

Deregulation of Proteasome Function Induces Abl-mediated Cell Death by Uncoupling p130^{CAS} and c-CrkII*

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Cell migration and survival are coordinately regulated through activation of c-Abl (Abl) family tyrosine kinases. Activated Abl phosphorylates tyrosine 221 of c-CrkII (Crk; Crk-Y221-P), which prevents Crk from binding to the docking protein p130^{CAS} (CAS). Disruption of CAS-Crk binding blocks downstream effectors of the actin cytoskeleton and focal adhesion assembly, inhibits cell migration, and disrupts survival signals leading to apoptosis. Here we show that inhibition of the 26 S proteasome and ubiquitination facilitates Abl-mediated Crk-Y221-P, leading to disassembly of CAS-Crk complexes in cells. Surprisingly, inhibition of these molecular interactions does not perturb cell migration but rather specifically induces apoptosis. Furthermore, we demonstrate that attachment to an extracellular matrix plays a key role in regulating the apoptotic machinery through caspase-mediated cleavage of Abl and Crk-Y221-P. Our findings indicate that regulated protein degradation by the proteasome specifically controls cell death through regulation of Abl-mediated Crk Tyr²²¹ phosphorylation and assembly of the CAS-Crk signaling scaffold.

The ubiquitin/proteasome pathway is the primary system for extralysosomal protein degradation. The 26 S proteasome is a large 2.4-MDa multicatalytic protease unit present in the nucleus and cytoplasm of all eukaryotic cells (1, 2). It consists of a 20 S core catalytic complex arranged as a cylinder capped at both ends by the 19 S regulatory subunits, which provide specificity to proteasome function. Ubiquitin is an abundant 76-amino acid polypeptide that is covalently attached to a target protein at lysine residues through a sequence of enzymatic reactions involving a ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, and ubiquitin ligase. Proteins targeted for degradation are polyubiquitinated by the addition of multiple ubiquitin molecules to lysine residues in the previous ubiquitin tag. The proteolytic fate of a given protein is a complex process that is tightly regulated by an intricate balance of ubiquitin ligases and deubiquitinases that control tagging of proteins with ubiquitin and targeting to the 26 S proteasome.

Regulated protein degradation through the ubiquitin system is necessary for maintaining normal cellular function. This regulation includes eliminating aberrant proteins as well maintaining the proper levels of transcriptional and cell cycle regulatory proteins, activated kinases, and signal

transduction molecules. Indeed, numerous important cellular proteins involved in cell proliferation, differentiation, and apoptosis have been found to undergo ubiquitination and processing by the 26 S proteasome, including p53, Bcl-2, cyclins A, D, and E, and β -catenin.

In recent years, protein ubiquitination and degradation by the 26 S proteasome has gained appreciation not only as a physiological regulator of normal cell function but also as a potential antineoplastic target of cancer, inflammation, and neurodegenerative diseases (3). The proteasome inhibitors lactacystin, MG132, and bortezomib have been shown to have efficacy against various human tumor cells, and clinical trials are in progress with these agents or derivatives of these compounds (3). Although the anti-cancer effects are not fully elucidated, they typically involve growth arrest of cells and induction of apoptosis due to cellular stress. Cancerous cells are more susceptible than normal cells to these inhibitors because of the burden placed on the proteasome due to the increased metabolic rate of proliferating tumor cells (3). It has also been shown that deregulated proteolysis of several tumor suppressors and oncoproteins, such as p53 and BCR-Abl, is associated with neoplastic proliferation, tumor progression, and poor patient prognosis. Although the ubiquitin/proteasome pathway has been linked to key cellular functions, the mechanisms that regulate degradation of targeted proteins in normal and transformed cells are largely unknown. Numerous ubiquitinated proteins remain to be deciphered and assigned a cellular function, including cell-adhesive and cytoskeletal regulatory proteins that contribute to malignancy and cancer progression.

We have previously shown that the molecular coupling of the adaptor protein c-CrkII (Crk)⁴ to the docking protein p130^{CAS} (Crk-associated substrate (CAS)) serves as a switch that induces cell migration and suppresses apoptosis in invasive cells (4–6). The formation of this molecular scaffold operates downstream of integrin and growth factor receptors in numerous cell types *in vitro* and *in vivo* (4, 7). Recently, Abl and its related partner Arg (Abl-related gene product) have emerged as key negative regulators of CAS-Crk coupling in migratory and apoptotic cells (5). When Abl phosphorylates Crk on tyrosine 221, it induces an intramolecular folding that binds the Crk Src homology 2 domain to the phosphorylated Tyr²²¹ in its C-terminal region. This intramolecular binding prevents the binding of Crk to phosphotyrosine residues present in the substrate domain of CAS, leading to decreased cell movement and induction of cell death (5, 7–10). Here we investigate the role of ubiquitination and protein degradation in regulating the Abl/CAS/Crk pathway that controls cell migration and survival. Deregulation of the

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⁴ The abbreviations and trivial name used are: Crk, cCrkII; Abl, c-Abl (Abelson family tyrosine kinase); Abl-TM, Abl triple mutant; Abl-WT, Abl wild type; CAS, p130^{CAS} (Crk-associated substrate); Crk-Y221-P, Crk phosphorylated at tyrosine 221; MEF, mouse embryonic fibroblast; PARP, poly(ADP-ribose)polymerase; TSA, trichostatin A; FAK, focal adhesion kinase; X-gal, 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside; PDGF, platelet-derived growth factor; ECM, extracellular matrix; MG132, benzoyloxycarbonyl-LLL.

proteasome promotes Abl-mediated phosphorylation of Crk, disassembly of CAS-Crk complexes, and cell death.

MATERIALS AND METHODS

Reagents—Expression vectors for ubiquitin were provided by Dr. Ron Kopito (Stanford University, Stanford, CA). Crk plasmids have been described previously (6). HA-tagged Abl was constructed as previously reported (5). Abl wild type and Abl-TM were constructed as previously described (11). Me₂SO, L- α -lysophosphatidic acid, and trichostatin A (TSA) were purchased from Sigma. Lactacystin, benzyloxycarbonyl-LLL (MG132), *cis*-diamminedichloroplatinum, and caspase inhibitor I (benzyloxycarbonyl-VAD-fluoromethylketone) were provided by Calbiochem. Human fibronectin, antibodies to CAS (for immunoblot), Crk, and paxillin were from BD Biosciences. PDGF-BB was purchased from R&D Systems Inc. STI-571 (Gleevec) was obtained from Novartis (La Jolla, CA). Antibodies to FAK, CAS (for immunoprecipitation), and c-Myc (9E10) were purchased from Santa Cruz Biotechnologies. Phosphospecific antibodies to paxillin phosphorylated at Tyr³¹ and FAK phosphorylated at Tyr³⁹⁷ were obtained from BIOSOURCE. Antibodies to Crk-Y221-P and cleaved PARP (Asp²¹⁴) were purchased from Cell Signaling. Ubiquitin antibody was from Chemicon. HA-tagged antibody was from Roche Applied Science, and Abl antibody was from Sigma.

Cell Culture, Transfection, Immunoprecipitation, and Western Blotting—NIH 3T3 fibroblasts were kindly provided by Dr. Tony Hunter (The Salk Institute, La Jolla, CA). COS-7 cells were from the American Tissue Culture Collection (Manassas, VA). All cell lines used were maintained in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal bovine serum (Gemini Bio-products), 200 mM L-glutamine, 50 μ g/ml gentamycin (Sigma), and 100 mM sodium pyruvate (Sigma). Suspension assays were performed using ultralow attachment 10-cm dishes from Corning, or a thin layer of agar was applied to prevent cell attachment. Cells were incubated at 37 °C with 5% CO₂. For transfection experiments, 100-mm dishes of cells 60–80% confluent were transfected using Lipofectamine (Invitrogen) according to the manufacturer's protocol. Briefly, 20 μ l of Lipofectamine was preincubated with 3.5 μ g of the appropriate plasmid DNA in 1 ml of transfection medium for 30 min. The volume was brought to 6 ml and layered over cells for 6–8 h at 37 °C. Cells were used for the appropriate assays within 48 h subsequent to serum starvation overnight. Immunoprecipitations and Western blots were performed as previously described (10).

Reconstitution of Abl Null Cells—c-Abl expression in Abl null cells (A. E. Kolesky, Yale University) was induced through retroviral mediated gene transfer using Bosc cell-generated retrovirus as previously described (5). To generate cells expressing Abl-WT and Abl-TM, Abl null cells were infected with retroviral vectors using the LIN XE packaging cell line (Dr. Peiqing Sun, The Scripps Research Institute) according to the manufacturer's instructions.

