An Activating Mutation in the ATP Binding Site of the ABL Kinase Domain*

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Patrick B. Allen† and Leanne M. Wiedemann§

From the Leukaemia Research Fund Centre, Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London, SW3 6J B United Kingdom.

A number of structural alterations have been shown to activate the leukemogenic potential of the ABL oncogene, but there is little understanding of the regulatory mechanisms that are subverted by such changes. We have used directed mutagenesis to examine a potential regulatory motif in cABL, which could directly influence ABL tyrosine kinase activity. A tyrosine to phenylalanine substitution within the ATP binding fold of the ABL kinase domain is sufficient to activate cABL enzymatic activity, and the mutant protein will alleviate growth factor dependence when expressed in the BA/F3 cell line. This growth promotion is dependent upon the structure of the amino terminus of the protein, and the ABL mutation will cooperate with certain BCR sequences in BCR/ABL fusion proteins to deregulate ABL kinase activity.

Tyrosine phosphorylation plays a key role in mediating cellular responses to various extracellular stimuli, including those that modulate cell growth and proliferation (1, 2). ABL is a member of the nonreceptor protein-tyrosine kinase family that has been strongly implicated in malignant transformation and is therefore presumed to participate in growth regulatory signaling. The BCR/ABL fusion proteins are associated with Philadelphia chromosome positive acute lymphoblastic and chronic myeloid leukemias (3–5). In addition, Abelson murine leukemia virus expresses a homologue of the endogenous abl gene and is the etiologic agent of a pre-B cell leukemia (6–10). These mutated ABL proteins display an elevated level of tyrosine kinase activity, which is thought to be central to the disease process, but although much is known concerning their transforming potential in various systems, the mechanism of oncogenic activation is unclear and very little is understood about the mode of the regulation of ABL kinase. The normal ABL protein is believed to function in the nucleus and appears to suppress cell growth (11). A large carboxyl terminus distinguishes the ABL subfamily of nonreceptor protein-tyrosine kinases. This region contains a sequence-specific DNA binding region that can be regulated by phosphorylation of serine residues in a cell cycle stage-dependent manner (12, 13). It is not known whether the growth suppression induced by ABL is dependent on this DNA binding activity. In contrast, activated ABL proteins appear to lose their partial localization to the cell nucleus (14), and the contribution of any nuclear function to the ABL transformation process is unclear at present.

Several putative regulatory domains of ABL have been identified, two of which are defined by SH2 and SH3. These regions are present in many of the nonreceptor protein-tyrosine kinases, as well as in other proteins involved in signaling, such as p21 ras GTPase-activating protein, phospholipase C-II, adapter proteins such as CRK, and protein tyrosine phosphatases (15). Mutation of the ABL SH3 domain can activate transforming ability, suggesting that this region has a negative regulatory function (16–18). A candidate ABL kinase inhibitor that binds to the SH3 domain has been purified, and this protein exhibits GTPase-activating protein homology, implying that ABL may interact with the ras related proteins (19). SH2 domains have been shown to facilitate protein-protein interactions among tyrosine phosphorylated proteins (20, 21), and the ABL SH2 domain appears to direct kinase activity to substrates relevant to transformation (22, 23). Additional regulation is presumably provided by separate amino termini, which are encoded by alternatively spliced ABL first exons (24, 25), although the 1α protein isoform is not routinely detected in cell lines (11). Only the human cABL type 1b has the potential for modification by amino-terminal myristoylation. This modification is thought to promote association with the plasma membrane, which in certain cell types is critical for transformation by ABL (26, 27).

Other members of the family of nonreceptor protein-tyrosine kinases, exemplified by proteins such as SRC and FPS, have themselves been shown to be regulated by tyrosine phosphorylation in either the kinase domain or at the carboxyl terminus (28–32). Phosphotyrosine has not been found in vivo in cABL; however, we noted the presence of a potential regulatory motif analogous to that present in certain cyclin-dependent kinases such as Schizosaccharomyces pombe CDC2. The CDC2 kinase has a regulatory mechanism that operates through the cell cycle-dependent phosphorylation of a tyrosine residue (Tyr-15) in the ATP binding site of the kinase domain. This phosphorylation has been proposed to act by inhibiting ATP binding, thereby down-regulating CDC2 (33), although more recent data suggest that the tyrosine phosphorylation does not interfere with nucleotide binding but may instead alter the catalytic function of the enzyme (34). The ABL proteins possess a tyrosine residue within the ATP binding motif at a position analogous to the regulatory CDC2 Tyr-15 (see Fig. 1a), whereas the vast majority of kinase family members have a phenylalanine at this site (35). These observations suggested that a regulatory mechanism similar to that of CDC2 may operate for cABL, in that phosphorylation of a tyrosine in the ATP binding site could down-regulate ABL kinase activity. Here we show that mutation of this tyrosine does indeed generate an activated ABL

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† Present address: The Rockefeller University, 1230 York Ave., New York, NY 10021-6399.

