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Variable expression of the translocated c-*abl* oncogene in Philadelphia-chromosome-positive B-lymphoid cell lines from chronic myelogenous leukemia patients

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ABSTRACT **The consistent cytogenetic translocation of chronic myelogenous leukemia (the Philadelphia chromosome, Ph^1) has been observed in cells of multiple hematopoietic lineages. This translocation creates a chimeric gene composed of breakpoint-cluster-region (*bcr*) sequences from chromosome 22 fused to a portion of the *abl* oncogene on chromosome 9. The resulting gene product ($P210^{c\text{-}abl}$) resembles the transforming protein of the Abelson murine leukemia virus in its structure and tyrosine kinase activity. $P210^{c\text{-}abl}$ is expressed in Ph^1-positive cell lines of myeloid lineage and in clinical specimens with myeloid predominance. We show here that Epstein–Barr virus-transformed B-lymphocyte lines that retain Ph^1 can express $P210^{c\text{-}abl}$. The level of expression in these B-cell lines is generally lower and more variable than that observed for myeloid lines. Protein expression is not related to amplification of the *abl* gene but to variation in the level of *bcr-abl* mRNA produced from a single Ph^1 template.**

Chronic myelogenous leukemia (CML) is a disease of the pluripotent stem cell (1). In greater than 95% of patients, the leukemic cells contain the cytogenetic marker known as the Philadelphia chromosome, or Ph^1 (2). This reciprocal translocation event between the long arms of chromosomes 9 and 22 has been used as a disease-specific marker for diagnosis and evaluation of therapy. Multiple hematopoietic lineages, including myeloid and B-lymphoid, contain Ph^1 in early or chronic phase, as well as in the more acute accelerated and blast crisis phases of the disease.

One molecular consequence of Ph^1 is the translocation of the chromosomal arm containing the c-*abl* gene on chromosome 9 into the middle of the breakpoint-cluster region (*bcr*) gene on chromosome 22 (3–6). Although the precise translocation breakpoints are variable, an RNA-splicing mechanism generates a very similar 8-kilobase (kb) mRNA in each case (5–9). The hybrid *bcr-abl* message encodes a structurally altered form of the *abl* oncogene product, called $P210^{c\text{-}abl}$ (10–13), with an amino-terminal segment derived from a portion of the exons of *bcr* on chromosome 22 and a carboxyl-terminal segment derived from a major portion of the exons of the c-*abl* gene on chromosome 9. The chimeric structure of *bcr-abl* and the resulting $P210^{c\text{-}abl}$ is similar to the structure of the Abelson murine leukemia virus *gag-abl* genome and resulting $P160^{v\text{-}abl}$ transforming gene product. Both proteins have very similar tyrosine kinase activities (10, 11, 14) which can be distinguished by their relative stability to denaturing detergents and by their ATP requirements from the recently described tyrosine kinase activity of the c-*abl* gene product (15).

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In concert with structural modification of the amino-terminal portion of the *abl* gene, increased level of expression has been implicated in activation of c-*abl* oncogenic potential. Myeloid and erythroid cell lines and clinical samples derived from acute-phase CML patients contain about 10-fold higher levels of the 8-kb *bcr-abl* mRNA and $P210^{c\text{-}abl}$ than the c-*abl* mRNA forms (6 and 7 kb) and $P145^{c\text{-}abl}$ gene product (5, 8, 9, 11). The higher level of expression of the chimeric *bcr-abl* message in acute-phase cells is not likely to be solely due to the presence of the *bcr* promoter sequences at the 5′ end of the gene, since the normal 4.5-kb and 6.7-kb *bcr*-encoded mRNA species are expressed at an even lower level than the normal c-*abl* messages (5, 6).

