The SH2 Domain of P210BCR/ABL Is Not Required for the Transformation of Hematopoietic Factor-Dependent Cells

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Src-homology region 2 (SH2) domains, by binding to tyrosine-phosphorylated sequences, mediate specific protein-protein interactions important in diverse signal transduction pathways. Previous studies have shown that activated forms of the Abi tyrosine kinase, including P210BCR/ABL, of human chronic myelogenous leukemia, require the SH2 domain for the transformation of fibroblasts. To determine whether SH2 is also required for Bcr/Abi to transform hematopoietic cells, we have studied two SH2 domain mutations in P210BCR/ABL, a point mutation in the conserved FLVRES motif (P210/R1053K), which interferes with phosphory-tyrosine-binding by SH2, and a complete deletion of SH2 (P210/ΔSH2). Despite a negative effect on intrinsic Abi kinase activity, both P210 SH2 mutants were still able to transform the hematopoietic factor-dependent cell lines Ba/F3 and FDC-P1 to growth factor independence. Unexpectedly, both mutants showed greater transforming activity than wild-type P210 in a quantitative transformation assay, probably as a consequence of increased stability of the SH2 mutant proteins in vivo. Cells transformed by both P210 SH2 mutants were leukemogenic in syngeneic mice, and P210/R1053K mice exhibited a distinct disease phenotype, reminiscent of that induced by v-Abi. These results demonstrate that while the Abi SH2 domain is essential for BCR/ABL transformation of fibroblasts, it is dispensable for the transformation of hematopoietic factor-dependent cell lines.

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MATERIALS AND METHODS

Construction of BCR/ABL SH2 Domain Mutants

The P210/R1053K mutant was constructed using site-specific mutagenesis using the MutaGene Phagemid system (Bio-Rad Laboratories). From the Division of Hematology/Oncology, Department of Medicine, Brigham and Women's Hospital and The Center for Blood Research, Department of Genetics, Harvard Medical School, Boston, MA.

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tories, Melville, NY), based on the method of Kunkel et al. The mutagenic primer (5' GCAGCTTTAAGAGGAGGTGAGAGTCTGACCAGAGATTCA 3') was hybridized to a single-stranded, uracil-containing, P210(BCR/ABL) HindIII fragment contained in the vector pTZ19U. The primer contained a new Hpa II site to identify mutant clones. The P210/R1053K mutant was confirmed by dyeoxy DNA sequencing, and the HindIII fragment was subcloned into the vector pGDDARI, which is identical to the vector pGD, but lacks the EcoRI site in the neomycin resistance gene cassette.

The P210/ΔSH2 mutant was generated through a series of restriction digestes in which the HindIII site at nucleotide #804 and the Kpn I site at #527 were blunt-ended and fused in-frame, resulting in the deletion of the SH2 domain from amino acid #1030 to #1120 in the K562 b3a2 cDNA. The mutation was verified by dyeoxy DNA sequencing and restriction mapping was performed to confirm preservation of the Kpn I site. The mutant cDNA was subcloned into pGDDARI for transfection.

**Cells and Cell Culture**

Ba/F3 and FDC-P1 cells were grown in liquid culture at 37°C and 5% CO₂ in Dulbecco’s modified Eagle’s medium (DME-high) containing 10% heat-inactivated fetal calf serum, penicillin/streptomycin, and 2 mM glutamine. Where indicated, the medium was supplemented with growth factor in the form of either 5% to 10% (vol/vol) WEHI-3B-conditioned medium as a source of interleukin 3 (IL-3) or (Ba/F3 and FDC-P1 cells), or murine recombinant granulocyte macrophage colony-stimulating factor (GM-CSF; Immunex, Seattle, WA) at a concentration of 30 U/mL (FDC-P1 cells). BOSC-23 cells were grown in DME-high containing 10% heat-inactivated fetal calf serum, penicillin/streptomycin, 2 mM glutamine, and nonessential amino acids.