‡ To whom correspondence should be addressed: Leukaemia Research Fund Centre at the Institute of Cancer Research, 237 Fulham Rd., London, SW3 6J B UK. Tel.: 44-171-352-8133; Fax: 44-171-352-3299; E-mail: Leanne@cr.ac.uk.
protein, although the mechanism of activation is not analogous to that of CDC2 kinase.

**EXPERIMENTAL PROCEDURES**

Mutagenesis and Plasmid Construction—Site-directed mutagenesis of ABL protein tyrosine kinase sequences was performed by polymerase chain reaction using the following oligomers: Y272F, 5'-TGGGCGGGCGGGCGGTGTACGAG; Y276F, 5'-TGGGCGGGCGGGCGGTGTACGAG; Y272F + Y276F, 5'-GGGGGCCAGTTCGGGGAGGTGTACGA; and minus strand primer, 5'-ATACGCCAATGCCCAG.

For the reverse transcription polymerase chain reaction fragments were cloned into Bluescript and sequenced to confirm accurate replication. Mutated fragments were digested with NlaIV/PstI and ligated into an 805-base pair A-sp718/Aattl fragment and replaced into a cDNA flanked by EcoRI and M1l sites encompassing the ABL1b or BCR/ABL constructs. The Bgl II site in the retroviral expression vector pMSneo (36) was converted to an M1l site with a linker fragment, and the cDNA fragments were ligated into the EcoRI/MluI-digested vector.

In the deletion constructs, BCR exon 1 sequences were modified as follows: p210 Δ41-242/p190 Δ41-242, digestion with Sall, Klenow polymerase blunt ending, digestion with Stul, and religation; p190 Δ2-168, digestion with NcoI, Klenow polymerase blunt ending, digestion with SmaI, and religation; p190 Δ41-133, digestion with Stul, T4 polynucleotide kinase, Stul digestion, with Stul, and religation; the Δ2-422, digestion with NcoI, Klenow polymerase blunt ending, and religation. These fragments were then used to replace the appropriate region in BCR/ABL DNAs using convenient restriction sites and cloned into the EcoRI/MluI-digested pMSNeo vector.

Cell Culture and Transfection—BA/F3 cells were cultured in RPMI medium plus 10% fetal calf serum and 5% WEHI 3B conditioned medium in a 5% CO2-gassed incubator. For electroporation 5 x 10^6 BA/F3 cells were washed and resuspended in 0.8 ml of serum-free RPMI medium plus 100 µg/ml sonicated salmon sperm DNA and 10 µg/ml plasmid DNA. 625 V/cm was discharged through the medium from a 25 cm electric field. Labeled cell populations were selected in bulk culture in medium plus 10% fetal calf serum and 5% WEHI 3B conditioned medium plus 100 µM sodium vanadate. Samples were lysed, immunoprecipitated, and electrophoresed and blotted as described above.

Immunoprecipitation—Cells were lysed on ice in buffer containing 10 mM phosphate, pH 7.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.05% SDS, 20 mM NaF, 1 mM sodium metavanadate, 0.005 mM molybdate, 60 µg/ml aprotinin, 60 µg/ml leupeptin, 60 µg/ml bestatin, 1 mM phenylmethylsulfonyl fluoride. The lysate was incubated on ice with anti-ABL Ab-3 monoclonal Ab-1 polyclonal antibodies (Oncogene Science, Uniondale, NY) for 2 h, followed by centrifugation at 10,000 x g for 10 min at 0°C. The immune complex was collected on protein A-Sepharose beads for 1 h, washed in buffer B containing 10 mM phosphate, pH 7.0, 150 mM NaCl, 5 mM EDTA, and 1% Triton X-100, and resuspended in sample buffer. Samples were run on a 6-8% SDS-polyacrylamide gel (37).