Cell Adhesion and Migration Assays—Migration and adhesion assays were performed as described previously (10). Briefly, Boyden chambers containing polycarbonate membranes (tissue culture-treated, 6.5-mm diameter, 10- μ m thickness, 8- μ m pores, Transwell®; Costar Corp. (Cambridge, MA) or Chemicon Inc.) were coated on the bottom (haptotaxis) with human fibronectin for 2 h at 37 °C. Serum-starved cells were allowed to migrate 3–5 h and then stained with crystal violet (Sigma). In some cases, migratory cells transfected with Crk constructs were co-transfected along with the reporter vector pCMV SPORT β -galactosidase (Invitrogen). Developing was completed using X-gal (Promega) as a substrate according to the manufacturer's recommendation and as previously described (10). For cell adhesion assays, an aliquot of cells used in the migration experiments were allowed to attach

for the indicated times to fibronectin (10 mg/ml)-coated cell culture wells at 37 °C and stained with crystal violet or X-gal reagent. Following migration, cells were fixed and stained with crystal violet, or transfected cells were fixed in β -galactosidase fixative and stained using X-gal according to the manufacturer's recommendations. To assess cellular adhesion/migration, the cells were directly counted or the stain eluted with 10% acetic acid and measured in an ELISA plate reader (A₅₆₀).

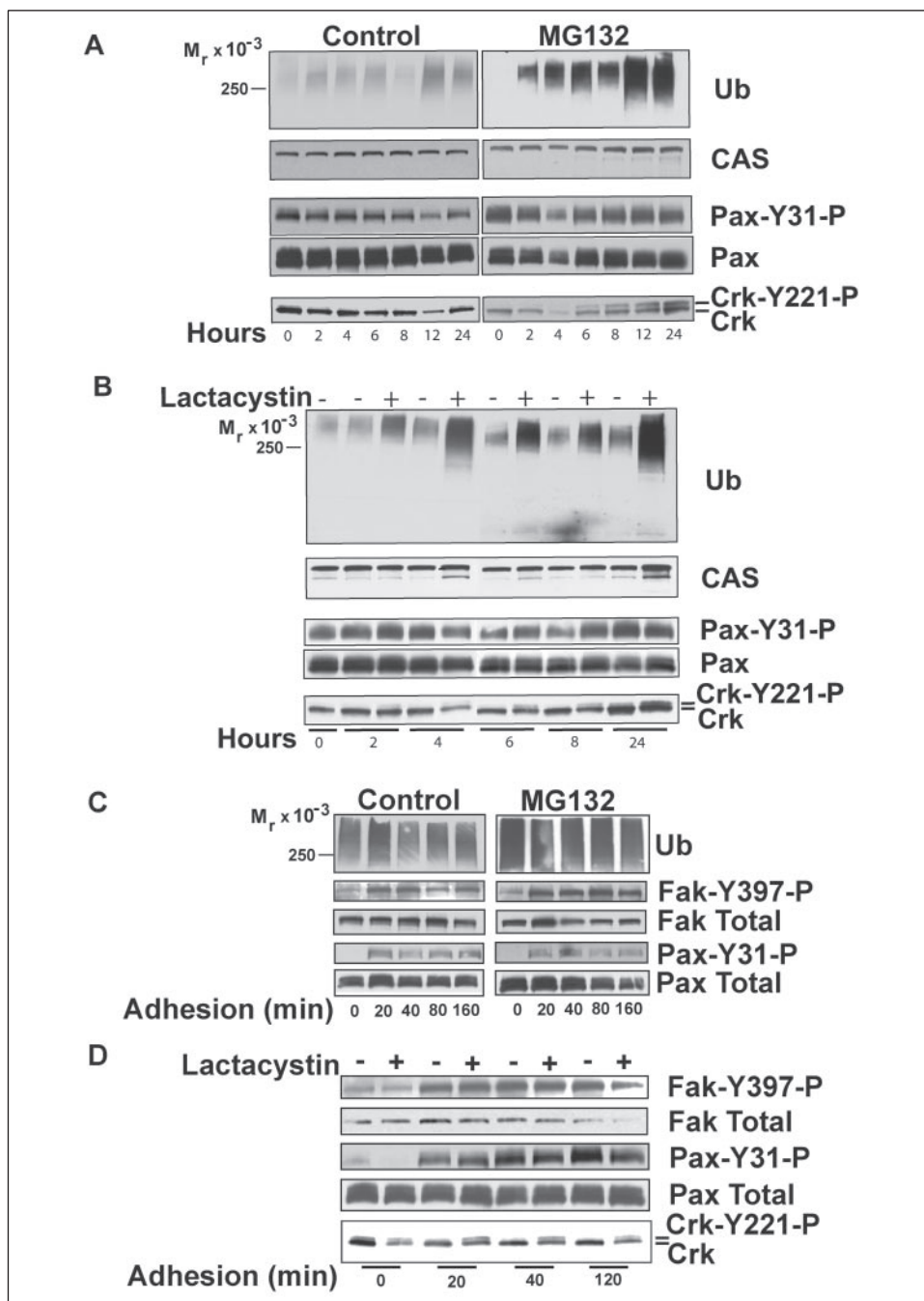
Apoptosis Assays—Cell apoptosis was determined by evaluating nuclear morphology and cell fragmentation as previously described with minor modifications (5). Briefly, cells were serum-starved overnight and then treated with the appropriate apoptotic agent or the Abl tyrosine kinase inhibitor STI-571, as indicated in the figure legend (Fig. 7), either on culture dishes coated with 10 μ g/ml fibronectin or culture dishes coated with a thin layer of agar to prevent cell adhesion (suspension conditions). Cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences), permeabilized with 10% SDS, and stained with propidium iodide (Sigma) to visualize nuclear morphology. The number of cells with condensed chromatin or fragmented nuclei were scored per microscopic field using a Leica DMIRE 2 microscope and SimplePCI Software program version 5.2 from Compix Inc. Imaging Systems. Percentage of apoptosis was determined by counting the number of apoptotic nuclei per field of at least five fields. All of the *graphs* (Figs. 4, 5, and 7) show the mean results of three experiments. In some cases, an aliquot of cells were lysed and subjected to Western blot for the cleaved, activated form of PARP (85 kDa) or stained with anti-terminal deoxynucleotidyl transferase-mediated 2'-deoxyuridine 5'-triphosphate nick end labeling antibodies (Roche Applied Science) and examined by fluorescence-activated cell sorting as an indicator of apoptosis.

RESULTS

Inhibition of Protein Degradation by the 26 S Proteasome Induces Crk-Y221-P and Disassembly of CAS-Crk Complexes—To determine whether Crk-Y221-P is regulated by ubiquitination and protein degradation, cells were treated for various times with MG132 or lactacystin, which block 26 S proteasome function through distinct mechanisms. Lactacystin is a natural, microbial product that acts as a pseudosubstrate that becomes covalently attached to hydroxyl groups on the active site threonine of β subunits (12). MG132 is a highly potent substrate analogue that inhibits the chymotryptic activity of the 26 S proteasome (13). As expected, both compounds inhibited proteasome function as indicated by the accumulation of high molecular weight, polyubiquitinated proteins in a time-dependent manner (Fig. 1, A and B). Importantly, cells treated with these inhibitors show a strong increase in the level of HSP70 (14). This specialized protein can easily be visualized using Ponceau stain prior to Western blotting of whole cell lysates (data not shown). Also associated with the inhibition of the 26 S proteasome was a reduction in Crk mobility in SDS-PAGE, which is due to phosphorylation of Crk at tyrosine 221, as previously reported (5, 9, 15). In cultured cells treated with proteasome inhibitors, Crk phosphorylation was first evident 4–6 h after treatment and appeared to maximize between 8 and 12 h (Fig. 1, A and B). There were no notable changes in the steady state levels of Crk, and no high molecular weight forms indicative of polyubiquitination were evident. Furthermore, we did not detect changes in the phosphorylation or protein levels of FAK, paxillin, or CAS in cultured cells or cells removed from the dishes and allowed to reattach to fibronectin coated dishes for various times (Fig. 1, C and D).

It is also possible to inhibit proteasome function through overexpression of free ubiquitin in cells (16, 17). Increased availability of unincorporated ubiquitin increases the level of total ubiquitinated proteins. The increased demand of ubiquitinated proteins to be processed leads to a

FIGURE 1. Inhibition of the 26 S proteasome induces CrkY221-P but not paxillin or FAK phosphorylation. COS-7 cells in culture were treated with either 5 $\mu\text{g/ml}$ MG132 (A) or 10 μM lactacystin or Me_2SO (vehicle control) (B) for the indicated times. Cells were lysed and subjected to Western blot for ubiquitin (Ub), CAS, Crk, paxillin (Pax), or paxillin phosphorylated at tyrosine 31 (Pax-Y31-P). The upper band of Crk represents Crk phosphorylated at Tyr²²¹, which causes reduced mobility during SDS-PAGE as previously reported (5). COS-7 cells were treated as described above for 16 h with either MG132 (C), lactacystin (+) (D), or Me_2SO (-). Cells were detached from the dish and allowed to reattach to dishes coated with fibronectin for the indicated times. Cells were lysed and subjected to Western blot for phosphorylated/activated FAK (FAK-Y397-P) and paxillin phosphorylated at Tyr³¹ and then stripped and reprobed for total FAK and paxillin.



reduction in proteasome function. Transient transfection of ubiquitin led to increased accumulation of polyubiquitinated proteins and increased Crk phosphorylation (Fig. 2A). Inhibition of the 26 S proteasome did not globally alter protein tyrosine phosphorylation in cells (data not shown), nor did it alter other focal adhesion proteins, including tyrosine phosphorylation and activation of FAK and paxillin in response to cell adhesion to the ECM (Fig. 2, A and B). Importantly, the increase in Crk Tyr²²¹ phosphorylation in response to inhibition of the 26 S proteasome was associated with a 50% decrease in CAS-Crk coupling (Fig. 2C). Thus, inhibition of ubiquitin-mediated protein degradation specifically facilitates Crk-Y221-P and decreases CAS-Crk coupling. It is noteworthy that the total level of focal adhesion proteins was not significantly altered, suggesting that regulated protein degradation through the 26 S proteasome is not the major mechanism used to con-

trol steady-state levels of Crk, FAK, CAS, or paxillin (Figs. 1 and 2). However, our findings do not exclude the possibility that they are ubiquitinated/deubiquitinated under specific conditions or in other cell types.

