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Cell lines and clinical isolates derived from Ph[1]-positive chronic myelogenous leukemia patients express c-*abl* proteins with a common structural alteration

(chromosome translocation/human leukemia/tyrosine kinase/oncogene)

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ABSTRACT **The Philadelphia chromosome (Ph[1]), observed in >90% of chronic myelogenous leukemia (CML) patients, results from a specific chromosomal translocation involving the c-*abl* gene. The translocation breakpoint occurs near c-*abl* and correlates with the production of an altered c-*abl* mRNA. In the CML-derived cell line K562, Ph[1] is accompanied by a structurally altered c-*abl* protein (P210[c-abl]) with *in vitro* tyrosine kinase activity not detected with the normal c-*abl* protein (P145[c-abl]). We have examined c-*abl* proteins in other Ph[1]-positive CML cell lines and found that they all express P210[c-abl]. P210[c-abl] was also detected in bone marrow cells from CML patients with Ph[1] in the accelerated and blast crisis phases of the disease. Comparison of the [^{35}S]methionine-labeled tryptic peptides generated from the normal P145[c-abl] and P210[c-abl] showed that they have closely related structures, but additional polypeptide sequences are present in P210[c-abl]. Based on these results we propose that translocation of c-*abl* in Ph[1]-positive CML results in the creation of a chimeric gene leading to the production of a structurally altered c-*abl* protein with activated tyrosine kinase activity. The altered P210 c-*abl* protein is strongly implicated in the pathogenesis of CML.**

The c-*abl* gene is the normal cellular homolog of the transforming gene of Abelson murine leukemia virus (Ab-MuLV) (1–3) and the Hardy–Zuckerman II feline sarcoma virus (4). Translocation of the c-*abl* gene from chromosome 9 to 22, resulting in the Philadelphia chromosome (Ph[1]), occurs in >90% of chronic myelogenous leukemia (CML) patients (5). The translocation breakpoint generally occurs within a limited region, *bcr*, on chromosome 22 and at a variable distance 5′ to the c-*abl* gene on chromosome 9 (6, 7). Analysis of c-*abl* RNA in Ph[1]-positive cell lines and patients has detected a unique 8-kilobase-pair (kb) c-*abl* mRNA (8, 9). In addition, a structurally altered c-*abl* protein (P210[c-abl]) has been detected in the CML cell line K562 (10) accompanying translocation (t9:22) and a 4–8× amplification of the c-*abl* gene in this cell line (11, 12). Amplification of the c-*abl* gene has only been observed in the K562 cell line.

Amino-terminal alteration of P210[c-abl] in K562 cells activates its tyrosine-specific protein kinase activity (10) similar to the results of viral transduction of c-*abl* (4, 13). The normal human c-*abl* protein (P145[c-abl]) (10) and murine c-*abl* protein (P150[c-abl]) (14, 15) lack detectable tyrosine kinase activity *in vitro*. Detailed comparison of the *in vitro* tyrosine kinase activities of P160[v-abl] and P210[c-abl] shows they are remarkably similar in terms of their substrates, stabilities, and optimal reaction conditions (16). The transforming activity of the v-*abl* protein is known to be mediated by its tyrosine kinase activity (13, 17–19). This suggests that the tyrosine kinase activity of P210[c-abl] may also have growth-promoting activity. Generation of Ph[1] may represent a common mechanism for activating the c-*abl* kinase activity. We have analyzed c-*abl* protein production in four different CML cell lines and in clinical isolates from CML patients and found that they also express P210[c-abl]. Since a similar P210[c-abl] was detected in CML samples derived from different individuals, some mechanism must override the variable position of the breakpoint. Possibly, the Ph[1] translocation results in creation of a chimeric gene. The variable breakpoint sequences could be removed from the primary transcript by splicing to generate an 8-kb c-*abl* mRNA that is of sufficient size to encode the P210[c-abl] protein. Creation of P210[c-abl] with active tyrosine kinase by this mechanism is functionally homologous to the results of viral transduction of the c-*abl* gene by Ab-MuLV.