For the RB interaction experiment (38), mock transfected COS cells and COS cells overexpressing ABL proteins were lysed in 0.5% Nonidet P-40, 1 mM EDTA, 50 mM Tris, pH 8.0, and 120 mM NaCl, plus protease inhibitors on ice for 30 min. 10 µg of anti-RB antibody (245 (PharMingen, San Diego, CA) was added to cell lysates containing 2 mg of total soluble protein. Antibody was subsequently collected on protein A-Sepharose beads. The immunoprecipitate was solubilized in sample buffer and run on an SDS-polyacrylamide gel. Control lanes of total soluble cell lysate protein (20 µg) were also loaded. The gel was blotted onto Immobilon and probed with anti-ABL Ab-3 followed by anti-mouse IgG/alkaline phosphatase conjugate (Sigma) and color development.

Immune Complex Kinase Assay—The immune complex kinase assay is based on the protocol of Konopka and Witte (1985). Briefly, the immune complex is digested with NlaIV/PstI and digested with Klenow polymerase blunt ending, digestion with SmaI, religation; and minus strand primer, 5'-ATACGCCAATGCCCAG.

Immunoblotting—For anti-phosphoysrinobosyn blotting, 6 x 10^6 cells were washed and lysed directly in hot sample buffer containing the phosphatase inhibitors sodium fluoride (20 mM), sodium metavanadate (1 mM), and molybdate (0.005 mM). Samples were run on a 7.5% SDS-polyacrylamide gel and transferred to nitrocellulose. The filter was blocked in 150 mM NaCl, 5 mM KCl, and 25 mM Tris, pH 7.4 with 3% bovine serum albumin. Blots were probed in blocking buffer plus 0.5% Tween 20 with anti-ABL monoclonal antibody Ab-3 followed by a horse-radish peroxidase-conjugated anti-mouse IgG antibody (Amersham Intl.). Secondary antibody was detected by enhanced chemiluminescence (Amersham Intl.). Blots were stripped in 5 mM sodium phosphate, pH 7.5, 2 mM 2-mercaptoethanol, and 2% SDS. Murine monoclonal anti-phosphotyrosine antibody (Upstate Technology, Inc., Lake Placid, NY) was used to reprobe the blots.

Samples for immunoblotting that had previously been labeled in an immune complex kinase assay were derived from 2 x 10^6 cells. Samples were electrophoresed on a 6% SDS-polyacrylamide gel and transferred to nitrocellulose. This filter was blocked with 3% nonfat milk and probed with anti-ABL Ab-3 in the presence of 0.5% Tween 20. Primary antibody was detected using alkaline phosphatase-conjugated anti-mouse IgG antibody (Promega, Madison, WI) and color staining with NBT/BCIP. The developed immunoblot was then autoradiographed overnight.

RESULTS

Mutational Analysis of the ABL ATP Binding Site—The hypothesis that ABL can be regulated by tyrosine phosphorylation was tested by introducing mutations into the relevant sites of the ABL ATP binding domain. The codons for the tyrosine residues within this region of cABL 1b (at positions 272 or 276) were substituted with those coding for the nonphosphorylatable phenylalanine to give the mutants cABL 1b Y272F and cABL 1b Y276F, respectively. In addition, in preliminary experiments, a third construct in which both sites were mutated resulted in the mutant cABL 1b Y272F/Y276F (Fig. 1a).

The biological activity of these mutants was tested in a transformation assay using the murine BA/F3 pro-B cell line, which is strictly dependent on the presence of IL-3 for viability and proliferation (40). Expression of activated ABL proteins in these cells has been shown to relieve the requirement for added growth factor and to render the cells tumorigenic in nude mice (41, 42). BA/F3 cells were transfected with either mutant or normal cABL 1b encoding constructs. Positive controls for BA/F3 cell transformation were provided by plasmids encoding vABL p160 and the BCR/ABL proteins p190 and p210. Transfected populations were selected in bulk culture in medium
supplemented with G418. In order to assess the ability of these constructs to convert BA/F3 cells to a growth factor-independent state, G418-resistant cells were washed in phosphate-buffered saline and transferred to medium lacking IL-3.

