UC Irvine
UC Irvine Previously Published Works

Title
Activation of c-Abl Kinase Activity and Transformation by a Chemical Inducer of Dimerization

Permalink
https://escholarship.org/uc/item/07t787t6

Journal
Journal of Biological Chemistry, 276(26)

ISSN
0021-9258 1083-351X

Authors
Smith, K. M
Van Etten, R. A

Publication Date
2001-04-24

DOI
10.1074/jbc.M100786200

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

MECHANISMS OF SIGNAL TRANSDUCTION:

Activation of c-Abl Kinase Activity and Transformation by a Chemical Inducer of Dimerization

Kristen M. Smith and Richard A. Van Etten

J. Biol. Chem. 2001, 276:24372-24379.
doi: 10.1074/jbc.M100786200 originally published online April 24, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100786200

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 26 of which can be accessed free at
http://www.jbc.org/content/276/26/24372.full.html#ref-list-1

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 26, Issue of June 29, pp. 24372–24379, 2001
© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

Activation of c-Abl Kinase Activity and Transformation by a Chemical Inducer of Dimerization*

Received for publication, January 29, 2001, and in revised form, March 26, 2001
Published, JBC Papers in Press, April 24, 2001, DOI 10.1074/jbc.M100786200

Kristen M. Smith and Richard A. Van Etten‡

From the Center for Blood Research, Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115-5717

c-Abl is a non-receptor tyrosine kinase that is activated in human leukemias by the fusion of Bcr or Tel sequences to the Abl NH_2 terminus. Although Bcr and Tel have little in common, both contain oligomerization domains. To determine whether oligomerization alone is sufficient to activate c-Abl, we have generated and characterized an Abl protein that can be activated selectively with the chemical inducer of dimerization, AP1510. Mutant Abl proteins with one (c4F1) or two (c4F2) copies of the AP1510 binding motif (FKBP) transformed NIH 3T3 cells in a ligand-dependent manner with the c4F2 protein 60-fold more potent than c4F1. Both chimeric proteins exhibited ligand-dependent dimerization *in vivo*, suggesting that the increased transformation efficiency of the c4F2 mutant reflects more effective dimerization rather than formation of higher order oligomers. In the absence of ligand, c4F2-expresssing fibroblasts morphologically reverted and arrested in G_1. In Ba/F3 cells, the c4F2 chimera exhibited ligand-dependent kinase activation, transformation to interleukin 3-independent growth, and relocalization of the fusion protein from nucleus to cytoplasm. These results demonstrate that dimerization alone is sufficient to activate the Abl kinase and provide a method to regulate conditionally c-Abl activity that will be useful for studying the normal physiological role of c-Abl and the mechanism of transformation and leukemogenesis.

c-Abl is a non-receptor tyrosine kinase of unknown function that is localized to the cell nucleus (1) and F-actin cytoskeleton (1–3). In mammalian cells, c-Abl kinase activity is increased by diverse physiological stimuli including S phase progression in the cell cycle (4), ionizing radiation (5), oxidative stress (6, 7), engagement of integrin adhesion receptors (8), and growth factor stimulation (5). Activation of c-Abl kinase activity is associated with a broad range of cellular responses including redistribution of Abl from cytoskeleton to nucleus (8), phosphorylation of RNA polymerase II (9), activation of c-Jun NH_2-terminal kinases (10), and induction of G_1 arrest (11, 12) and apoptosis (12–14). These observations collectively support a role for c-Abl in the integration of adhesion signals with cell cycle control and in the cellular response to DNA damage and oxidative stress (15).

The *in vivo* mechanism of regulation of c-Abl kinase activity is not understood completely but appears to be distinct from that of Src family members. c-Src is regulated negatively by phosphorylation of the COOH-terminal tyrosine 527 by Csk and other cellular kinases and assumes an inactive conformation when this phosphotyrosine is bound by the Src SH2[1] domain in an intramolecular fashion (16, 17). In this structure, the Src SH3 domain interacts with a single proline (Pro-250) in the linker region between the SH2 and catalytic domains. Activation of c-Src by dephosphorylation or mutation of Tyr-527 (18) or mutation of the SH2 or SH3 domain (19, 20) results in increased Src kinase activity *in vitro* and *in vivo* and induces cellular transformation in many cases. In contrast, c-Abl is not tyrosine phosphorylated in its inactive state (21), and truncation of the Abl COOH terminus (1, 21) or mutation of the SH2 domain (22) does not activate Abl *in vivo*. Mutation of the Abl SH3 domain or a proline residue homologous to Src Pro-250 increases the catalytic activity of purified c-Abl (23) and induces cellular transformation (24, 25), suggesting that c-Abl is likewise regulated intramolecularly by its SH3 domain. c-Abl kinase activity is also stimulated by phosphorylation of a catalytic domain tyrosine residue (Tyr-412) via autophosphorylation (23) or transphosphorylation by c-Src (5), and possibly upon phosphorylation of Ser-465 by the Atm kinase (26). However, purified unphosphorylated c-Abl has high intrinsic kinase activity relative to inactive c-Src (23), implying that a cellular inhibitor may contribute to suppression of c-Abl kinase activity *in vivo* (27). Pag/MSP23, a novel c-Abl SH3-binding protein, specifically suppresses c-Abl tyrosine kinase activity upon coexpression *in vivo*, suggesting it is a physiological inhibitor of c-Abl (6).

Naturally occurring oncogenic forms of Abl implicate another mechanism for activation of Abl kinase activity. The t(9;22) and t(9;12) chromosomal translocations in human leukemias activate Abl by fusing sequences from the Bcr and Tel proteins, respectively, to the NH_2 terminus of c-Abl. Although Bcr, a cytoplasmic protein with RacGAP activity (28), and Tel, a nuclear transcription factor of the Ets family (29), appear to have little in common, the translocated portions of both proteins contain domains implicated in self-association or oligomerization. Disruption of a leucine zipper-like coiled-coil domain in Bcr by insertion of proline-containing peptides (30) or deletion of a helix-loop-helix oligomerization domain in Tel (29) results in Abl fusion proteins that are defective for transformation and exhibit greatly decreased or absent *in vivo* kinase activity, as assessed by cellular phosphotyrosine levels. These observations suggest that oligomerization of c-Abl might be sufficient to activate Abl kinase activity. However, in the case of the Bcr-Abl fusion protein, additional sequences in Bcr are re-

* This work was supported by National Institutes of Health Grants CA72465 (to R. A. V.) and T32-HL07623 (to K. M. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Scholar of the Leukemia and Lymphoma Society and the Carl and Margaret Walter Scholar in Blood Research at Harvard Medical School. To whom correspondence should be addressed: Center for Blood Research, 200 Longwood Ave., Boston, MA 02115-5717. Tel.: 617-278-3250; Fax: 617-278-3030; E-mail: vanetten@cbr.med.harvard.edu.

[1] The abbreviations used are: SH2, Src homology 2; SH3, Src homology 3; ER, estrogen receptor; IL-3, interleukin 3; PIPES, 1,4-piperazinediethanesulfonic acid; Epo-R, erythropoietin receptor.

This paper is available on line at http://www.jbc.org

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

quired for cellular transformation because Bcr-Abl mutants containing just the extreme NH_2 terminus of Bcr including the amphipathic coiled-coil domain are defective for transformation of fibroblasts (30, 31).

In support of this model of kinase activation, fusion of the estrogen receptor (ER) hormone binding domain to the COOH terminus of wild-type or SH3-deleted c-Abl generates chimeric proteins capable of inducing estradiol-dependent increases in cellular phosphotyrosine and transformation of fibroblasts (32), presumably mediated by hormone-dependent dimerization. Interestingly, in the absence of hormone, cells expressing both types of fusion exhibit low or absent phosphotyrosine levels and morphologically revert to an untransformed state, but they arrest in the cell cycle in early G_1 (33). The fusion of ER to SH3-deleted Abl dominantly inactivates the transforming ability of this protein in the absence of ligand by formation of an inactive complex with Hsp90 (34). The SH3-containing Abl-ER fusion protein requires increased time, new protein synthesis, and a higher estradiol dose for reinduction of transformation in reverted cells, implying that the Abl-ER fusion must overcome the inhibitory function of SH3 upon addition of hormone.

