INTERACTION OF HEMATOPOIETIC PROGENITOR KINASE 1 (HPK1) AND c-ABL
TYROSINE KINASE IN RESPONSE TO GENOTOXIC STRESS

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ABSTRACT

The c-Abl protein tyrosine kinase is activated by certain DNA-damaging agents and regulates induction of the stress-activated c-Jun N-terminal protein kinase (SAPK/JNK). The hematopoietic progenitor kinase (HPK1) has also been shown to act upstream to the SAPK/JNK signaling pathway. We report here that exposure of hematopoietic Jurkat T cells to genotoxic agents is associated with activation of HPK1. The results demonstrate that exposure of Jurkat cells to DNA-damaging agents is associated with translocation of active c-Abl from nuclei to cytoplasm and binding of c-Abl to HPK1. Our findings also demonstrate that c-Abl phosphorylates HPK1 in cytoplasm and stimulates HPK1 activity. The functional significance of the c-Abl-HPK1 interaction is supported by the demonstration that this complex regulates SAPK/JNK activation. Overexpression of c-Abl(K-R) inhibits HPK1-induced activation of SAPK/JNK. Conversely, the dominant negative mutant of HPK1 blocks c-Abl-mediated induction of SAPK/JNK. These findings indicate that activation of HPK1 and formation of HPK1/c-Abl complexes are functionally important in the stress response of hematopoietic cells to genotoxic agents.
INTRODUCTION

The cellular response to ionizing radiation (IR) and other genotoxic agents includes cell cycle arrest, activation of DNA repair and apoptosis or programmed cell death. However, the intracellular signals that control these events are largely unclear. The available evidence supports a role for the c-Abl protein tyrosine kinase in the induction of apoptosis (1,2). Transient transfection studies with wild-type, but not kinase-inactive, c-Abl have demonstrated induction of an apoptotic response (2). Also, cells that stably express the dominant negative c-Abl(K-R) mutant exhibit resistance to induction of apoptosis by IR and other DNA-damaging agents (2,3). Similar results have been obtained in Abl−/− fibroblasts (2,3). The apoptosis resistant phenotype is more pronounced in cells expressing c-Abl(K-R) compared to c-Abl null cells. In addition, a pro-apoptotic role for c-Abl is supported by c-Abl-dependent induction of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) in the response to genotoxic stress (4-6).

The SAPK/JNK signaling cascade plays a critical role in the responses stimulated by DNA damage, heat shock, interleukin-1, tumor necrosis factor α (TNFα) and Fas (4-15). SAPK is phosphorylated and activated by immediate upstream mitogen activated protein kinase-kinases (MAPKKs), MAPK kinase 4(MKK4)/SEK1 (8,16) and MKK7 (17). These MAPKKs are activated, in turn, by the upstream MAPK kinase kinases (MAPKKKs) including MAPKK/ERK kinase kinase 1(MEKK1)(18), mixed lineage kinase 3(MLK3)(19), TGF-β-activated kinase 1 (TAK1)(20), tumor
progression locus 2 (Tpl-2)(16), MAPK upstream kinase (MUK)(21) and apoptosis signal-regulating kinase 1 (ASK1)(22). Furthermore, several Ste20-related protein kinases which activate SAPK through MAPKKKs have been identified as MAPKKKKs, including hematopoietic progenitor kinase 1 (HPK1) (23,24), germinal center kinase (GCK) (25,26), HPK1/GCK-like kinase (HGK)/Nck interacting kinase (NIK)(27,28), GCK-like kinase (GLK)(29) and kinase homologous to Ste20/Sps1 (KHS)/GCK-related kinase (GCKR) (30,31).

HPK1, a 97-kDa serine/threonine kinase, is restricted to hematopoietic tissues in adults (23,24). Studies have shown that HPK1 interacts with MEKK1 (23), MLK3 (24) and TAK1 (32), which, in turn, can activate MKK4/SEK1 and thereby result in activation of the SAPK signaling pathway. Previous studies have demonstrated that four proline-rich motifs in HPK1 are potential binding sites for SH3 domain-containing proteins. HPK1 interacts with the SH2/SH3 domain-containing adaptor proteins Crk and CrkL (33). Using yeast two-hybrid analysis, HPK1 has also been shown to associate with the c-Abl SH3 domain (24). The demonstration that Abl-/- cells exhibit a defective SAPK response in response to certain DNA damaging agents has provided support for c-Abl as an upstream effector in SAPK pathway (5,6) and has raised the possibility of a functional interaction between c-Abl and HPK1.

The present studies demonstrate that exposure of Jurkat cells to ionizing radiation (IR) is associated with activation of HPK1. Similar results were obtained with another genotoxic
agent, 1-β-D-arabinosylcytosine (ara-C). The results also demonstrate that activated HPK1 forms a complex with cytoplasmic c-Abl in the cellular response to genotoxic agents. The functional significance of the c-Abl/HPK1 interaction is supported by the finding that HPK-1-induced activation of SAPK is inhibited by a dominant negative c-Abl and that kinase-inactive mutants of HPK1 block c-Abl-mediated induction of SAPK activity.
MATERIALS AND METHODS

Cell culture and reagents. Human Jurkat T cells (American Type Culture Collection, Rockville, MD) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma), 100 units/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine. Human embryonic kidney 293T cells were cultured in DMEM containing 10% heat-inactivated FBS, antibiotics. MCF-7/neo and MCF-7/c-Abl(K-R) (34) cells were cultured in DMEM containing 10% FBS, antibiotics and 500 µg/ml geneticin sulfate (GIBCO/BRL). Cells were seeded at a density of 2.5 x 10^5/ml for 24 h before treatment with 20 Gy IR or 10 µM ara-C (Sigma). Irradiation was performed at room temperature with a γ-ray source (Cs^{173}, Gamma Cell 1000, Atomic Energy of Canada, Ontario) at a fixed dose rate of 13 Gy/min.

Isolation of the cytosolic fraction. Cytosolic fractions were prepared as described (35). Cells were washed twice with PBS and then suspended in ice cold buffer (20 mM HEPES, pH 7.5, 1.5 mM MgCl_2, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, and 10 µg/ml leupeptin and aprotinin) containing 250 mM sucrose. The cells were disrupted by five strokes in a Dounce homogenizer. Following centrifugation of the lysate at 10,000 x g for 5 min at 4°C, the supernatant fraction was centrifuged at 105,000 x g for 30 min at
4°C. The resulting supernatant was used as the soluble cytosolic fraction.

