Regulation of cell migration and survival by focal adhesion targeting of Lasp-1

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Large-scale proteomic and functional analysis of isolated pseudopodia revealed the Lim, actin, and SH3 domain protein (Lasp-1) as a novel protein necessary for cell migration, but not adhesion to, the extracellular matrix (ECM). Lasp-1 is a ubiquitously expressed actin-binding protein with a unique domain configuration containing SH3 and LIM domains, and is overexpressed in 8–12% of human breast cancers. We find that stimulation of nonmotile and quiescent cells with growth factors or ECM proteins facilitates Lasp-1 relocalization from the cell periphery to the leading edge of the pseudopodium, where it associates with nascent focal complexes and areas of actin polymerization. Interestingly, although Lasp-1 dynamics in migratory cells occur independently of c-Abl kinase activity and tyrosine phosphorylation, c-Abl activation by apoptotic agents specifically promotes phosphorylation of Lasp-1 at tyrosine 171, which is associated with the loss of Lasp-1 localization to focal adhesions and induction of cell death. Thus, Lasp-1 is a dynamic focal adhesion protein necessary for cell migration and survival in response to growth factors and ECM proteins.

Introduction

The direction of cell migration is controlled by chemokine and ECM gradients, and is exhibited during wound healing, angiogenesis, embryonic development, and immune function (Lauffenburger and Horwitz, 1996). Cells respond by local activation and amplification of signaling events on the side facing the attractant (Parent and Devreotes, 1999). This facilitates localized actin polymerization leading to morphological polarity and establishment of a dominant-leading pseudopodium and rear cell body compartment (Lauffenburger and Horwitz, 1996; Parent and Devreotes, 1999). Interestingly, the initial protrusion of a pseudopodium at the cell surface is independent of integrins and the ECM (Wyckoff et al., 2000). However, integrins tether the extending membrane to the substratum, which supports sustained and directional growth of a single dominant pseudopodium. Indeed, a pseudopodium that does not attach to the ECM rapidly retracts back to the cell body (Bailly et al., 1998). This suggests that formation of new integrin focal complexes at the leading front of the extending membrane provides necessary signals to fine-tune and maintain directional growth, while suppressing retraction mechanisms. Cell movement then commences as the cell undergoes repeated cycles of membrane extension and integrin ligation at the front and cell body retraction at the rear (Lauffenburger and Horwitz, 1996). The specific signaling proteins that regulate the spatial adhesive changes necessary for morphological polarity and directional cell translocation are poorly understood.

To help unravel the spatiotemporal organization of signaling cascades involved in cell polarization, including mechanisms of protein translocation, activation, post-translational modifications, and formation of complex multi-protein scaffolds, we developed a biochemical method to selectively isolate the pseudopodium and cell body of cells polarized toward a chemoattractant gradient using a microporous filter system (Cho and Klemke, 2002; Brahmbhatt and Klemke, 2003). Pseudopodia extension in this system, like traditional pseudopodia formation on two-dimensional surfaces, requires Cdc42 and Rac activity and shows normal actin cytoskeletal organization and focal adhesions. Cells extend pseudopodia projections through small openings in the vasculature and

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Abbreviations used in this paper: Lasp-1, Lim, actin, and SH3 domain; MudPIT, multidimensional protein identification technology; SH3, Src homology domain 3; siRNA, small interfering RNA; TSA, trichostatin A.

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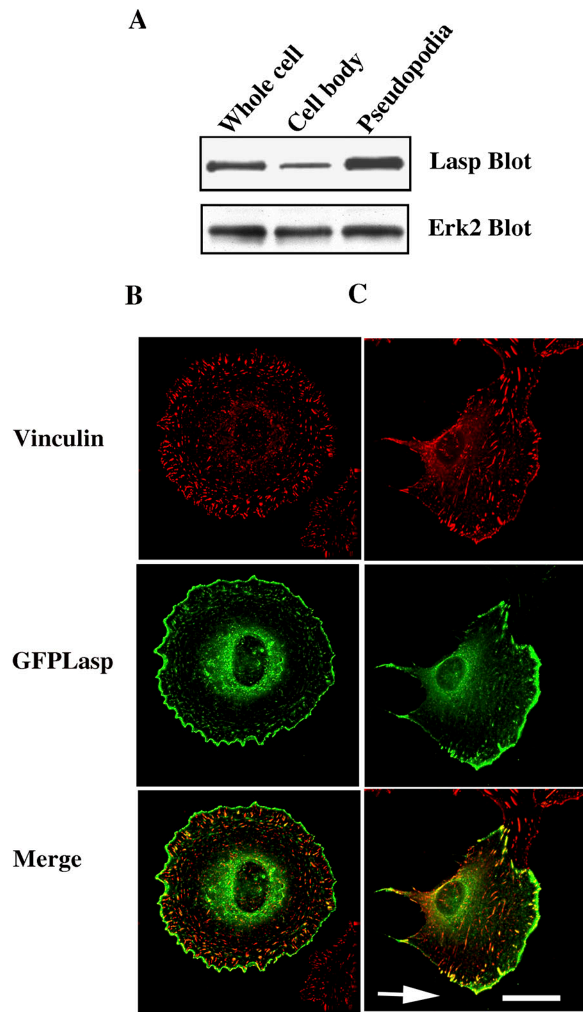