MATERIALS AND METHODS

CML Patients, Cells, and Cell Labelings. EM-2 and EM-3 (20) are Ph[1]-positive cell lines isolated from a patient who relapsed with CML following bone marrow transplantation. BV173 (G. Pegararo and C. Croce, personal communication) and K562 (12) were isolated from separate patients during the blast crisis phase of CML. KG-1 (21) is a Ph[1]-negative myeloid leukemia cell line. Exponentially growing cells from established cell lines ($1–3 \times 10^7$) were washed twice with 150 mM NaCl and then labeled in 2 ml of Dulbecco's modified Eagle's medium (DME medium) lacking either phosphate or methionine and containing 5% dialyzed fetal calf serum. Labelings were started with the addition of [^{32}P]orthophosphate (ICN; 1 mCi/ml; 1 Ci = 37 GBq) or [^{35}S]methionine (Amersham; 1 mCi/ml) and incubated for 3–4 hr at 37°C. Bone marrow aspirates of CML patients in Seattle were frozen in serum plus 5% dimethyl sulfoxide and stored in liquid N_2. Where designated, cells were first fractionated on a Percoll gradient to enrich for light density cells. Patient no. 2543 was a Ph[1]-positive 28-year-old female in the accelerated phase. Patient no. 2518 was a Ph[1]-positive 16-year-old male in accelerated phase. Patient no. 2500 was a Ph[1]-positive 41-year-old female in blast crisis. Frozen cells ($1–3 \times 10^7$) were incubated in RPMI medium plus 20% fetal calf serum overnight prior to analysis. Patient samples were labeled as above except that they were washed and labeled in DME medium lacking phosphate and containing 20% dialyzed fetal calf serum.

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Abbreviations: Ab-MuLV, Abelson murine leukemia virus; kb, kilobase pair(s); CML, chronic myelogenous leukemia.

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Immunoprecipitations and Protein Analysis. Cells ($1–3 \times 10^7$) were washed with phosphate-buffered saline and then extracted into 3 ml of PL buffer (phosphate lysis buffer: 1% Triton X-100/0.1% $NaDodSO_4$/0.5% deoxycholate/10 mM Na_2HPO_4/NaH_2PO_4, pH 7.5/100 mM NaCl) with 5 mM EDTA and 5 mM phenylmethylsulfonyl fluoride (Sigma). Cells obtained directly from CML patients were boiled for 3 min in 0.75 ml of 0.5% $NaDodSO_4$/1 mM dithiothreitol/5 mM EDTA/10 mM Na_2HPO_4/NaH_2PO_4, pH 7.0, and leupeptin at 75 μg/ml and then diluted with 4 vol of PL buffer. Extracts were clarified by centrifugation and then incubated overnight with 5 μl of normal or anti-*abl* sera per ml of extract. As previously described (22), anti-pEX-2 cross-reacts with the internal *src*-homologous domain and anti-pEX-5 cross-reacts with the carboxyl terminus of the v-*abl* protein. The immune complexes were collected by the addition of 50 μl of a 10% (wt/vol) suspension of formalin-fixed *Staphylococcus aureus* (23) per ml of extract. Autophosphorylation (10), phosphoamino acid analysis (10), partial proteolysis using *S. aureus* V-8 protease (24), and two-dimensional analysis of [^{35}S]methionine-labeled tryptic peptides (25) for the P210 c-*abl* protein were performed essentially as described.

RESULTS

Independent Ph^1-Positive CML Cells Produce Indistinguishable Altered c-*abl* Proteins. To determine whether synthesis of an altered c-*abl* protein is a common feature of all Ph^1-positive CML cells, their c-*abl* proteins were examined by immunoprecipitation. Because the translocation breakpoints on chromosome 9 were likely to vary (6), we also wanted to determine if the size of the abnormal c-*abl* proteins would vary. Four Ph^1-positive CML cell lines and a control Ph^1-negative myeloid leukemia cell line were metabolically labeled with [^{32}P]orthophosphate, extracted, immunoprecipitated with anti-*abl* sera, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography as shown in Fig. 1. Analysis of the control Ph^1-negative myeloid leukemia cell line KG-1 (Fig. 1*A*) demonstrates the precipitation of the normal M_r 145,000 (P145$^{c\text{-}abl}$) c-*abl* protein with two different anti-*abl* sera (lanes 1 and 2) but not with control serum (lane C). This pattern of normal c-*abl* protein production has also been seen in control murine and human cells, including the HL-60, CCRF-CEM, 293, and IM9 cell lines (refs. 10, 15; unpublished data). Similar analysis of the Ph^1-positive CML cell lines EM-2 (Fig. 1*B*), EM-3 (Fig. 1*C*), BV173 (Fig. 1*D*), and K562 (Fig. 1*E*) demonstrates the precipitation of an altered c-*abl* protein of $M_r \approx 210{,}000$ (P210$^{c\text{-}abl}$). The similar high molecular weight P210$^{c\text{-}abl}$ detected in different Ph^1-positive CML cells correlates with the expression of a unique 8-kb mRNA in Ph^1-positive CML cells (8, 9).