Isolation of the nuclear fraction. Nuclear proteins were isolated as described (36). In brief, cells were washed three times with PBS and resuspended in 4 cell volumes of hypotonic lysis buffer (10 mM HEPES, pH 7.5, 2 mM MgCl₂, 10 mM KCl, 10 µg/ml leupeptin, and 10 µg/ml aprotinin). After incubation on ice for 30 min to allow swelling, the cells were disrupted in a Dounce homogenizer (15-20 strokes). The homogenate was layered on a cushion of 1 M sucrose in hypotonic solution and subjected to centrifugation for 10 min. The nuclei were then suspended in lysis buffer containing 0.5% Nonidet P-40. After incubation at 4°C for 30 min, the suspension was centrifuged at 12,000 x g and the supernatant was used as the nuclear fraction.

Immunoprecipitation and immunoblot analysis. Total cell lysates were prepared as described in lysis buffer containing 1% Nonidet P-40 (37). Equal amounts of total, cytosolic or nuclear proteins were subjected to immunoprecipitation with anti-c-Abl (K-12; Santa Cruz Biochemistry) or anti-HPK1 (32). Immune complexes were recovered by incubation with protein A-Sepharose for 1 h at 4°C, washed three times with lysis buffer, separated by SDS-PAGE and then transferred to nitrocellulose filters. After blocking with 5% dried milk in PBS-Tween, the filters were incubated with anti-HPK1, anti-c-Abl (Ab-3; Oncogene Research Products), anti-Flag M2 (Sigma), anti-P-Tyr (4G10, Upstate...
Biotechnology), anti-Lamin A (Santa Cruz), anti-β-actin (Santa Cruz) or anti-SAPK (Santa Cruz). The filters were analyzed by ECL (Amersham).

**Plasmids and peptides.** The pSRα-c-Abl wild-type and pSRα-c-Abl(K-290R) have been described (6,34). HA-c-Abl was provided by Dr. Jean Y.J. Wang (University of California, San Diego, CA); GST-Jun(1-102) (38); pEBG-SAPK, pEBG-SEK1(6); pCIneo-Flag-HPK1 wild-type, pCIneo-Flag-HPK1(M46), GST-HPK1KD, GST-HPK1CD (23). The plasmid GST-Crk 120-225 was provided by Dr. Stethan Feller (Bavarian Julius-Maximilians University, Germany). The peptides PR1: H2N-PELPPAIPRR-COOH; PR2: H2N-PPPLPPKPK-COOH; PR3: H2N-PPPNSPRPFPPPP-COOH and PR4: H2N-KPPLLPPKE-COOH were prepared as described (33).

**Fusion protein binding assays and peptide competition assays.** Glutathione S-transferase (GST) and GST-Abl SH3 (39) were purified by affinity chromatography using glutathione-sepharose beads and equilibrated in lysis buffer. Cell lysates were incubated with 5 µg of immobilized GST or GST-Abl SH3 for 2 h at 4°C. The resulting protein complexes were washed three times with lysis buffer and boiled for 5 min in SDS sample buffer. The complexes were then separated by SDS-PAGE and subjected to immunoblot analysis with anti-HPK1. GST-c-Abl SH3 fusion protein was incubated with proline-rich sequences 2 (PR2) (33), PR3 or PR4. The fusion protein-peptide mixtures were separately incubated with cell lysates for 30 min at room temperature.
temperature. Following washing, bound proteins were analyzed by immunoblotting.

*c-Abl and HPK1 kinase assays.* Cell lysates were subjected to immunoprecipitation with anti-HPK1 or anti-c-Abl as described (35). The protein complexes were washed and incubated in kinase buffer (20 mM HEPES, pH 7.4, 10 mM MgCl2) containing 2.5 μCi [γ-\(^{32}\)P]ATP and either GST-Crk (120-225) (40) or myelin basic protein (MBP, Sigma) as substrates for 15 min at 30°C. The reaction products were analyzed by SDS-PAGE and autoradiography.

*c-Jun kinase assays.* 293T cells were transfected with pEBG-SAPK, pEBG-SEK-1, Flag-HPK1, Flag-HPK1(M46) and c-Abl or c-Abl(K-R). After 12 h of incubation at 37°C, the medium was replaced and the cells were incubated for another 24 h. Cell lysates were prepared as described and 200-250 μg soluble proteins were incubated with 5 μg immobilized GST for 30 min at 4°C. The protein complexes were washed with lysis buffer and then incubated in kinase buffer containing [γ-\(^{32}\)P]ATP and GST-c-Jun (2-100)(38) for 15 min at 30°C. Reactions were terminated by addition of SDS-PAGE sample buffer and boiling. Phosphorylated proteins were resolved by SDS-PAGE and analyzed by autoradiography.

*Transient transfections and immunoprecipitations.* 293T cells were cotransfected by the calcium phosphate method with HA-
c-Abl and Flag-HPK1. After incubation for 36 h, the cells were lysed in lysis buffer containing 1% Nonidet P-40 and then subjected to immunoprecipitation with anti-HA (Boehringer and Mannheim) and the immunoprecipitates were analyzed by immunoblotting with anti-Flag. 293T cells were also transiently transfected by the calcium phosphate method with Flag-HPK1 or Flag-HPK1 M46 in the presence of c-Abl or c-Abl(K-R). The cells were cotransfected with pEBG-SAPK or pEBG-SEK1. After incubation for 36 h, the cells were lysed in lysis buffer containing 1% Nonidet P-40 and then subjected to HPK1 kinase assay or immunoblot analysis with anti-Flag. Total cell lysates were also separately subjected to incubation with GST and the protein precipitates were assayed for GST-c-Jun phosphorylation. MCF-7/neo or MCF-7/c-Abl(K-R) cells were transiently transfected with Flag-HPK1 by lipofectamine (GIBCO/BRL). Total cell lysates were subjected to immunoprecipitation with anti-Flag and then subjected to immunoblot analysis with anti-P-Tyr. Autoradiograms were scanned by laser densitometry and the intensity of the signals were quantitated with the ImageQuant program (Molecular Dynamics, Sunnyvale, Calif.).

In vitro phosphorylation of HPK1. Recombinant c-Abl protein was incubated with GST-HPK1-KD or GST-HPK1-CD fusion proteins in the presence of [γ-32P]ATP for 30 min at 30°C. Phosphorylation of the reaction products was assessed by SDS-PAGE and autoradiography. 293T cells were transiently transfected with
pCI.neo-Flag-HPK1. Cell lysates were subjected to immunoprecipitation with anti-Flag and the precipitates were incubated with recombinant purified c-Abl or kinaseinactive c-Abl(K-R) in the presence [γ-32P]ATP for 30 min at 30°C. Phosphorylation of the reaction products was assessed by SDS-PAGE and autoradiography.
RESULTS

Genotoxic stress induces the interaction of c-Abl and HPK1 in Jurkat cells.