Figure 1. **Subcellular localization of Lasp-1 in spreading and migrating cells.** (A) Pseudopodia and cell body fractions were purified as described in Materials and methods. 10 μg of proteins were analyzed for relative expression of Lasp-1 by Western blotting using anti-Lasp-1 and anti-Erk2 antibodies, which served as a loading control. (B) Deconvolution images of NIH 3T3 cells transfected with GFP Lasp. Cells were allowed to attach and spread on fibronectin-coated coverslips for 120 min or (C) to migrate into a wound for 18 h. Cells were fixed and stained with anti-vinculin antibodies to visualize focal adhesions. Merged images show colocalization (yellow) of focal adhesions (red) and GFP Lasp (green). Note that GFP Lasp strongly localized to focal adhesions proximal to the cell edge at the leading front of the pseudopodium. Arrow indicates the direction of cell migration. Bar, 28 μm.

ECM in vivo as a necessary process of immune cell intravasation as well as pathological processes associated with cancer cell metastasis (Wyckoff et al., 2000). Therefore, this model recapitulates physiological events associated with cell migration and is ideal for unraveling the spatial and temporal signaling mechanisms responsible for focal adhesion changes leading to cell polarity and directional movement.

In this report, we used this technique along with a new protein sequencing method called multidimensional protein identification technology (MudPIT; Washburn et al., 2001) for rapid and large-scale proteome analysis of purified pseudopodia. By using multidimensional liquid chromatography, tandem mass spectrometry, and database searching with SEQUEST algorithm, it is possible to identify large numbers of proteins (>1,000) directly from a complex protein lysate (Washburn et al., 2001). Here, we isolated pseudopodia and cell body proteins for comparison by MudPIT to reveal proteins uniquely present in these different cellular compartments. The novel cytoskeletal-associated Lim, actin, and SH3 protein (Lasp-1; GenBank/EMBL/DDBJ accession no. X82456) was identified as a component of the pseudopodium and further characterized for functional significance. Lasp-1 was initially identified from a breast cancer–derived metastatic lymph node cDNA library and is overexpressed in 8–12% of breast cancer (Tomasetto et al., 1995a,b). It is a ubiquitously expressed actin-binding protein with a unique domain configuration (Tomasetto et al., 1995a; Schreiber et al., 1998) that includes a LIM domain (*Lin*11, *Isl*-1, and *Mec*-3) in the NH_2-terminal region followed by two actin-binding repeats (R1, R2), and an Src homology 3 (SH3) domain in the COOH-terminal region. There are also two tyrosine phosphorylation sites (Y52 and Y152) corresponding to SH2-binding consensus motifs (YXXP; Tomasetto et al., 1995a), suggesting that Lasp-1 is tyrosine phosphorylated and may facilitate binding of SH2 effector proteins and their downstream signals. However, the biological function and regulation of this molecule has not yet been identified. Our findings demonstrate that Lasp-1 is a dynamic, spatially regulated protein necessary for cell migration. Furthermore, we show that the cytoskeletal regulatory

Table I. **Proteins identified in purified pseudopodia**[a]

Cytoskeletal-associated proteins	Number of identified peptides
α-Actinin	7
Tropomyosin 1	16
β-4-Tubulin	10
β-Actin	29
Transgelin 2	9
Lim and SH3 protein (Lasp-1)	1
Myosin 1B and C	1
Cofilin 1 and 2	1
Arp1 and 3	1
Caldesmon	1
Drebrin 1, isoform a and b	1
Filamin	1
Fascin	1
Adenomatous polyposis coli–binding protein EB1	1
Signaling proteins	
Rho guanine nucleotide exchange factor 1	1
PI 3-kinase–related kinase SMG-1	1
Guanine nucleotide binding protein, β-type	1
RAB5A and C	1
A-kinase anchor protein 3	1
Oligophrenin 1, Rho-activating protein	1
Ca^{++}/calmodulin-dependent protein kinase 2 delta subunit	1
Insulin receptor substrate 1	1
Neuregulin 1: c-neu receptor	1

[a]Complete list of all identified peptides and identified proteins in pseudopodia and cell body fractions is shown in Tables S1–S4 and Fig. S1 (available at http://www.jcb.org/cgi/content/full/jcb.200311045/DC1).