Bone marrow cells from Ph^1-positive CML patients were also assayed for c-*abl* protein expression. P210$^{c\text{-}abl}$ can be detected in clinical isolates following metabolic labeling with [^{32}P]orthophosphate and immunoprecipitation analysis (Fig. 1*F*). Detection of the c-*abl* proteins from Ph^1-positive CML patient samples is hindered by their poor cell viability and lower metabolic activity *in vitro*. However, better results were obtained by using Percoll gradient fractionation to enrich for light density blast cells (Fig. 1 *G* and *H*). P210$^{c\text{-}abl}$ has been detected in fractionated light density cells from two accelerated-phase CML patients and one of two blast crisis patients (Fig. 1 *G* and *H*; also data not shown). However, the sensitivity of detection of P210$^{c\text{-}abl}$ must be improved before we can rigorously determine if P210$^{c\text{-}abl}$ is present in every CML patient.

The Normal P145$^{c\text{-}abl}$ Is Not Detected in Some Ph^1-Positive Cells. In the K562 cell line, both P145$^{c\text{-}abl}$ and P210$^{c\text{-}abl}$ are detected (Fig. 1*E*). However, the EM-2, EM-3, and BV173 CML cell lines lack detectable expression of P145$^{c\text{-}abl}$ (Fig. 1 *B–D*). EM-2 cells have been shown to lack detectable expression of the normal 6- and 7-kb c-*abl* mRNAs (9). The lack of normal c-*abl* protein in the EM-2 and EM-3 cell lines correlates with the lack of a normal chromosome 9 containing a normal c-*abl* allele (20). The only c-*abl* allele present in these cells is presumably altered by reciprocal translocation between chromosomes 9 and 22. This suggests that the generation of Ph^1 may result in the expression of P210$^{c\text{-}abl}$ but not the expression of P145$^{c\text{-}abl}$. These results also suggest that P145$^{c\text{-}abl}$ may not be essential for growth or that its function can be fulfilled by P210$^{c\text{-}abl}$. More sensitive assay conditions to detect c-*abl* protein in clinical isolates will be necessary to examine the possibility that loss of normal c-*abl* protein expression is involved in the pathogenesis of CML.

Altered c-*abl* Proteins Have Similar *in Vitro* Kinase Activities. The normal c-*abl* protein lacks detectable *in vitro* tyrosine kinase activity but P210$^{c\text{-}abl}$ isolated from K562 cells has been shown to have *in vitro* kinase activity similar to the v-*abl* protein (10, 16). To determine whether *in vitro* kinase activity is a common property of the altered c-*abl* proteins, different CML cell lines were extracted, immunoprecipitated with anti-*abl* sera, and then incubated with [γ-^{32}P]ATP under conditions previously used to detect the autophosphorylation of P210$^{c\text{-}abl}$ from K562 cells (10). The reaction products were analyzed by polyacrylamide gel electrophoresis as shown in Fig. 2. A M_r 210,000 species was not detected for the control Ph^1-negative myeloid leukemia cell line KG-1 (Fig. 1*A*), which correlates with its lack of detectable P210$^{c\text{-}abl}$ following metabolic labeling (Fig. 1*A*). In addition,