The above studies collectively suggest that dimerization or oligomerization of c-Abl might be sufficient to activate Abl kinase activity *in vivo*. However, a requirement for additional functions in Bcr, Tel, or ER is difficult to exclude. In addition, the use of the Abl-ER fusion as a conditional form of Abl is compromised by the lack of COOH-terminal c-Abl sequences likely to be important for physiological function of Abl (35) and by the pleiotropic effects of estradiol in mouse model systems. We have therefore employed a chemical inducer of dimerization (36) to investigate the effect of oligomerization on Abl kinase activity and transformation. This system utilizes a bifunctional adduct of the fungal immunosuppressive drug FK506, which is lipid-soluble, metabolically stable, and binds with subnanomolar affinity to its cellular receptor, FKBP12. FKBP12 is an abundant and ubiquitous 108-amino acid polypeptide with prolyl isomerase activity that binds to and inhibits calcineurin when complexed with FK506, resulting in immunosuppression by blocking T cell activation. Introduction of the FKBP module into a heterologous protein allows ligand-dependent homo- and heterodimerization of the target proteins (37). Because the ligand is engineered to lack the calcineurin binding site, there is no inhibition of calcineurin. Using this system, we show here that dimerization of c-Abl is sufficient to activate c-Abl kinase activity *in vivo* and induce transformation of fibroblasts and hematopoietic cells. This system will be a useful tool for understanding the regulation of c-Abl and for investigating the primary consequences of activation of Abl kinase *in vivo*.

EXPERIMENTAL PROCEDURES

Constructs—The pPLcIV construct has been described previously (24). To make the pPLc4F1 and pPLc4F2 constructs, the cIV *abl* cDNA was opened at the unique *Stu*I site at nucleotide 42 and fitted with a 12-base pair *Bam*HI linker. This resulted in insertion of four amino acids (RGSA) at this position, which does not affect Abl kinase activity or regulation (data not shown). The *Xba*I-*Spe*I fragment from the plasmids pMF1E and pMF2E (36), containing one or two tandem copies of FKBP, was blunted with T4 polymerase, fitted with an 8-base pair *Bam*HI linker, and cloned into the *Bam*HI site in Abl to generate c4F1 and c4F2, respectively. The resulting fusion proteins have an additional three amino acids (underlined) on either side of the FKBP domain(s) (NH_2-MGQQPGKVLGDQRR<u>RGS</u>(FKBP domain)<u>GSA</u>P . . .). This vector was used to construct all the other mutants in this study, including the pcDNA3 and MINVneo (38) vectors. The Abl P131L (24), K290M (21), Y412F (23), and $1^Q2^Q3^Q$ and ΔSal (12) mutations have been described previously.

Cell Culture, Transfection, and Retroviral Transduction—For coprecipitation studies, 293T cells were transfected with 5 μg of c4F1 K290M and 5 μg of c4F1 K290M ΔSal (or 5 μg of c4F2 K290M and 5 μg of c4F2 K290M ΔSal) in the pcDNA3 vector (Invitrogen) using a modified calcium phosphate method (39). For generation of retroviral stocks, 293T cells were transfected with 10 μg of pPL or MINVneo retroviral vector DNA and 5 μg of the ecotropic packaging construct pMCVecopac (39). Supernatant was harvested 48 h later and titered by G418 resistance or Southern blot analysis of proviral copy number in transduced fibroblasts. To assay fibroblast transformation, NIH 3T3 cells were transduced with pPL retrovirus, plated in soft agar with or without 0.5 μM AP1510, and colonies counted at 21 days. To correct the soft agar colony numbers for differences in transduction efficiency, genomic DNA was isolated from an aliquot of the transduced cells and proviral copy number determined by Southern blotting, as described (24). Results were expressed as soft agar colonies/10^4 cells plated, normalized to one proviral copy/cell. To derive transformed fibroblast cell lines, pPL- or MINVneo-transduced NIH 3T3 cells were grown in monolayers in the presence of AP1510, populations of transformed cells were dislodged by squirting with medium, cloned by limiting dilution in the presence of 1 μM AP1510, and assayed for ligand-dependent morphological transformation. To determine the dose response of the clonal cell lines to AP1510, cells were plated in soft agar with increasing concentrations of AP1510 and scored for colony growth after 21 days. Ba/F3 cells were transduced with c4F2 MINVneo virus, selected for G418 resistance, and maintained in the absence of AP1510 and with supplemental WEHI-3B-conditioned medium as a source of IL-3. To determine the dose response of Ba/F3 cells expressing c4F2 to AP1510, cells were washed and replated in medium lacking IL-3 and containing increasing concentrations of AP1510. Viable cell counts were determined by trypan blue exclusion.

Antibodies—Anti-Abl monoclonal antibodies (8E9, Pharmingen and Ab3, Oncogene Research), anti-phosphotyrosine monoclonal antibody (4G10, Upstate Biotechnology), and rhodamine-conjugated anti-mouse and anti-rabbit IgG antibodies (Jackson Immunoresearch) were purchased from the respective vendors. Rabbit anti-Abl (GEX5) antiserum has been described previously (24).

Immunoprecipitation and Immunoblotting—293T cells were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris-HCl at pH 7.4, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate, 40 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 1% aprotinin), with 0.5 μM AP1510 included where indicated. Lysates were incubated overnight with polyclonal anti-Abl GEX5 antiserum, immune complexes collected with protein A-Sepharose, washed in RIPA buffer, high salt buffer (500 mM NaCl, 20 mM Tris-HCl at pH 7.4, 1% Triton X-100), and low salt buffer (20 mM PIPES at pH 7.2), then subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting with the indicated antibodies, as described (24).

Sucrose Gradient Centrifugation—NIH 3T3 cell extracts were prepared in phosphate-buffered saline with 0.2% Triton X-100, 1 mM Na_3VO_4, 40 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1% aprotinin, with 0.5 μM AP1510 added to extracts of cells grown in the presence of ligand. Cell lysates were layered onto a 2-ml 10–30% sucrose gradient in similar buffer without detergent and centrifuged at 200,000 × *g* for 2.5 h at 4 °C. Fractions were collected from the bottom of the gradient, boiled in sample buffer under reducing conditions, fractionated by 5–20% SDS-polyacrylamide gel electrophoresis, and analyzed by immunoblotting as described above. Parallel gradients were run with visible molecular weight markers (Amersham Pharmacia Biotech).

Reversion Assays—NIH 3T3 clones transformed by c4F1, c4F2, or c4F2 $1^Q2^Q3^Q$ were plated in the presence or absence of 0.5 μM AP1510. At various times after plating, cells were treated with trypsin, washed in phosphate-buffered saline, and fixed in 70% ethanol. Fixed cells were treated with RNase A, stained with propidium iodide, and the cellular DNA content analyzed by fluorescence-activated cell sorting utilizing a FACScan flow cytometer and CellQuest software (Becton Dickinson). Ligand-dependent survival of cloned cell lines was analyzed by plating 10^5 cells/6-cm plate in the presence or absence of 0.5 μM AP1510 and counting viable cells at 24, 48, and 72 h after plating. Cell survival was also examined by terminal nucleotidyl transferase staining (*In Situ* Cell Death Detection Kit, Roche) at the same time points, according to the manufacturer's instructions. To assess ligand-dependent activation of Abl kinase activity *in vivo*, Ba/F3 cells expressing c4F2 were grown for several generations in the absence of AP1510 and the presence of IL-3. Cells were washed extensively and plated at 10^6 cells/ml in medium lacking both IL-3 and AP1510. After 30 min, 0.5 μM AP1510 was added to the medium (time = 0). Cycloheximide (20 μg/ml) was added to duplicate samples. Whole cell extracts were prepared from 10^6 cells at various times post-AP1510 addition and processed for immunoblotting as described above.