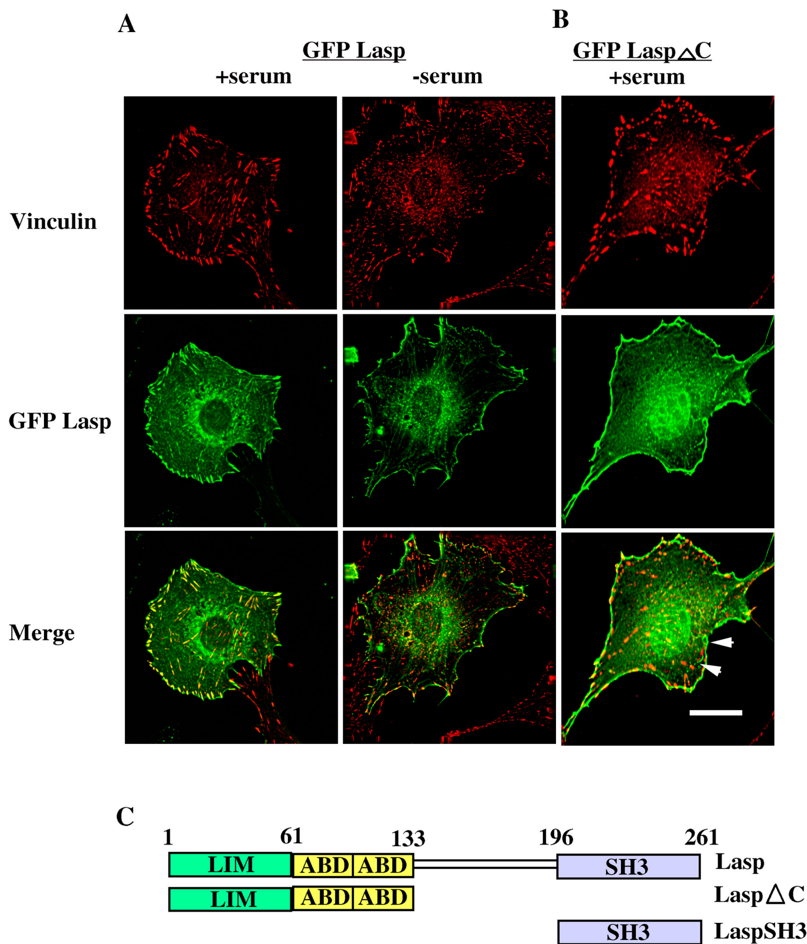

Figure 2. **Lasp-1 is a dynamic protein that transits from the cell periphery to focal adhesions upon stimulation with growth factors.** (A) Deconvolution images of NIH 3T3 cells transfected with GFP Lasp and stained for vinculin-positive focal adhesions as described in Materials and methods. Cells were serum starved overnight (−serum) and stimulated with 10% FBS for 15 min. Merged images represent colocalization (yellow) of focal adhesions (red) and GFP Lasp (green). (B) GFP Lasp with its COOH-terminal region truncated (GFP LaspΔC) was examined for localization to focal adhesions upon serum stimulation as described in Materials and methods. Arrows indicate membrane ruffles. GFP LaspΔC cells without serum showed a similar pattern of expression as of cells with serum (not depicted). (C) Schematic representation of the LaspΔC and SH3 domain mutants (LaspSH3) used in this experiment and the experiments shown in Fig. 4 C. Bar, 28 μm.

protein c-Abl tyrosine kinase (Woodring et al., 2003) directly phosphorylates Lasp-1, which regulates its localization to focal adhesions in apoptotic (but not migratory) cells.

Results

Characterization of the pseudopodial proteome and identification of Lasp-1

The ability to differentially isolate proteins from the leading pseudopodium and cell body and the recent development of MudPIT technology allowed us undertake large-scale proteomics to characterize the pseudopodium and cell body proteomes. Five independent cell body and pseudopodial fractions were prepared and examined by MudPIT. From the tandem mass spectroscopy data, we generated 4,000 peptides leading to the identification of 980 total proteins. Although mass spectroscopy and MudPIT systems are limited in their ability to directly quantify proteins, they can be used to identify relative differences in protein profiles of two separate protein samples because an enriched protein will result in higher peptide identification. We considered a protein to be enriched if at least one peptide was identified in a fraction or at least 50% more peptides were sequenced in a given fraction. Comparison of the 980 proteins using DTA-select and Contrast software (Tabb et al., 2002) revealed 119 enriched proteins in the pseudopodial fraction and 203 enriched proteins in the cell body component. Notable proteins previously shown to be enriched in the pseudopodium of cells are shown in Table I, demonstrating the feasibility of this approach (a comparative list of all pseudopodia and cell body–associated proteins is shown in Tables S1–S4 and Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200311045/DC1). Of the proteins identified, Lasp-1 was further characterized for functional significance. Western blot analysis and densitometry confirmed that Lasp-1 protein was increased (approximately ninefold) in the pseudopodium compared with the cell body (Fig. 1 A). A Lasp-1 GFP fusion also revealed striking localization to the leading pseudopodial edge of spreading and migrating cells (Fig. 1, B and C). A similar pattern of Lasp-1 localization was observed using immunofluorescent staining and Lasp-1 antibodies (unpublished data; see also Chew et al., 2002). These findings confirm the purification and identification of this protein from the MudPIT system and demonstrate its localization to the leading pseudopodium of polarized cells.

Cell adhesion and exposure to growth factors induce the translocation of Lasp-1 from the cell periphery to focal complexes in migrating cells

It is notable that GFP Lasp colocalized to vinculin-positive focal adhesions primarily at the leading edge of the spreading membrane and was either absent or reduced in focal adhesions in the cell body proper (Fig. 1). In nonmotile serum-starved cells, Lasp-1 localized to the peripheral edge of