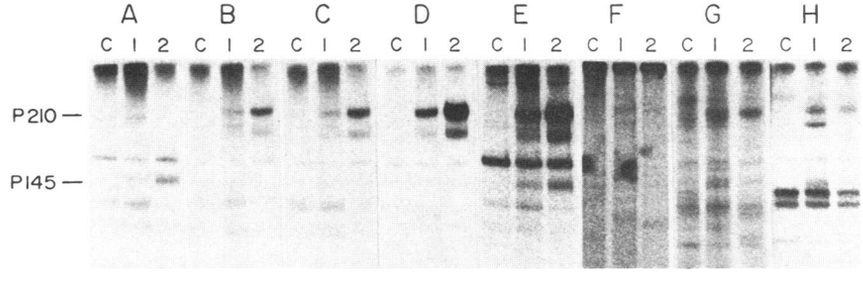

FIG. 1. Altered c-*abl* proteins are detected in independently derived Ph^1-positive CML cells. Control Ph^1-negative myeloid leukemia cell line KG-1 (*A*), Ph^1-positive CML cell lines EM-2 (*B*), EM-3 (*C*), BV173 (*D*), and K562 (*E*), bone marrow cells from accelerated-phase CML patient no. 2543 (*F*), and Percoll gradient (26) fractionated light density bone marrow cells from accelerated-phase CML patient no. 2518 (*G*) and blast crisis phase CML patient no. 2500 (*H*) were metabolically labeled with [^{32}P]orthophosphate and then extracted. Clarified extracts were immunoprecipitated with control normal serum (lanes C) or anti-v-*abl* sera anti-pEX-2 (lanes 1) and anti-pEX-5 (lanes 2). Samples were electrophoresed on an 8% $NaDodSO_4$/polyacrylamide gel and dried and bands were detected by autoradiography with an intensifying screen for 2 days (*A–E*) or 3 weeks (*F–H*).

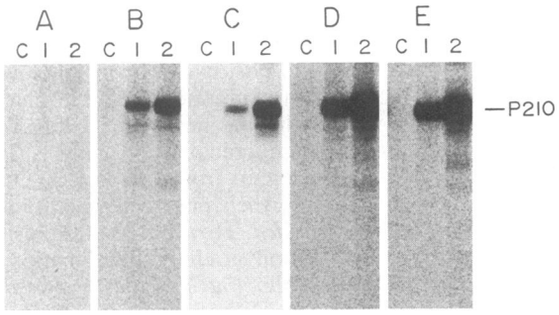

FIG. 2. *In vitro* phosphorylation of P210$^{c\text{-}abl}$. Cell extracts prepared from the control Ph^1-negative myeloid leukemia cell line KG-1 (*A*) and the Ph^1-positive CML cell lines EM-2 (*B*), EM-3 (*C*), BV173 (*D*), and K562 (*E*) were immunoprecipitated with control normal serum (lanes C) or anti-v-*abl* sera anti-pEX-2 (lanes 1) and anti-pEX-5 (lanes 2). Immunoprecipitates were washed twice with PL buffer, washed once with 50 mM Tris (pH 7.0), and then resuspended in 45 μl of Pipes (pH 7.0) with 10 mM $MnCl_2$. Reactions were started with the addition of 5 μCi of [γ-^{32}P]ATP (ICN; 7000 Ci/mmol) and then incubated at 30°C for 2 min. Samples were then washed with PL buffer and analyzed by electrophoresis on an 8% $NaDodSO_4$/polyacrylamide gel and autoradiography with an intensifying screen for 1 day.

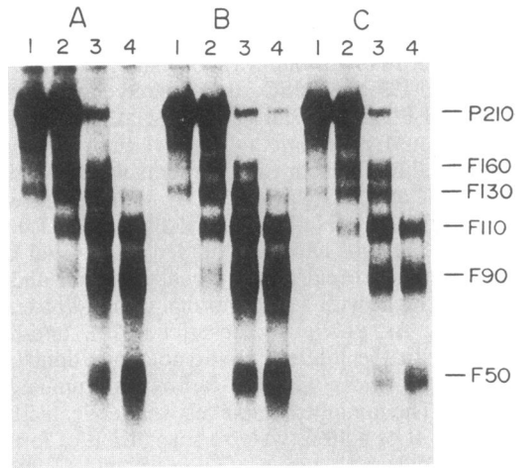

FIG. 3. Partial proteolytic analysis of P210$^{c\text{-}abl}$ from independently derived Ph^1-positive CML cell lines. P210$^{c\text{-}abl}$s from the EM-2 (*A*), BV173 (*B*), and K562 (*C*) Ph^1-positive CML cell lines were phosphorylated *in vitro* by using [γ-^{32}P]ATP as described in the legend to Fig. 2. The proteins were eluted from the immune complex with 40 μl of gel sample buffer per 5 μl of v-*abl* antisera used. Twenty-five-microliter aliquots of each were incubated with an equal volume of 50 mM Tris (pH 6.8) (lanes 1) or *S. aureus* V-8 protease at 0.1 μg/ml (lanes 2), 0.3 μg/ml (lanes 3), and 0.5 μg/ml (lanes 4) for 30 min at 37°C. Reactions were terminated with the addition of 25 μl of 2× concentrated sample buffer and incubation at 95°C for 5 min. Samples were electrophoresed on an 8% $NaDodSO_4$/polyacrylamide gel and autoradiographed for 2 days with an intensifying screen. Apparent molecular weights of proteolytic fragments were estimated relative to stained molecular weight standards.