Immunofluorescence—Ba/F3 cells expressing c4F2 or c4F2 $1^Q2^Q3^Q$ were deposited on glass coverslips by cytocentrifugation (Shandon) at

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

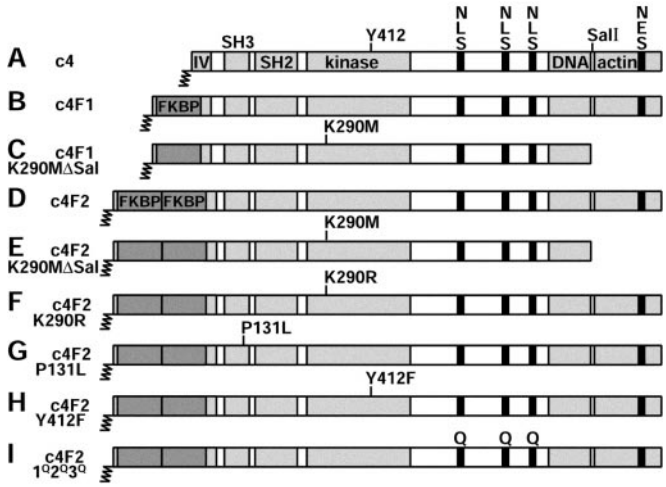

FIG. 1. **Structure of the Abl-FKBP chimeric proteins used in this study.** *Panel A*, schematic representation of murine type IV (myristoylated form) c-Abl protein. Depicted from *left* to *right* are the NH_2-terminal myristate group (*zigzag line*), type IV first exon, SH3 and SH2 domains, kinase domain with location of activation loop tyrosine 412, three nuclear localization signals (*NLS*), DNA and actin binding domains, *Sal*I recognition site, and nuclear export signal (*NES*). *Panel B*, c4F1 chimera containing a single copy of the FKBP motif inserted into the Abl first exon. *Panel C*, c4F1 K290M ΔSal mutant containing the K290M kinase-inactivating point mutation and COOH-terminal truncation at the *Sal*I site. *Panel D*, c4F2 chimera containing two tandem copies of the FKBP motif. *Panel E*, c4F2 K290M ΔSal mutant containing the K290M mutation and ΔSal truncation. *Panel F*, c4F2 K290R, containing a kinase-inactivating mutation. *Panel G*, c4F2 P131L, containing an activating SH3 domain point mutation. *Panel H*, c4F2 Y412F, containing a Tyr → Phe mutation at the major autophosphorylation site. *Panel I*, c4F2 $1^Q2^Q3^Q$, containing inactivating point mutations in the three COOH-terminal nuclear localization signals.

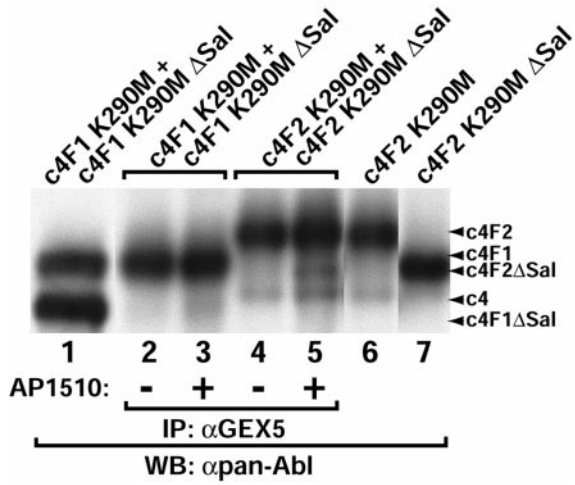

FIG. 2. **Ligand-dependent oligomerization of Abl-FKBP chimeric proteins *in vivo*.** c4F1 and c4F2 chimeras with the kinase-inactivating K290M mutation were coexpressed in 293T cells with the respective ΔSal truncation mutants. *Lane 1*, lysate from cells coexpressing c4F1 K290M and c4F1 K290M ΔSal. *Lanes 2* and *3*, anti-COOH-terminal Abl (GEX5) immunopreciptate (*IP*) from cells coexpressing c4F1 K290M and c4F1 K290M ΔSal in the absence or presence of AP1510, respectively. *Lanes 4* and *5*, anti-GEX5 immunoprecipitate from cells coexpressing c4F2 K290M and c4F2 K290M ΔSal in the absence or presence of AP1510, respectively. *Lanes 6* and *7*, lysates from cells expressing either c4F2 K290M or c4F2 K290M ΔSal, respectively. Abl proteins were detected by immunoblotting with a pan-reactive Abl antibody and enhanced chemiluminescence.

600 rpm for 1 min. The cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 for 30 min at room temperature, blocked with 5% normal donkey serum in phosphate-buffered saline, incubated with a mixture of 1 μg/ml each of the mouse anti-Abl monoclonal antibodies 8E9 and Ab3, and stained with rhodamine-conjugated donkey anti-mouse antibody. Coverslips were mounted with Fluoromount G (Fisher Scientific) and photographed on a Zeiss epifluorescence microscope with Fuji Neopan ASA 1600 black and white film.

RESULTS

Ligand-dependent Oligomerization of c-Abl-FKBP Proteins—To create a c-Abl molecule that could be inducibly dimerized by addition of an exogenous ligand, we utilized the FKBP oligomerization system. This system is comprised of two parts: a membrane-diffusible bivalent ligand and a ligand binding domain that is fused to the protein of interest. We constructed the chimeric Abl-FKBP molecule by inserting one or two tandem copies of the FKBP domain after the initial 14 amino acids of murine type IV (myristoylated) c-Abl (Fig. 1*A*), generating c4F1 and c4F2 chimeric proteins (Fig. 1, *B* and *D*). The c-Abl-FKBP chimeras were predicted to form homo-oligomers in the presence of AP1510.

To confirm the ligand-dependent oligomerizatioin of the Abl-FKBP chimeras, we generated two additional constructs that were truncated at the COOH-terminal *Sal*I site, c4F1 K290M ΔSal and c4F2 K290M ΔSal (Fig. 1, *C* and *E*). We introduced the K290M kinase-inactivating point mutation into both full-length and truncated chimeras to eliminate the possibility of oligomerization mediated by tyrosine phosphorylation and SH2 binding. The full-length and truncated constructs were coexpressed in 293T cells and immunoprecipitated with an antibody (GEX5) that recognizes only the full-length protein. In the presence of AP1510, the c4F1 truncation mutant demonstrated weak coprecipitation with the full-length version, whereas the c4F2 truncation mutant exhibited stronger coassociation (Fig. 2). No coprecipitation was seen in the absence of AP1510. The Abl-FKBP chimeras therefore self-associate *in vivo* in a ligand-dependent fashion.

Ligand-dependent Fibroblast Transformation by Abl-FKBP Chimeras Requires the Abl Autophosphorylation Site—To determine the consequences of oligomerization on c-Abl kinase activity, we tested the ability of the c4F1 and c4F2 chimeras to transform fibroblasts. NIH 3T3 cells were transduced with retroviruses expressing wild-type c-Abl, c4F1, or c4F2 and plated in soft agar with or without AP1510. A kinase-inactive mutant (c4F2 K290R) and a constitutively active Abl SH3 domain mutant (c4F2 P131L) were included as controls. Addition of the FKBP domain to c-Abl resulted in ligand-dependent soft agar colony formation (Fig. 3*A*). The c4F1 construct produced about 10-fold more soft agar colonies in the presence of AP1510 than wild-type c-Abl, whereas the c4F2 construct was even more potent and produced more than 100 more colonies than wild-type c-Abl, comparable to transformation by Abl with the activating SH3 point mutation (c4 P131L). Both c4F1 and c4F2 showed only background levels of transformation in the absence of AP1510. c4F2 K290R (Fig. 1*F*), a chimera with an inactive kinase domain, was nontransforming under all conditions (Fig. 3*A*), demonstrating that Abl-FKBP was not acting merely to induce the dimerization of another pro-oncogenic protein that bound to Abl. Fusion of the ER hormone binding domain to SH3-deleted Abl dominantly inactivates the transforming ability of this kinase in the absence of estradiol (32). In contrast, the c4F2 P131L construct (Fig. 1*G*) was equally transforming in the presence or absence of ligand (Fig. 3*A*). This suggests that addition of the FKBP domain does not grossly affect Abl kinase activity or function in the absence of ligand.