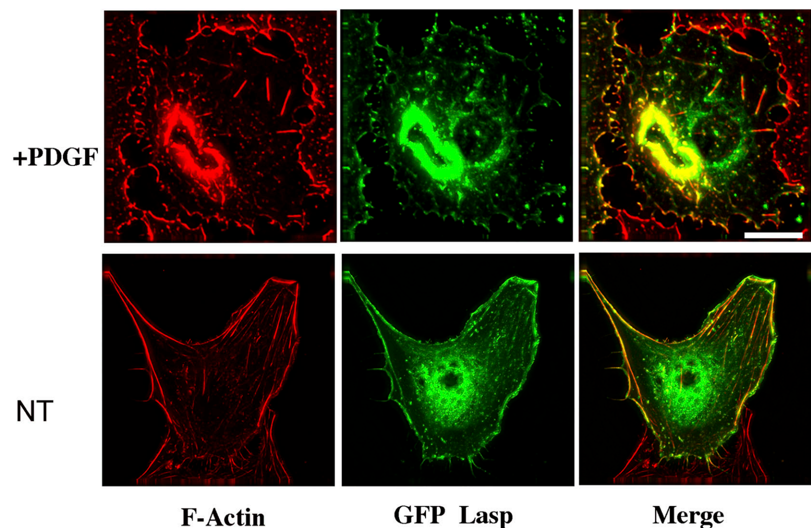

Figure 3. **Lasp-1 localizes to cortical F-actin and actin cables in quiescent cells and dorsal membrane ruffles in serum stimulated cells.** Deconvolution images of NIH 3T3 cells transfected with GFP Lasp and stained for F-actin with rhodamine-phalloidin. Cells were serum starved overnight (NT) and then stimulated with PDGF-BB for 30 min. Merged images represent colocalization (yellow) of F-actin (red) and GFP Lasp (green). Bar, 28 μm.

cells, where it colocalized with cortical actin structures and weakly with F-actin cables (Fig. 2 A and Fig. 3), but not focal adhesions. However, exposure of these cells to growth factors to activate cell migration caused the rapid (1–2 min) relocalization of GFP Lasp from the cell periphery to focal adhesions (Fig. 2 A). At later times after stimulation (>15 min), GFP Lasp strongly localized to actin-rich membrane ruffles on the cell surface (Fig. 3).

Importantly, truncation of the COOH-terminal region of Lasp-1 (GFP LaspΔC) prevented translocation of Lasp-1 to focal adhesions (Fig. 2 B), but not to membrane ruffles, actin cables, or cortical actin structures in response to serum (Fig. 2 B) or cell spreading on the ECM (unpublished data). Thus, the COOH terminus of Lasp-1 is specifically responsible for translocation and targeting to focal adhesions, but not ruffles, in response to ECM proteins and growth factors. Expression of GFP fused to either the LIM or SH3 domains showed only diffuse cytoplasmic staining, suggesting these domains are not sufficient by themselves to target Lasp-1 to actin or focal adhesion structures under these conditions (unpublished data).

Lasp-1 is necessary for cell migration (but not adhesion) to the ECM

Although the function of Lasp-1 is not known, the above findings suggest that it is important for cell adhesion and migration on the ECM. To directly examine this possibility, we depleted cells of Lasp-1 using small interfering RNA (siRNA) technology (Elbashir et al., 2001). Cells exposed to anti-Lasp-1 siRNA showed an ~80–90% reduction in Lasp-1 protein compared with cells mock treated with control siRNA directed to GL2 luciferase. Total protein staining and Western blotting for either Lasp-1 or actin revealed that only Lasp-1 was reduced and not other cellular proteins (Fig. 4 A). Interestingly, although cells depleted of Lasp-1 showed little difference in adhesion and spreading, they did show a significantly impaired ability to migrate on the ECM (Fig. 4, A and B). These findings indicate that Lasp-1 protein is necessary for cell migration, but not cell adhesion and spreading. Similar findings were obtained with NIH 3T3 fibroblast cells depleted of Lasp-1 protein (unpublished data). Interestingly, Lasp-1 expression has been reported to be increased in metastatic breast cancers, suggesting that protein amplification may contribute to the migratory properties of these cells (Tomasetto et al., 1995b). To investigate this, we ectopically expressed Lasp-1 in cells and monitored their ability to migrate in response to growth factors. Surprisingly, Lasp-1 amplification inhibited basal and growth factor–stimulated cell migration (Fig. 4 C) without affecting attachment to the ECM (unpublished data). Similar findings were obtained with HEK 293 (Fig. 4 D) and MCF-7 cells (unpublished data). Importantly, expression of LaspΔC failed to inhibit cell migration or adhesion, indicating that the COOH-terminal region of Lasp-1 is required for this process. Expression of the SH3 domain alone strongly inhibited cell migration, which supports this notion (Fig. 4 C). It is not yet clear why both depletion and amplification of Lasp-1 inhibits cell migration. However, it is possible that global amplification of Lasp-1 throughout the cell body disrupts signaling polarity and the normal restricted localization of this protein, as has been shown for Cdc42 (Allen et al., 1998). In this regard, disruption of signaling polarity by constitutive relocalization of Lasp-1 to the plasma membrane caused dramatic membrane blebbing and detachment from the ECM leading to cell death, whereas targeting of LaspΔC to the membrane failed to induce this response (Fig. 4 E). In any case, these findings demonstrate that Lasp-1 plays an important spatial role in cell migration, and that this process is regulated by the COOH-terminal region of Lasp-1 containing the SH3 domain.

