* transcript detectable 30 min to 1 h after actinomycin D treatment (Fig. 7). In addition to the aberrant *bcr-abl* hybrid transcript, the KCL-22 cell line expressed normal *abl* transcripts of 7.4, 6.6, and 5 kb, with the latter two transcripts predominating (Fig. 7B). (The 5-kb *abl* transcript was reproducibly observed in KCL-22 and other CML cells and may correspond to a similar-sized *abl*-related transcript recently observed in adult mouse testes [20]). When compared with these chromosome 9 *abl* transcripts, the Ph¹ 8-kb *bcr-abl* transcript appears to be significantly more stable (Fig. 7) in these actinomycin D-treated CML cells.

We also did similar studies assessing *bcr* versus *abl* mRNA stability in the non-Ph¹-containing cell line KG-1 (Fig. 8). In general, the half-lives of the *bcr*, *abl* and *myc* messages appear to be prolonged in this cell line compared with those in KCL-22. However, this may be an artifact secondary to differential uptake of the actinomycin D by these two cell lines. Nevertheless, it is clear from Fig. 8 that in KG-1 cells, the *bcr* transcripts were more stable than the *abl* and *myc* transcripts were, and this parallels the findings noted in the KCL-22 cell line (Fig. 7).

DISCUSSION

The presence of Ph¹ in over 90% of cases of CML and the well-documented association of this abnormal chromosome with the *bcr-abl* hybrid transcript suggests that the expression of this aberrant transcript is critical to the pathogenesis of CML. Since the 5' end of this aberrant transcript consists of *bcr* sequences (22), it would seem that factors important in controlling normal *bcr* gene expression might also be involved in the expression of the aberrant *bcr-abl* hybrid transcript. For this reason, we investigated the patterns of expression of the normal *bcr* gene.

Initial observations of *bcr* expression indicated that there was differential expression of this gene, with some cells exhibiting relatively high levels of *bcr* transcripts, while

FIG. 6. Slot blot analysis of Ph¹-positive CML samples. Poly (A)⁺ RNA (1 μ g) from individual CML samples was blotted onto nitrocellulose and hybridized to the indicated probes. The lanes correspond exactly to those described in the legend to Fig. 5. The BCR-22 probe is specific for normal chromosome 22 *bcr* transcripts (Fig. 1).

FIG. 7. Relative stability of KCL-22 RNA transcripts. KCL-22 CML cells were incubated for the indicated times in actinomycin D (7.5 μ g/ml), and poly(A)⁺ RNA was extracted. For each sample, 5 μ g of poly(A)⁺ RNA was fractionated on a 1% agarose formaldehyde gel, transferred to nitrocellulose and hybridized to the indicated probes. (A) Hybridization to *bcr* and *myc* probes. The aberrant 8-kb *bcr-abl* hybrid transcript, the normal 7-, 4.5-, and 3-kb *bcr* transcripts, and the normal 2.5-kb *c-myc* transcript are indicated. Exposure time was 6 h. (B) Hybridization to *v-abl* probe. The aberrant 8-kb *bcr-abl* hybrid transcript, as well as the 7.4-, 6.6-, and 5-kb *abl* transcripts are indicated. Exposure time was 20 h.

other cells expressed little or none (22). Since CML is a hematopoietic stem cell disorder (9), we were particularly interested in determining whether there was preferential *bcr* expression in hematopoietic versus nonhematopoietic cells; whether there was a differential expression of *bcr* in myeloid versus erythroid cells, T cells versus B cells, etc.; and whether *bcr* expression varied in resting versus actively proliferating cells. By Northern blot hybridization, we demonstrated no such differential expression of *bcr* transcription and in fact observed approximately equal amounts of *bcr* transcripts in all the cells we examined, including cultured hematopoietic cells of T lymphoid, B lymphoid, myeloid, and erythroid lineages; cultured HL-60 cells terminally differentiated to mature granulocytes and macrophage-like cells; skin and bone marrow fibroblasts; and both resting and actively proliferating peripheral blood lymphocytes. There did not appear to be preferential *bcr* expression in one cell type versus another or in actively proliferating versus nonproliferating cells. Moreover, we found the *bcr* gene to be evolutionarily conserved, and we identified distinct *bcr*-related fragments in Southern blots of mouse and chick DNA and specific *bcr* transcripts in chick RNA. The widespread expression of the *bcr* gene as well as its evolutionary conservation suggests that this gene may play an important though presently unknown housekeeping role in normal cell physiology.

Our Northern blot analysis of *bcr* expression in Ph¹-positive CML cells revealed the presence of both the aber-

FIG. 8. Relative stability of KG-1 RNA transcripts. KG-1 cells were incubated for the indicated times in actinomycin D (7.5 μ g/ml, and poly(A)⁺ RNA was extracted. For each sample, 5 μ g of poly(A)⁺ RNA was fractionated on a 1% agarose formaldehyde gel, transferred to nitrocellulose, and hybridized to the indicated probes exactly as described in the legend to Fig. 7. Approximate sizes of the transcripts are shown in kilobases.

rant 8-kb *bcr-abl* transcript arising from the Ph¹ and the normal 7- and 4.5-kb *bcr* transcripts arising from the normal intact chromosome 22. Thus, there was no allelic exclusion of normal *bcr* transcription in any of these CML samples. This is consistent with the notion that the *bcr* gene product may provide an essential cellular function. Interestingly, we noted in these Ph¹-positive CML samples that although there was variation in the amount of both normal *bcr* and aberrant *bcr-abl* transcripts in different Ph¹-positive CML samples, the relative amounts of *bcr-abl* hybrid transcripts and normal chromosome 22 *bcr* transcripts were similar within individual CML samples. That is, individual CML samples with relatively large amounts of *bcr-abl* transcripts also displayed relatively large amounts of the normal *bcr* transcripts. A notable exception to this observation was the K-562 CML blast crisis cell line which had amplified the *bcr-abl* fusion gene at least four- to eightfold and subsequently demonstrated a higher level of the fusion gene transcript compared with the level of the normal *bcr* transcript.