We have analyzed a series of Epstein–Barr virus-immortalized B-lymphoid cell lines derived from CML patients (16). With such *in vitro* clonal cell lines, we can evaluate whether the presence of Ph^1 always results in synthesis of the chimeric *bcr-abl* message and protein, and whether the quantitative expression varies for cells of B-lymphoid lineage as compared to previously examined myeloid cell lines. Our results show that cell lines that retain Ph^1 do express *bcr-abl* message and protein, but that the level is generally lower and more variable than previously seen for myeloid cell lines. The demonstration that the Ph^1 chromosomal template can vary in its level of expression of $P210^{c\text{-}abl}$ suggests that secondary mechanisms, beyond the translocation itself, contribute to the regulation of the *bcr-abl* gene in different cell types or subclones that derive from the affected stem cell.

MATERIALS AND METHODS

Cells and Cell Labelings. Epstein–Barr virus-transformed B-lymphoid cell lines were established from peripheral blood samples of chronic- and acute-phase CML patients as reported (16). The cell lines are designated according to patient number, karyotype, and lineage. For example, SK-CML7Bt(9,22)-33 refers to CML patient 7, B-lymphoid cell line, 9;22 translocation (Ph^1), cell line 33; and SK-CML7BN-2 refers to B-cell line 2 with a normal karyotype derived from the same patient. Repeat karyotype analysis was performed to verify the retention of Ph^1 just prior to analysis for *abl* protein and RNA. Cells were maintained in RPMI 1640 medium with 20% fetal bovine serum. We have not observed any consistent pattern of *in vitro* growth rate that correlates to the stage of disease at the time of transformation with Epstein–Barr virus. Cells (1.5×10^7) were washed twice with Dulbecco's modified Eagle's medium lacking phosphate and

Abbreviations: *bcr*, breakpoint-cluster region; CML, chronic myelogenous leukemia; kb, kilobase(s).

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supplemented with 5% dialyzed fetal bovine serum. Cells were then resuspended in 2 ml of the minimal medium. Labeling was started with the addition of [^{32}P]orthophosphate (1 mCi/ml; ICN; 1 Ci = 37 GBq) and continued at 37°C for 3–4 hr.

Immunoprecipitation and Immunoblotting. Immunoprecipitations were carried out as described (10). Cells (1.5×10^7) were washed with phosphate-buffered saline and extracted with 3–5 ml of phosphate lysis buffer (1% Triton X-100/0.1 $NaDodSO_4$/0.5% deoxycholate/10 mM Na_2HPO_4, pH 7.5/100 mM NaCl) with 5 mM EDTA and 5 mM phenylmethylsulfonyl fluoride. Extracts were clarified by centrifugation and precipitated with normal or rabbit anti-abl sera (anti-pEX-2 or anti-pEX-5) (17). The precipitated proteins were electrophoresed in a $NaDodSO_4$/8% polyacrylamide gel. ^{32}P-labeled proteins were detected by autoradiography. Alternatively, *abl* proteins were detected by immunoblotting. Extracts from unlabeled cells were clarified, and proteins were concentrated by immunoprecipitation with rabbit antisera against *abl*-encoded proteins [anti-pEX-2 and anti-pEX-5 combined (17)] and then fractionated in 8% acrylamide gels. The proteins were transferred from the gel to nitrocellulose filters, using protease-facilitated transfer (18). The *abl*-encoded proteins were detected using murine monoclonal antibodies as a probe and peroxidase-conjugated goat anti-mouse second stage antibody (Bio-Rad) for development. Rabbit antisera and mouse monoclonal antibodies to *abl* proteins were prepared using bacterially expressed regions of the v-*abl* protein as immunogens (17, 19). Anti-pEX-2 antibodies react with the internal tyrosine kinase domain and anti-pEX-5 antibodies react with the carboxyl-terminal segment of the *abl* proteins.

RNA Analysis. RNA was extracted from 10^8 cells by the $NaDodSO_4$/urea/phenol method (20). Polyadenylylated RNA was purified by oligo(dT) affinity chromatography. Samples were electrophoresed in a 1% agarose/formaldehyde gel and transferred to nitrocellulose. *abl* RNA species were detected by hybridization with a nick-translated v-*abl* fragment probe (21).