Abl kinase associates with the SH3 domain of Lasp-1 and phosphorylates tyrosine 171 in vivo

Next, we performed a database and motif screen for potential regulatory proteins associated with Lasp-1 using the SH3 domain and putative tyrosine phosphorylation sites in the COOH terminus as targets. Lasp-1 was found to contain a conserved Abl consensus phosphorylation sequence at tyrosine 171 (VYxxP) and an SH3 domain that is predicted to bind to the proline-rich region of Abl family kinase (Liu et al., 1996; Wang et al., 1997; Barila et al., 2000). Furthermore, the fact that Abl and Arg regulate the actin cytoskele-

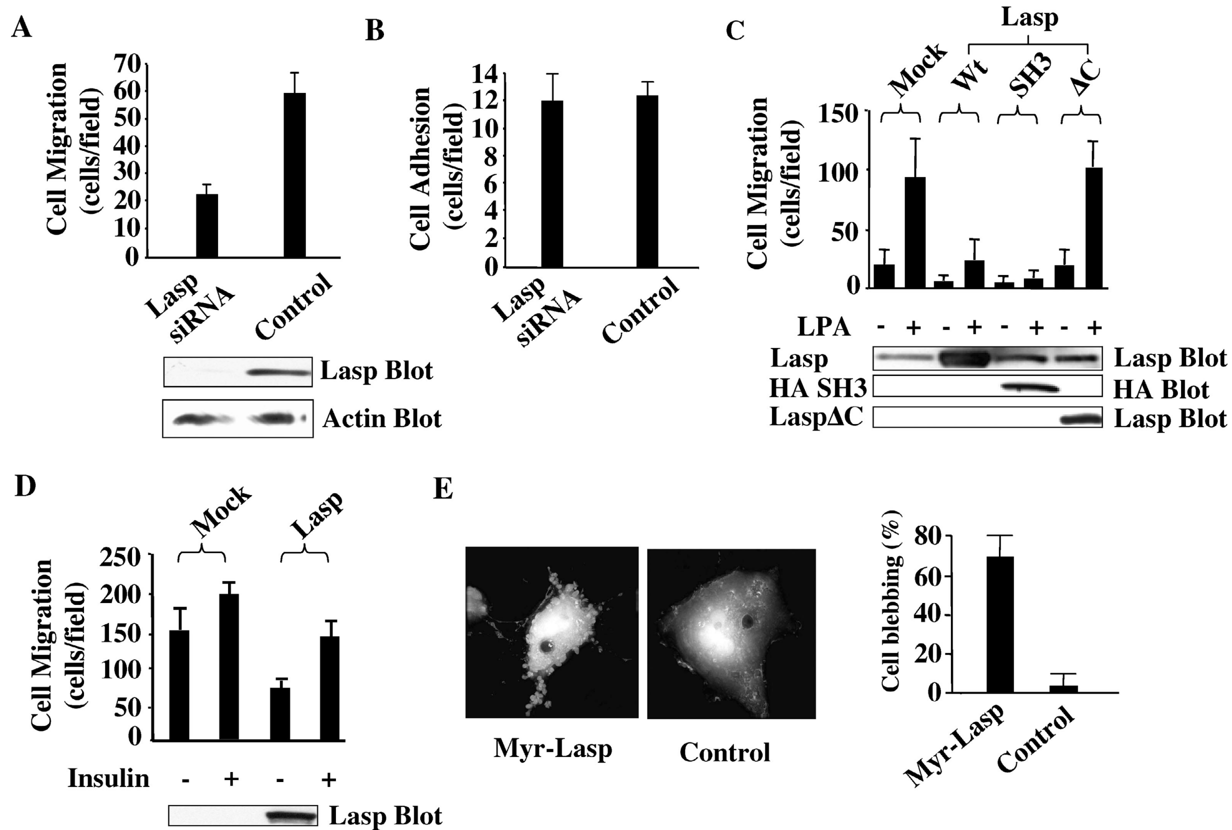

Figure 4. **Lasp-1 is necessary for cell migration, but not adhesion to the ECM.** (A) Cos-7 cells were depleted of endogenous Lasp-1 protein using siRNA specific for Lasp-1 or control siRNA to GL2 luciferase. Cells were allowed to either migrate using Boyden chambers coated on the bottom with 10 μg/ml fibronectin or (B) to attach to culture dishes coated with the same ECM as described in Materials and methods. The number of migratory or adherent cells per microscopic field was counted. Each bar represents the mean ± SEM of cells in triplicate migration/adhesion chambers of three independent experiments. An aliquot of cell lysates prepared from cells treated as described above were Western blotted for expression of Lasp-1 or actin, which served as a loading control. (C) COS-7 cells were transfected with the empty vector (Mock), wild-type Lasp-1 (Wt), LaspΔC (ΔC), or the SH3 domain tagged with HA (SH3) along with a β-galactosidase reporter construct to mark transfected cells. Cells were allowed to chemotax with or without LPA in the lower chamber for 3 h. Migratory cells per microscopic field on the underside of the membrane were stained and counted. Each bar represents the mean ± SEM of blue cells in triplicate migration chambers of three independent experiments. An aliquot of cell lysates prepared from cells treated as described above were Western blotted for the expression of the appropriate protein or tag as indicated. (D) HEK 293 cells stably expressing the empty vector (mock) or the vector encoding full-length Lasp-1 were tested for chemotaxis with or without 10 μg/ml insulin in the lower chamber as indicated in C. Lasp-1 protein was Western blotted to confirm the protein expression. HEK 293 cells show low levels of endogenous Lasp-1 protein, which is not detected in the short exposure time to the film. (E) Deconvolution images of COS-7 cells transfected with either Lasp-1 or LaspΔC fused with the membrane-targeting sequence (myr) along with a GFP reporter construct. Cells were then fixed and examined for membrane blebs 16 h after transfection, and the number of transfected cells with prominent blebs were counted per microscopic field relative to the total number of GFP-positive cells.