No factor-independent progeny were derived from repeated transfections with the construct encoding normal cABL 1b or from BA/F3 cells transfected with the expression vector alone. Furthermore, cells expressing the mutant cABL 1b Y272F did not give rise to factor-independent progeny. In contrast, factor-independent progeny were derived from cultures transfected with plasmids encoding cABL 1b Y272F, cABL 1b Y272F + Y276F, BCR/ABL p190 and p210, and vABL p160 (Table I). The expression of these ABL proteins was confirmed by Western blotting with anti-ABL antibody (Fig. 1a, upper panel) whereas an anti-phosphotyrosine antibody was used to examine the cellular proteins that contain phosphotyrosine-protein content of these cells is high (Fig. 2). In addition, the mutant BCR/ABL proteins exhibit high level expression of the mutant ABL proteins for conversion to factor independence (data not shown). The frequency for this conversion was lower than that observed for the vABL p160 transfectants (Table I).

Y272F Will Cooperate with BCR Sequences in Deregrulating ABL Kinase Activity—BCR exon 1 sequences are implicated in the deregulation of ABL activity by virtue of their inclusion in both the p190 and p210 forms of the BCR/ABL fusion protein. The contribution of BCR sequences to ABL deregulation was assessed by introducing deletions into the coding sequence of BCR exon 1 (Table I). In accordance with the findings of others, these deletions severely compromise the growth promoting effects of BCR/ABL. BA/F3 cells expressing the BCR/ABL deletion mutants p190 D41–242, p190 D41–333, and p190 D2–422 did not give rise to factor-independent progeny. Examination of the phosphotyrosine-containing proteins in cells expressing these mutant BCR/ABL proteins reveals reduced levels with respect to cells expressing BCR/ABL p190 and p210 (Fig. 2a). In addition, the mutant BCR/ABL proteins exhibit reduced levels of autophosphorylation as measured in an immune complex (Fig. 2b). Some of the lines expressing construct p190 D2–168 give rise to factor-independent cells at low frequency (Table II), indicating that this mutant retains partial transforming ability. In accordance with this finding, the phosphotyrosine-protein content of these cells is high (Fig. 2a).

We next examined the ability of the Y272F ABL kinase domain mutation to rescue the growth promoting potential of the BCR/ABL deletion mutants. Double mutant ΔBCR/ABL plus Y272F constructs were generated and assessed in BA/F3 cells. Transfectants expressing the double mutant constructs p190 Δ2–422 + Y272F and p190 Δ41–333 + Y272F did not give rise to factor-independent progeny. In contrast, p210 Δ41–242 + Y272F, p190 Δ41–242 + Y272F, and p190 D2–168 + Y272F expression gave rise to factor-independent cells with high effi-
ciency (Table II). Incorporation of the Y276F mutation into construct p190 Δ41–242 did not produce a protein that would relieve BA/F3 factor dependence. These results indicate that certain BCR sequences, when fused upstream of the mutant ABL Y272F kinase domain, will cooperate to produce chimeric proteins with growth promoting potencies similar to that of full-length BCR/ABL.

The phosphotyrosine-protein content of BA/F3 cells expressing these double mutant proteins was invariably higher than that of cells expressing BCR/ABL proteins lacking the Y272F mutation (Fig. 2). In some cases the moderate elevation in kinase activity is not sufficient to confer growth factor independence; a certain threshold level is required for the conversion of BA/F3 cells to growth factor independence. Examination of the in vitro kinase activity of the various BCR/ABL deletion mutants with and without the Y272F mutation did not reveal notable differences in activity with respect to BCR/ABL p190 (Fig. 2b). Likewise, the cABL Y272F mutant did not appear to exhibit elevated activity with respect to cABL in this assay (Fig. 2b).

Phosphorylation Status of cABL 1b—The above observations indicate that mutation of the ABL ATP binding domain at tyrosine residue 272 can produce a moderately activated ABL variant. Comparison with the CDC2 kinase suggests that ABL residue 272 may be a target for phosphorylation and that the loss of a phosphorylatable residue at this site results in a loss of negative regulation.

To test this possibility for cABL 1b, we re-examined the phosphorylation status of cABL directly. This has previously been performed with antibodies to phosphotyrosine and by acid hydrolysis of the labeled protein but has failed to reveal any in vivo tyrosine phosphorylation of cABL. In contrast, the transforming variants of ABL are all phosphorylated on tyrosine in vivo (16, 17, 45, 46). In order to re-evaluate the in vivo phosphoamino acid content of cABL 1b, we varied the labeling conditions to include up to a 20-h incubation period with [32P]phosphate to permit good incorporation of 32P into the intracellular ATP pool. This was followed by the isolation and brief acid hydrolysis of ABL proteins prior to phosphoamino acid analysis. BCR/ABL p190, cABL1b Y272F, and cABL 1b were expressed in BA/F3 cells and examined for phosphotyrosine content (Fig. 3a). Phosphorylation is readily detectable in BCR/ABL p190, and a low level is detectable in cABL1b Y272F, reflecting the activation of this mutant. However, no phosphotyrosine is hydrolyzed from the cABL 1b preparation.