To assess whether c-Abl and HPK1 associate in cells, lysates from human Jurkat T cells were subjected to immunoprecipitation with anti-HPK1 and the protein precipitates were analyzed by immunoblotting with anti-c-Abl. Immunoblot analysis of precipitates using a control antibody or preimmune rabbit serum demonstrated little, if any, detection of c-Abl (Fig. 1a and data not shown). However, a similar analysis of anti-HPK1 immunoprecipitates demonstrated the coprecipitation of HPK1 and c-Abl (Fig. 1a). To assess interactions between c-Abl and HPK1 in response to genotoxic agents, Jurkat cells were treated with 10 μM ara-C and harvested at 3 h. Analysis of anti-HPK1 immunoprecipitates by immunoblotting with anti-c-Abl demonstrated induction of HPK1-c-Abl complexes (Fig. 1b). Whereas ara-C incorporates into DNA and inhibits DNA replication (41), IR induces single and double DNA strand breaks. The finding that exposure of Jurkat cells to IR is also associated with increased binding of c-Abl and HPK1 indicated that this response is induced by diverse types of genotoxic stress (Fig. 1b).

To further determine interaction of c-Abl with HPK1, we transiently overexpressed Flag-HPK1 with HA-c-Abl in 293T cells and analyzed anti-HA immunoprecipitates with anti-Flag. Reactivity of anti-Flag with a 100 kDa protein supported the
coprecipitation of HPK1 with c-Abl (Fig. 2a). In the reciprocal experiment, anti-Flag immunoprecipitates were subjected to immunoblot analysis with anti-HA. The results confirmed the detection of complexes containing HPK1 and c-Abl (data not shown). Lysates from transfected 293T cells were also subjected to immunoprecipitation with anti-HA. Analysis of protein precipitates by immunoblotting with anti-HA demonstrated equal levels of c-Abl (Fig. 2a). Taken together, these findings indicate that c-Abl associate with HPK1 in cells. To assess whether interaction between c-Abl and HPK1 is induced by genotoxic stress in conditions overexpressing c-Abl and HPK1, 36 h after the transfection with Flag-HPK1 and HA-c-Abl, cells were treated with 10 \( \mu \)M ara-C for 3 h. Analysis of anti-HA immunoprecipitates by immunoblotting with anti-Flag demonstrated significant induction of HPK1-c-Abl complex in response to ara-C (Fig. 2b). Four proline-rich sequences (PR1-PR4) are present in the C-terminal region of HPK1 (33). One of these proline-rich sequences [SGPPPNSPRPGPPPS, aa 430-444] displays homology with motifs located in the C-terminal domains of 3BP1, 3BP2 and ST5 that bind c-Abl SH3. To determine whether the c-Abl SH3 domain binds to HPK1, lysates from irradiated Jurkat cells were incubated with GST or GST-Abl SH3 and the resulting precipitates were analyzed by immunoblotting with anti-HPK1. The results demonstrate that, in contrast to GST, HPK1 was detectable in the adsorbates to GST-Abl SH3 (Fig. 2c). Because the HPK1 proline-
rich motif PR3, but not PR1, 2 or 4, match the c-Abl SH3-binding consensus motif (PXXXXPXP), we examined the ability of HPK1 proline-rich peptides to block the formation of c-Abl/HPK1 complexes. The results demonstrate that the PR3 proline-rich peptide efficiently blocks the interaction of HPK1 with c-Abl, while PR2 and PR4 had at best a marginal effect on c-Abl/HPK1 complex (Fig. 2d). These findings collectively indicate that the interaction between HPK1 and c-Abl likely involves c-Abl-SH3 and HPK1-Pro. It is possible that the coprecipitation of HPK1 and c-Abl is due to interactions of each kinase with other molecules.

HPK1 is primarily localized in the cytoplasm (23). To define the subcellular localization of the interaction between c-Abl and HPK1, we subjected nuclear and cytoplasmic lysates from control and ara-C-treated cells to immunoprecipitation with anti-HPK1. The immunoprecipitates were then analyzed by immunoblotting with anti-c-Abl. Signal intensities from the anti-c-Abl immunoblotting experiments (n=3) were analyzed by densitometric scanning. Immunoblot analysis of the immunoprecipitates from control and ara-C-treated cells demonstrated little if any reactivity with anti-c-Abl in the nuclear fraction (Fig. 3a). Formation of HPK1-c-Abl complexes was significantly increased in the cytoplasm, but not in the nucleus, of ara-C-treated cells (Fig. 3a). Studies have shown that although c-Abl contains three nuclear localization signals (NLS), it is not exclusively localized to the nucleus (42,43). c-Abl contains a functional nuclear export signal (NES) and the
subcellular localization of c-Abl is determined by a balance of nuclear import and export (44). To assess whether c-Abl translocates to the cytoplasm in response to genotoxic stress, Jurkat cells were treated with ara-C for different intervals of time. Nuclear and cytoplasmic fractions were isolated and analyzed by immunoblotting with anti-c-Abl. As controls, nuclear and cytoplasmic fractions were also analyzed by immunoblotting with anti-Lamin A and anti-β-actin, respectively. The results demonstrate that treatment of Jurkat cells with ara-C was associated with significant decreases in nuclear c-Abl levels (Fig. 3b, left panel). Moreover, levels of cytoplasmic c-Abl were increased in response to ara-C (Fig. 3b, right panel).

Translocation of c-Abl from nucleus in the response to genotoxic stress may initiate formation of complexes with multiple molecules in cytoplasm. Indeed, densitometric scanning of autoradiograms and quantitative analysis demonstrate that the formation of c-Abl complexes with HPK1 in cytoplasm is significantly less than the translocation of c-Abl from nucleus to cytoplasm. These findings support a model in which c-Abl is activated in the nucleus in response to genotoxic stress, translocates to the cytoplasm and thereby interacts with HPK1.