ton, focal adhesions, and cell migration (Woodring et al., 2003) suggests these proteins may be part of a common signaling pathway. To begin to determine whether Abl and Lasp-1 associate in vivo, cells were transfected with HA-tagged Abl and full-length Lasp-1. HA-tagged Abl was then immunoprecipitated from these cells and immunoblotted for the presence of Lasp-1. In the reciprocal experiment, GST Lasp was precipitated from transfected cells and immunoblotted for HA-Abl. In both cases, Abl associated with and promoted increased tyrosine phosphorylation (>25-fold) of Lasp-1 in cells (Fig. 5 A). Tyrosine phosphorylation of Lasp-1 was accompanied by reduced mobility in SDS-PAGE, as indicated by the appearance of a higher molecular weight form of Lasp-1. This was also the case when purified activated Abl was allowed to phosphorylate bacterial purified Lasp-1 in vitro (Fig. 5 E). Importantly, LaspΔC without the Abl binding and phosphorylation site Y171 failed to associate with Abl and showed no tyrosine phosphorylation as the result of Abl activation (Fig. 5, A and B). As expected, Abl with a truncated proline-rich domain (AblΔPro; Smith et al., 1999) failed to interact and phosphorylate Lasp-1 in cells (Fig. 5 C). Wild-type Abl and AblΔPro become auto-activated in cells due to protein amplification as previously reported (Smith et al., 1999; Kain and Klemke, 2001; Kain et al., 2003). In this regard, the constitutively activated oncogenic form of Abl (Bcr-Abl; Daley et al., 1990) tyrosine phosphorylates GST Lasp in cells (Fig. 5 D). K562 leukemic cells derived from a CML patient with endogenous Bcr-Abl (Grosveld et al., 1986) also show increased Lasp-1 tyrosine phosphorylation (unpublished data). As expected, exposure of cells to the Abl kinase inhibitor STI 571 (Buchdunger et al., 1996) inhibited Lasp-1 tyrosine phosphorylation, indicating that kinase activity is necessary for this event (Fig. 5 D). Most importantly, substitution of tyrosine 171 to phe-