DNA Analysis. DNA was prepared from 5×10^7 cells of each cell line and processed for Southern blots with a v-*abl* probe as described (21).

RESULTS

Variable Levels of P210$^{c\text{-}abl}$ Are Detected in Ph^1-Positive Cell Lines. Ph^1-positive and Ph^1-negative, Epstein–Barr virus-transformed B-lymphocyte cell lines derived from the same patient were examined for P210$^{c\text{-}abl}$ synthesis by immunoprecipitation of [^{32}P]orthophosphate-labeled cell extracts with anti-abl sera (Fig. 1). The normal c-*abl* protein P145$^{c\text{-}abl}$ was detected at a similar level in multiple Ph^1-positive and Ph^1-negative cell lines. P210$^{c\text{-}abl}$ was only detected in the Ph^1-positive cell lines because the *bcr-abl* chimeric gene which encodes P210$^{c\text{-}abl}$ resides on the Ph^1 (4, 5, 11, 13). The level of P210$^{c\text{-}abl}$ was about 4- to 5-fold higher than the level of P145$^{c\text{-}abl}$ in the SK-CML7Bt-33 cell line (Fig. 1*A*, +). The Ph^1-positive erythroid-progenitor cell line K562 (*C*) showed a level of P210$^{c\text{-}abl}$ about 10-fold higher than P145$^{c\text{-}abl}$. However, the level of P210$^{c\text{-}abl}$ was about one-fifth that of P145$^{c\text{-}abl}$ in the Ph^1-positive SK-CML16Bt-1 cell line (Fig. 1*B*, +). Comparison of different autoradiographic exposures roughly indicated that the level of P210$^{c\text{-}abl}$ varies over a 20-fold range between these Ph^1-positive B-cell lines. Analysis of four additional Ph^1-positive B-cell lines demonstrated that the level of P210$^{c\text{-}abl}$ fell into two general classes; some cell lines had a level of P210$^{c\text{-}abl}$ similar to SK-CML7Bt-33 and others had the low level similar to SK-CML16Bt-1 (Table 1). This differs from previous studies with Ph^1-positive myeloid cell lines and patient samples derived from acute-phase CML patients, in which P210$^{c\text{-}abl}$ was detected at a 10-fold higher level than P145$^{c\text{-}abl}$ (refs. 10 and 11; Table 1). There was no large difference in level of chimeric mRNA and P210$^{c\text{-}abl}$ expressed in four myeloid/erythroid-lineage Ph^1-positive cell lines (K562, EM2, EM3, CML22, and BV173; refs. 9 and 11), despite a 4- to 5-fold amplification of *abl*-related sequences in the K562 cell line.