To determine whether the kinase function of HPK1 is necessary for the interaction with c-Abl, we transiently cotransfected Flag-HPK1 or a kinase-inactive Flag-HPK1 M46 mutant with c-Abl in 293T cells. Following treatment with ara-C, anti-
c-Abl immunoprecipitates were analyzed by immunoblotting with anti-Flag. The results demonstrate that, in contrast to HPK1 M46, overexpression of wild-type HPK1 is associated with an increase in binding with c-Abl (Fig. 3c). As c-Abl is also activated by ara-C and IR (11), we asked whether the association of HPK1 with c-Abl is also dependent on the c-Abl kinase function. To address this issue, 293T cells were transiently transfected with Flag-HPK1 and c-Abl or dominant negative c-Abl(K-R) mutant and then treated with ara-C. Anti-c-Abl immunoprecipitates were analyzed by immunoblotting with anti-Flag. The results demonstrate that the interaction between HPK1 with c-Abl is significantly increased in cells overexpressing wild-type c-Abl (Fig. 3d). Taken together, these findings suggest that the kinase function of c-Abl and HPK1 may be necessary for their interaction. Although, our data do not rule out the possibility that the loss of interaction between c-Abl and HPK1 might also be due to improper folding of these mutants. Further studies using purified recombinant c-Abl and HPK1 proteins are required to delineate this issue.

To assess in part the functional significance of the interaction of c-Abl and HPK1, we incubated purified recombinant c-Abl with GST-HPK1-KD [HPK1 kinase domain; aa 1-291] or GST-HPK1-CD [carboxy terminal domain; aa 292-833] (Fig. 4a) fusion proteins in the presence of [γ-32P]ATP. Analysis of the reaction products demonstrated little, if any, phosphorylation of either...
GST-HPK1-KD or GST-HPK1-CD (Fig. 4b, left panel). Unavailability of full length GST-HPK1 protein precludes us to use it as a substrate. The potential HPK1 binding sequence for the c-Abl SH3 domain [SGPPPNSPRGPPPS, aa 430-444] is present in the HPK1-CD, while the c-Abl phosphorylation site [YXXP] (39) in HPK1 [Y^{232}QPP; aa 232-235] is localized in HPK1-KD. To determine whether full length HPK1 acts as a substrate for c-Abl, 293T cells were transiently transfected with Flag-HPK1 full length. Cell lysates were subjected to immunoprecipitation with anti-Flag and the precipitates were incubated with recombinant c-Abl in the presence of [γ-^32P]ATP. As control, anti-Flag immunoprecipitates were separately incubated with recombinant c-Abl (K-R) protein in the presence of [γ-^32P]ATP. Analysis of the reaction products demonstrated c-Abl-mediated phosphorylation of full length HPK1 (Fig. 4b, right panel). As control, anti-Flag immunoprecipitates were separately analyzed by immunoblotting with anti-Flag. The results demonstrate equal expression of Flag-HPK1 in multiple transfections (data not shown). To confirm c-Abl-mediated tyrosine phosphorylation of HPK1, 293T cells were cotransfected with Flag-HPK1 with increasing amounts of c-Abl. Lysates were subjected to immunoprecipitation with anti-Flag and analyzed by immunoblotting with anti-P-Tyr. Signal intensities from anti-P-Tyr immunoblotting experiments were analyzed by densitometric scanning. The results from quantitation of signal intensities demonstrate 3.2 ± 0.4-fold induction in tyrosine phosphorylation.
of HPK1 by c-Abl (Fig. 4c).

Since c-Abl phosphorylates HPK1 in vitro, we asked whether HPK1 is tyrosine phosphorylated in the cellular response to genotoxic stress. Jurkat cells were treated with ara-C or exposed to IR. Total cell lysates were subjected to immunoprecipitation with anti-HPK1 and the precipitates were analyzed by immunoblotting with anti-P-Tyr. As control, anti-HPK1 immunoprecipitates were also analyzed by immunoblotting with anti-HPK1. The results demonstrate a 2-3-fold induction in tyrosine phosphorylation of HPK1 in response to genotoxic agents (Fig. 5a and data not shown). Exposure of cells to genotoxic agents is associated with activation of c-Abl (4-6). We therefore investigated whether genotoxic stress affects c-Abl-mediated tyrosine phosphorylation of HPK1. MCF-7 cells expressing neo cassette (MCF-7/neo) and MCF-7 expressing c-Abl(K-R) [MCF-7/c-Abl(K-R)] cells were transiently transfected with Flag-HPK1. After transfection, cells were exposed to IR or treated with ara-C and harvested after 3 h. Cytoplasmic lysates were subjected to immunoprecipitation with anti-Flag antibody and the protein precipitates were analyzed by immunoblotting with anti-P-Tyr. As control, anti-Flag immunoprecipitates were also analyzed by immunoblotting with anti-Flag. Exposure of MCF-7/neo cells to IR was associated with increases (3 ± 0.5-fold; Fig. 5b, lower panel) in tyrosine phosphorylation of HPK1 (Fig. 5b). Moreover, IR had no detectable effect on tyrosine phosphorylation of HPK1 in MCF-7/c-Abl(K-R) cells (Fig. 5b). Similar results
were obtained when MCF-7/neo or MCF-7/c-Abl(K-R) cells were treated with ara-C (Fig. 5b). Since MCF-7/c-Abl(K-R) cells stably overexpress c-Abl(K-R), and because of very minimal basal activity of c-Abl(K-R), the background level of tyrosine phosphorylation of c-Abl(K-R) is high to that compared with endogenous levels in MCF/neo. Collectively, these findings demonstrate that genotoxic stress induces tyrosine phosphorylation of HPK1 by a c-Abl-dependent mechanism. c-Abl activates HPK1 in vitro and in the response to genotoxic stress.

To further assess the functional significance of the interaction between c-Abl and HPK1, we investigated whether c-Abl affects HPK1 activity. 293T cells were cotransfected with Flag-HPK1 and empty vector or c-Abl. Anti-Flag immunoprecipitates were assayed for HPK1 kinase activity using MBP as a substrate. Analysis of the reaction products by autoradiography demonstrated that overexpression of c-Abl is associated with an increase (approximately 3-fold) in the kinase activity of HPK1 (Fig. 6a). Since HPK1 is a serine/threonine kinase, we next assessed whether HPK1 activates c-Abl. To address this issue, 293T cells were transfected with HA-c-Abl and HPK1 or empty vector and anti-HA Immunoprecipitates were assayed for phosphorylation of GST-Crk 120-225 (40). The results demonstrate that co-expression of HPK1 and c-Abl had no detectable effect on c-Abl kinase activity (Fig. 6b).