analysis of the control Ph^1-negative cell line KG-1 (Fig. 2*A*) demonstrates the lack of detectable *in vitro* phosphorylation for the normal P145$^{c\text{-}abl}$. For each of the Ph^1-positive CML cell lines examined (Fig. 2 *B–E*) the *in vitro* phosphorylation of a M_r ≈210,000 species was detected with anti-*abl* sera. The altered c-*abl* proteins were all phosphorylated predominantly on tyrosine *in vitro* as described for P210$^{c\text{-}abl}$ from K562 cells (ref. 10; data not shown).

Altered c-*abl* Proteins Have Similar Structures. The similar high molecular weight gel migration position of the altered c-*abl* proteins suggests they have identical structural alterations. However, small differences in size may not be detectable for proteins in the high molecular weight range on $NaDodSO_4$/polyacrylamide gels. To further compare the P210$^{c\text{-}abl}$ they were analyzed by partial proteolytic analysis. *In vitro* phosphorylated P210$^{c\text{-}abl}$s from the EM-2, BV173, and K562 cell lines (Fig. 3 *A–C*) were digested with varying doses of *S. aureus* V-8 protease. This method of analysis compares the size and number of proteolytic fragments generated from each P210$^{c\text{-}abl}$ species. The results demonstrate that all of the P210$^{c\text{-}abl}$ proteins examined produce very similar proteolytic fragments. In addition, a naturally occurring proteolytic fragment of M_r ≈190,000 is occasionally detected in P210$^{c\text{-}abl}$ immunoprecipitates (Fig. 1; also unpublished data). Previously, we have shown that the structural alteration in P210$^{c\text{-}abl}$ is likely to be amino-terminal (10). Since the sites of *in vitro* phosphorylation of P210$^{c\text{-}abl}$ are likely near the amino-terminal region of the *abl* sequences (10), this analysis should be sensitive to variations in this region. These results indicate that the P210$^{c\text{-}abl}$s expressed in independently derived Ph^1-positive CML cell lines have very similar structures.

Additional Polypeptide Sequences Are Present in P210$^{c\text{-}abl}$. The structural alteration of P210$^{c\text{-}abl}$ may be due to post-translational modification or addition of polypeptide sequences. Glycosylation is known to cause large shifts in apparent molecular weight but we have not found evidence for N-linked glycosylation of P210$^{c\text{-}abl}$ using the inhibitor tunicamycin (unpublished data). To demonstrate that P210$^{c\text{-}abl}$ contains additional polypeptide sequences relative to P145$^{c\text{-}abl}$ we compared their [^{35}S]methionine-labeled tryptic peptides by two-dimensional analysis on thin-layer plates (Fig. 4). The resulting display of [^{35}S]methionine-labeled tryptic peptides for P145$^{c\text{-}abl}$ (Fig. 4*A*) and P210$^{c\text{-}abl}$ (Fig. 4*B*) are generally similar (shaded spots in Fig. 4*C*). This is in agreement with previous results demonstrating that P145$^{c\text{-}abl}$ and P210$^{c\text{-}abl}$ generate similar phosphopeptides (10). However, two tryptic peptides detected in P145$^{c\text{-}abl}$ were not detected in P210$^{c\text{-}abl}$ (open spots in *C*). This indicates that this sequence is either altered or not present in P210$^{c\text{-}abl}$. Four of the tryptic peptides detected for P210$^{c\text{-}abl}$ are not present in P145$^{c\text{-}abl}$, suggesting that the structural alteration of P210$^{c\text{-}abl}$ is due to additional polypeptide sequences that have been fused onto the P145$^{c\text{-}abl}$ sequences (dark spots in *C*).