**Gene Transfer**

Retroviral infection. Ten micrograms of P210 or SH2 domain mutant DNA in the retroviral expression vector pGDDARI, which contains a gene encoding resistance to neomycin, was introduced into the retroviral packaging cell line BOSC-23 by calcium phosphate transfection, as described. Twelve to 16 hours later, 6 x 10⁶ Ba/F3 cells were gently added to each 10 cm BOSC-23 plate in the nonessential amino acids.

Analysis of Clones for Growth Factor Independence

Neomycin resistant populations of Ba/F3 or FDC-P1 cells, containing P210 or one of the SH2 domain mutants, were cloned by limiting dilution into 96-well plates. Clones were isolated in the presence of growth factor and neomycin, with fresh medium added every 3 days to prevent any possibility of growth factor depletion. After approximately 10 days the wells were sufficiently confluent to be harvested. The cells were gently pelleted in 1.6 mL eppendorf tubes, washed twice in PBS, and distributed equally into parallel 24-well plates containing medium with or without supplemental growth factor. After 48 to 72 hours, the 24-well plates were scored by counting the number of clones capable of factor-independent growth. A growth factor-independent clone was defined as a clone that was able to proliferate both in the presence and absence of supplemental growth factor.

**Autocrine Growth Analysis**

(A) Conditioned media obtained from growth factor-independent populations of Ba/F3 and FDC-P1 cells expressing P210 or one of the SH2 mutants was concentrated 25- to 30-fold using centrifrip-10 ultrafiltration (Amicon, Beverly, MA), and added to duplicate samples of 10⁶ parental Ba/F3 or FDC-P1 cells at a concentration of 10% (vol/vol) in standard medium without growth factor. The viable cell count was determined daily. (B) Neutralizing monoclonal anti-IL-3 antibody (Genzyme, Cambridge, MA) was added to growth factor-independent populations of Ba/F3 or FDC-P1 cells expressing P210 or one of the SH2 mutants at a concentration (10 µg/mL) 10-fold higher than was required to cause growth arrest of Ba/F3 cells and death of FDC-P1 cells growing in 1% WEHI-3B-conditioned medium. Viable cells were counted daily and their growth rate compared with cells grown in the absence of neutralizing antibody.

RNA Analysis

Total RNA was prepared from populations of neomycin-resistant Ba/F3 cells transfected with P210 or the SH2 mutants, before and after selection for the growth factor-independent phenotype, using the guanidinium isocyanate method. Equal amounts of total RNA were subjected to denaturing agarose gel electrophoresis, transferred to a nylon membrane, and hybridized with a 32P-labeled DNA probe generated from a 1,100-bp Cτa 1 fragment from the neomycin gene cassette contained in the pGDDARI vector.

**Electroporation.** Because FDC-P1 cells were relatively resistant to BOSC-23 cell retroviral infection, electroporation was used. Approximately 8 to 12 x 10⁶ exponentially growing FDC-P1 cells were washed twice in ice cold phosphate-buffered saline lacking calcium and magnesium chloride (PBS-), and resuspended in 800 µL of PBS-. All DNA was twice purified by buoyant density centrifugation in cesium chloride. Fifty micrograms of linearized P210 or SH2 domain mutant DNA in pGDDARI was then added to the cells. Electroporation conditions were 220 volts and 960 µfarads (time constant: 17 to 22.5 msec), using a Bio-Rad Genepulser apparatus. After electroporation, the cells were placed on ice for 5 to 10 minutes and then allowed to recover in nonselective medium for 24 hours at 37°C. Selection was then performed in the presence of growth factor in 0.3 mg/mL neomycin (absolute) in a 75 cm² tissue culture flask. By day 9 to 11 a confluent population of neomycin resistant cells was obtained for subsequent cloning by limiting dilution.