To determine whether HPK1 is activated in the response to
genotoxic stress, lysates from Jurkat cells treated with ara-C were subjected to immunoprecipitation with anti-HPK1. The immunoprecipitates were assayed for phosphorylation of MBP. The results demonstrate an increase ($5 \pm 1.1$-fold) in phosphorylation of MBP by HPK1 in ara-C-treated, as compared to control, cells (Fig. 7a). Similar results were obtained when Jurkat cells were exposed to IR (Fig. 7b). To define the subcellular localization of HPK1 activation, we assayed nuclear and cytoplasmic lysates from control and ara-C-treated cells for HPK1 activity. The results demonstrate increased activation of HPK1 in cytoplasmic, but not nuclear, lysates of the ara-C-treated cells (Fig. 7c). Similar results were obtained when Jurkat cells were exposed to IR (data not shown). We next determined the role of c-Abl in the regulation of HPK1 activity in response to genotoxic agents. Studies have shown that HPK1 is expressed in Jurkat and other cell types that are predominantly hematopoietic. Due to the extremely low transfection efficiency of Jurkat cells, we transiently transfected 293T cells with Flag-HPK1 and empty vector, c-Abl or c-Abl(K-R). Following transfections, cells were treated with 10 µM ara-C and harvested after 3 h. Anti-Flag immunoprecipitates were analyzed for phosphorylation of MBP. As controls, anti-Flag immunoprecipitates and total cell lysates were separately analyzed by immunoblotting with anti-Flag and anti-c-Abl, respectively. The results demonstrate increased phosphorylation of MBP in cells overexpressing wild-type c-Abl,
but not Abl(K-R) (Fig. 7d). Moreover, to demonstrate whether the kinase function of HPK1 is necessary for HPK1 activation in response to genotoxic stress, we transiently overexpressed empty vector, Flag-HPK1 or Flag-HPK1 M46 and treated with ara-C. Anti-Flag immunoprecipitates were analyzed for phosphorylation of MBP. The results demonstrate increased phosphorylation of MBP in cells overexpressing wild-type HPK1, but not HPK1 M46 (Fig. 7e).

To further determine activation of HPK1 in response to IR, we transiently overexpressed Flag-HPK1 in 293T cells and then exposed with IR. Anti-Flag immunoprecipitates were analyzed for phosphorylation of MBP. The results demonstrate that exposure of cells with IR is associated with an increase in activation of HPK1 (Fig. 7f).

c-Abl and HPK1 synergistically activate SAPK/JNK

Treatment of cells with diverse genotoxic agents activates SAPK (5,7-15). We next asked whether SAPK is activated in Jurkat cells in the response to genotoxic stress. To assess SAPK activation, Jurkat cells were either treated with 10 μM ara-C or exposed to 20 Gy IR and harvested after different times. Total cell lysates were subjected to immunoprecipitation with anti-SAPK and assayed for phosphorylation of GST-c-Jun. In concert with previous findings, treatment of Jurkat cells with ara-C or IR was also associated with activation of SAPK (Figs. 8a and b). Studies have shown that both HPK1 and c-Abl are upstream activators of SAPK (5,6,23,24,33,45-47). To assess whether c-Abl and HPK1 cooperate in the activation of SAPK, 293T cells were transiently cotransfected with pEBG-SAPK and SEK1 in the presence and
absence of HPK1 and/or c-Abl. Lysates were incubated with glutathione-beads and the precipitates were assayed for GST-c-Jun phosphorylation. Transient expression of c-Abl induced a 4- to 5-fold increase in SAPK activity as compared to empty vector. Overexpression of HPK1 with SEK1 and JNK was associated with 13- to 15-fold activation of SAPK. Moreover, HPK1 and c-Abl together induced SAPK activity 18- to 20-fold over basal level (Fig. 8c). These findings suggest that c-Abl interacts with HPK1 to activate SAPK. To determine whether a dominant-negative mutant of c-Abl [c-Abl(K-R)] affects HPK1-induced SAPK activation, 293T cells were transiently transfected with HPK1 and c-Abl(K-R). Cells were also cotransfected with SEK1 and pEBG-SAPK. Lysates were subjected to protein precipitation with glutathione-beads, and the precipitates were assayed for phosphorylation of GST-c-Jun. The results demonstrate that c-Abl(K-R) significantly inhibits HPK1-induced SAPK activation (Fig. 8c). Conversely, overexpression of HPK1 M46 also inhibited c-Abl-induced activation of SAPK (Fig. 8c). Taken together, these findings indicated that c-Abl and HPK1 synergize for activation of SAPK.

Studies have shown that mixed lineage kinase-3 (MLK-3) acts as a substrate for HPK1 and that the HPK1-induced SAPK activation is inhibited by overexpression of a dominant-negative mutant of MLK-3 (24). Our recent studies have shown that c-Abl phosphorylates and activates MEKK-1 in nuclei (48). Moreover, c-Abl-induced activation of SAPK is inhibited by overexpression of a dominant-negative mutant of MEKK-1 (48). MEKK-1 stimulates SEK1/JNKK, which in turn activates SAPK (8,18,49). The finding that MEKK-1(K-R) fails to completely block c-Abl-induced SAPK activation further indicates that c-Abl also stimulates
the SAPK/JNK pathway by MEKK-1-independent mechanisms (48). To determine whether c-Abl and HPK1 function upstream to MLK-3, 293T cells were transiently cotransfected with c-Abl or HPK1 and MLK-3(K-R). Cells were also cotransfected with SEK1 and pEBG-SAPK. Total cell lysates were subjected to protein precipitation with glutathione beads and assayed for GST-c-Jun phosphorylation. The results demonstrate that transfection of MLK-3(K-R) also inhibits c-Abl-induced activation of SAPK (data not shown). The results further demonstrate that overexpression of MLK-3(K-R) blocks HPK-1-induced activation of SAPK (data not shown). Taken together, these findings indicate that c-Abl/HPK1 complex function upstream to MLK-3 and induce SAPK activation in the response to genotoxic stress at least in Jurkat cells.
DISCUSSION

Eukaryotic cells respond to DNA damage with cell cycle arrest, activation of DNA repair and in the event of irreparable damage, the induction of apoptosis. The signals that determine cell fate, that is repair of DNA damage and survival, or activation of cell death mechanisms, remain unclear. The c-Abl tyrosine kinase is activated in the cellular response to certain DNA-damaging agents (5,6,14,47,50,51). Previous studies have also demonstrated that c-Abl functions upstream to activation of the SAPK/JNK pathway in the response of cells to genotoxic stress (5,6,14,47,51). The exposure of diverse types of mammalian cells to genotoxic agents is associated with SAPK activation (5-7,11,48,52-54). Other studies have demonstrated that activation of SAPK in the DNA damage response is associated with the induction of apoptosis (7,17,52,53). While c-Abl has also been linked to DNA damage-induced apoptosis, the precise role for c-Abl as an upstream effector of the SAPK pathway has been controversial. In this context, other work has indicated that c-Abl is not required for the activation of SAPK by genotoxic agents (51). The discrepancy between findings may be related to the demonstration that c-Abl is necessary for activation of SAPK in proliferating, but not growth-arrested, cells (1). As further support for c-Abl involvement, recent work has shown that c-Abl directly activates MEKK-1, an upstream effector in the SEK1->SAPK cascade, in the DNA damage response (48). The present findings extend the role of c-Abl in the activation of SAPK signaling by demonstrating that c-Abl interacts with HPK1 in transducing signals to SEK1 and SAPK.