We also tested the requirement for several functional domains of Abl for ligand-dependent transformation by the Abl-FKBP chimera. An Abl-FKBP chimera with inactivation of the three COOH-terminal nuclear localization signals (c4F2 $1^Q2^Q3^Q$, Fig. 1*H*) was still transforming in the presence of ligand but somewhat less efficient than the parental c4F2,

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

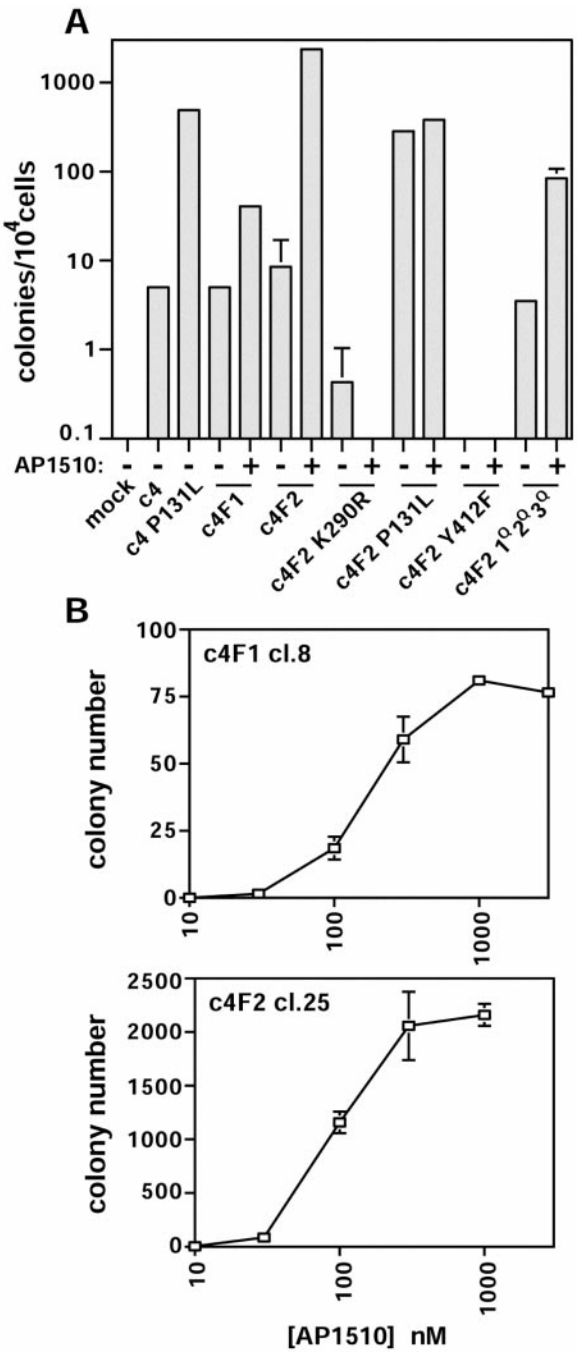

FIG. 3. **Ligand-dependent fibroblast transformation by the Abl-FKBP chimeric proteins.** *Panel A*, soft agar colony formation by NIH 3T3 fibroblasts transduced with pPL retrovirus expressing the indicated Abl proteins and plated in the presence or absence of AP1510, normalized to one proviral copy/cell as described under "Experimental Procedures." Note that the *y axis* is a logarithmic scale. S.D. values are indicated by the *bars* but are unapparent for most samples because of small values. *Panel B*, dose-response curve for soft agar colony formation by clones of NIH 3T3 cells expressing c4F1 (*top*) or c4F2 (*bottom*) in varying concentrations of AP1510 ligand. Note the difference in the scale of the *y axis* between the two *panels*.

confirming previous observations that nuclear localization increases transformation by oncogenic forms of Abl (12). c-Abl can undergo intermolecular autophosphorylation at Tyr-412 in the catalytic domain that stimulates kinase activity over 8-fold (23). Interestingly, mutation of Tyr-412 to phenylalanine (c4F2 Y412F, Fig. 1*I*) completely abolished ligand-dependent transformation by the Abl-FKBP chimera (Fig. 3*A*), suggesting that dimerization-induced autophosphorylation is critical for transformation by this fusion protein.

Increased Efficiency of Dimerization Explains the Superior Transformation by the c4F2 Chimera—We isolated transformed clones stably expressing c4F1 or c4F2 by limiting dilution in the presence of AP1510 and assessed the transformation dose response to AP1510 for each cell line. Both c4F1 and c4F2 cell lines showed a sigmoidal response to increasing concentrations of AP1510 (Fig. 3*B*). The half-maximal concentration of AP1510 for c4F1 was ~2.5-fold higher than for c4F2 (90 μM *versus* 200 μM).

Unlike the c4F1 chimera, the c4F2 chimera can theoretically form higher order oligomers such as homotrimers or homotetramers. The increased transformation ability of the c4F2 chimera relative to c4F1 could be a result of this property or might simply reflect more efficient dimerization at a given concentration of ligand. To examine the ligand-dependent association of the Abl-FKBP chimera *in vivo*, lysates from the c4F1 and c4F2 cell lines grown in the presence or absence of AP1510 were analyzed by sucrose gradient centrifugation. The c4F2 protein sedimented at about 300 kDa apparent molecular mass when cells were grown in AP1510 but sedimented at about 150 kDa from cells grown without AP1510 (Fig. 4*A*). The change in sedimentation pattern is consistent with c4F2 existing as a dimer in the presence of AP1510 and as a monomer in its absence, and there was no evidence of higher order oligomers. Similar results were obtained for the c4F1 chimera (Fig. 4*B*). These observations suggest that the Abl-FKBP chimeras exist predominantly as dimers in the presence of the exogenous ligand, AP1510, and that a second copy of the FKBP domain increases the efficiency of dimerization by AP1510.

Transformed Cells Expressing Abl-FKBP Chimeras Exhibit Morphological Reversion and G_1 Arrest upon Withdrawal of Ligand—We next examined whether transformation by the Abl-FKBP chimeras was reversible upon withdrawal of AP1510. In the presence of 1 μM AP1510, cell lines expressing c4F1, c4F2, or c4F2 $1^{Q}2^{Q}3^{Q}$ were morphologically transformed. The cells were highly refractile with a rounded or spindly appearance, lacked contact inhibition, and formed large foci when grown at high density. Within 24 h after ligand withdrawal, the cells had uniformly flattened and showed typical nonrefractile morphology (Fig. 5*A*). The cells failed to proliferate in the absence of AP1510 (data not shown), and examination of cellular DNA content by propidium iodide staining demonstrated that the reverted cells had arrested predominantly in the G_1 stage of the cell cycle with 2n DNA content (Fig. 5*B*). G_1 arrest has also been observed in cells that overexpress c-Abl after transfection (12) or from an inducible promoter (11), and this cytostatic effect absolutely requires nuclear localization by Abl (12). However, G_1 arrest was still observed upon withdrawal of AP1510 from transformed cells expressing c4F2 $1^{Q}2^{Q}3^{Q}$ (Fig. 5*B*), which is restricted to the cytoplasm, demonstrating that the mechanism of growth arrest by the Abl-FKBP chimeras under these conditions is different. In addition to its cytostatic effect, transfection of c-Abl is also associated with prominent cytotoxicity with induction of apoptosis (12, 13), which also requires nuclear localization (12). However, the reverted and growth-arrested fibroblasts expressing the Abl-FKBP chimeras demonstrated only background levels of apoptosis, as assessed by terminal nucleotidyl transferase staining (data not shown) and the lack of significant sub-2n DNA content (Fig. 5*B*).

Western blot analysis demonstrated that the Abl-FKBP chimeras were highly expressed in the transformed cells at levels greater than 20-fold over endogenous c-Abl (Fig. 5*C*). In the presence of AP1510, there was prominent tyrosine phosphorylation of the Abl-FKBP chimera and many other cellular pro-