**In Vitro Kinase Assay**

P210, P210/R1053K, or P210/ΔSH2, contained in the vector P3I71 were introduced into 293T cells by transient transfection; alternatively, growth factor-independent Ba/F3 cells were used as the source of Bcr/Abl protein. Lysates were made and normalized by western blot for the amount of Bcr/Abl protein using the anti-Abl monoclonal antibody 19-84. Equal amounts of Bcr/Abl proteins were subjected to immunoprecipitation with anti-GE4 antisera (recognizing COOH-terminal Abl sequences), and an immune complex kinase assay performed as previously described, with emulsase added as an exogenous substrate. The proteins were then separated on a 5% to 9% gradient sodium dodecyl sulfate (SDS)-polyacrylamide gel, detected by autoradiography, and quantitated using a phosphorimager (GS-250; Bio-Rad Laboratories).
TRANSFORMATION BY Bcr/Ab1 SH2 MUTANTS

Leukemogenesis Assay

Polyclonal populations of Ba/F3 cells transformed by wild-type P210, P210/R1053K, or P210/ΔSH2 were propagated in liquid culture as described above, without a source of IL-3. Cells were washed twice in PBS, counted, and resuspended at a density of 10⁶ cells per 0.5 mL PBS. Recipient BALB/c BYJ female mice (Jackson Laboratories, Bar Harbor, ME), between 6 and 8 weeks of age and having 17 to 20 g body weight, received 10⁶ cells intravenously by tail vein injection. Injected mice were subsequently evaluated on a daily basis for weight loss, failure to thrive, splenomegaly, or hind-limb paralysis. Mice that appeared pre-morbid were killed. Hind-limb paralysis was scored if mice exhibited an inability to use hind limbs for ambulation on a smooth surface such as a countertop.

RESULTS

Abl SH2 Mutations Attenuate Bcr/Ab1 Kinase Activity In Vitro

To examine the effect of mutations in SH2 on the tyrosine kinase activity of P210, we expressed P210 and SH2 mutant proteins in 293T cells and performed an immune complex kinase assay. Addition of Bcr sequences to the NH₂-terminus of Ab1 increases the intrinsic tyrosine kinase activity of the protein. Relative to c-Ab1, P210 kinase activity was increased approximately six-fold, while P190, the form of Bcr/Ab1 most commonly associated with Philadelphia chromosome-positive ALL, was elevated almost ten-fold, consistent with previous observations (Fig 1). The mutation disrupting the phosphotyrosine-binding function of P210, P210/R1053K, resulted in a modest decrease in kinase activity relative to P210, to 4.4-fold over c-Ab1. However, complete deletion of the SH2 domain abrogated the elevated kinase activity of P210, essentially to the level of c-Ab1 (0.7-fold). The impact of these mutations on Bcr/Ab1 autophosphorylation paralleled their effects on the phosphorylation of the exogenous substrate enolase. Similar results were obtained when these proteins were immunoprecipitated from Ba/F3 cells (data not shown).

Neither Phosphotyrosine-Binding nor Other Functions of SH2 Are Required for the Transformation of Factor-Dependent Hematopoietic Cell Lines by BCR/ABL

Quantitation of differences between wild-type and mutant forms of BCR/ABL in fibroblast transformation have relied on comparisons in focus formation or the number of colonies formed in soft agar. Because both Ba/F3 and FDC-P1 cells grow in liquid culture, we compared the transforming ability of the P210/R1053K and P210/SH2 mutations to wild-type P210 by the analysis of individual transfected clones. The neomycin-containing vector pGDΔRI, containing either wild-type P210, P210/R1053K, or P210/ΔSH2, was introduced into the factor-dependent cell lines Ba/F3 and FDC-P1 by helper-free retroviral infection or electroporation. After neomycin selection in the presence of growth factor, individual clones were isolated by limiting dilution and assessed for growth in medium with or without growth factor (WEHI-3B conditioned medium as source of IL-3 for Ba/F3 cells, and WEHI-3B or recombinant murine GM-CSF for FDC-P1 cells). Analysis of the site of chromosomal integration of proviral or plasmid DNA confirmed that independent clones were analyzed (data not shown). In parallel experiments where transfected cells were cloned directly in neomycin, the efficiency of recovery of neomycin-resistant clones was similar with either parental pGDΔRI vector alone, P210, or the SH2 mutants, suggesting there was no significant lethal or cytotoxic effect of transfection of Bcr/Ab1 in these cells. Cells incapable of growth in the absence of IL-3 or GM-CSF generally died within 24 hours of growth factor deprivation. No Ba/F3 or FDC-P1 cells spontaneously acquired the ability to grow without growth factor (data not shown). Further, neither pGDΔRI vector alone nor a kinase-inactive mutant of Ab1 supported any factor-independent growth (data not shown), confirming that the tyrosine kinase activity of Ab1 is required for the transformation of factor-dependent hematopoietic cells.