The inability to detect phosphotyrosine in cABL in this assay could potentially be due to rapid dephosphorylation of the protein upon cell lysis. We therefore repeated the labeling experiments with a 6-h incubation in the presence of 100 mM vanadate, a potent phosphatase inhibitor. A significant quantity of phosphotyrosine is detected in both ABL 1b and ABL 1b Y272F under these conditions (Fig. 3b), indicating that the phosphorylation status of ABL is normally under tight regulation by phosphotyrosine phosphatases.

In order to examine the phosphorylation status of tyrosine 272 in this vanadate-treated sample, we performed tryptic
digests of labeled ABL1b Y272F and ABL1b Y276F followed by two-dimensional phosphopeptide mapping and compared the migration patterns of these phosphopeptides with each other and with that of an in vitro phosphorylated synthetic peptide corresponding to the predicted tyrosine 272 containing tryptic fragment of ABL1b Y276F (Fig. 4). The peptide was synthesized with phenylalanine at the 276 position to ensure that only the 272 tyrosine is phosphorylated in vitro during preparation of the phosphopeptide. Migration of this phosphopeptide would be directly comparable with the tryptic fragment of ABL1b Y276F phosphorylated on tyrosine at position 272. The maps indicate that although the phosphorylated synthetic peptide does co-migrate with a tryptic fragment of ABL1b Y276F, the same fragment is also present in the ABL1b Y272F map. This fragment was scraped from the ABL1b Y276F map and subjected to phosphoamino acid analysis. This revealed that the label was largely due to phosphoserine, although a very low signal due to phosphothreonine and phosphotyrosine was detectable. In conclusion, using this methodology, we were unable
to show that ABL tyrosine 272 is phosphorylated in vivo at a stoichiometry that could account for the altered biological activity of the kinase.

Interaction with RB—A recent report showed that nuclear cABL kinase activity can be regulated by RB, the product of the retinoblastoma gene (38, 47). This regulation is thought to occur due to cell cycle stage-specific interaction of RB with the ATP binding domain of cABL. The appearance of the complex correlates with decreased nuclear ABL kinase activity, suggesting that the inhibition is direct. Loss of RB interaction due to the Y272F mutation in the ABL ATP binding site could potentially result in the observed elevation in ABL kinase activity. We therefore measured the ability of ABL1b Y272F to interact with RB in a communoprecipitation assay. Fig. 5 shows that both overexpressed cABL and ABL1b Y272F are found in RB immunoprecipitates at levels similar to those observed by Welch and Wang (38). Because the degree of RB binding by the two proteins is indistinguishable, the interaction of ABL1b Y272F with RB appears to be similar to that of cABL, and altered RB interaction is not likely to account for the elevated kinase activity of this mutant.

**DISCUSSION**

Overexpression of cABL 1b in the BA/F3 cell line will not relieve the strict requirement these cells display for added growth factor. In addition, no cABL autophosphorylation activity is detected in these cells, and the kinase is therefore presumed to be largely inactive. We have described a mutant ABL protein, with a tyrosine to phenylalanine substitution in the ATP binding site, that displays an elevated tyrosine kinase activity and can relieve IL-3 dependence when expressed in BA/F3 cells. This suggested that cABL may be down-regulated by tyrosine phosphorylation. However, we were unable to detect phosphorylation of the tyrosine in this position, in cABL, indicating that the protein is not generally maintained in the inactive state in cells by phosphorylation of the ATP binding site.