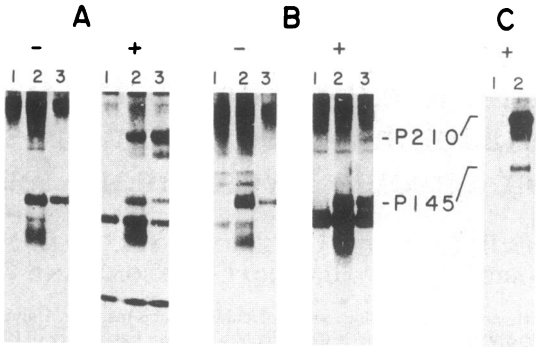

FIG. 1. Detection of variable levels of P210$^{c\text{-}abl}$ in Ph^1-positive B-cell lines. Production of P145$^{c\text{-}abl}$ and P210$^{c\text{-}abl}$ in Epstein–Barr virus-transformed B-cell lines derived from a blast-crisis (*A*) and a chronic-phase (*B*) CML patient was examined by metabolic labeling with [^{32}P]orthophosphate and immunoprecipitation. Ph^1-negative (−) and Ph^1-positive (+) cell lines derived from each patient were analyzed. The Ph^1-negative cell line in *A*,− is SK-CML7BN-2 and in *B*,− is SK-CML16BN-1. The Ph^1-positive cell line in *A*,+ is SK-CML7Bt-33 and in *B*,+ is SK-CML16Bt-1. The K562 cell line, a Ph^1-positive erythroid progenitor cell line spontaneously derived from a blast-crisis patient (33), is represented in *C*. Cells (1.5×10^7) were metabolically labeled with 2 mCi of [^{32}P]orthophosphate for 3–4 hr and then were extracted and clarified by centrifugation. Samples were immunoprecipitated with control normal serum (lanes 1), anti-pEX-2 (lanes 2), or anti-pEX-5 (lanes 3) and analyzed by $NaDodSO_4$/8% PAGE followed by autoradiography with an intensifying screen (3 days for *A* and *C*, 10 days for *B*).

Detection of different levels of P210$^{c\text{-}abl}$ in Fig. 1 could be due to decreased phosphorylation of P210$^{c\text{-}abl}$, a lower level of P210$^{c\text{-}abl}$ synthesis, or altered stability of the protein. To help distinguish among these possibilities, the steady-state level of P210$^{c\text{-}abl}$ in the cell lines was assayed by immunoblotting. The results show that SK-CML7Bt-33 (Fig. 2*A*, +) had a higher level of P210$^{c\text{-}abl}$ than P145, similar to the results with metabolic labeling (Fig. 1). We did not detect P210$^{c\text{-}abl}$ by immunoblotting with 2×10^7 cells of line SK-CML8Bt-3 (Fig. 2*B*, +). Reconstruction experiments using dilutions of cell extracts showed that we could detect about 5–10% the level of P210$^{c\text{-}abl}$ expressed in the K562 cell line (data not shown). We infer that the steady-state level of P210$^{c\text{-}abl}$ in SK-CML8Bt-3 is lower than the level in SK-CML7Bt-33 by a factor of at least 10. The level of P210$^{c\text{-}abl}$ detected in these assays correlated with the amount of P210$^{c\text{-}abl}$ tyrosine kinase activity that could be detected *in vitro* (data not shown).

Different Levels of P210$^{c\text{-}abl}$ Are Reflected in the Amount of Stable *bcr-abl* mRNA. To identify the basis for detection of variable levels of P210$^{c\text{-}abl}$, we examined the production of the *abl* RNA. RNA blot hybridization analysis using a v-*abl* probe (Fig. 3) showed that the normal 6- and 7-kb c-*abl* mRNAs were present at a similar level in Ph^1-positive and -negative cell lines derived from different patients. However, the 8-kb mRNA that encodes P210$^{c\text{-}abl}$ was detected at a 10-fold higher level in SK-CML7Bt-33 (Fig. 3*A*, +) than in SK-CML16Bt-1 (*B*, +), which correlated with the relative level of P210$^{c\text{-}abl}$ detected in each cell line. Analysis of additional cell lines demonstrated that the level of 8-kb RNA directly correlated with the level of P210$^{c\text{-}abl}$ (Table 1). The variation in level of 8-kb RNA detected in these cell lines was not due to loss or gain of Ph^1, because cytogenetic analysis confirmed the presence of Ph^1 in these cell lines (ref. 16 and

Table 1. Relative levels of *bcr-abl* expression in Epstein–Barr virus-immortalized B-cell lines and myeloid CML lines

Cell line*	CML phase†	Ph¹‡	P210§	8-kb mRNA¶
SK-CML7BN-2	BC	−	−	−
SK-CML8BN-10	Chronic	−	−	−
SK-CML8BN-12	Chronic	−	−	−
SK-CML16BN-1	Chronic	−	−	−
SK-CML35BN-1	Chronic	−	−	−
SK-CML7B5-33	BC	+	+++	+++
SK-CML21Bt-1	Acc	+	+++	+++
SK-CML21Bt-6	Acc	+	+++	+++
SK-CML8Bt-3	Chronic	+	+	±
SK-CML16Bt-1	Chronic	+	+	+
SK-CML35Bt-2	Chronic	+	+	+
K562	BC	+	+++++	+++++
BV173	BC	+	+++++	+++++
EM2	BC	+	+++++	+++++