Abl Tyrosine Kinase Is Necessary for Crk-Y221-P Induced by Inhibition of the 26 S Proteasome—Our previous findings show that Abl and Arg are the primary tyrosine kinases responsible for Crk-Y221-P in cells (5, 9). Endogenous Abl serves as a negative feedback mechanism that controls cell migration through phosphorylation of Crk Tyr²²¹, leading to inhibition of CAS-Crk coupling in cells. Abl is responsible for Crk-Y221-P in MG132- and lactacystin-treated cells, since mouse embryonic fibroblast (MEF) cells deficient in Abl and Arg (Abl null) did not show Crk-Y221-P in response to these compounds, whereas wild type MEFs or Abl-null cells reconstituted with Abl did (Fig. 3, A and B). Also, embryonic fibroblast cells isolated from mice deficient in Abl, but not

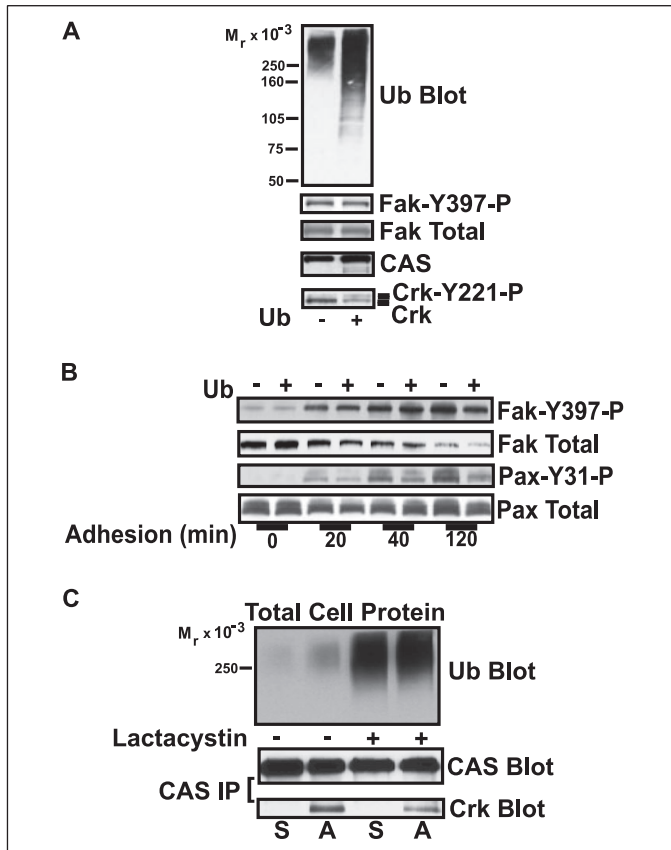


FIGURE 2. Inhibition of the 26 S proteasome reduces CAS-Crk coupling in cells upon attachment to ECM proteins. *A*, COS-7 cells transiently transfected with plasmids encoding ubiquitin (+) or the empty vector (-) were lysed and subjected to Western blot for the indicated proteins. *B*, COS-7 cells transfected as described in *A* were detached and then allowed to reattach to fibronectin-coated dishes for the indicated times. Cell lysates were then subjected to Western blot for the indicated proteins. *C*, COS-7 cells were treated with 10 μ M lactacystin (+) or Me₂SO (-) for 16 h and then detached from the plate and either allowed to reattach to fibronectin-coated dishes for 60 min (*A*) or held in suspension (*S*). Cells were lysed and then subjected to Western blot for ubiquitin (*Ub*), or CAS was immunoprecipitated (*IP*) and blotted for CAS or Crk.

Arg (*abl*^{-/-arg}+/+), showed increased Crk-Y221-P in response to treatment with lactacystin (Fig. 3C). Thus, Abl and/or Arg can facilitate Crk-Y221-P in MEF cells treated with lactacystin. Furthermore, treatment of COS-7 (data not shown), MEF, and MDA-435 cells with the Abl and Arg kinase inhibitor STI-571 prevented Crk-Y221-P induced by MG132, indicating that Abl/Arg kinase activity is necessary for this process (Fig. 7, *A* and *B*). Again, inhibition of the proteasome did not alter paxillin phosphorylation (Fig. 3A) or global phosphorylation of proteins as indicated by Western blotting of whole cell lysates with anti-phosphotyrosine antibodies (data not shown). These findings demonstrate that Abl/Arg are necessary for Crk-Y221-P in response to inhibition of the proteasome.

Inhibition of the 26 S Proteasome Does Not Affect CAS-Crk-mediated Cell Migration—We next wanted to determine whether the uncoupling of Crk from CAS in response to proteasome inhibition altered cell attachment and migration on the ECM. Surprisingly, it did not alter cell adhesion or basal haptotaxis migration of NIH 3T3 or COS-7 cells, nor did it affect haptotaxis migration induced by overexpression of Crk. We have previously shown this overexpression induces strong migration in numerous cell types (5, 9, 18) (Fig. 4, *A–C*). Overexpression of Crk also did not affect chemotaxis migration induced by PDGF-BB (Fig. 4, *E* and *F*). Similar findings were obtained with cells in which the 26 S proteasome function was inhibited by overexpression of free ubiquitin (Fig. 4,

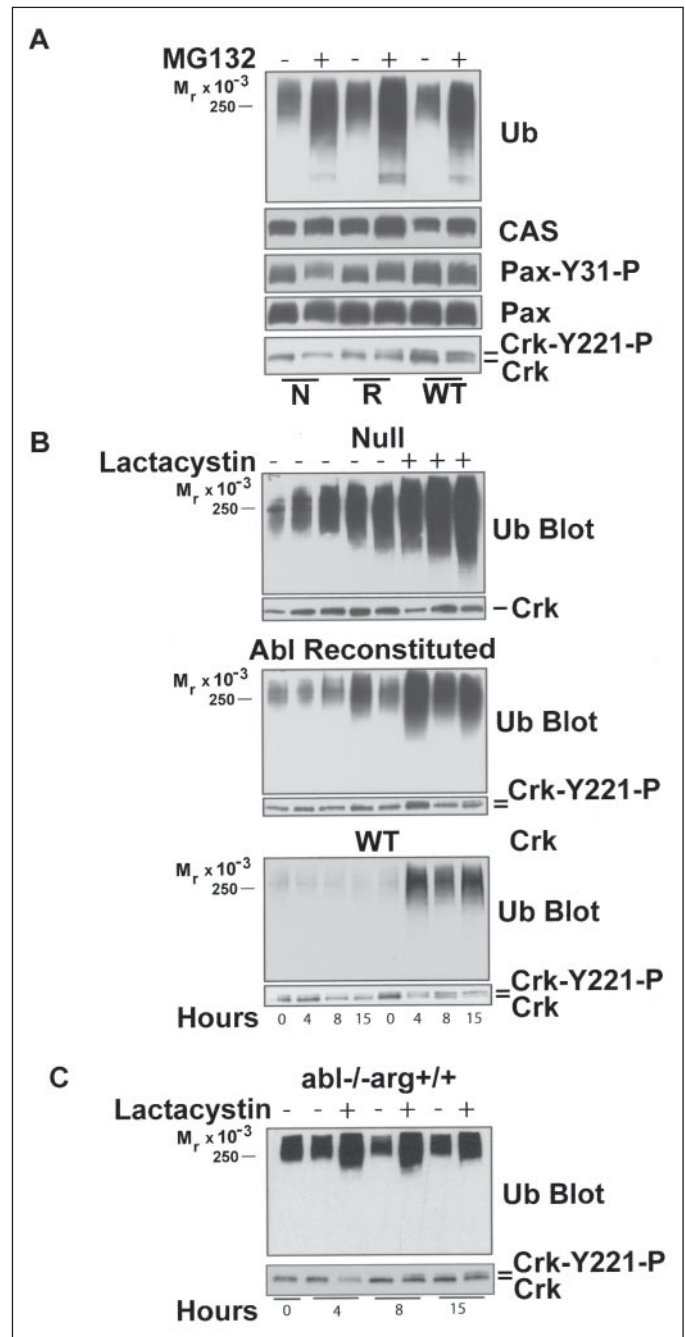


FIGURE 3. Abl and Arg tyrosine kinases are necessary for Crk-Y221-P induced by inhibition of the 26 S proteasome. Embryonic fibroblast cells were isolated from wild type mice (*WT*) or mice deficient in Abl and Arg tyrosine kinases (*Null*). Null cells were also stably reconstituted with Abl kinase (*R*) as previously reported (5). *A*, cells in culture were treated with 10 μ M MG132 (+) or Me₂SO (-) for 12 h and then lysed and subjected to Western blot for the indicated proteins. *B*, cells in culture were treated for the indicated times with 10 μ M lactacystin (+) or Me₂SO (-) and then lysed and subjected to Western blot for the indicated proteins. *C*, embryonic cells deficient in Abl kinase (*abl*^{-/-arg}+/+) were treated with 10 μ M lactacystin (+) or MeSO (-) for the indicated times and then lysed and subjected to Western blot for the indicated proteins.