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

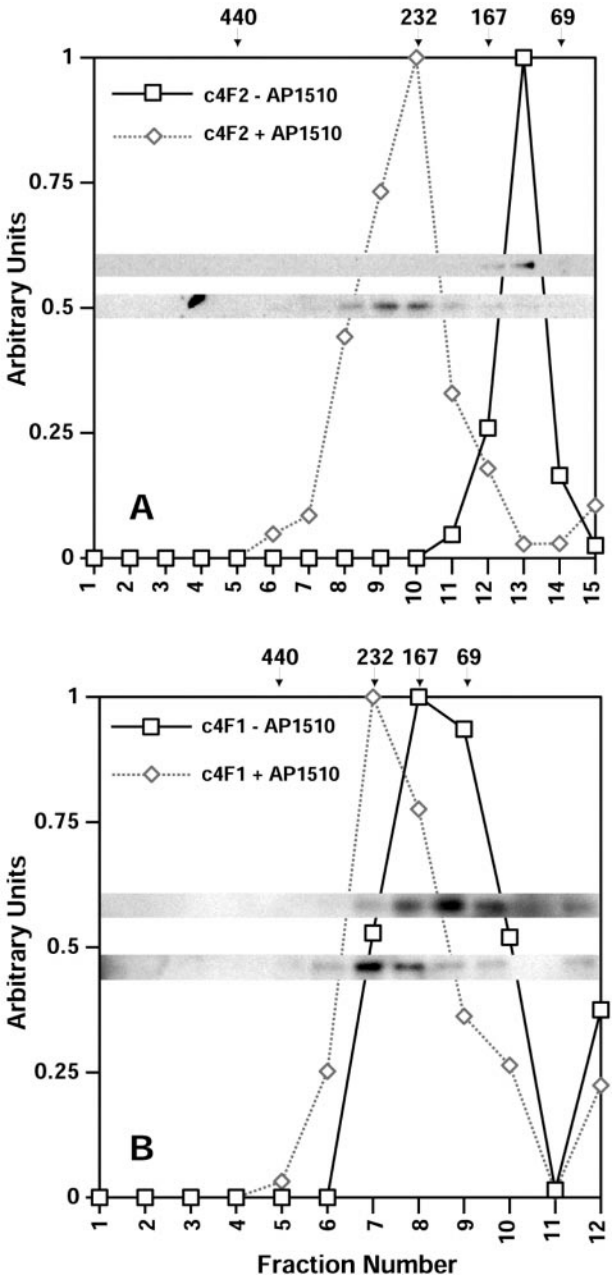

FIG. 4. **The c4F1 and c4F2 proteins exist predominantly as dimers in the presence of ligand *in vivo*.** *Panel A*, lysates from an NIH 3T3 clone expressing the c4F2 chimera grown in the presence (◇) or absence (□) of AP1510 were subjected to sucrose gradient centrifugation as described under "Experimental Procedures," and individual fractions were assayed for content of c4F2 protein by immunoblotting (*insets*), with the relative amounts quantitated by densitometry and plotted with the maximal fraction arbitrarily assigned a value of 1. The fractions of a parallel gradient containing protein standards of different molecular mass are indicated by the *markers* at the *top*. *Panel B*, identical to *panel A* but with a clone expressing c4F1.

teins which was similar among the different Abl-FKBP-expressing clones (Fig. 5*C*). Upon withdrawal of AP1510, the level of tyrosine phosphorylation decreased but remained significantly above that of control NIH 3T3 cells, particularly in the c4F1-expressing cells, which had the highest levels of Abl-FKBP expression. This demonstrates that although the transformed morphology of cells expressing the Abl-FKBP chimeras reverts to normal in the absence of AP1510, the kinase activity of the Abl fusion is not suppressed completely. This may be a consequence of the very high levels of Abl protein expression in these cell lines.

Expression of the Abl-FKBP Chimera in Hematopoietic Cells Results in Ligand-dependent Activation of Kinase Activity and Transformation—To express the c4F2 protein at levels similar to endogenous c-Abl, we transduced the murine IL-3-dependent pro-B cell line Ba/F3 with a c4F2 MINVneo retrovirus (38) that coexpresses c4F2 and a neomycin resistance gene via an internal ribosome entry site. The transduced cells were selected for G418 resistance and tested for growth in varying concentrations of AP1510 in the absence of IL-3. As the concentration of AP1510 was increased, a corresponding increase in IL-3-independent survival and proliferation was observed (Fig. 6*A*). The proliferation dose response of c4F2-expressing Ba/F3 cells for AP1510 was superimposable on the soft agar colony transformation curve observed for NIH 3T3 cells expressing c4F2 (Fig. 6*A*, *inset*). These results demonstrate that c4F2 is able to transform Ba/F3 cells to IL-3 independence in a ligand-dependent manner.

Upon ligand withdrawal, c4F2-expressing Ba/F3 cells underwent G_1 arrest and apoptosis (data not shown) but could be rescued by the addition of IL-3 to the culture medium (Fig. 6*A*). This allowed us to examine transformation and reversion of these cells in more detail. We first examined the time course of Abl kinase activation. Ba/F3 cells expressing c4F2 were grown in the absence of AP1510 and presence of IL-3 for several generations, then washed and replated in medium lacking IL-3. Upon addition of AP1510, we observed a rapid increase in tyrosine phosphorylation levels which was detectable by 15 min and nearly maximal by 1 h (Fig. 6*B*). The observed increase in tyrosine phosphorylation did not require new protein synthesis, demonstrating that the existing c4F2 protein was quickly dimerized and activated by ligand.

We also examined the localization of the c4F2 protein in the presence or absence of AP1510. When cells were grown in AP1510, the c4F2 protein was exclusively cytoplasmic (Fig. 7, *A* and *B*). The chimeric protein moved to the nucleus upon ligand withdrawal (Fig. 7, *C* and *D*). This mimics the difference in subcellular localization between transforming and nontransforming Abl proteins in fibroblasts (1). In contrast, the c4F2 $1^{Q}2^{Q}3^{Q}$ mutant was localized to the cytoplasm in the presence or absence of AP1510, as expected (Fig. 7, *E–H*).

DISCUSSION

The mechanism of regulation of c-Abl tyrosine kinase activity and the primary effects of activation of Abl kinase *in vivo* are not well understood. Fusion of c-Abl with proteins containing oligomerization domains such as Bcr and Tel causes constitutive activation of Abl kinase and oncogenic transformation. However, the oligomerization domain of Bcr alone is insufficient to activate c-Abl (30, 31). In previous studies, fusion of c-Abl with the hormone binding domain of ER (32) or the extracellular and transmembrane portions of the erythropoietin receptor (Epo-R) generated chimeric Abl proteins (40) whose kinase activity and transforming properties were regulated by estradiol and erythropoietin ligands, respectively. The underlying mechanism of activation was presumed to be ligand-dependent oligomerization, but this was not demonstrated directly in either case. To test the hypothesis that oligomerization alone is sufficient to activate Abl and to provide a method to control Abl kinase activity specifically, independent of other stimuli, we developed a chimeric Abl protein that can be dimerized inducibly in response to an exogenous non-hormone ligand. We chose to use the FKBP-AP1510 oligomerization system for several reasons. The FKBP domain is small and does not alter the regulation or subcellular localization of c-Abl by itself. AP1510, the bivalent ligand responsible for dimerization, is a synthetic membrane-soluble compound with high affinity for FKBP12 yet is nontoxic and has no effect on cellular sig-