These findings may yet be reconciled with the proposed mechanism for cABL kinase down-regulation, which involves noncovalent association of ABL with a cytoplasmic inhibitor distinct from the nuclear RB protein. Evidence for this is provided by the activation of cABL after the removal of lysate components in the immune complex kinase assay, by hyperexpression in eukaryotic cells, or by expression in bacteria (48–50). A negative regulator may interact with ABL at the SH3 domain, because mutation of this region can activate ABL (16, 17). In light of this, it remains a possibility that cABL 1b tyrosine 272 is indeed a target for regulatory phosphorylation but that this target is only disclosed after cABL has been activated by dissociation from this putative inhibitor. Phosphorylation of the ABL ATP binding site would then represent a second level of regulation, which would be deficient in cABL Y272F, and the stoichiometry of cABL tyrosine phosphorylation in BA/F3 cells would be too low for detection in the assay employed. It is noteworthy that the regulation of CDC2 kinase activity has been shown to be mediated by associated factors such as cyclin and the CDK inhibitor proteins (51, 52). The CDC2 tyrosine 15 phosphorylation was originally proposed to inhibit ATP binding, thus down-regulating CDC2 kinase activity (33). However, examination of the ATP binding characteristics of CDC2 have shown that the tyrosine 15 phosphorylated molecule binds ATP with an affinity similar to that of the nonphosphorylated protein (34); therefore by analogy, the regulation of ABL kinase activity by tyrosine phosphorylation would be unlikely to involve a mechanism of stearic inhibition of ATP binding.

An alternative possibility is that phosphorylation plays no role in regulating ATP recruitment and that activation of cABL Y272F is due to the amino acid substitution producing a conformational change in the protein leading to altered catalysis, altered affinity for substrates, or altered affinity for an ABL inhibitor. Interestingly, activation of the avian c-erbB growth factor receptor tyrosine kinase by a single point mutation within the ATP binding site has been documented (53). This mutation replaces the highly conserved valine residue in the motif Gly-Xaa-Gly-Xaa-Xaa-Gly-Xaa-Val with isoleucine. This mutant kinase also displays an activity that is not greatly elevated in an in vitro assay but that is substantially increased in vivo.

Deletion of sequences present in BCR exon 1 will compromise the growth promoting effects of BCR/ABL fusion proteins in BA/F3 cells. Pendergast et al. (50) have identified two regions present within BCR exon 1 (shown in Table II as A and B regions) that are necessary but not sufficient for transformation by BCR/ABL. These regions will independently mediate the binding of BCR to the ABL SH2 domain, and they postulate that this interaction interferes with the binding of a cellular inhibitor to ABL regulatory domains. The amino-terminal region of BCR is also required for transformation by BCR/ABL (Ref. 43 and Table II) and acts by promoting homodimerization (44, 54). Deletion of this region of BCR (residues 2-168) produces a protein that retains a weak ability to induce factor-independent BA/F3 proliferation. The residual growth promoting activity of this mutant may be due to the fact that the deletion spares a tyrosine residue at position 177. When phosphorylated, this tyrosine mediates the binding of BCR/ABL to the adapter protein GRB-2 (55); therefore GRB-2-mediated activation of the Ras signaling pathway may explain the partial activation of this mutant. Deletion of BCR residues 41–242 interrupts the oligomerization region, tyrosine 177, and the A box (Table II). The growth promoting effects of both of these proteins (p190 Δ41–242 and p190 Δ2-168) are compromised but can be restored to high efficiency by the Y272F mutation. This cooperativity indicates that with Y272F present, the integrity of the BCR oligomerization region, the GRB-2 binding site, and one of the ABL SH2 binding regions are dispensable for growth factor abrogation in BA/F3 cells. Extending the deletion to interrupt the B box produces a protein that will not cooperate with the Y272F mutation, indicating that the elevated ABL kinase activity cannot functionally substitute for both the BCR A and B boxes in ABL deregulation.

Our findings show that the amino terminus is critical for the...
growth promotion elicited by an activated ABL kinase. Construct p190 Δ2-422-Y272F encodes a nontransforming protein that is effectively an activated ABL kinase lacking a first exon. The addition of either ABL exon 1b or certain BCR sequences produces a protein that will promote cell growth. ABL exon 1b is presumably important in dictating an association with specific substrates relevant to growth regulation. Although the amino-terminal myristoylation specified by exon 1b is important for fibroblast transformation (17, 26), this modification does not appear to be critical for BA/F3 transformation (27). This implies that the amino acid sequence encoded by the first exon is key. Likewise, the sequences encoded by BCR exon 1 that cooperate with the ABL Y272F mutation may influence association with specific substrates in addition to their role in deregulating ABL kinase activity.

In conclusion, these observations show that a single amino acid mutation in the ABL kinase domain can promote cell growth and that this signal is dependent upon the integrity of the amino-terminal, noncatalytic domain of the protein. This highlights the delicate regulation and sensitivity of endogenous substrates to the ABL kinase activity.

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