The ratio of growth factor-independent clones to total number of clones was calculated for each of the P210 mutants, and compared with wild-type P210 (Table 1). P210/R1053K conferred growth factor independence on approximately 25% and 23% of transfected Ba/F3 and FDC-P1 clones, respectively. Surprisingly, both SH2 domain mutants not only retained the ability to confer growth factor independence on these cells, but also exhibited approximately twice the transforming activity of wild-type P210;
approximately 46% to 50% of clones containing the point mutation that severely impairs phosphoryrosine binding (P210/R1053K), and 47% to 49% of clones lacking the Abl SH2 domain entirely (P210/ΔSH2) were capable of growth-factor-independent growth. The differences between P210 and the SH2 domain mutants were statistically significant (P < .02, using a χ² test comparing two proportions). In the case of the FDC-P1 cells, there was no significant difference in the ability of clones to grow in the absence of either IL-3 (WEHI-3B-conditioned medium) or GM-CSF (data not shown). Therefore, in contrast to fibroblast transformation, neither the phosphotyrosine-binding function of SH2 nor the SH2 domain itself was required for factor independence, consistent with a threshold requirement for transformation. These results suggest that the elevated expression of the Bcr/Abl SH2 mutant proteins may be due to increased protein stability. We could not demonstrate any significant decrease in the level of mutant or wild-type Bcr/Abl protein even after over 24 hours of treatment with cycloheximide (data not shown), consistent with the known long half-life of P210.

To investigate the possibility that autocrine secretion of IL-3 was responsible for induction of factor-independence by P210 or the SH2 mutants, we tested the ability of conditioned medium from transformed cells to support the growth of the parental Ba/F3 and FDC-P1 cells, and the effect of neutralizing anti-IL-3 antibodies on their growth. High concentrations of conditioned medium from Ba/F3 or FDC-P1 cells expressing P210/R1053K supported the growth of parental FDC-P1 cells, consistent with secretion of IL-3 or GM-CSF (data not shown). Therefore, in contrast to fibroblast transformation, neither the phosphotyrosine-binding function of SH2 nor the SH2 domain itself was required for BCR/ABL to transform these hematopoietic factor-dependent cells to growth factor-independent proliferation.

P210/R1053K, P210 containing an FLVRES mutation (P210/R1053K), or P210 containing an in-frame deletion of the entire SH2 domain (P210/ΔSH2) were introduced into the hematopoietic factor-dependent cell lines Ba/F3 and FDC-P1 by retroviral gene transfer or electroporation. Individual clones were isolated by limiting dilution in the presence of growth factor (GF+), and then analyzed by the ability to grow in medium lacking supplemental growth factor (GF−).

<table>
<thead>
<tr>
<th>Mutant/</th>
<th>Cell Type</th>
<th>No. of Clones</th>
<th>Percent</th>
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<tbody>
<tr>
<td>P210/R1053K</td>
<td>Ba/F3</td>
<td>27/54</td>
<td>50.01</td>
</tr>
<tr>
<td>P210/ΔSH2</td>
<td>Ba/F3</td>
<td>26/53</td>
<td>49.11</td>
</tr>
<tr>
<td>P210</td>
<td>Ba/F3</td>
<td>13/53</td>
<td>24.5</td>
</tr>
<tr>
<td>P210/R1053K</td>
<td>FDC-P1</td>
<td>33/72</td>
<td>45.81</td>
</tr>
<tr>
<td>P210/ΔSH2</td>
<td>FDC-P1</td>
<td>33/71</td>
<td>46.51</td>
</tr>
<tr>
<td>P210</td>
<td>FDC-P1</td>
<td>16/71</td>
<td>22.5</td>
</tr>
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*P210BCWABL, P210 containing an FLVRES mutation (P210/R1053K), or P210 containing an in-frame deletion of the entire SH2 domain (P210/ΔSH2) were introduced into the hematopoietic factor-dependent cell lines Ba/F3 and FDC-P1 by retroviral gene transfer or electroporation. Individual clones were isolated by limiting dilution in the presence of growth factor (GF+), and then analyzed by the ability to grow in medium lacking supplemental growth factor (GF−). †P < .02, for SH2 mutants compared to wild-type P210, using a χ² test comparing two proportions.