HPK1 is a mammalian Ste20/PAK-like serine/threonine kinase which is primarily found in hematopoietic cells (23,24). While little is known about the signals responsible for activation
of HPK1, studies have shown that HPK1 activity is induced in cells treated with TGFβ (55). Notably, HPK1 functions as an upstream effector of TGFβ-induced activation of SAPK (55). Other studies have demonstrated that HPK1 is phosphorylated by the epidermal growth factor receptor (EGFR) (56). EGF stimulation induces the binding of HPK1 with the Grb2 adaptor protein and recruitment of these complexes to the autophosphorylated EGFR (56). HPK1 also associates with the Crk and CrkL adaptor proteins in signaling that results in activation of the SAPK pathway (33). The present results demonstrate that HPK1 is activated in the response of cells to DNA-damaging agents. In this context, IR exposure is associated with the accumulation of single and double DNA strand breaks (57). By contrast, ara-C is a nucleoside analog that incorporates into DNA and causes arrest of DNA replication by functioning as a relative chain terminator (41,58-60). The finding that both IR and ara-C activate HPK1 indicates that this response is induced by diverse types of DNA damage. In addition, the findings that IR and ara-C induce the activation of SAPK (5-7,11,48,52-54) suggest that HPK1 could contribute to SAPK-mediated signals induced by these genotoxic agents. Indeed, expression of a kinase-inactive HPK1 mutant abrogated in part IR- and ara-C-induced SAPK activation.

The present findings provide further support for an interaction between c-Abl and HPK1 in the DNA damage response. The results demonstrate that genotoxic stress induces the
association of c-Abl and HPK1. In vitro studies indicate that
the c-Abl SH3 domain interacts directly with a proline-rich motif
in the HPK1 C-terminal region. Moreover, studies in cells
cotransfected to express c-Abl and HPK1 demonstrate that c-Abl
phosphorylates HPK1. The finding that HPK1 is phosphorylated on
tyrosine in IR- or ara-C-treated cells expressing wild-type c-
Abl, but not in cells expressing c-Abl(K-R), further indicates
that HPK1 is phosphorylated by a c-Abl-dependent mechanism in the
dNA damage response. The functional significance of the c-Abl-
HPK1 interaction is supported by the finding that c-Abl activates
HPK1. Conversely, the results indicate that HPK1 has no apparent
effect on c-Abl activity. These findings support a model in
which HPK1 is a downstream effector of the c-Abl response to
Genotoxic stress. The recent demonstration that c-Abl is also
activated in cells exposed to hydrogen peroxide has supported a
role for c-Abl in the response to diverse types of stress (61).
The available evidence, however, indicates that the interaction
between c-Abl and HPK1 is induced by genotoxic, and not
oxidative, stress (data not shown).

Previous work had shown that nuclear c-Abl functions as an
Upstream effector of the MEKK1->SEK1->SAPK pathway in the
response of cells to DNA damage in non-hematopoietic cells (48).
The incomplete abrogation of DNA damage-induced SAPK activation
by the kinase-inactive MEKK1(K-R) mutant, however, indicates that
c-Abl can also stimulate the SAPK pathway by a MEKK1-independent
mechanism (48). HPK1 is predominantly a cytoplasmic kinase and
DNA damage-induced formation of c-Abl-HPK1 complexes is detectable in the cytoplasm and not in the nucleus. In concert with these findings, the results demonstrate that, in response to DNA damage, nuclear c-Abl translocates to the cytoplasm. Therefore, while nuclear c-Abl interacts with MEKK-1 (48), following translocation to the cytoplasm, c-Abl associates with HPK1. Coexpression of c-Abl and HPK1 was associated with a synergistic effect on SAPK activation and that this activation of SAPK is sensitive to MLK-3. Moreover, expression of either c-Abl(K-R) or HPK1 M46 blocked DNA damage-induced SAPK activation. Thus the kinase functions of both cytoplasmic c-Abl and HPK1 are required in the induction of SAPK activity at least in hematopoietic cells. These findings support a model in which c-Abl mediates activation of HPK1 and confers an additional signal which is also necessary for SAPK activation.
ACKNOWLEDGEMENTS

This work was supported by PHS Grants CA75216 (SK) and CA 55241 and CA 29431 (DK) awarded by the National Cancer Institute, DHHS; and AI 8738649 and AI 42532 (THT) awarded by the National Institute of Allergy and Infectious Diseases, DHHS. We thank Drs Leonard Zon, John Kyriakis, Joseph Avruch, Charles Sawyer, Stephen Feller, Ruibao Ren, Jean Y.J. Wang, Dennis Templeton, Melanie Cobb, Hawa Avraham, Bruce Meyer and Jim Woodgett for providing necessary reagents.
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FIGURE LEGENDS

Fig. 1. **c-Abl associates with HPK1.**  

**a.** Total cell lysates from Jurkat cells were subjected to immunoprecipitation with anti-c-Abl, anti-HPK1 or preimmune rabbit serum (PIRS). The protein precipitates were separated by SDS-PAGE and transferred to nitrocellulose filters. The filters were analyzed by immunoblotting with anti-c-Abl antibody.  

**b.** Jurkat cells were treated with 10 µM ara-C or exposed to 20 Gy ionizing radiation (IR) and harvested after 3 h. Cell lysates (approx. 150 µg total protein) were subjected to immunoprecipitation with anti-HPK1 and analyzed by immunoblotting with anti-c-Abl (upper panel). As control, anti-HPK1 immunoprecipitates were analyzed by immunoblotting with anti-HPK1 (lower panel). Total cell lysates (10 µg total protein; +ve) were also analyzed by immunoblotting with anti-c-Abl or anti-HPK1.