*Cell lines derived from CML patients by transformation with Epstein–Barr virus as described (16). Names of cell lines indicate patient number and Ph¹ status: SK-CML7Bt indicates a cell line derived from patient 7 that carries the 9;22 Ph¹ translocation; N indicates a normal karyotype. Myeloid-erythroid cell lines (K562, EM2, and BV173) are described in previous publications (9, 11, 22, 33).

†Status of patient at the time cell line was derived. BC, blast crisis; Acc, accelerated phase.

‡Presence (+) or absence (−) of Ph¹ as demonstrated by karyotypic or Southern blot analysis.

§$P210^{c\text{-}abl}$ detected as described in legend to Fig. 1. B-cell lines derived from blast-crisis and accelerated-phase patients had levels of P210 3- to 5-fold higher (+++) than levels of P145. Chronic-phase-derived cell lines had P210 levels lower than or just equivalent (+) to the level of P145. Myeloid and erythroid lines had levels of P210 5- to 10-fold higher than P145 (+++++).

¶Eight-kilobase *bcr-abl* mRNA detected as described in legend to Fig. 2. Symbols: ±, borderline detectable; +++++, level of 8-kb mRNA 5- to 10-fold higher than that of the 6- and 7-kb c-*abl* mRNA species; +++, level of 8-kb mRNA 3- to 5-fold higher than that of the 6- and 7-kb species; +, a level approximately equivalent to that of the 6- and 7-kb messages.

data not shown). There was no difference in the copy number of *abl*-related sequences as judged by Southern blot analysis (Fig. 4). Only the K562 cell line control showed an amplification of *abl* sequences, as previously reported (22, 23). These combined data suggest that differential *bcr-abl* mRNA expression from a single gene template is responsible for the variable levels of $P210^{c\text{-}abl}$ detected. This could be mediated

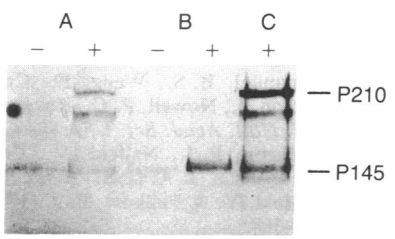

FIG. 2. Analysis of steady-state *abl* protein levels by immunoblotting. Cell extracts prepared from 2×10^7 cells of lines SK-CML7BN-2 (*A*,−), SK-CML7Bt-33 (*A*,+), SK-CML8BN-10 (*B*,−), and SK-CML8Bt-3 (*B*,+) were concentrated by immunoprecipitation with anti-pEX-2 plus anti-pEX-5. Samples were then electrophoresed in a $NaDodSO_4$/8% polyacrylamide gel and transferred to nitrocellulose, using protease-facilitated transfer (18). *abl* proteins were detected using a mixture of two monoclonal antibodies directed against the pEX-2 and pEX-5 *abl*-protein fragments produced in bacteria (19) as a probe and a peroxidase-conjugated goat anti-mouse second-stage antibody (Bio-Rad) for development.

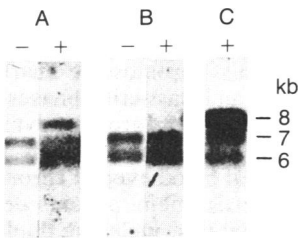

FIG. 3. Comparison of *abl* RNA levels in Ph¹-positive and -negative B-cell lines. The levels of the normal 6- and 7-kb c-*abl* RNAs and the 8-kb *bcr-abl* RNA were analyzed by blot hybridization using a v-*abl* probe. RNA was extracted from Ph¹-negative lines SK-CML7BN-2 (*A*,−) and SK-CML16BN-1 (*B*,−), from Ph¹-positive lines SK-CML6Bt-33 (*A*,+) and SK-CML16Bt-3 (*B*,+), and from line K562 (*C*,+) by the $NaDodSO_4$/urea/phenol method (20). Polyadenylylated RNA was purified by oligo(dT) affinity chromatography, and 15 μg of each sample was electrophoresed in a 1% agarose/formaldehyde gel and then transferred to nitrocellulose. The blotted RNAs were hybridized with a nick-translated v-*abl* fragment probe (21) and then autoradiographed for 4 days.

by factors influencing the transcription rate of the *bcr-abl* gene or the stability of the mRNA.