The overall pattern of phosphotyrosinated proteins was quite similar between P210 and the SH2 domain mutants, except that R1053K-expressing clones frequently demonstrated decreased tyrosine phosphorylation of proteins migrating at 120 and 60 kd, which are likely p120 rasGAP (GTPase-activating protein) and p62 rasGAP-associated protein, respectively. These proteins have been shown to be tyrosine-phosphorylated in cells transformed by various tyrosine kinase oncoproteins, including BCR/ABL. Decreased tyrosine phosphorylation of p62 has also been reported in fibroblasts transformed by a BCR/ABL SH2 point mutant. The overall pattern of Bcr/Abl expression and protein tyrosine phosphorylation seen in P210 and the SH2 domain mutations did not differ significantly between Ba/F3 and FDC-P1 cells (data not shown), suggesting that these findings are not peculiar to one hematopoietic lineage.

The P210/R1053K Mutant Exhibits a Distinct Disease Phenotype in an In Vivo Leukemogenesis Assay

The transformation of Ba/F3 cells to growth factor independence by P210 has been previously shown to correlate with their ability to form tumors after subcutaneous inoculation into athymic (nu) nude mice. Because the Ba/F3 cell line was originally derived from BALB/c mice, these animals provide a convenient in vivo model to explore P210BCWABL-mediated leukemogenicity. Intravenous injection of 10⁵ P210-transformed Ba/F3 cells causes death in all recipient hosts within 7 weeks.
BALB/c mice within 16 days (Van Etten et al, in preparation). Autopsy of diseased animals reveals an overwhelming leukemia, with diffuse replacement of bone marrow, liver, and spleen with cells that morphologically resemble the input Ba/F3 cells (data not shown). Injections of factor-dependent parental Ba/F3 cells have yielded no disease in recipient mice followed for up to 3 months.

The clinical manifestations or latency of BCR/ABL-associated illness did not differ significantly between mice that received Ba/F3 cells transformed by P210 or P210/ΔSH2, with all animals dying secondary to a diffuse leukemic process between days 15 and 16. In contrast, mice that received cells transformed by the SH2 point mutant P210/R1053K demonstrated a significantly longer latency, with all animals succumbing by day 26 (Fig 3). This was unlikely to be due to a difference in growth rate, because all three cell populations grew at similar rates in culture (data not shown). In addition to the longer latency period, mice receiving Ba/F3 cells transformed by P210/R1053K exhibited a distinct disease phenotype, with approximately two-thirds of animals developing a hind-limb paralysis syndrome before death. This condition, which is characteristic of adult BALB/c mice infected with Abelson murine leukemia virus and reflects compression of the lumbar spinal cord nerve roots due to leukemic involvement of the paraspinous lymph node chain, was never observed in recipients of P210- or P210/ΔSH2-transformed Ba/F3 cells.

DISCUSSION

The interaction of SH2 domain-containing proteins with specific phosphotyrosinated sequences has been shown to be important for signal transduction involving both receptor and nonreceptor protein-tyrosine kinases. Previous studies have shown that the SH2 domain of activated forms of Abl is required for fibroblast transformation. We have studied two P210BCR/ABL SH2 domain mutants, P210/R1053K, which contains a point mutation interfering with phosphotyrosine-binding by SH2, and P210/ΔSH2, lacking the entire domain. In
Others have also recently found FDC-P1 cells to be trans-
kinase, while complete deletion of SH2 abrogated the activa-
phorylation of the ASH2 mutant was more similar to that
transformed hematopoietic cells the degree of tyrosine phos-
oligomerization,” may be important for transformation
lines. These findings suggest that other functions of Bcr, such
between Ab1 kinase activity in vitro and transformation abil-
transfusion assay. 10\(^6\) Ba/F3 cells
were injected into BALB/c BYJ female mice intravenously by tail vein
injection. Survival was assessed from time of injection. The curve
depicted represents one of three independent experiments, all of
which obtained similar results.