G and *H*). Together, these findings indicate that perturbation of protein degradation by the proteasome does not impact cell adhesion or migration on the ECM, nor does it impact the activity of FAK and paxillin, which regulate these processes (Figs. 1 and 2).

Inhibition of the 26 S Proteasome Induces Cell Apoptosis through Abl-mediated Disruption of Crk Signaling—Although regulated protein degradation by the proteasome does not appear to be necessary for cell

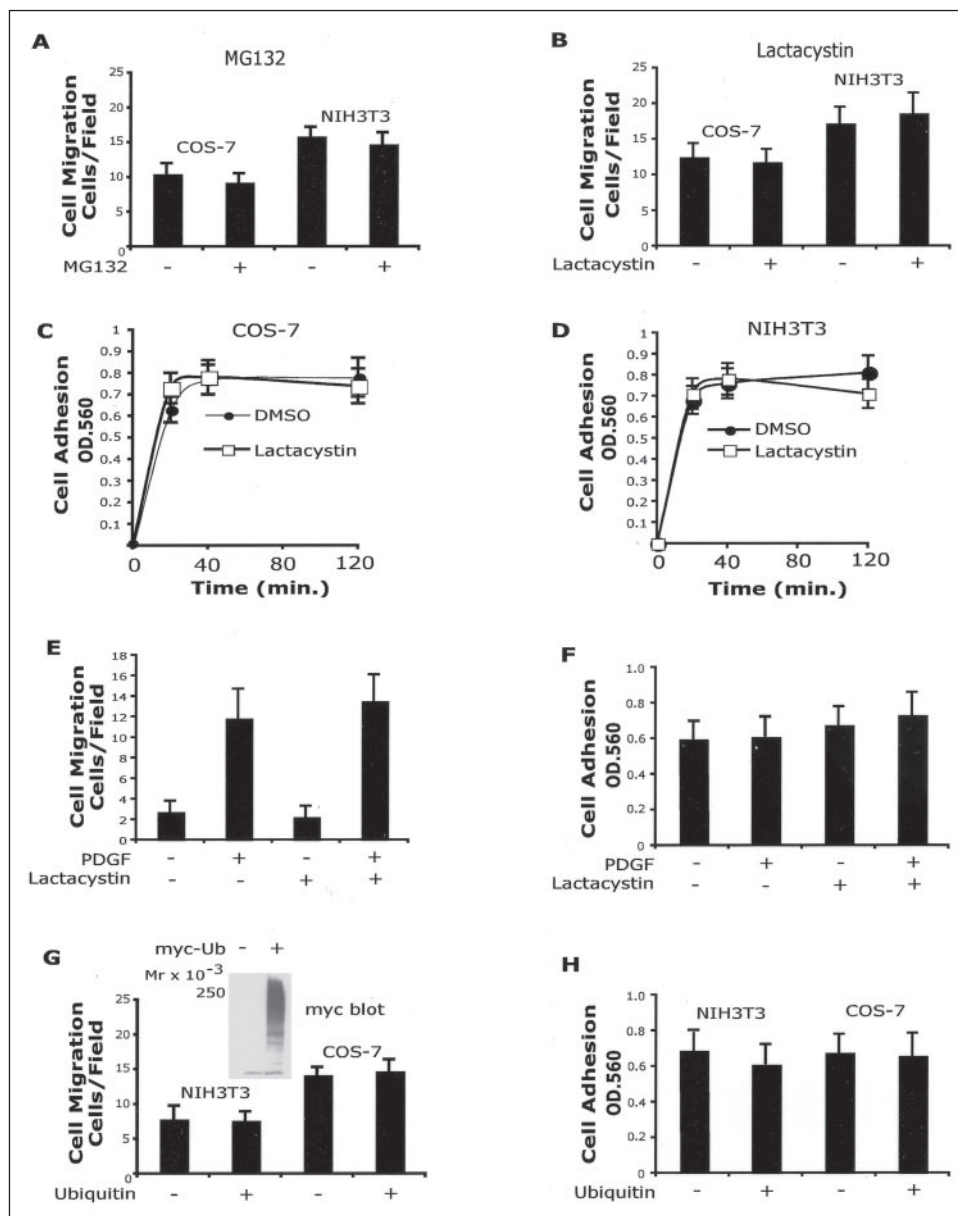


FIGURE 4. Blocking protein degradation by the 26S proteasome does not affect CAS-Crk or PDGF-BB-induced cell migration or adhesion to the ECM. COS-7 or NIH 3T3 cells in culture were treated for 6 h with either 10 μ g/ml MG132 (A) or 10 μ M lactacystin (+) or Me₂SO (DMSO) (-) (B) and then examined for their ability to migrate for 3 h in a haptotaxis assay (A and B) or attach to fibronectin-coated dishes for the indicated times in the continued presence of the compounds (C and D). E, NIH 3T3 cells were treated in culture with 10 μ M lactacystin (+) or Me₂SO (-) for 6 h then examined for chemotaxis toward 20 ng/ml PDGF-BB (+) or buffer only (-) for 3 h or allowed to attach to fibronectin-coated dishes for 60 min in the continued presence of the compound (F). Shown is haptotaxis cell migration (G) or adhesion of COS-7 cells transiently transfected with plasmids encoding Myc-tagged ubiquitin (+) or the empty vector (-) (H). Cells were allowed to migrate for 3 h or attach to fibronectin-coated dishes for 60 min. An aliquot of the cells was lysed and subjected to Western blot with anti-Myc antibodies to reveal Myc-tagged ubiquitinated proteins (*myc-Ub*) in transfected cells (G, inset). The number of transfected migratory or adherent cells was determined as described under "Materials and Methods." Each bar or point represents the mean \pm S.E. of cells in triplicate migration/adhesion chambers of three independent experiments.

attachment or migration mediated through an Abl/CAS/Crk pathway, it may play a distinct role in the regulation of survival mechanisms controlled by this pathway (5, 6). To investigate whether Abl and Crk-Y221-P are involved in apoptosis induced by inhibition of the proteasome, we examined cell death in wild type, Abl null cells, and Abl null cells reconstituted with Abl after treatment with MG132 or lactacystin for either 8 or 24 h. We did not observe any significant differences in apoptosis in cells treated for 8 h with the proteasome inhibitors (Fig. 5A). However, after 24 h, Abl null cells showed significantly less apoptosis in response to MG132 and lactacystin compared with Abl null cells reconstituted with Abl and wild type MEFs (Fig. 5A). Furthermore, cells overexpressing Crk showed decreased apoptosis in response to inhibition of the proteasome, indicating that Crk plays an important role in the death response (Fig. 5B). Our previous work demonstrates that overexpression of Crk promotes increased CAS-Crk assembly, resulting in increased cell migration and survival as well as increased cancer cell metastasis in animal models (5, 6). Interestingly, cells overexpressing Crk and treated with the DNA-damaging agent cisplatin, which activates Abl (19–21) but does not promote Crk-Y221-P, showed similar

levels of apoptosis as control cells (Fig. 5B). As expected, treatment of cells with either cisplatin or proteasome inhibitors for 8 h did not alter cell migration, since these pathways are distinct from apoptotic regulation pathways (Fig. 5C). These findings indicate that Abl and Crk contribute to apoptosis induced by inhibition of the proteasome.