DISCUSSION

Several lines of evidence suggest that formation of Ph¹ is not the primary event that affects the stem cell in CML. Patients have been identified that present with the clinical picture of CML but only later develop Ph¹ (1). This observation, coupled with studies of *G6PD* (glucose-6-phosphate dehydrogenase)-heterozygous females with CML that demonstrate stem-cell clonality by isozyme analysis among cell

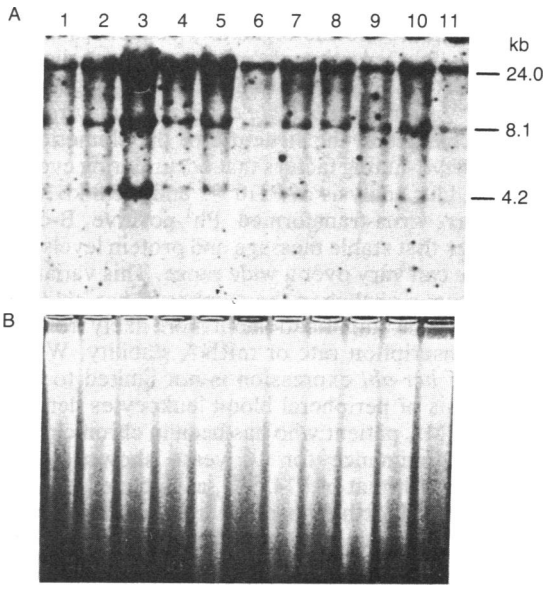

FIG. 4. Southern blot analysis of *abl* sequences in Ph¹-positive and -negative B-cell lines. High molecular weight DNA (15 μg) was digested with restriction endonuclease *Bam*HI, separated in a 0.8% agarose gel, and then transferred to nitrocellulose. The blotted DNA fragments were hybridized with a nick-translated, 2.4-kb *Bgl* II v-*abl* fragment (1.5×10^8 cpm/μg; ref. 21) and exposed for 4 days. (*A*) Autoradiogram of *abl*-specific fragments in cell lines HL-60 (lane 1), EM2 (lane 2), K562 (lane 3), SK-CML7Bt-33 (lane 4), SK-CML8Bt-3 (lane 5), SK-CML16Bt-1 (lane 6), SK-CML21Bt-6 (lane 7), SK-CML35Bt-2 (lane 8), SK-CML7BN-2 (lane 9), SK-CML8BN-2 (lane 10), and SK-CML35BN-1 (lane 11). (*B*) Ethidium bromide staining of agarose gel prior to transfer to nitrocellulose, showing the level of variation in amount of DNA loaded per lane.

populations that lack the Ph^1 marker, supports a secondary or complementary role for Ph^1 in the progression of the disease (24, 25). This chromosome marker is found in chronic, accelerated, and blast-crisis phases of the disease. It is likely that Ph^1 confers some growth advantage, since cells with the marker chromosome eventually predominate the marrow and peripheral blood even in chronic phase. During the phase of blast crisis, many patients develop additional chromosome abnormalities, including duplication of Ph^1, a variety of trisomies, and complex translocations (26). This is suggestive evidence for Ph^1 being a necessary but not sufficient genetic change for the full evolution of the disease.