DISCUSSION

Mechanism for Production of P210$^{c\text{-}abl}$. The similar size and structure of the altered c-*abl* proteins detected in the Ph^1-positive CML patients and cell lines suggest that there is a common mechanism for their genesis. The detection of P210$^{c\text{-}abl}$ but not P145$^{c\text{-}abl}$ in cell lines lacking a normal chromosome 9 (EM-2, EM-3) further suggests that it is the altered c-*abl* gene on the Ph^1-positive chromosome that encodes P210$^{c\text{-}abl}$. Groffen *et al.* (6) analyzed the translocation breakpoints in Ph^1-positive CML patients and found that they occurred within a limited 5-kb region on chromosome 22 but at variable distances 5′ to the v-*abl* homologous sequences of the human c-*abl* gene. In spite of this variability, P210$^{c\text{-}abl}$ was detected in Ph^1-positive CML cell lines and patients. Ph^1-positive cells have also been shown to express an 8-kb c-*abl* mRNA distinct from the normal 6- and 7-kb c-*abl* mRNAs (8, 9). The structural alteration of the P210$^{c\text{-}abl}$ is likely to be fusion of new protein sequences near the amino-terminal region of P145$^{c\text{-}abl}$ (Fig. 4). These results suggest that translocation of the c-*abl* gene results in the creation of a chimeric gene leading to the production of a hybrid protein, P210$^{c\text{-}abl}$. Possibly P210$^{c\text{-}abl}$ is encoded on chromosome 9 sequences and its expression is activated by

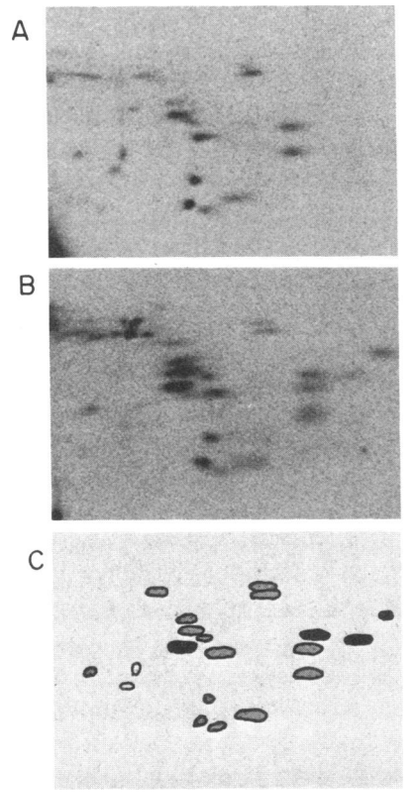

FIG. 4. Two-dimensional analysis of [^{35}S]methionine-labeled tryptic peptides generated from P145$^{c\text{-}abl}$ and P210$^{c\text{-}abl}$. The P145$^{c\text{-}abl}$ (*A*) and P210$^{c\text{-}abl}$ (*B*) were isolated from K562 cells metabolically labeled with [^{35}S]methionine. The c-*abl* proteins were immunoprecipitated, released from the immune complex, diluted into PL buffer, reimmunoprecipitated, and electrophoresed on a 7% NaDodSO$_4$/polyacrylamide gel. The c-*abl* proteins were localized by autoradiography and eluted from the gel in 50 mM NH_4HCO_3/ 0.1% NaDodSO$_4$/5% 2-mercaptoethanol at 37°C for 40 hr. The proteins were then precipitated with trichloroacetic acid with 50 μg of bovine serum albumin as carrier. Samples were then performic acid oxidized for 2 hr on ice, lyophilized, and then digested with 50 μg of tosyl-L-phenylalanine chloromethyl ketone-trypsin in 50 mM NH_4HCO_3. The tryptic digests were then concentrated by lyophilization and then loaded onto silica gel thin-layer plates. Samples were then electrophoresed for 1.5 hr at 400 V in formic acid/acetic acid/H_2O (5:15:80) in the first dimension followed by ascending chromatography in *n*-butanol/pyridine/acetic acid/H_2O (15:10:3:12) in the second dimension. The origin is in the lower left corner and electrophoresis was toward the cathode on the right. The plates were sprayed with 20% 2,5-diphenyloxazole in dimethyl sulfoxide and fluorographed for 60 days. (*C*) The peptides present only in P145$^{c\text{-}abl}$ (open spots), the peptides present only in P210$^{c\text{-}abl}$ (black spots), and the peptides present in both (shaded spots).

chromosome 22 sequences. However, owing to the variability of translocation breakpoints it seems likely that P210$^{c\text{-}abl}$ is encoded by an mRNA that initiates on chromosome 22 sequences, extends through the v-*abl* homologous sequences on chromosome 9, and has the breakpoint region removed by splicing (see Fig. 5). This would also place expression of the altered c-*abl* gene under the control of a new promoter region on chromosome 22. One consequence of this is that P210$^{c\text{-}abl}$ is detected at an approximately 5- to 10-fold higher level than the P145$^{c\text{-}abl}$ but at an approximately 10- to 20-fold lower level than the v-*abl* protein (10). The 4- to 8-fold amplification of the c-*abl* gene in the K562 cell line does not lead to higher levels of P210$^{c\text{-}abl}$ than other Ph^1-positive cell lines, indicating its expression is regulated or that the extra copies of the gene may not encode P210$^{c\text{-}abl}$. This is in agreement with results indicating that the level of the 8-kb c-*abl* mRNA is similar between the K562 and EM-2 Ph^1 CML cell lines (8).