Cell Adhesion to the ECM Regulates Abl-mediated Crk-Y221-P and Apoptosis in Response to Inhibition of the 26S Proteasome—The context of the ECM and formation of integrin contacts can determine whether a cell responds to a given apoptotic signal such as DNA damage (22). For example, in some cell types, detachment from the ECM promotes caspase-mediated apoptosis or anoikis death (23–26). Therefore, we next determined whether adhesion to the ECM plays a role in regulating the Abl/CAS/Crk-mediated apoptotic response to inhibition of the 26S proteasome. We first examined COS-7 cells, which undergo cell death upon detachment from the ECM. We reasoned that increased Crk-Y221-P from disassembled focal adhesions may provide proapoptotic signals. However, basal Crk-Y221-P was reduced in the apoptotic cells detached from the ECM, suggesting that Crk-Y221-P is not involved in this death response (Fig. 6A). Furthermore, exposure of cells in suspen-

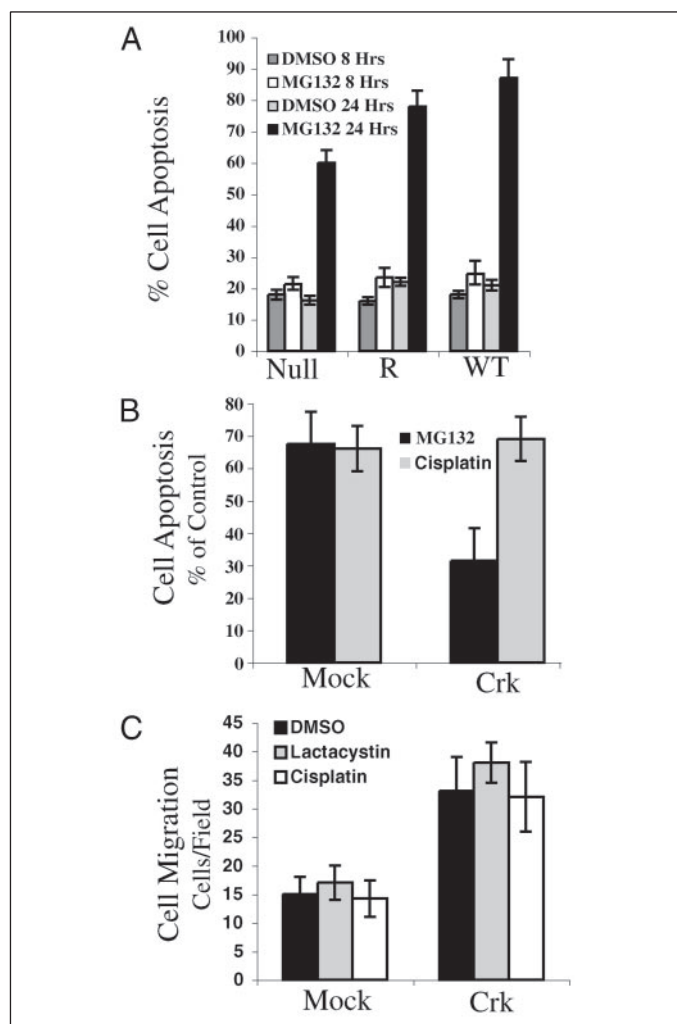


FIGURE 5. Inhibition of the 26 S proteasome induces apoptosis through Abl-mediated disruption of Crk signaling. *A*, embryonic fibroblast cells with and without Abl kinases as described in the legend to Fig. 3A were treated with 10 $\mu\text{g/ml}$ MG132 or Me_2SO (DMSO) for either 8 or 24 h. The percentage of cells with apoptotic nuclei were determined as described under "Materials and Methods." *B*, COS-7 cells were transiently transfected with plasmids encoding Crk or the empty vector (*mock*) and then 48 h later were treated with 10 $\mu\text{g/ml}$ MG132 or 25 μM cisplatin for 24 h and examined for the percentage of apoptotic cells. The number of apoptotic cells is relative to Me_2SO -treated control cells and is represented as the percentage of control cells. *C*, COS-7 cells transfected as in *B* were treated with 10 μM lactacystin, 25 μM cisplatin, or a similar amount of vehicle (Me_2SO) for 6 h and then allowed to migrate in a haptotaxis assay toward fibronectin for 3 h in the continued presence of the compounds. The number of migratory cells was determined as described under "Materials and Methods." Each bar or point represents the mean \pm S.E. of cells in triplicate migration chambers of three independent experiments.

sion to proteasome inhibitors failed to promote Crk-Y221-P, whereas adherent cells showed a significant increase in this event (Fig. 6A). The increase in Crk-Y221-P in adherent cells required Abl activity, since STI-571 prevented this response (data not shown). The increased Crk-Y221-P is specific to proteasome-mediated death, since the deacetylase inhibitor TSA did not alter Crk-Y221-P in adherent cells, although it is a potent apoptotic agent (Fig. 6A) (27).

Cell Detachment from the ECM Promotes Abl Cleavage and Dephosphorylation of Crk—The above findings suggest that Abl is not capable of coupling to Crk when cells are detached from the ECM. Remarkably, Western blot comparison of Abl protein levels in COS-7 cells revealed that detachment from the ECM promotes cleavage of full-length Abl (140 kDa) into a major 60-kDa fragment and minor fragments of 120 and 22 kDa (data not shown), which were first detected 8 h after cell

detachment (Fig. 6, A–D). Exposure of cells in suspension to the general caspase inhibitor benzylloxycarbonyl-VAD-fluoromethylketone prevented Abl and PARP cleavage and apoptosis, indicating that caspases are required for these processes (Fig. 6C). Immunoblot analysis of cells transfected with full-length HA-tagged Abl showed a similar cleavage pattern as endogenous Abl in suspension cells, confirming that Abl is cleaved *in vivo* (Fig. 6B). In contrast, Abl and PARP were not cleaved in cells that do not undergo apoptosis when detached from the ECM (MEF fibroblast, RAW macrophages, FG pancreatic carcinoma, MDA-435 and MCF-7 breast adenocarcinoma cells) (Table 1)(Fig. 6E). Thus, cell detachment from the ECM does not facilitate Abl cleavage *per se* but rather is associated with the specific activation of the apoptotic machinery in cells. Importantly, Abl cleavage is not a global response that occurs in all dying cells, since TSA-induced cell apoptosis does not involve Abl cleavage (Fig. 6A).

Recent reports indicate that Abl can be cleaved by caspases 3, 6, 7, 8, and 10 in apoptotic cells in response to tumor necrosis factor- α , DNA-damaging agents, and Fas activation (11, 28). Two caspase cleavage sites have been identified in human c-Abl type 1 *in vivo* at Asp⁵⁶⁵ and Asp⁹⁵⁸ that generate a similar profile of Abl cleavage products (120, 60, 22 kDa) as when apoptosis is induced by detachment of cells from the ECM (Fig. 6F). The first cleavage occurs at the C terminus at Asp⁹⁵⁸ and generates a 22-kDa fragment consisting of the actin binding domain and the nuclear exclusion sequence and a 120-kDa fragment. A second cleavage of the 120-kDa fragment at Asp⁵⁶⁵ generates a 60-kDa fragment that is similar to Src kinase with Src homology 2 and 3 domains as well as a functional kinase domain. Taken together with our findings, it seems that similar caspase activities are involved in Abl cleavage under different apoptotic conditions.

Apoptosis Induced by Blocking 26 S Proteasome Function Is Regulated by Distinct Signaling Mechanisms in Response to Cell Adhesion to the ECM—Apoptosis induced by proteasome inhibitors did not increase PARP cleavage in attached cells as opposed to cells detached from the ECM (Fig. 6A). Thus, detachment from the ECM involves activation of apoptosis through caspase cleavage of Abl and PARP, whereas apoptosis of adherent cells in response to inhibitors of the 26 S proteasome involve Abl phosphorylation of Crk Tyr²²¹, which is independent of caspases. Importantly, TSA potently induced both PARP cleavage and apoptosis of attached COS-7 cells, indicating that the PARP cleavage machinery is fully functional in attached cells (Fig. 6A). These findings suggest that COS-7 cells can utilize distinct mechanisms to regulate apoptosis in response to proteasome inhibition and cell/ECM interactions through modulation of Crk-Y221-P and PARP cleavage, respectively. In support of this, overexpression of HA-Abl, which becomes autoactivated in cells, induced strong Crk-Y221-P and apoptosis in attached cells without induction of PARP cleavage (Fig. 6B). Furthermore, detachment of cells from the ECM induced cleavage of HA-Abl and PARP, leading to decreased Crk-Y221-P. This supports the notion that these are distinct pathways and that cell adhesion is necessary for coupling of Abl and Crk. As expected, overexpression of Crk does not change the apoptotic response in suspension cells (data not shown), whereas it does in attached cells (Fig. 5B). However, it is notable that cells in suspension and treated with inhibitors of the 26 S proteasome show significantly increased levels of Abl cleavage products (120 and 60 kDa) as well as the appearance of several additional fragments at 55 and 85 kDa compared with control cells. These results suggest that once Abl is cleaved by caspases, the resulting fragments are regulated by ubiquitination and the 26 S proteasome. However, the accumulation of Abl fragments in these cells is not associated with a change in the level of Crk-Y221-P, indicating that they do not couple to Crk under these conditions (Fig. 6A).