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

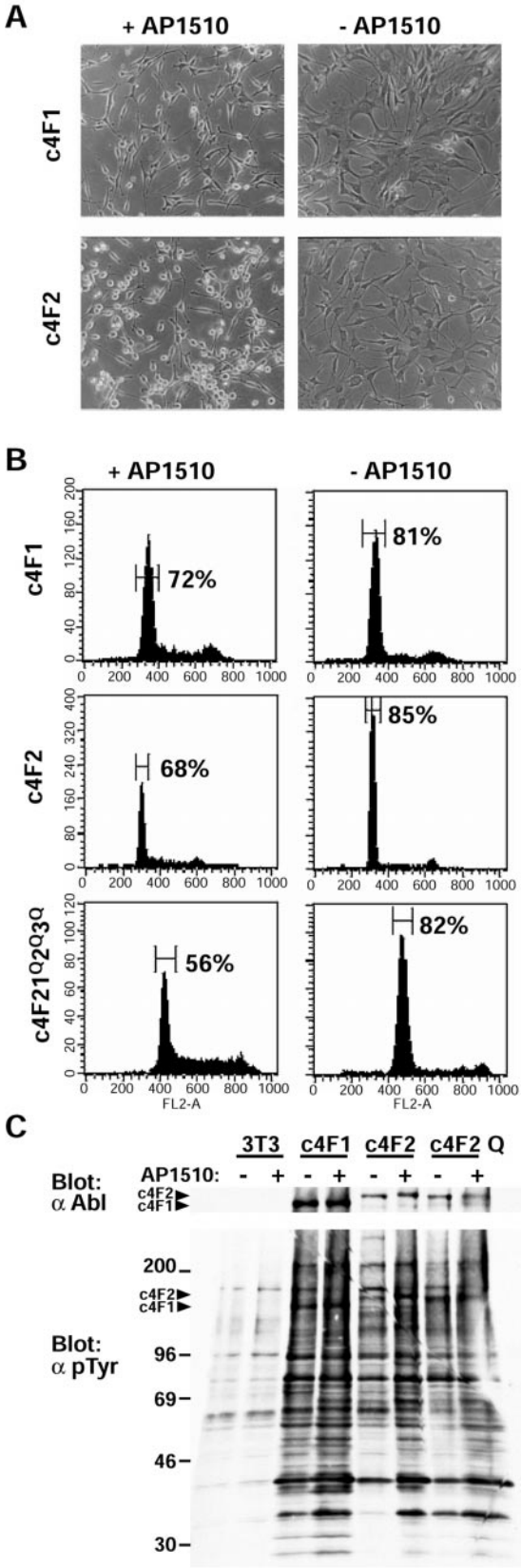

FIG. 5. **Transformed fibroblasts expressing the c4F1 and c4F2 chimeras undergo morphological reversion and G_1 arrest upon withdrawal of ligand.** *Panel A*, photomicrographs of NIH 3T3 clones expressing c4F1 (*top squares*) or c4F2 (*bottom squares*) grown in the presence of 0.5 μM AP1510 (*left squares*) or deprived of AP1510 for 72 h (*right squares*). *Panel B*, DNA content assessed by flow cytometric analysis of propidium iodide-stained populations of NIH 3T3 clones expressing the c4F1 (*top squares*), c4F2 (*middle squares*), and c4F2 $1^{Q}2^{Q}3^{Q}$ (*bottom squares*) proteins in the presence (*left squares*) or absence (*right squares*) of AP1510. The percentage of total cells with 2n DNA content (*bracketed* region) in each *square* is indicated. *Panel C*, anti-Abl (*top section*) and anti-phosphotyrosine (*bottom section*) immunoblot of lysates from parental NIH 3T3 cells (3T3) or clones expressing the c4F1, c4F2, and c4F2 $1^{Q}2^{Q}3^{Q}$ (*c4F2 Q*) chimeras grown in 0.5 μM AP1510 (+) or deprived of AP1510 for 72 h (−). Endogenous c-Abl was detectable in the 3T3 cells but only upon gross overexposure of the blot (data not shown). The positions of the c4F1 and c4F2 chimeras are indicated by the *arrowheads* on the *left*.

naling. The FKBP-AP1510 chemical dimerization system has been used previously for controlling the activity of genes, proteins, and intracellular signal transduction pathways. Our results demonstrate conclusively that oligomerization is sufficient to activate Abl kinase activity and induce cellular transformation, and we can now add Abl to the list of proteins that can be regulated by a chemical inducer of dimerization. Although this technology has been used to control localization-dependent signaling by Src kinases (41), this is the first example of conditional oncogenic transformation using this system.

In Ba/F3 hematopoietic cells, activation of Abl-FKBP kinase activity by AP1510 was rapid, reversible, and did not require new protein synthesis. Chimeric Abl proteins containing either one or two copies of the FKBP moiety transformed fibroblasts in a ligand-dependent manner. The c4F2 chimera was more efficient than c4F1, both in terms of the dose response to AP1510 and in the maximal number of anchorage-independent colonies. Sedimentation analysis demonstrated that both the c4F1 and c4F2 chimeras exist predominantly as dimers in the presence of AP1510 *in vivo*, despite the potential of the c4F2 chimera to form higher order oligomers. This suggests that the greater potency of c4F2 is caused by an increased efficiency of dimerization at a given concentration of ligand. The interaction of FK506 or related bivalent dimerizer compounds to FKBP12 is characterized by a high equilibrium binding constant with a significant k_{off} rate but even higher k_{on}. The presence of a second ligand binding domain in close proximity to the first increases the probability that a dissociated ligand will be rebound before the two FKBP-containing proteins have diffused apart. In support of this model, two or even three tandem copies of the FKBP motif have increased biological potency over a single copy when introduced into several other signaling proteins (37).

In the case of the Bcr-Abl and Tel-Abl leukemia fusion proteins, it is not known with certainty whether the activation of Abl by these fusion partners requires simple dimerization or the formation of higher order multimers. A peptide including the Bcr leucine zipper-like domain can undergo tetramerization *in vitro* (30), while a fraction of Bcr-Abl from leukemia cells can be found in complexes of very large molecular mass (42). The stoichiometry of the oligomers formed by Tel fusion proteins *in vivo* has not been defined (43), but structural studies of the SAM domain found in Ets transcription factors and other signaling proteins suggest the possibility of formation of large oligomers (44). Our studies imply that dimerization may be the critical process that dysregulates these leukemogenic Abl fusion proteins and that small molecules that interfere with Abl oligomerization may have therapeutic activity in treatment of these malignancies.

Our results predict a molecular mechanism for the dysregulation of Abl kinase activity upon dimerization. Recent mutagenic (25, 45) and enzymological (23) studies strongly suggest that c-Abl is regulated in part through intramolecular binding of SH3 domain to the linker region between the SH2 and kinase domains. Because the Abl-FKBP chimera was properly inhibited in Ba/F3 cells in the absence of ligand, introduction of the FKBP module into the Abl NH_2 terminus does not by itself negate the regulatory function of the SH3 domain. Interestingly, although SH3-deleted c-Abl does not require the major autophosphorylation site at Tyr-412 for fibroblast transformation (46), the transforming activity of the c4F2

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

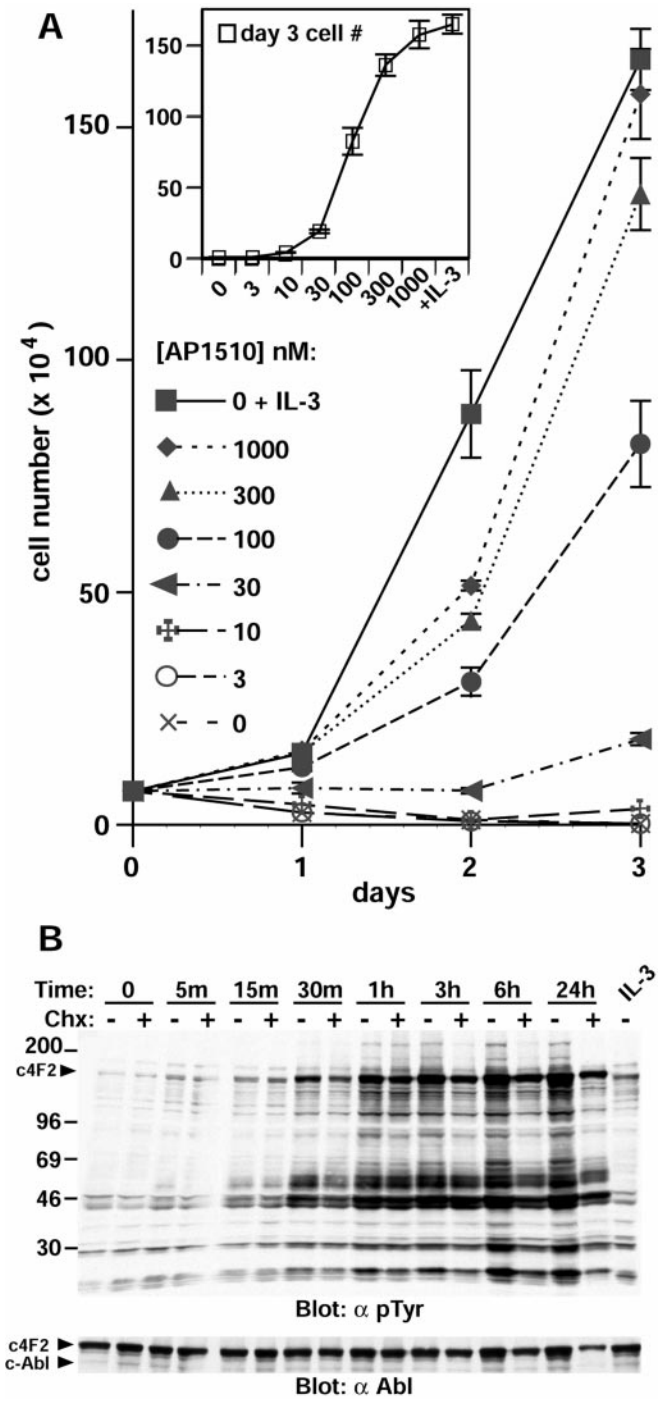

FIG. 6. **Ba/F3 cells expressing the c4F2 chimera exhibit ligand-dependent activation of kinase activity and proliferation.** *Panel A*, Ba/F3 cell populations expressing the c4F2 chimera were generated by retroviral transduction with a c4F2-MINVneo retrovirus and selection for G418 resistance. Cells were then grown in the absence of IL-3 and presence of the indicated concentration of AP1510, and viable cell counts of triplicate wells were determined daily for 3 days. The mean day 3 cell number for the different concentrations of AP1510 (in nM) is shown in the *inset*. *Bars* indicate S.D. values. *Panel B*, anti-phosphotyrosine (*top section*) and anti-Abl (*bottom section*) immunoblots of lysates from c4F2-expressing Ba/F3 cells grown in IL-3 (*far right lane*) or starved of IL-3 and AP1510, followed by stimulation with 0.5 μM AP1510 at time 0. Duplicate cultures (+) had 20 μg/ml cycloheximide added to prevent new protein synthesis. The positions of the c4F2 chimera and endogenous c-Abl are indicated by the *arrowheads* on the *left*.