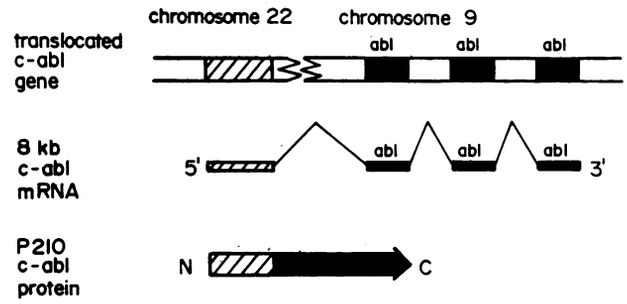

FIG. 5. Model for proposed mechanism of P210$^{c\text{-}abl}$ expression. This diagram illustrates the proposed model to explain the mechanism of P210$^{c\text{-}abl}$ expression. Generation of Ph^1 results in creation of a chimeric gene containing unknown sequences from chromosome 22 (hatched block) and *abl* sequences from chromosome 9 (solid block). The variable translocation breakpoint region is then removed from the mature mRNA by splicing, resulting in expression of the 8-kb mRNA detected in Ph^1-positive cells. Note, the number and size of the exons composing the gene are not known. The 8-kb mRNA that encodes the P210 protein has genetic information from chromosome 22 fused to the c-*abl* sequences from chromosome 9. Amino-terminal alteration of the c-*abl* protein by this mechanism activates the c-*abl* tyrosine kinase.

Role of P210$^{c\text{-}abl}$ in CML. CML is a clonal disorder of the pluripotent stem cell in which >90% of the patients have Ph^1 (5). The chronic phase is marked by an increase in the number of myeloid cells but later progresses to a more acute phase (blast crisis) in which cells no longer differentiate and a subclonal outgrowth of an early myeloid or pre-B-lymphoid cell type dominates (27). Some patients develop the clinical symptoms of blast crisis prior to the increase of blast cells in the blood and bone marrow (accelerated phase) (27). This differs from the general pattern of pathogenesis of Ab-MuLV, which is characterized by an acute, pre-B-cell leukemia *in vivo* (28). However, the v-*abl* gene can affect a broad range of hematopoietic cell types under certain experimental conditions (29). The similarity between the v-*abl* and P210$^{c\text{-}abl}$ tyrosine kinase activities suggest that P210$^{c\text{-}abl}$ may be involved in the pathogenesis of CML. Altered *abl* expression may have different consequences in CML and Ab-MuLV-induced leukemia due to different promoters regulating the expression of the v-*abl* and P210$^{c\text{-}abl}$ genes.

Activation of the c-*abl* tyrosine kinase in Ph^1 CML contrasts with activation of the c-*myc* gene by specific chromosomal translocations observed in Burkitt lymphoma and in murine plasmacytomas (30, 31). The c-*myc* protein is not always altered but analysis of c-*myc* mRNA levels indicates regulation of c-*myc* gene expression is disturbed (32). Both mechanisms of c-*onc* activation are similar in that normal regulation of a gene product activity is altered.

P210$^{c\text{-}abl}$ has not been directly shown to cause CML. Other oncogenes such as N-*ras* and c-*sis* may also be involved in CML. The N-*ras* gene has activated transforming potential in one Ph^1-negative CML cell line (33) and the c-*sis* gene is translocated from chromosome 22 to chromosome 9 as part of the reciprocal translocation that generates Ph^1 (7). However, the c-*sis* gene is not located near the translocation breakpoint and is not detectably expressed in Ph^1-positive cells (7, 9). Analysis of the effect of P210$^{c\text{-}abl}$ on normal hematopoietic cell proliferation by using gene transfer experiments will help to define the precise role of P210$^{c\text{-}abl}$ in the pathogenesis of CML.

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