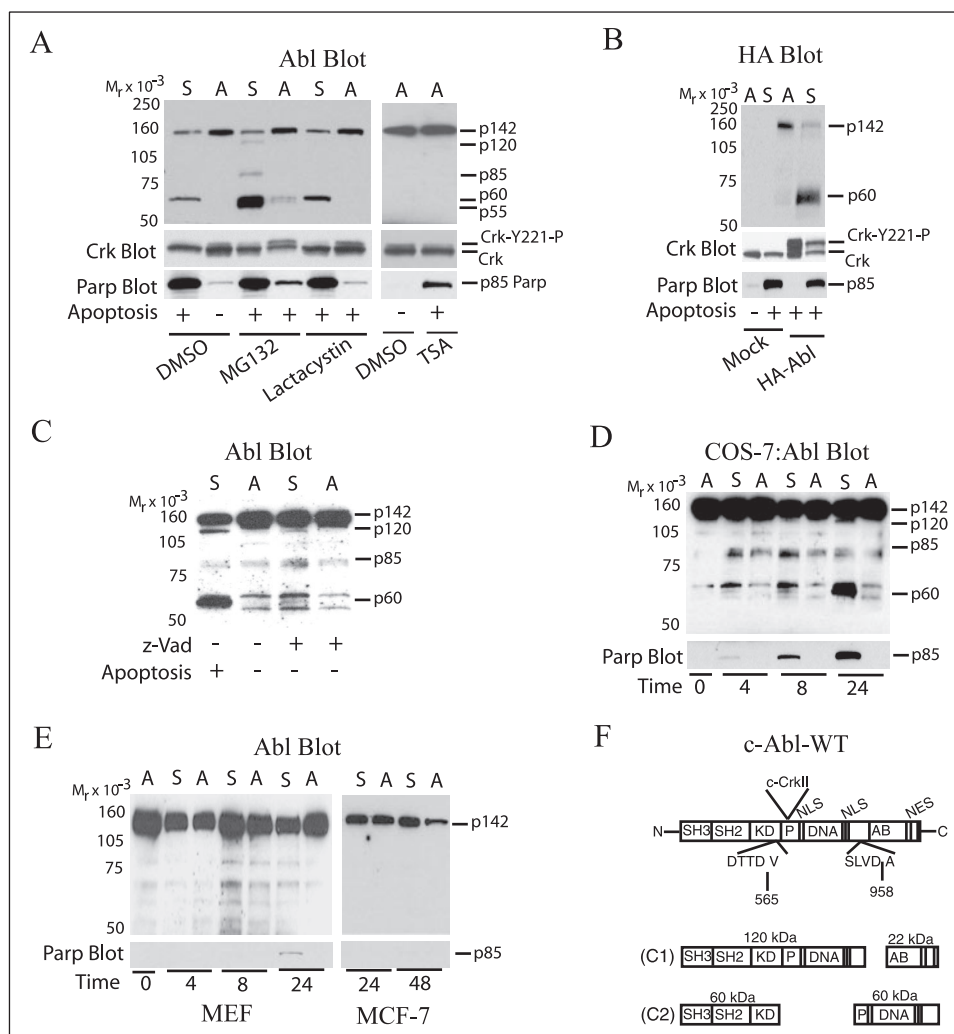


FIGURE 6. Cell adhesion to the ECM regulates Abl cleavage by caspases and Crk-Y221-P in response to inhibition of the 26 S proteasome. *A*, COS-7 cells were detached from culture dishes and then either held in suspension (S) or reattached (A) to fibronectin-coated dishes for 24 h in the presence of 10 μ g/ml MG132, 10 μ M lactacystin, 100 ng/ml trichostatin A (TSA), or a similar amount of the vehicle (Me₂SO; DMSO). Cells were lysed in detergent, and equivalent amounts of protein were subjected to Western blot for Abl kinase, Crk, and the cleaved, activated form of PARP (p85). In addition, an aliquot of cells was examined for apoptosis by determining the number of cells with apoptotic nuclei as described under "Materials and Methods." +, greater than 60% apoptotic cells. Note the reduction in the p142 form of Abl and the appearance of low molecular weight forms at p120, p85, and p60 in suspension cells only. *B*, COS-7 cells were transfected with plasmids encoding HA-tagged Abl or the empty vector (*mock*) and, after 48 h, detached from the dishes and either held in suspension or reattached to fibronectin-coated dishes for 24 h. Cells were lysed and subjected to Western blot for HA, activated PARP, and Crk or examined for cell apoptosis. Note that overexpression of Abl in cells leads to autoactivation and increased kinase activity, which promotes robust Crk-Y221-P as previously reported (5). *C*, COS-7 cells were detached from dishes and then either held in suspension or reattached to fibronectin-coated dishes in the presence of the caspase inhibitor benzyloxycarbonyl-VAD-fluoromethylketone (100 μ M) for 24 h. Cells were lysed and subjected to Western blot for Abl or examined for cell apoptosis. *D*, COS-7 cells were detached from culture dishes and either held in suspension or reattached to fibronectin-coated dishes for the indicated times. Cells were lysed and subjected to Western blot for Abl and activated PARP. *E*, MEF or MCF-7 breast adenocarcinoma cells were detached from culture dishes and either held in suspension or reattached to fibronectin-coated dishes for the indicated times and then examined for Abl expression and activated PARP as described above. *F*, schematic showing the known caspase cleavage sites (positions 565 and 928) in human Abl and the resulting protein fragments (11). *In vivo* Abl is first cleaved (C1) at site Asp⁹²⁸ to generate 120- and 22-kDa fragments. A second cleavage (C2) then occurs at Asp⁵⁶⁵ to generate two 60-kDa fragments. SH3 and SH2, Src homology 3 and 2 domain, respectively; P, polyproline domain and Crk binding region; KD, kinase domain; DNA, DNA binding domain; AB, actin binding domain. NLS, nuclear localization sequence; NES, nuclear exclusion sequence.

MG132 Induces Abl Cleavage and Crk-Y221-P in a Cell Type-specific Manner in Response to Cell Adhesion to the ECM—We next wanted to determine if inhibition of the 26 S proteasome would induce Abl cleavage and apoptosis in MDA-435 adenocarcinoma cells, which survive when detached from the ECM (Fig. 7A). Indeed, MG132 did induce apoptosis in suspension as well as attached cells (Fig. 7A). However, Abl and PARP cleavage occurred only in suspension and not in adherent cells. Therefore, cell adhesion to the ECM prevents Abl cleavage by caspases in response to inhibition of the 26 S proteasome in both COS-7 (Fig. 6A) and MDA-435 tumor cells (Fig. 7A). Thus, MG132-induced death utilizes distinct mechanisms to kill the cell, depending on its adhesion status in both COS-7 and MDA-435 cells. It is notable that, in contrast to COS-7 cells, detachment of MDA-435 cells from the ECM

does not decrease Crk-Y221-P in response to MG132 treatment, nor does it reduce the steady-state levels of full-length Abl (Fig. 7A). The inability to fully cleave the Abl pool in suspension cells may be responsible for the sustained level of Crk phosphorylation in response to inhibition of the 26 S proteasome. Indeed, inhibition of Abl kinase activity with STI-571-inhibited Crk-Y221-P in response to MG132 in suspension and attached MDA-435 cells (Fig. 7A). Furthermore, STI-571 significantly reduced the level of Abl cleavage, suggesting that Abl kinase activity is necessary for caspase cleavage of this protein. These findings demonstrate that detachment of MDA-435 cells from the ECM does not uncouple Abl from promoting Crk-Y221-P in response to inhibition of the 26 S proteasome, which is different from COS-7 cells.

TABLE 1
Cell type specificity of Abl cleavage in response to apoptotic agents in suspension and adherent cells

Listed are various cell types showing Abl cleavage/apoptosis in suspension (Sus.) and attached (Att.) cells following treatment with the proapoptotic agents TSA, MG132, and H₂O₂ for 24 h. Cleavage of full-length Abl (140 kDa) to 120- and 60-kDa fragments was determined by Western blot analysis. Cell apoptosis was determined by nuclear and cell morphology as indicated under "Materials and Methods." +, greater than 50% of the cells displaying apoptosis. COS-7, monkey epithelial; MDA-435, human adenocarcinoma; MEF, mouse embryonic fibroblast; RAW, mouse macrophage; FG, human pancreatic adenocarcinoma; MCF-7, human adenocarcinoma; SW480 human colon carcinoma; HT1080, human fibrosarcoma; NIE-115, mouse neuroblastoma.