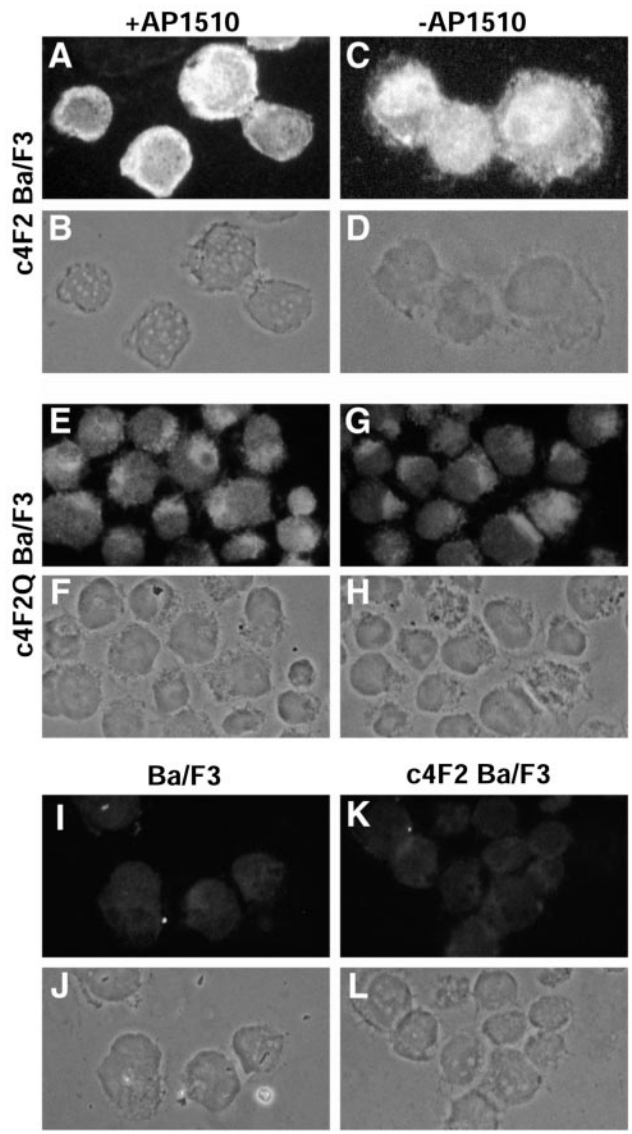

FIG. 7. **Ligand-dependent activation of the Abl-FKBP chimera relocates the protein from predominantly nuclear to cytoplasmic.** *Panels A* and *C*, anti-Abl staining of c4F2-expressing Ba/F3 cells grown in the presence of AP1510 (*A*) or grown in IL-3 without AP1510 (*C*). *Panels B* and *D*, phase-contrast images of the cells in *panels A* and *C*, demonstrating the location of cell nuclei. *Panels E* and *G*, anti-Abl staining of c4F2 $1^{Q}2^{Q}3^{Q}$-expressing Ba/F3 cells in the presence (*E*) or absence (*G*) of AP1510. *Panels F* and *H*, phase-contrast images of the cells in *E* and *G*. *Panel I*, anti-Abl staining of parental Ba/F3 cells, with corresponding phase-contrast image (*panel J*). *Panel K*, c4F2-expressing Ba/F3 cells stained with rhodamine-conjugated secondary antibody alone and phase-contrast image (*panel L*).

chimera was abolished completely by the Y412F mutation. This strongly suggests that the principal effect of dimerization of the Abl-FKBP chimera is to induce intermolecular phosphorylation of Tyr-412. This stimulates the catalytic activity of Abl up to 9-fold and rapidly induces secondary phosphorylation of Tyr-245 in the SH2 kinase domain linker region which may functionally disrupt SH3 binding (23). It would be of interest to assess the effect of a Y245F mutation on the ligand-dependent transformation by c4F2. The dysregulation of Abl-FKBP by dimerization and subsequent autophosphorylation are analogous to activation of receptor tyrosine kinases by ligand-induced dimerization (47, 48). These results also raise the question of whether oligomerization might be a physiological mechanism of c-Abl activation.

Withdrawal of dimerizing ligand from Abl-FKBP-expressing Ba/F3 cells and NIH 3T3 cells had dramatically different consequences. c4F2-expressing Ba/F3 cells lost detectable phosphotyrosine and reverted to IL-3 dependence for survival and growth, whereas c4F2-transformed 3T3 cells morphologically

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014

reverted but arrested in G_1 with significant residual levels of intracellular phosphotyrosine. These distinct responses likely reflect the different levels of Abl-FKBP expression in the two cell types. Previous studies have shown that transformation of fibroblasts by oncogenic tyrosine kinases requires a high threshold level of expression of the kinase (49), whereas alleviation of IL-3 dependence in Ba/F3 cells can occur at lower levels of activated Abl expression (50). In selecting initially for anchorage-independent growth in the presence of ligand, we isolated fibroblasts that expressed much higher levels of Abl-FKBP protein than seen in IL-3-independent Ba/F3 cells. Consistent with this, expression of c4F2 in fibroblasts at levels similar to endogenous c-Abl does not cause prominent growth inhibition in the absence of ligand.[2] The cytostatic effect of the Abl-FKBP chimera when expressed at high levels in fibroblasts without ligand is reminiscent of the growth arrest induced by transfection of fibroblasts with c-Abl, which requires nuclear localization and the p53 and Rb tumor suppressor proteins (12). However, the lack of requirement for nuclear localization for growth arrest by Abl-FKBP and the minimal apoptosis observed suggest that a distinct mechanism is involved. The Abl-ER fusion (33) and v-Abl (12, 51) proteins are cytoplasmically localized and also have prominent cytostatic effects, but the presence of potentially functional nuclear localization signals in these proteins (52, 53) made it impossible to determine if growth inhibition was independent of nuclear localization. The growth inhibition requires Abl kinase activity in all cases, but the mechanism is otherwise poorly understood. A plausible explanation is that the high level of expression of Abl required for transformation of fibroblasts leads to persistent dysregulation of Abl-FKBP kinase activity upon withdrawal of ligand, as evidenced by the increased intracellular phosphotyrosine despite monomeric Abl-FKBP protein. This may be caused in part by impaired suppression of Abl kinase activity by a cellular inhibitor protein such as Pag/MSP23 (6) or Rb (4).

The system we have described here allows, for the first time, the controlled activation of Abl kinase activity *in vivo* without subjecting cells to stimuli (such as integrin engagement or ionizing radiation) that have many pleiotropic effects on cell signaling, survival, and growth which are independent of Abl activation. The Abl-FKBP protein is correctly localized to the nucleus in the absence of ligand and responds to cellular transformation by moving to the cytoplasm, in contrast to the Abl-ER and Epo-R-Abl proteins, which are constitutively localized to the cytoplasm and plasma membrane, respectively. This feature makes the Abl-FKBP protein a valuable tool for investigating the primary cellular responses to and substrates of c-Abl. The Abl-FKBP protein could also be targeted to different locations in the cell by appropriate mutations in nuclear import/export signals, actin or DNA binding domains, and the physiological consequences of Abl activation assessed. The Abl-FKBP chimera may have applications in transgenic mice, where conditional mutants that rely on hormone treatment or temperature shifts are not feasible. The strategy employed here could also be extended to generate conditional mutants of the Bcr-Abl and Tel-Abl fusion proteins which may be useful for analyzing signaling pathways in leukemogenesis.

Acknowledgments—We thank Drs. Jane Amara and Victor Rivera (ARIAD Pharmaceuticals) for the pMF1E and pMF2E plasmids and the generous gift of AP1510 dimerizer compound, Dr. Peter Jackson (Stanford University) for critically reading the manuscript, and the members of the Van Etten laboratory for many helpful suggestions.