The realization that one molecular result of Ph^1 is the generation of a chimeric *bcr-abl* protein with functional characteristics and structure analogous to the *gag-abl* transforming protein of the Abelson murine leukemia virus strengthens the argument for an important role of Ph^1 in the pathogenesis of CML. Although the Abelson virus is generally considered a rapidly transforming retrovirus, its effects can range from overcoming growth factor requirements, to cellular lethality, to induction of highly oncogenic tumors in a number of hematopoietic cell lineages (27, 28). Even in the transformation of murine cell targets, there are several lines of evidence that suggest that the growth-promoting activity of the v-*abl* gene product is complemented by further cellular changes in the production of the malignant-cell phenotype (29–31).

The regulation of *bcr-abl* gene expression is complex because the 5′ end of the gene is derived from the non-*abl* sequences, *bcr*, normally found on chromosome 22 (6). The level of stable message for the normal *bcr* gene and the normal *abl* gene are both much lower than the level of the *bcr-abl* message and protein from cell lines and clinical specimens derived from myeloid blast-crisis patients (5, 6, 11). Therefore, the high level of *bcr-abl* expression cannot simply be attributed to the regulatory sequences associated with *bcr*. Possibly, creation of the chimeric gene disrupts the normal regulatory sequences and results in a higher level of expression. Variation in *bcr-abl* expression may result from secondary changes in the structure of the chimeric gene or function of *trans*-acting factors that occur during evolution of the disease. Our analysis of $P210^{c\text{-}abl}$ and the 8-kb mRNA in Epstein–Barr virus-transformed Ph^1-positive B-cell lines demonstrates that stable message and protein levels from the *bcr-abl* gene can vary over a wide range. This variation does not result from a change in the number of *bcr-abl* templates secondary to gene amplification but more likely from changes in either transcription rate or mRNA stability. We suspect this range of *bcr-abl* expression is not limited to lymphoid cells. Analysis of peripheral blood leukocytes derived from an unusual CML patient who has been in chronic phase with myeloid predominance for 16 years showed a level of $P210^{c\text{-}abl}$ one-fifth that of $P145^{c\text{-}abl}$, as detected by metabolic labeling with [^{32}P]orthophosphate and immunoprecipitation (S.C., O.N.W., and P. Greenberg, unpublished observations). Lower levels of expression of the chimeric mRNA have been demonstrated in clinical samples from chronic-phase CML patients compared to acute-phase CML patients (9). Others have reported chronic-phase patients with variable but, in some cases, relatively high levels of the *bcr-abl* mRNA (32). The sampling variation and the heterogenous mixture of cell types in clinical samples complicate such analyses. Further work is needed to evaluate whether there is a defined change in $P210^{c\text{-}abl}$ expression during the progression of CML. It is interesting to note that among the limited sample of Ph^1-positive B-cell lines we have examined (Table 1), we have seen higher levels of $P210^{c\text{-}abl}$ in those derived from patients at more advanced stages of the disease. It will be important to search for cell-type-specific mechanisms that might regulate expression of *bcr-abl* from Ph^1.