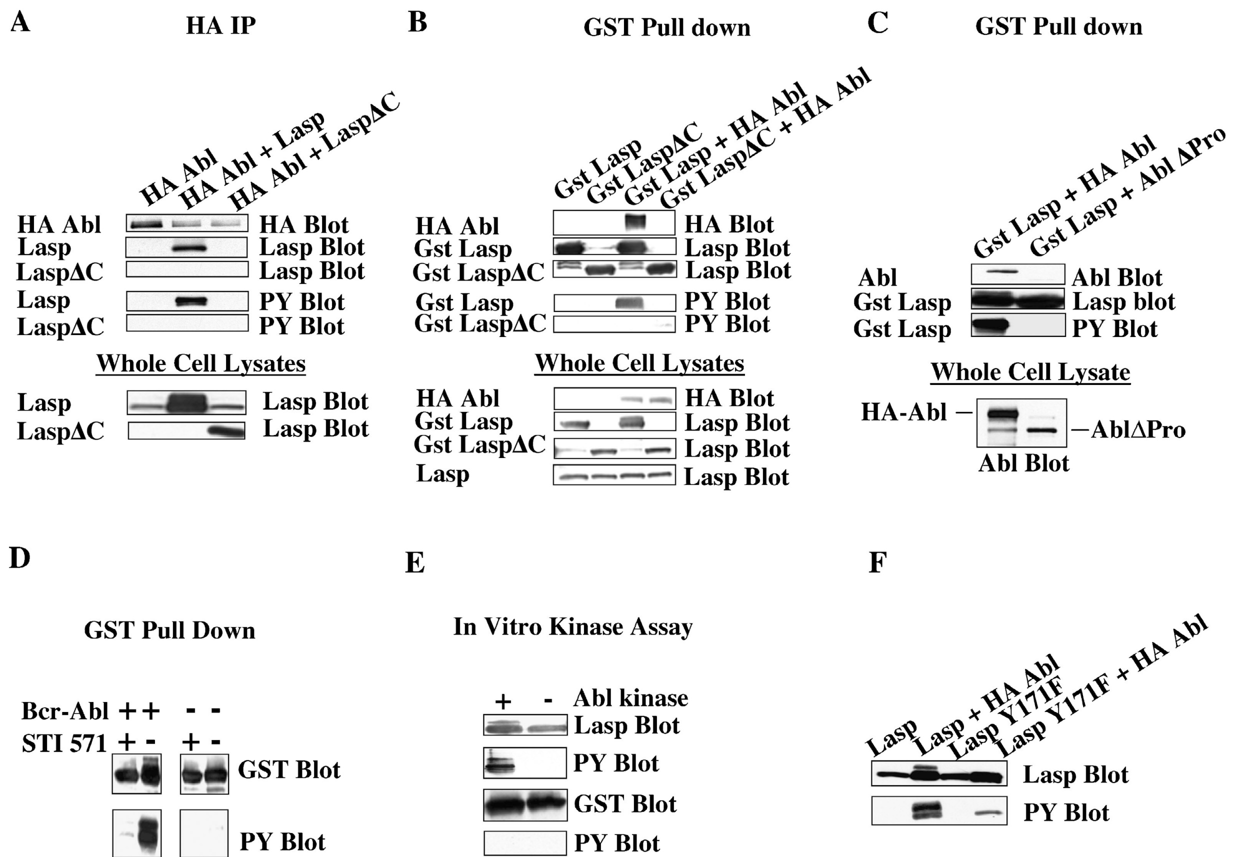

Figure 5. **Abl kinase associates with the COOH terminus of Lasp-1 and tyrosine phosphorylates Y171.** (A) COS-7 cells were transfected with HA-Abl together with Lasp-1 or LaspΔC. HA-Abl was immunoprecipitated and Western blotted for associated Lasp-1 protein with anti-Lasp-1 antibodies. The blot was then stripped and reprobed for phosphotyrosine (PY). Western blots of whole-cell lysates are included to confirm expression of the appropriate protein (bottom). (B) GST-tagged Lasp or GST LaspΔC was precipitated with glutathione beads and Western blotted for associated Abl protein with anti-HA antibodies from COS-7 cells expressing these constructs. Blots were stripped and reprobed for PY as indicated above, and whole-cell lysates were included to confirm protein expression. (C) GST Lasp was precipitated and Western blotted for associated Abl or Abl without its polyproline domain (AblΔPro) using anti-Abl antibodies. The blot was then stripped and blotted for PY to detect Lasp-1 phosphorylation. The expression levels of wild-type Abl and AblΔpro in whole-cell lysates are shown below. (D) Bcr-Abl–transformed NIH 3T3 cells (+) and mock control NIH 3T3 cells (−) transfected with GST Lasp were starved and treated with 2 μM STI 571 or buffer only for 16 h. GST Lasp was precipitated and Western blotted using anti-phosphotyrosine antibodies and then was stripped and reprobed with GST-specific antibodies. (E) Purified activated Abl was incubated with bacterial purified GST Lasp in the presence of ATP and kinase reaction buffer or purified GST alone as a control. Western blotting with phosphotyrosine, GST, or Lasp-1 was performed as indicated. (F) Cos-7 cells with or without HA-Abl were transfected along with Lasp-1 or Lasp with the Abl consensus tyrosine phosphorylation site Y171 substituted with phenylalanine (LaspY171F). Lasp-1 from these cells was Western blotted using anti-phosphotyrosine or Lasp-1-specific antibodies.

nylalanine (LaspY171F) prevented Lasp-1 tyrosine phosphorylation and mobility shift by Abl activation (Fig. 5 F). The remaining phosphoprotein band seen in the LaspY171F and HA-Abl cells likely represents endogenous Lasp-1 phosphorylated by Abl. These findings demonstrate the in vivo association of Abl with the COOH-terminal region of Lasp-1, and confirms tyrosine 171 is the site of phosphorylation induced by Abl activation in cells.

Abl specifically phosphorylates Lasp-1 in apoptotic (but not migratory) cells, and this prevents Lasp-1 translocation to focal complexes

Abl is activated by exposure of cells to growth factors and through integrin activation processes that occur during cell spreading on the ECM (Lewis et al., 1996; Plattner et al., 1999). Like Lasp-1, Abl is a dynamic protein that translocates to focal adhesions and membrane ruffles under these conditions (Woodring et al., 2003). Therefore, we investigated whether Lasp-1 was tyrosine phosphorylated in cells treated with growth factors (FBS, EGF, and PDGF-BB) or in cells spreading on the ECM. However, although these stimuli caused Lasp-1 translocation to focal adhesions and membrane ruffles (Figs. 1–3), they did not induce tyrosine phosphorylation of Lasp-1 (unpublished data). Furthermore, STI 571 did not inhibit Lasp-1 translocation to focal complexes under these conditions (see Fig. 7). Together, these findings demonstrate that Abl activation and tyrosine phosphorylation of Lasp-1 by themselves do not play a primary role in Lasp-1 localization to actin structures and focal adhesions under these conditions.

Interestingly, Abl also induces cell apoptosis in response to DNA-damaging agents (cisplatin; Gong et al., 1999) and oxidative stress (H_2O_2; Sun et al., 2000). Although the mechanisms are not yet understood, the death process in-