REFERENCES

1. Van Etten, R. A., Jackson, P., and Baltimore, D. (1989) *Cell* **58,** 669–678
2. McWhirter, J. R., and Wang, J. Y. J. (1993) *EMBO J.* **12,** 1533–1546
3. Van Etten, R. A., Jackson, P. K., Baltimore, D., Sanders, M. C., Matsudaira, P. T., and Janmey, P. A. (1994) *J. Cell Biol.* **124,** 325–340
4. Welch, P. J., and Wang, J. Y. J. (1993) *Cell* **75,** 779–790
5. Plattner, R., Kadlec, L., DeMali, K. A., Kazlauskas, A., and Pendergast, A. M. (1999) *Genes Dev.* **13,** 2400–2411
6. Wen, S.-T., and Van Etten, R. A. (1997) *Genes Dev.* **11,** 2456–2467
7. Sun, X., Majumder, P., Shioya, H., Wu, F., Kumar, R., Weichselbaum, R., Kharbanda, S., and Kufe, D. (2000) *J. Biol. Chem.* **275,** 17237–17240
8. Lewis, J. M., Baskaran, R., Taagepera, S., Schwartz, M. A., and Wang, J. Y. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 15174–15179
9. Baskaran, R., Dahmus, M. E., and Wang, J. Y. J. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 11167–11171
10. Kharbanda, S., Pandey, P., Ren, R., Mayer, B., Zon, L., and Kufe, D. (1995) *J. Biol. Chem.* **270,** 30278–30281
11. Sawyers, C. L., McLaughlin, J. L., Goga, A., Havlik, M., and Witte, O. (1994) *Cell* **77,** 121–131
12. Wen, S.-T., Jackson, P. K., and Van Etten, R. A. (1996) *EMBO J.* **15,** 1583–1595
13. Yuan, Z.-M., Huang, Y., Ishiko, T., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 1437–1440
14. Gong, J., Costanzo, A., Yang, H. Q., Meliino, G., Kaelin, W. G., Levrero, M., and Wang, J. Y. J. (1999) *Nature* **399,** 806–809
15. Van Etten, R. A. (1999) *Trends Cell Biol.* **9,** 179–186
16. Xu, W., Harrison, S. C., and Eck, M. J. (1997) *Nature* **385,** 595–602
17. Sicheri, F., Moarefi, I., and Kuriyan, J. (1997) *Nature* **385,** 602–609
18. Piwinica-Worms, H., Saunders, K., Roberts, T., Smith, A., and Cheng, S. (1987) *Cell* **49,** 75–82
19. Hirai, H., and Varmus, H. E. (1990) *Mol. Cell. Biol.* **10,** 1307–1318
20. Seidel-Dugan, C., Meyer, B. E., Thomas, S. M., and Brugge, J. S. (1992) *Mol. Cell. Biol.* **12,** 1835–1845
21. Jackson, P., and Baltimore, D. (1989) *EMBO J.* **8,** 449–456
22. Mayer, B. J., Jackson, P. K., Van Etten, R. A., and Baltimore, D. (1992) *Mol. Cell. Biol.* **12,** 609–618
23. Brasher, B. B., and Van Etten, R. A. (2000) *J. Biol. Chem.* **275,** 35631–35637
24. Van Etten, R. A., Debnath, J., Zhou, H., and Casasnovas, J. M. (1995) *Oncogene* **10,** 1977–1988
25. Barila, D., and Superti-Furga, G. (1998) *Nat. Genet.* **18,** 280–282
26. Baskaran, R., Wood, L. D., Whitaker, L. L., Canman, C. E., Morgan, S. E., Xu, Y., Barlow, C., Baltimore, D., Wynshaw-Boris, A., Kastan, M. B., and Wang, J. Y. J. (1997) *Nature* **387,** 516–519
27. Pendergast, A. M., Muller, A. J., Havlik, M. H., Clark, R., McCormick, F., and Witte, O. N. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5927–5931
28. Diekmann, D., Brill, S., Garrett, M. D., Totty, N., Hsuan, J., Monfries, C., Hall, C., Lim, L., and Hall, A. (1991) *Nature* **351,** 400–402
29. Golub, T. R., Goga, A., Barker, G. F., Afar, D. E. H., McLaughlin, J., Bohlander, S. K., Rowley, J. D., Witte, O. N., and Gilliland, D. G. (1996) *Mol. Cell. Biol.* **16,** 4107–4116
30. McWhirter, J. R., Galasso, D. L., and Wang, J. Y. J. (1993) *Mol. Cell. Biol.* **13,** 7587–7595
31. Muller, A. J., Young, J. C., Pendergast, A., Pondel, M., Landau, N. R., Littman, D. R., and Witte, O. N. (1991) *Mol. Cell. Biol.* **11,** 1785–1792
32. Jackson, P., Baltimore, D., and Picard, D. (1993) *EMBO J.* **12,** 2809–2819
33. Mattioni, T., Jackson, P. K., Bchini-Hooft van Huijsduinnen, O., and Picard, D. (1995) *Oncogene* **10,** 1325–1333
34. Picard, D., Salser, S. J., and Yamamoto, K. R. (1988) *Cell* **54,** 1073–1080
35. Schwartzberg, P. L., Stall, A. M., Hardin, J. D., Bowdish, K. S., Humaran, T., Boast, S., Harbison, M. L., Robertson, E. J., and Goff, S. P. (1991) *Cell* **65,** 1165–1176
36. Spencer, D. M., Wandless, T. J., Schreiber, S. L., and Crabtree, G. R. (1993) *Science* **262,** 1019–1024
37. Belshaw, P. J., Ho, S. N., Crabtree, G. R., and Schreiber, S. L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 4604–4607
38. Hawley, R. G., Lieu, F. H. L., Fong, A. Z. C., Goldman, S. J., Leonard, J. P., and Hawley, T. S. (1996) *Ann. N. Y. Acad. Sci.* **795,** 341–345
39. DuBridge, R. B., Tang, P., Hsia, H. C., Leong, P. M., Miller, J. H., and Calos, M. P. (1987) *Mol. Cell. Biol.* **7,** 379–387
40. Okuda, K., D'Andrea, A., Van Etten, R. A., and Griffin, J. D. (1997) *J. Clin. Invest.* **100,** 1708–1715
41. Spencer, D. M., Graef, I., Austin, D. J., Schreiber, S. L., and Crabtree, G. L. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9805–9809
42. Li, W., Kloetzer, W. S., and Arlinghaus, R. B. (1988) *Oncogene* **2,** 559–566
43. Jousset, C., Carron, C., Boureux, A., Quang, C. T., Oury, C., Dusanter-Fourt, I., Charon, M., Levin, J., Bernard, O., and Ghysdael, J. (1997) *EMBO J.* **16,** 69–82
44. Thanos, C. D., Goodwill, K. E., and Bowie, J. U. (1999) *Science* **283,** 833–836
45. Brasher, B. B., Roumiantsev, S., and Van Etten, R. A. (2001) *Oncogene*, in press
46. Jackson, P. K., Paskind, M., and Baltimore, D. (1993) *Oncogene* **8,** 1943–1956
47. Heldin, C.-H., Ernlund, A., Rorsman, C., and Ronnstrand, L. (1989) *J. Biol. Chem.* **264,** 8905–8912
48. Ullrich, A., and Schlessinger, J. (1990) *Cell* **61,** 203–212
49. Jakobovits, E. B., Majors, J. E., and Varmus, H. E. (1984) *Cell* **38,** 757–765
50. Klucher, K. M., Lopez, D. V., and Daley, G. Q. (1998) *Blood* **91,** 3927–3934
51. Chen, Y., Knudsen, E. S., and Wang, J. Y. J. (1996) *J. Biol. Chem.* **271,** 19637–19640
52. Picard, D., and Yamamoto, K. R. (1987) *EMBO J.* **6,** 3333–3340
53. Birchenall-Roberts, M. C., Ruscetti, F. W., Kasper, J. J., Bertolette, D. C., III, Yoo, Y. D., Bang, O.-K., Roberts, M. S., Turley, J. M., Ferris, D. K., and Kim, S.-J. (1995) *Mol. Cell. Biol.* **15,** 6088–6099

[2] K. M. Smith and R. A. Van Etten, unpublished data.

Downloaded from http://www.jbc.org/ at Univ of California - Irvine on December 17, 2014