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1. Champlin, R. E. & Golde, D. W. (1985) *Blood* **65,** 1039–1047.
2. Rowley, J. D. (1973) *Nature (London)* **243,** 290–291.
3. Heisterkamp, N., Stephenson, J. R., Groffen, J., Hansen, P. F., de Klein, A., Bartram, C. R. & Grosveld, G. (1983) *Nature (London)* **306,** 239–242.
4. Bartram, C. R., de Klein, A., Hagemeijer, A., van Agthoven, T., van Kessel, A. G., Bootsma, D., Grosveld, G., Ferguson-Smith, M. A., Davies, T., Stone, M., Heisterkamp, N., Stephenson, J. R. & Groffen, J. (1983) *Nature (London)* **306,** 277–280.
5. Shtivelman, E., Lifshitz, B., Gale, R. P. & Canaani, D. (1985) *Nature (London)* **315,** 550–554.
6. Heisterkamp, N., Stam, K. & Groffen, J. (1985) *Nature (London)* **315,** 758–761.
7. Groffen, J., Stephenson, J. R., Heisterkamp, N., de Klein, A., Bartram, C. R. & Grosveld, G. (1984) *Cell* **36,** 93–99.
8. Gale, R. P. & Canaani, E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5648–5652.
9. Collins, S., Kubonishi, L., Miyoshi, I. & Groudine, M. T. (1984) *Science* **225,** 72–74.
10. Konopka, J. B., Watanabe, S. M. & Witte, O. N. (1984) *Cell* **7,** 1035–1042.
11. Konopka, J. B., Watanabe, S. M., Singer, J., Collins, S. & Witte, O. N. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1810–1814.
12. Kloetzer, W., Kurzrock, R., Smith, L., Talpaz, M., Spiller, M., Gutterman, J. & Arlinghaus, R. (1985) *Virology* **140,** 230–238.
13. Kozbor, D., Giallongo, A., Sierzega, M. E., Konopka, J. B., Witte, O. N., Showe, L. C. & Croce, C. M. (1985) *Nature (London)*, in press.
14. Davis, R. L., Konopka, J. B. & Witte, O. N. (1985) *Mol. Cell Biol.* **5,** 204–213.
15. Konopka, J. B. & Witte, O. N. (1985) *Mol. Cell. Biol.* **5,** 3116–3123.
16. Nitta, M., Kato, Y., Strife, A., Wachter, M., Fried, J., Perez, A., Jhanwar, S., Duigou, R., Chaganti, R. S. K. & Clarkson, B. (1985) *Blood* **66,** 1053–1061.
17. Konopka, J. B., Davis, J. L., Watanabe, S. M., Ponticelli, A. S., Schiff-Maker, L., Rosenberg, N. & Witte, O. N. (1984) *Virology* **51,** 223–232.
18. Gibson, W. (1981) *Anal. Biochem.* **118,** 1–3.
19. Schiff-Maker, L., Konopka, J. B., Clark, S., Witte, O. N. & Rosenberg, N. (1986) *J. Virol.* **57,** 1182–1186.
20. Schwartz, R. C., Sonenshein, G. E., Bothwell, A. & Gefter, M. L. (1981) *J. Immunol.* **126,** 2104–2108.
21. Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. (1980) *Cell* **22,** 777–785.
22. Collins, S. J. & Groudine, M. T. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4813–4817.
23. Selden, J. R., Emanuel, B. S., Wang, E., Cannizzaro, L., Palumbo, A., Erikson, J., Nowell, P. C., Rovera, G. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7289–7292.
24. Fialkow, P. J., Martin, P. J., Najfeld, V., Penfold, G. K., Jacobson, R. J. & Hansen, J. A. (1981) *Blood* **58,** 158–163.
25. Martin, P. J., Najfeld, V. & Fialkow, P. J. (1982) *Can. Gen. Cytogenet.* **6,** 359–368.
26. Rowley, J. D. (1980) *Annu. Rev. Genet.* **14,** 17–40.
27. Whitlock, C. A. & Witte, O. N. (1984) *Adv. Immunol.* **37,** 74–98.
28. Pierce, J. H., Di Fiore, P. P., Aaronson, S. A., Potter, M., Pumphrey, J., Scott, A. & Ihle, J. N. (1985) *Cell* **41,** 685–693.
29. Whitlock, C. A., Ziegler, S. & Witte, O. N. (1983) *Mol. Cell. Biol.* **3,** 596–604.
30. Wolf, D., Harris, N. & Rotter, V. (1984) *Cell* **38,** 119–126.
31. Klein, G. & Klein, E. (1985) *Nature (London)* **315,** 190–195.
32. Stam, K., Jr., Heisterkamp, N., Grosveld, G., de Klein, A., Verma, R., Colemen, M., Dosik, H. & Groffen, J. (1985) *N. Engl. J. Med.* **313,** 1429–1433.
33. Lozzio, C. B. & Lozzio, B. B. (1975) *Blood* **45,** 321–334.