![Graph](image)

**Fig 3.** P210 SH2 mutant leukemogenesis assay. 10\(^6\) Ba/F3 cells
transformed either by (A) wild-type P210, (Δ) the SH2 domain point
mutation P210/R1053K, or (Δθ) the SH2 deletion mutant P210/ΔSH2
were injected into BALB/c BYJ female mice intravenously by tail vein
injection. Survival was assessed from time of injection. The curve
depicted represents one of three independent experiments, all of
which obtained similar results.

In contrast to the findings in fibroblasts, both SH2 mutants are
more highly transforming than wild-type P210 in the hematopoietic factor-dependent cell lines Ba/F3 and FDC-
P1, despite decreased intrinsic kinase activity. These results
confirm a previous report that noted that a population of Ba/F3 cells transfected with an SH2-deleted form of BCR/
ABL was able to grow in the absence of growth factor.\(^{24}\)
Others have also recently found FDC-P1 cells to be trans-
formed by BCR/ABL lacking an SH2 domain.\(^{49}\)

The tyrosine kinase function of Abl is required for Bcr/
Ab1 transformation of hematopoietic factor-dependent cell
lines\(^{30}\) (data not shown). The mechanism by which Bcr acti-
vates the Ab1 kinase is unknown, but in fibroblasts may
be related to its interactions with Ab1 SH2. Bcr first exon
sequences have been shown to bind Ab1 SH2 in vitro in a
phosphotyrosine-independent fashion, and deletion of these
regions in Bcr abolished fibroblast transformation.\(^{50}\) We
have found that the tyrosine-binding function of SH2 (P210/
R1053K) is not required for the activation of the Ab1 tyrosine
kinase, while complete deletion of SH2 abrogated the activation
of the Ab1 kinase by Bcr in vitro, consistent with this
model. However, deletion of SH2 did not interfere with Bcr/
Ab1 transformation of factor-dependent hematopoietic cell
lines. These findings suggest that other functions of Bcr, such
as oligomerization,\(^{31}\) may be important for transformation
of hematopoietic cells by BCR/ABL. A lack of correlation
between Ab1 kinase activity in vitro and transformation abil-
ity in vivo has also been observed for SH3-deleted c-Ab1,
which is potent in cellular transformation despite the same kinase activity as c-Ab1 in vitro.\(^{32,33}\) Although the P210/
ΔSH2 mutant showed about sixfold lower in vitro autophos-
phorylation activity than wild-type P210, when isolated from
transformed hematopoietic cells the degree of tyrosine phos-
phorylation of the ΔSH2 mutant was more similar to that
of wild-type P210 (about twofold lower). This suggests that
the tyrosine kinase activity of P210/ΔSH2 is higher in vivo
than in vitro, or that the susceptibility of the ΔSH2 protein
to tyrosine phosphatases in vivo is reduced.

Hematopoietic cells and fibroblasts have been shown to
require distinct functions of Abl for transformation. Acti-
vated forms of Abl lacking the membrane-targeting myrist-
tylation domain,\(^{35}\) or containing a point mutation in an
autophosphorylation site within the P210(BCR/ABL) kinase
domain (Y1294F)\(^{19}\) are defective in fibroblast transformation,
but retain the ability to transform factor-dependent cell lines.
Similarly, while the SH2 domain is essential for fibroblast
transformation by Abl, it is dispensable for the BCR/ABL
transformation of Ba/F3 or FDC-P1 cells. BCR/ABL signal
transduction may involve different pathways, or emphasize
particular mediators, depending on cell type. In fibroblasts
the Abl SH2 domain may bind specific tyrosine-phosphory-
lated proteins that transduce signals required for transforma-
tion, while in hematopoietic factor-dependent cell lines this
SH2-mediated signaling pathway is not required, or is regu-
lated differently. Alternatively, the Ab1 SH2 domain might
serve to protect phosphotyrosine sites on substrates from the
action of phosphatases,\(^{30}\) which might have different conse-
quences in fibroblasts and hematopoietic cells.