One possible explanation for the coordinate expression of the normal *bcr* and the *bcr-abl* fusion transcripts is the presence of cellular factors which interact with specific regulatory sequences common to both the normal chromosome 22 *bcr* gene and the Ph¹ chromosome *bcr-abl* gene. This would result in equal transcription of *bcr* and *bcr-abl*

hybrid genes within a given CML sample. However, since the normal and rearranged *bcr* and *abl* genes were expressed in these CML cells, we could not directly compare the relative rates of transcription of these genes by nuclear run-off assays.

Actinomycin D chase experiments performed on the KCL-22 CML blast crisis cell line indicate that the three normal *bcr* transcripts, as well as the aberrant *bcr-abl* hybrid transcript, shared prolonged, virtually identical half-lives (Fig. 7A). Moreover, the *bcr-abl* hybrid transcript appears to be more stable than the normal *abl* transcripts in this cell line (Fig. 7B). Thus, the half-life of the hybrid *bcr-abl* message approximates that of the normal *bcr* transcripts rather than that of the normal *abl* transcripts, suggesting that the 5' *bcr* sequences are the dominant factor in determining the relative stability of the *bcr-abl* hybrid transcript. It is also formally possible that the absence of specific 5' *abl* sequences in the *bcr-abl* fusion transcript is responsible for the prolonged half-life of the rearranged compared with the normal *c-abl* RNA. Regardless of the precise basis for the altered half-life, it appears that one consequence of the fusion of *bcr* sequences to the 5' end of the *abl* transcript is enhanced stabilization of the message, making the *bcr-abl* hybrid transcripts more resistant to degradation compared with the normal *abl* transcripts. Moreover, the similar half-lives and amounts of the normal *bcr* and the *bcr-abl* fusion transcripts in CML cells suggest that the rates of transcription of the normal *bcr* and the *bcr-abl* fusion genes must be similar. This is consistent with the above-stated hypothesis that cellular factors regulating transcription from the 5' region of the normal *bcr* gene also regulate transcription of the *bcr-abl* fusion gene.

Our data indicate that the *bcr* gene is widely expressed in both hematopoietic and nonhematopoietic cell types in different vertebrate species. It is interesting that in the chick tissue survey, the highest levels of *bcr* expression were noted in chick brain, the lowest levels were seen in liver, and a truncated *bcr* RNA was noted in testes. The significance of this pattern of expression and the normal function of this gene remain unknown. The 3' portion of *bcr* cDNA has been sequenced, and the deduced amino acid sequences have been compared by computer search with other known protein sequences without revealing any homologous proteins (11). Similarly, the normal cell function of the *c-abl* oncogene, which also is expressed in a wide variety of cell types (25), and which is a member of the tyrosine kinase family of oncogenes, is presently unknown.

It is also perplexing why the fusion of the *abl* and *bcr* genes brought about by the Ph¹ translocation is manifest predominantly in the neoplastic hematopoietic stem cells of CML rather than in other types of malignancies. In the case of the *myc* oncogene translocation to different immunoglobulin loci observed in certain mouse and human B-cell plasmacytomas and lymphomas (7, 21, 24), it is thought that the specificity of these *myc*-immunoglobulin translocations to B-cell malignancies is related to the rearrangement and transcription of the immunoglobulin loci that occur specifically in B-cell development and which may predispose these particular cells to an aberrant recombination event. By analogy, one might predict that the Ph¹ translocation would involve translocation of the *c-abl* oncogene into a locus which is specifically transcribed and undergoes rearrangement in certain hematopoietic stem cells. However, our data indicate no such transcriptional specificity of the *bcr* gene, and there is presently no evidence that the *bcr* gene normally undergoes genomic rearrangement in any cell type. Never-

theless, it is possible that such events might occur in an as yet unidentified cell in an early compartment of the hematopoietic lineage.

From these studies we conclude the following. (i) The normal *bcr* gene is evolutionarily conserved and is expressed in a wide variety of actively proliferating and nonproliferating vertebrate tissues. A truncated *bcr* mRNA is noted in chick testes. (ii) Within individual CML samples, the relative amounts of *bcr-abl* hybrid transcripts and normal chromosome 22 *bcr* transcripts are similar. (iii) The *bcr-abl* hybrid transcript is more stable than the normal *abl* transcripts, and its prolonged half-life is virtually identical to that of the normal *bcr* transcripts. (iv) The similar half-lives and amounts of the *bcr* and *bcr-abl* fusion RNAs in CML cells suggest that the rates of transcription of these genes are the same.

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