Cell type	Me ₂ SO		MG132		Lactacystin		TSA		Peroxide	
	Sus.	Att.	Sus.	Att.	Sus.	Att.	Sus.	Att.	Sus.	Att.
COS-7	+/+	-/-	+/+	-/+	+/+	-/+	-/+	-/+	-/+	-/+
MDA-435	-/-	-/-	+/+	-/+	+/+	-/+	-/+	-/-	-/+	-/+
MEF	-/-	-/-	+/+	+/+	+/+	+/+	-/+	-/+	-/+	-/+
RAW	-/-	-/-	+/+	+/+	+/+	+/+	+/+	+/+	-/+	-/+
FG	-/-	-/-	+/+	+/+	+/+	+/+	-/+	-/+	-/-	-/-
MCF-7	-/-	-/-	-/+	-/+	-/+	-/-	-/+	-/-	-/+	-/+
SW480	-/+	-/-	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
HT1080	-/-	-/-	+/+	+/+	+/+	+/+	-/+	-/+	+/+	+/+
NIE-115	-/+	-/-	+/+	+/+	+/+	+/+	-/+	-/+	-/+	-/+

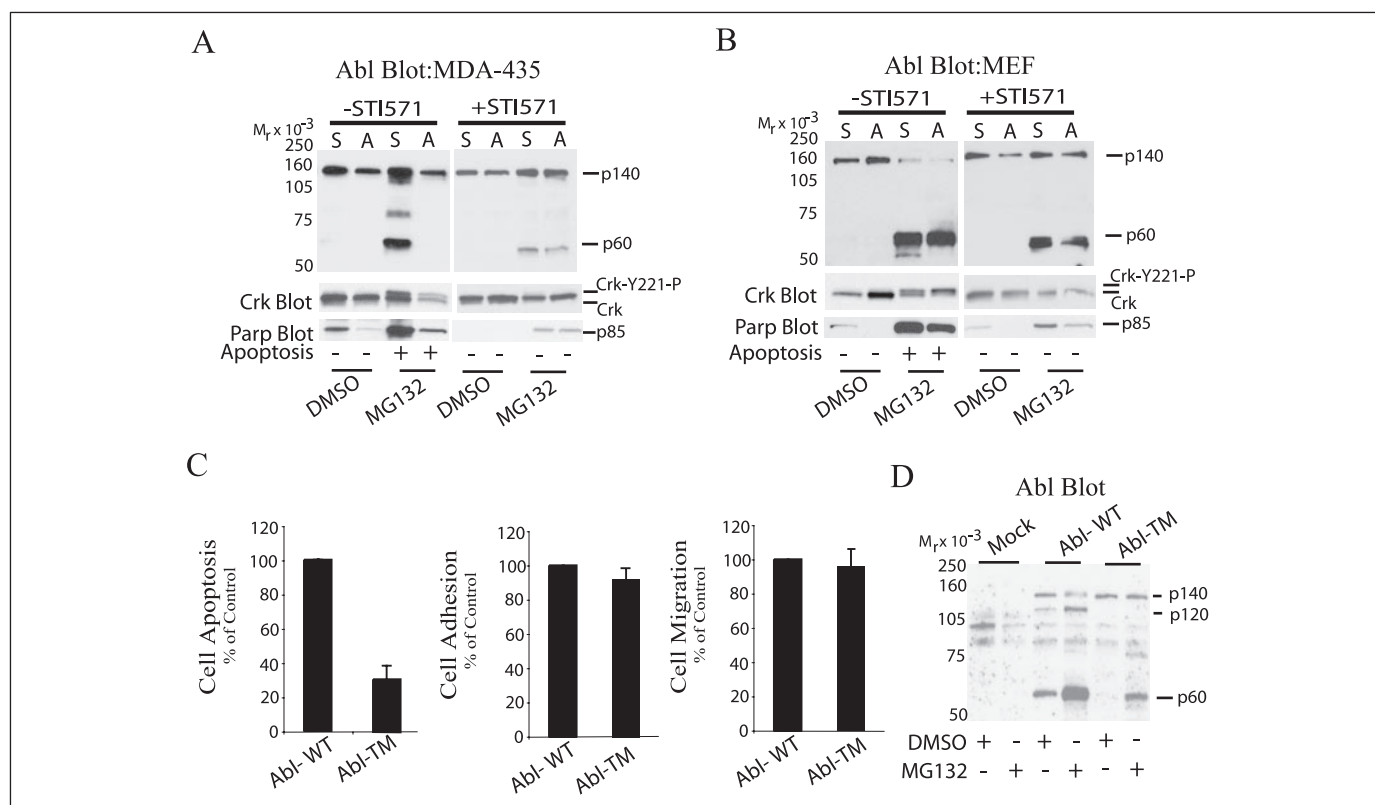


FIGURE 7. Abl cleavage by caspases requires kinase activity and contributes to MG132-induced cell apoptosis. MDA-435 (A) or MEF cells (B) were detached from culture dishes and either held in suspension (S) or reattached (A) to fibronectin-coated dishes for 24 h in the presence or absence of a 5 μ M concentration of the Abl tyrosine kinase inhibitor STI-571 along with either 10 μ g/ml MG132 or an equivalent amount of the vehicle Me₂SO (DMSO). Cells were either evaluated for apoptosis or lysed in detergent, and equivalent amounts of protein were subjected to Western blot for Abl kinase, Crk, and the cleaved, activated form of PARP (p85). C, Abl null cells reconstituted with either the empty vector, wild type Abl (WT), or Abl with the caspase cleavage sites mutated (TM) were placed in suspension and treated with 10 μ g/ml MG132 for 24 h. Cells were then examined for apoptosis as described under "Materials and Methods." Also, untreated cells were examined for cell adhesion and migration on fibronectin as described under "Materials and Methods" (middle and right graphs). D, an aliquot of Abl null cells treated as in C were lysed and subjected to Western blot for Abl kinase.

We next examined Abl cleavage in MEF cells, which do not undergo cell death upon detachment from the ECM (Fig. 7B). MG132 strongly induced apoptosis and the cleavage of Abl into 120-, 60-, and 22-kDa fragments in both suspension and attached cells (Fig. 7B). In this case, MG132 promoted strong Crk-Y221-P in suspension and attached cells, although it was slightly reduced in suspension cells (Fig. 7B). Again, Abl kinase activity was required for Abl cleavage and Crk-Y221-P, since these processes were blocked with STI-571 (Fig. 7B). Thus, MEF cell adhesion to the ECM does not protect Abl from cleavage, nor does it uncouple Abl from Crk in response to MG132 treatment in suspension cells, which is different from COS-7 and MDA-435 cells. Together these

findings suggest that regulation of Abl cleavage by ECM attachment and proteasome inhibition occur in a cell type-specific manner. To investigate this further, we examined nine different cell lines for Abl cleavage following proteasome inhibition under suspension and adherent conditions (Table 1). We also examined the ability of TSA and H₂O₂ to facilitate Abl cleavage, which induces cell apoptosis through Abl-independent and -dependent mechanisms, respectively (5, 20–22, 29–32). Treatment of suspension and adherent cells with proteasome inhibitors induced Abl cleavage in the majority of the cell lines tested, whereas TSA and H₂O₂ did not. These findings indicate that inhibition of the proteasome is a potent and selective inducer of Abl cleavage, regardless

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of the adhesion status of the cell. However, COS-7 and MDA-435 cells are notable exceptions, since they show no Abl cleavage when attached to the ECM (Table 1) (Figs. 6A and 7A).

Abl Cleavage by Caspases Contributes to MG132-induced Cell Apoptosis—To determine whether Abl cleavage contributes to MG132-induced death, we reconstituted Abl null cells with either Abl-WT or Abl-TM, which prevents its cleavage by caspases (11). These cells were then treated with MG132 and examined for cell apoptosis and Abl cleavage. Null cells expressing Abl-TM showed significantly reduced sensitivity to MG132-induced apoptosis compared with cells expressing full-length Abl-WT (Fig. 7C). Western blot analysis confirmed that Abl-WT, but not Abl-TM, is cleaved under these conditions (Fig. 7D). Cells expressing Abl-TM showed an ability to attach and migrate on the ECM similar to that of cells expressing Abl-WT, indicating that Abl-TM specifically alters apoptosis but not other Abl functions in the cell (Fig. 7C). These findings demonstrate that cleavage of Abl contributes to MG132-induced apoptosis in these cells.

DISCUSSION

Role of the 26 S Proteasome in the Regulation of Cell Spreading and Migration—The molecular mechanisms that control migration and apoptosis are coordinately regulated through interactions with ECM proteins and growth factors present in the extracellular environment. Regulation of these processes is critical during development, when cells must move long distances to populate newly developing tissues. However, deregulation of these processes during cancer progression allows cells to invade into the surrounding tissue and survive in a foreign environment. Although the molecular mechanisms that facilitate these processes are poorly understood, they probably involve coordinate interactions of growth factors and integrin adhesion receptors (23, 24, 26).

In this report, we provide evidence that polyubiquitination and regulated proteolysis are not necessary for COS-7 and NIH 3T3 cell attachment, spreading, or migration on the ECM. First, cells treated with pharmacological inhibitors of the proteasome readily attach, spread, and migrate in response to ECM proteins and PDGF. Second, inhibition of the proteasome by overexpression of free ubiquitin in cells does not perturb these processes. Third, proteasome inhibition does not inhibit Crk-induced cell migration. Finally, blocking the 26 S proteasome does not perturb the steady-state levels or activity of key signals that regulate spreading and motility, including FAK and paxillin. A recent report also showed that FAK is not ubiquitinated in adherent COS-7 cells treated with proteasome inhibitors (33). Together, these findings indicate that regulated protein degradation by the proteasome is not a major pathway that controls cells spreading and migration.