Fig. 2. **c-Abl associates with HPK1 via its SH3 domain.**  

**a.** 293T cells were transiently transfected with Flag-HPK1 in the presence of HA-c-Abl or empty vector. Lysates were subjected to immunoprecipitation with anti-HA and the precipitates were analyzed by immunoblotting with anti-Flag (upper panel). As a positive control (+ve), total cell lysate was also analyzed by immunoblotting with anti-Flag. As controls, anti-HA and anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-HA (middle panel) and anti-Flag (lower panel), respectively.
IgH: Immunoglobulin heavy chain. b. 293T cells were transiently transfected with Flag-HPK1 and HA-c-Abl. Thirty-six hours after the transfection cells were treated with 10 µM ara-C for 3 h. Total cell lysates were subjected to immunoprecipitation with anti-HA. The precipitates and lysate were analyzed by immunoblotting with anti-Flag (upper panel) or anti—HA (middle panel). As control, anti-Flag immunoprecipitates were also analyzed by immunoblotting with anti-Flag (lower panel). c. Jurkat cell lysates (150 µg total protein) were incubated with GST or GST-c-Abl SH3 fusion proteins. The protein adsorbates and lysate (10 µg total protein; +ve) were analyzed by immunoblotting with anti-HPK1. d. 293T cells were transiently transfected with Flag-HPK1 and total lysates were divided in three equal portions. GST-c-Abl SH3 fusion protein was incubated with proline-rich sequences 2 (PR2) (32), PR3 or PR4. The fusion protein-peptide mixtures were separately incubated with lysates for 1 h at 4°C. Following washing, bound proteins were analyzed by immunoblotting with anti-Flag.

Fig. 3. Kinase activities of c-Abl and HPK1 are required for their optimal interaction. a. Jurkat cells were treated with 10 µM ara-C for 6 h. Nuclear and cytoplasmic fractions were subjected to immunoprecipitation with anti-HPK1. The precipitates were analyzed by immunoblotting with anti-c-Abl.
Signal intensities from the anti-c-Abl immunoblotting experiments were analyzed by densitometric scanning. The data represent the fold increase in signal intensities compared with untreated controls. The results are expressed as the mean ± S.D. from three independent experiments. b. Jurkat cells were treated with 10 µM ara-C for the indicated times. Nuclear (left panels) and cytoplasmic (right panels) fractions were isolated and analyzed by immunoblotting with anti-c-Abl (upper panels), anti-Lamin A (left lower panel) or anti-β-actin (right lower panel) antibodies. c. 293T cells were transiently cotransfected with c-Abl and Flag-HPK1 or kinase-inactive mutant Flag-HPK1 M46. Following treatment of cells with ara-C, anti-c-Abl immunoprecipitates were analyzed by immunoblotting with anti-Flag (upper panel). As control, anti-c-Abl immunoprecipitates were analyzed by immunoblotting with anti-c-Abl (middle panel). Total cell lysates were also analyzed by immunoblotting with anti-Flag (lower panel). d. 293T cells were transiently cotransfected with Flag-HPK1 and c-Abl or dominant negative mutant c-Abl(K-R). Following treatment of cells with ara-C, anti-c-Abl immunoprecipitates were analyzed by immunoblotting with anti-Flag (upper panel). As control, anti-c-Abl immunoprecipitates were analyzed by immunoblotting with anti-c-Abl (middle panel). Total cell lysates were also analyzed by immunoblotting with anti-Flag (lower panel).
Fig. 4. **c-Abl phosphorylates HPK1 in vitro.**  

**a.** A schematic diagram displays various HPK1 constructs, including wild-type HPK1, the kinase domain (HPK1-KD; amino acids 1 to 291) and the carboxy terminal domain (HPK1-CD; amino acids 292 to 833).  

**b.** (left panel) GST-HPK1-KD (KD) and GST-HPK1-CD (CD) fusion proteins were separately incubated with purified c-Abl in the presence of [\(\gamma^{-32}P\)]ATP. As control, GST-Crk 120-225 fusion protein was separately incubated with purified c-Abl in the presence of [\(\gamma^{-32}P\)]ATP. Following kinase reactions, the products were analyzed by SDS-PAGE and autoradiography (left panel). The GST-HPK1-KD and GST-HPK1-CD proteins were visualized by commassie blue staining (not shown). 293T cells were transiently transfected with Flag-HPK1 wild-type. Lysates were subjected to immunoprecipitation with anti-Flag, and the precipitates were incubated with recombinant c-Abl or recombinant c-Abl(K-R) proteins in the presence of [\(\gamma^{-32}P\)]ATP. As control, GST-Crk 120-225 fusion protein was incubated with purified c-Abl in the presence of [\(\gamma^{-32}P\)]ATP. The phosphorylated products were analyzed by SDS-PAGE and autoradiography (right panel).  

**c.** 293T cells were transfected with Flag-HPK1 (1 \(\mu\)g) and increasing concentrations (0, 2, 4 and 8 \(\mu\)g) of c-Abl. Cell lysates were subjected to immunoprecipitation with anti-Flag and the precipitates were analyzed by immunoblotting with anti-P-Tyr.
Fig. 5. **c-Abl-mediated tyrosine phosphorylation of HPK1 in response to genotoxic stress.**  
**a.** Jurkat cells were treated with 20 Gy ionizing radiation and harvested after different times. Total cell lysates were subjected to immunoprecipitation with anti-HPK1 and the precipitates were analyzed by immunoblotting with anti-P-Tyr (upper panel) or anti-HPK1 (lower panel).  
**b.** MCF-7/neo and MCF-7/c-Abl(K-R) cells were transiently transfected with Flag-HPK1 and treated with 10 µM ara-C for 3h or exposed to 20 Gy IR and harvested after 3h. Total cell lysates were subjected to immunoprecipitation with anti-Flag and analyzed by immunoblotting with anti-P-Tyr (upper panel) or anti-Flag (middle panel). **The bottom panel depicts the fold increase in tyrosine phosphorylation and expressed as the mean ± S.D. from three independent experiments.**

Fig. 6. **c-Abl activates HPK1.**  
**a.** 293T cells were transfected with Flag-HPK1 (1 µg) in the presence of different concentrations (0, 1, 2, and 5 µg) of c-Abl or empty vector. Total cell lysates were subjected to immunoprecipitation with anti-Flag and assayed for phosphorylation of MBP in the presence of [γ-32P]ATP. The reaction products were separated by SDS-PAGE and analyzed by autoradiography (upper panel). **The fold-increase in MBP phosphorylation is shown in the bottom panel.**
phosphorylation is described as the mean of three independent experiments. As control, total lysates were also analyzed by immunoblotting with anti-Flag (middle panel) or anti-c-Abl (lower panel). b. 293T cells were transiently cotransfected with HA-c-Abl (1 µg) in the presence of increasing concentrations (0, 1, 2 and 5 µg) of HPK1 or empty vector. Following transfection, anti-c-HA immunoprecipitates were assayed for phosphorylation of GST-Crk 120-225 in the presence of [γ-32P]ATP. The reaction products were separated by SDS-PAGE and analyzed by autoradiography (upper panel). As control, total lysates were also analyzed by immunoblotting with anti-Flag (middle panel) or anti-HA (lower panel).