Using a quantitative transformation assay we have found
that both P210 SH2 domain mutants are actually more trans-
forming than wild-type P210. There are several possible ex-
planations for this finding. One possibility is that P210 SH2
mutants have decreased cytotoxicity. A growth inhibitory or
toxic effect has been observed with expression of c-Abl, \(v\)-
Ab1, and Bcr/Ab1 in fibroblasts,\(^{36-38}\) which may be dependent
on SH2.\(^{39}\) Therefore, mutations in SH2 may decrease this
toxic effect of Bcr/Ab1 expression and allow increased recov-
er of transformed clones. However, we found no significant
difference in the frequency of neomycin-resistant clones
transformed with vector DNA alone or with wild-type or
SH2 mutant forms of P210, suggesting that Bcr/Ab1 does
not exhibit prominent toxicity in hematopoietic cells (data
not shown). A more likely possibility is related to our obser-
vation that there is a threshold level of Bcr/Ab1 necessary
for Ba/F3 transformation, analogous to Src transformation.
We found that populations of Ba/F3 cells transfected with
the SH2 mutants expressed higher levels of Bcr/Ab1 protein
even before selection for growth factor independence, which
could explain their greater transforming ability in this assay.
Because P210 and the SH2 mutants had similar BCR/ABL
mRNA levels by northern blot, this suggests a post-transla-
tional effect, such as increased stability of the SH2 mutant
proteins, was responsible for the increased expression. We
were not able to demonstrate any significant decrease in the
level of mutant or wild-type Bcr/Ab1 proteins even with
prolonged cycloheximide treatment, which probably reflects
the relatively long half-life of P210, as has been noted by
others.\(^{31}\) The loss of SH2 function may affect the interaction
of Bcr/Ab1 with another protein(s), which normally serves
to regulate its stability.

The Ab1 SH2 domain is also not required for leukemoge-
nicity of P210-Ba/F3 cells in vivo. Mice inoculated intrave-
nously with Ba/F3 cells transformed by P210 of the ΔSH2
mutant die from an overwhelming leukemia by day 16, with infiltration of bone marrow, liver, and spleen. Interestingly, the SH2 domain point mutant P210/R1053K demonstrated a distinct disease phenotype, manifested by a greater latency of illness (death by day 25) and the frequent development of a syndrome of lower extremity paresis due to paraspinal lymph node infiltration, not seen in wild-type or P210/ΔSH2 disease, but reminiscent of classic disease induced by the Abelson virus. These findings raise the possibility that interference with the phosphotyrosine-binding function of P210<sub>BCR/ABL</sub> may affect cell homing in vivo. The possible mechanisms for this remain speculative, but may reflect alternative signal transduction pathways used by P210/ R1053K, perhaps related to the differences in protein tyrosine phosphorylation of p120 rasGAP and p62 rasGAP-associated proteins seen on Western blot.

In summary, while the Abl SH2 domain is essential for BCR/ABL transformation of fibroblasts, it is dispensable for the transformation of hematopoietic factor-dependent cell lines. Transformation of fibroblasts may require a specific signal mediated by the Abl SH2 domain, which is not required for induction of growth factor independence in hematopoietic factor-dependent cell lines. Compared with wild-type P210, the P210/R1053K mutant demonstrated differences in both the pattern of protein tyrosine phosphorylation and leukemic phenotype in vivo. Whether these two phenomena are related, or reflect a physiologic difference between phosphotyrosine binding and other functions of Abl SH2, remains to be investigated. It will be important to determine whether the Abl SH2 domain is required for BCR/ ABL-induced leukemia in humans. This issue is particularly relevant if the Abl SH2 domain is to be considered a target for rational drug design for the therapy of patients afflicted with these diseases. Experimental models that permit the analysis of P210 SH2 domain mutants in the context of early hematopoietic stem cells<sup>89</sup> may help resolve this issue.

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The SH2 domain of P210BCR/ABL is not required for the transformation of hematopoietic factor-dependent cells

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