However, it is noteworthy that FAK can be targeted for degradation by SOCS (suppressors of cytokine signaling). FAK- and PDGF-induced cell migration is inhibited in cells replated on fibronectin and treated with growth factors to induce the *de novo* expression of SOCS proteins (33). In contrast, our findings demonstrate that blockage of the proteasome does not inhibit PDGF-induced cell migration. In fact, we observed a small but reproducible increase in cell motility. Inhibition of the proteasome may increase PDGF signaling by preventing the degradation of the PDGF receptor. Additional work will be necessary to clarify the role of ubiquitination in growth factor-mediated cell migration and to define the molecular events that control this process.

Recently, the RING finger protein RNF5 was found to promote the polyubiquitination of paxillin through the Lys⁶³ topology. This mechanism of ubiquitination does not target proteins to the proteasome for destruction but rather regulates protein function and sig-

naling (1, 2, 34). Indeed, it was reported in this study that treatment of cells with proteasome inhibitors does not change the steady levels of paxillin, which corroborates our findings. Thus, our collective findings indicate that paxillin is not regulated by Lys¹⁴ ubiquitination and degradation by the proteasome system in migratory cells. Interestingly, ubiquitinated paxillin was found to relocalize from focal adhesions to the cytoplasm, which inhibited cell migration (34). These results indicate that ubiquitination of paxillin through the RNF5/Lys⁶³ mechanism is specifically important for paxillin-mediated cell migration.

Role of Abl Activation in Mediating Cell Apoptosis Induced by Proteasome Inhibitors—Our findings indicate that the Abl/CAS/Crk signaling module is a specific apoptotic pathway that is targeted during proteasome inhibition and is not a general response that occurs in cells undergoing apoptosis. For example, the proapoptotic agent TSA strongly induced cell apoptosis without altering Abl/CAS/Crk signaling. Our previous findings indicate that Abl and Arg play a key housekeeping role by modulating Crk activity through phosphorylation of Tyr²²¹. Indeed, Abl/Arg null cells show little or no basal Crk-Y221-P compared with wild type MEF cells. Whereas the upstream activator of Abl is unknown, Src family kinases are good candidates, since they can phosphorylate and activate Abl (35). Once Abl and/or Arg is activated, it may be ubiquitinated and targeted for degradation by the proteasome, as recently reported (36, 37). This suggests that the proteasome may play a central role in maintaining the proper level of activated Abl/Arg kinases and Crk-Y221-P in healthy cells. Perturbation of Abl/Arg degradation by proteasome inhibitors could lead to the accumulation of activated Abl, increased Crk-Y221-P, and apoptosis. However, we were unable to detect changes in the steady-state levels of endogenous Abl, and we did not observe high molecular weight forms of polyubiquitinated Abl in cells treated with proteasome inhibitors. Alternatively, it is known that proteasome inhibition induces cellular stress due to excess accumulation of ubiquitinated proteins in the cell (2, 3, 16). This could promote activation of Abl and Crk-Y221-P as part of the suicide signal. Our data demonstrate that Abl kinase activity is necessary for Crk-Y221-P and apoptosis, since the Abl kinase inhibitor STI-571 inhibited these events in response to proteasome inhibition. Also, death effectors such as c-Jun N-terminal kinase, NF- κ B, p53, and p73, which are targeted by Abl and the ubiquitin system, are likely to be involved in mediating cell apoptosis under these conditions.

Role of the ECM in Regulating Abl-mediated Crk-Y221-P in Response to Inhibition of the Proteasome—Accumulating evidence indicates that Abl kinases serve as biological sensors that relay extracellular signals to subcellular compartments, including focal adhesions and the actin cytoskeleton. An interesting question is how Abl regulates such diverse cellular processes as proliferation, apoptosis, and cell migration. We have observed that cell stress is associated with persistent (>2 h) activation of Abl kinase, Crk-Y221-P, and activation of the apoptotic machinery. However, exposure of cells to growth- and/or motility-promoting factors and ECM proteins induces transient Abl activation and Crk-Y221-P. In our current work, we found that proteasome inhibitors induce cell stress and are associated with increased levels of Crk-Y221-P and persistent Abl activity (>12 h). We believe that prolonged Abl activity disrupts survival signals that normally emanate from focal adhesions and the actin cytoskeleton, including those provided by CAS-Crk coupling and the focal adhesion protein Lasp-1 (29). For example, in the case of COS-7 cells, the increased Crk-Y221-P induced by proteasome inhibitors occurred only in cells that were attached to the ECM and not in suspension cells. This apoptotic response was not associated with PARP or Abl cleavage. On the other hand, cell detachment from the

ECM induced Abl and PARP cleavage by caspases and decreased Crk-Y221-P, leading to cell apoptosis. Thus, proteasome inhibitors kill cells using caspase-dependent and independent pathways, depending on the adhesion status of the cell. Although it is not yet known how cell adhesion protects Abl from caspase cleavage and degradation, it may be related to its ability to bind to the actin cytoskeleton and focal adhesion structures (4, 5, 38, 39).

There is significant heterogeneity in the role of the ECM in dictating which suicide program will be used by a given cell. For example, in contrast to COS-7 and MDA-435 cells, proteasome inhibitors induced the cleavage of Abl and PARP in both suspension and adherent MEF cells. These findings indicate that cells are armed with different apoptotic mechanisms and that the ECM plays a role in dictating which suicide program is used in some cell types but not others. This and the fact that proapoptotic signals also cause Abl to translocate to the nucleus and mitochondria suggest that cell death mediated by this tyrosine kinase family is a multifaceted process that targets numerous substrates in a temporal and spatial manner (20, 40–43).

The Role of Abl Cleavage in Regulation of CAS-Crk Coupling and Cell Apoptosis Induced by Proteasome Inhibitors—Detachment of cells from the ECM and/or treatment of cells with proteasome inhibitors trigger Abl cleavage and apoptosis. We demonstrate that this process requires kinase activity of Abl, since treatment of cells with STI-571 prevented Abl cleavage and apoptosis. Furthermore, cells expressing Abl with the caspase cleavage sites mutated showed impaired apoptosis in response to proteasome inhibitors, indicating that this process contributes to the death mechanism. Whereas it is not yet known how these proteolytic products couple to the death machinery, based on the domain structure and alignment of the caspase cleavage sites, they may relocate to different regions of the cell, where they target distinct effectors like Crk and CAS. For example, the 120-kDa fragment, which has lost its actin binding domain and its nuclear exclusion sequence, can relocate to the nucleus (11, 28). On the other hand, the 60-kDa fragment has lost the nuclear localization sequences but retains the Src-like structures, including the kinase and Src homology 2/3 domains, suggesting that this fragment targets cytoplasmic substrates. Previous work has shown that a recombinant form of this Src-like fragment retains kinase activity toward an exogenous substrate (28). However, additional work is necessary to determine the functionality of these fragments and to identify their *in vivo* targets.

It is noteworthy that Abl cleavage products may be regulated by ubiquitination and the proteasome. We found that cells treated with proteasome inhibitors show additional high molecular mass forms of Abl fragments (80–85 kDa) as well as increased levels of the 60- and 120-kDa proteolytic products (Fig. 6A). This finding suggests that once Abl is cleaved by caspases, the resulting products are regulated by ubiquitination and the proteasome. Monitoring the level of Abl cleavage products may be an additional mechanism utilized by the cell as it decides to attempt a rescue program or commit to a death pathway.

Based on our work and the work of others, we propose a model in which proteasome inhibitors induce apoptosis through modulation of the Abl/CAS/Crk signaling module and the cytoskeleton. Proteasome inhibitors like MG132 and lactacystin prevent the degradation of ubiquitinated proteins that are targeted for degradation, including cell cycle regulatory proteins. Initially, the loss of proteasome function does not affect cell migration on the ECM. However, the abnormal accumulation of these proteins over time induces cellular stress and the decision to commit suicide. As part of the apoptotic program, Abl kinases are persistently activated in response to cell stress. Abl then phosphorylates Crk Tyr²²¹, leading to disassembly of CAS-Crk complexes. This

response is regulated by cell adhesion to the ECM in a cell type-specific manner through Abl activation and cleavage by caspases. Also, Abl and its cleavage products probably modulate additional downstream death effector pathways that operate in the nucleus and cytoplasm as previously demonstrated (20, 40–43). Together, these molecular events contribute to apoptosis in response to cellular stress induced by inhibition of proteasome function. In this model, the proteasome and regulated protein degradation play an important role in maintaining cell homeostasis through modulation of Abl and the cytoskeleton. Our finding that blockage of the proteasome induces Abl-mediated cell death by disrupting CAS-Crk signaling helps to provide a better understanding of how regulated proteolysis controls normal and abnormal growth and motility of cells.

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Deregulation of Proteasome Function Induces Abl-mediated Cell Death by Uncoupling p130^{CAS} and c-CrkII

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