Fig. 7. Genotoxic agents induced activation of HPK1. a. Jurkat cells were treated with 10 µM ara-C and harvested at 3 h. Total cell lysates were subjected to immunoprecipitation with anti-HPK1. The immunoprecipitates were analyzed in immune complex kinase assays using MBP as a substrate (upper panel). As control, total cell lysates were analyzed by immunoblotting with anti-HPK1 (lower panel). The fold-increase in MBP phosphorylation is described as the mean of two independent experiments. b. Jurkat cells were exposed to 20 Gy IR and harvested after 3 h. Total cell lysates were subjected to immunoprecipitation with anti-HPK1 and assayed as described.
above.  

**c.** Jurkat cells were treated with 10 µM ara-C and harvested after 3 h. Nuclear (left panels) and cytoplasmic (right panels) lysates were subjected to immunoprecipitation with anti-HPK1. The immunoprecipitates were analyzed in immune complex kinase assay using MBP as a substrate (upper panels). As control, total cell lysates were analyzed by immunoblotting with anti-HPK1 (lower panels).  

**d.** 293T cells were transiently transfected with Flag-HPK1 and empty vector, c-Abl or c-Abl(K-R) in the presence of Flag-HPK1. Following treatment of cells with ara-C for 3 h, anti-Flag immunoprecipitates were assayed for phosphorylation of MBP (upper panel). As control, anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-Flag (middle panel). Total cell lysates were also analyzed by immunoblotting with anti-c-Abl (lower panel).  

**e.** 293T cells were transiently transfected with empty vector, Flag-HPK1 or Flag-HPK1 M46. Following treatment of cells with ara-C, anti-Flag immunoprecipitates were assayed for phosphorylation of MBP (upper panel). As control, total cell lysates were analyzed by immunoblotting with anti-Flag (lower panel).  

**f.** 293T cells were transiently transfected with Flag-HPK1. Following exposure of cells with IR for different time intervals, anti-Flag immunoprecipitates were assayed for phosphorylation of MBP (upper panel). As control, total cell lysates were separately analyzed by immunoblotting with anti-Flag (lower panel).
Fig. 8. c-Abl and HPK1 co-operate to synergistically activate JNK. a and b. Jurkat cells were either treated with 10 μM ara-C (a) or exposed with 20 Gy IR (b) and harvested after indicated times. Total cell lysates were subjected to immunoprecipitation with anti-SAPK. The immunoprecipitates were analyzed in immune complex kinase assay using GST-c-Jun as a substrate (upper panels). As control, total cell lysates were analyzed by immunoblotting with anti-SAPK (lower panels). c. 293T cells were transiently transfected in duplicate with the indicated cDNAs. Total cell lysates were subjected to protein precipitation with glutathione-beads. The adsorbates were assayed in immune complex kinase assay using GST-c-Jun as a substrate (upper panel). As control, total lysates were separately analyzed by immunoblotting with anti-SAPK (middle panel). The bottom panel depicts the fold increase in GST-c-Jun phosphorylation and expressed as the mean ± S.D of two independent experiments performed in duplicate.
b.

**Nucleus**

| Jurkat | ara-C | 3h | 6h |
|--------|-------|----|----|
|        |       |    |    |

- **IB:** anti-c-Abl
- **IB:** anti-Lamin A

**Cytoplasm**

| Jurkat | ara-C | 3h | 6h |
|--------|-------|----|----|
|        |       |    |    |

- **IB:** anti-c-Abl
- **IB:** anti-β-Actin

c.

| HPK1M46 | HPK1 | c-Abl |
|---------|------|-------|
| -       | +    | +     |
| +       | -    | +     |
| +       | +    | +     |

- **IP:** anti-c-Abl
- **IB:** anti-Flag
- **IB:** anti-c-Abl

| Flag-HPK1 |
|-----------|

| HPK1(K-R) | c-Abl |
|-----------|-------|
| -         | +     |
| +         | -     |
| +         | +     |

- **IP:** anti-c-Abl
- **IB:** anti-Flag
- **IB:** anti-c-Abl

| Flag-HPK1 |
|-----------|
### a.

|      | 1   | 1   | 1   | 1   |
|------|-----|-----|-----|-----|
| 0    | 1   | 2   | 5   |     |
| 5    | 4   | 3   | 0   |     |
| **Fold:** 1.0 2.1 2.5 3.2 |

**Flag-HPK1**

**c-Abl vector**

**MBP**

**IB:** anti-Flag

**c-Abl**

**IB:** anti-c-Abl

### b.

|      | 1   | 1   | 1   | 1   |
|------|-----|-----|-----|-----|
| 0    | 1   | 2   | 5   |     |
| 5    | 4   | 3   | 0   |     |
| **Fold:** 1.0 1.1 0.9 1.2 |

**HA-c-Abl**

**Flag-HPK1 vector**

**GST-Crk**

**IB:** anti-Flag

**Flag-HPK1**

**IB:** anti-HA

**HA-c-Abl**
a. Jurkat cells were treated with ara-C for 1h, 3h, and 6h. Western blots show an increase in GST-c-Jun and SAPK levels with time.

b. Jurkat cells were irradiated (IR) for 1h, 3h, and 6h. Western blots show an increase in GST-c-Jun and SAPK levels with time.

c. A table showing the expression levels of various proteins under different conditions. The proteins include c-Abl(K-R), HPK1M46, c-Abl, HPK1, SEK1, and SAPK. The lanes indicate the time points (1-7) for each condition.

A bar graph shows the fold change in GST-c-Jun phosphorylation in each lane, with lane 7 showing the highest increase.
Interaction of hematopoietic progenitor kinase 1 (HPK1) and c-ABL tyrosine kinase in response to genotoxic stress
Yasumasa Ito, Pramod Pandey, Pradeep Sathyanarayana, Pin Ling, Ajay Rana, Ralph Weichselbaum, Tse-Hua Tan, Donald Kufe and Surender Kharbanda

J. Biol. Chem. published online January 30, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M007294200

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