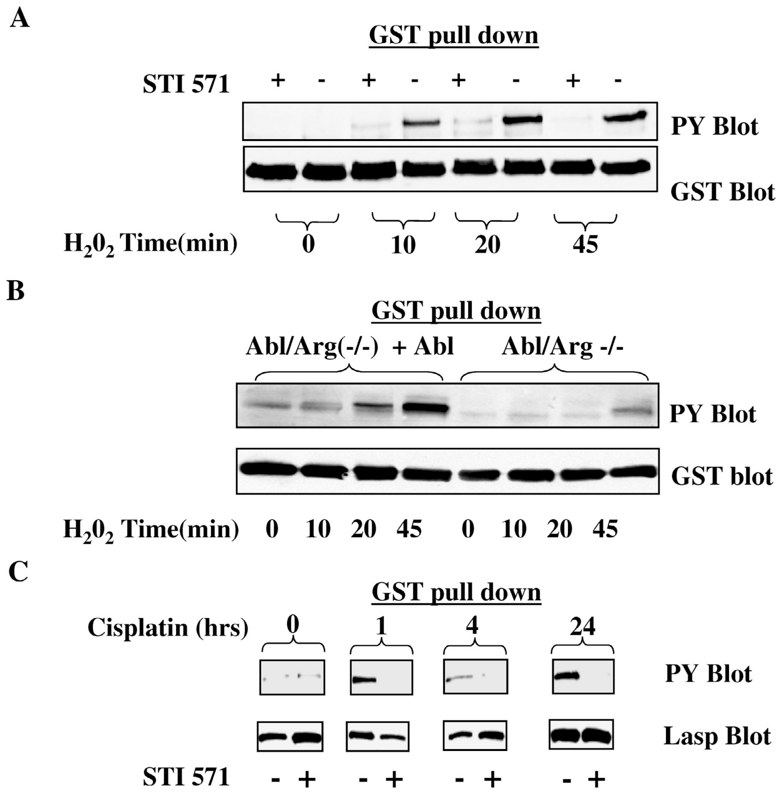

Figure 6. **Apoptotic agents induce tyrosine phosphorylation of Lasp-1, and this requires Abl and Arg kinase activity.** (A) Cos-7 cells transfected with GST Lasp were treated with 1 mM H_2O_2 for the indicated times in the presence or absence of 5 μM STI 571. GST Lasp was precipitated and Western blotted using anti-phosphotyrosine or GST antibodies. (B) Embryonic fibroblast cells isolated from *abl*$^{-/-}$*arg*$^{-/-}$ animals or these cells stably reconstituted with Abl were transfected with GST Lasp and were then serum starved and treated with 1 mM H_2O_2 for the indicated times. GST Lasp expression and tyrosine phosphorylation were determine as described above. (C) Cos-7 cells transfected with GST Lasp were incubated with 25 μM cisplatin for the indicated times in the presence or absence of 2 μM STI 571. Lasp-1 expression and tyrosine phosphorylation were determined as described above.

volves deregulation of the actin cytoskeleton and focal adhesions during the execution phase of death (Huot et al., 1998; Houle et al., 2003). Indeed, exposure of cells to either H_2O_2 or cisplatin caused strong Lasp-1 tyrosine phosphorylation that required endogenous Abl kinase activity (Fig. 6). Moreover, H_2O_2-induced Lasp-1 phosphorylation was significantly impaired in embryonic mouse fibroblast cells isolated from *abl*$^{-/-}$*arg*$^{-/-}$ animals, compared with these cells stably reconstituted with Abl (Fig. 6 B). However, at later times (>45 min) a small level of Lasp-1 phosphorylation is detected, suggesting that another kinase(s) may phosphorylate Lasp-1. Similar findings were obtained with cells treated with pervanadate, which strongly (>17-fold) activates Abl (unpublished data; Woodring et al., 2003). These findings

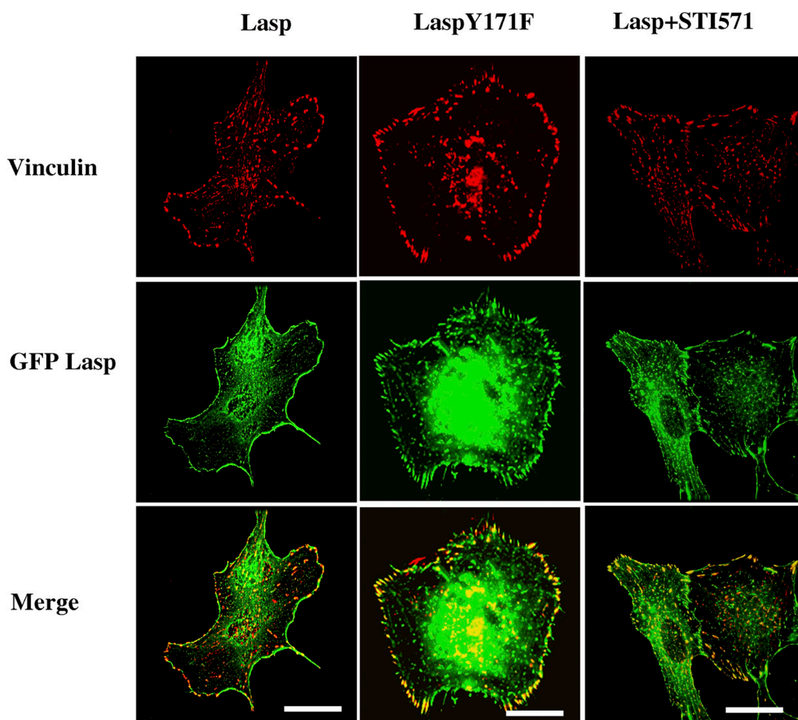

Figure 7. **Tyrosine phosphorylation Y171 of Lasp-1 prevents its translocation from the cell periphery to focal adhesions in response to growth factor stimulation.** Deconvolution images of NIH 3T3 cells expressing GFP Lasp or GFP LaspY171F were treated for 30 min with 1 mM H_2O_2 and for 15 min with FBS. Cells were fixed and stained with anti-vinculin antibodies to visualize focal adhesions as described above. In some cases, GFP Lasp transfected cells were preincubated with 5 μM STI 571 before H_2O_2 and FBS treatment to block Abl kinase activity as described in Materials and methods. Merged images represent colocalization (yellow) of focal adhesions (red) and GFP Lasp (green). Bars: 28 μm (right and left panels) and 20 μm (middle panel).

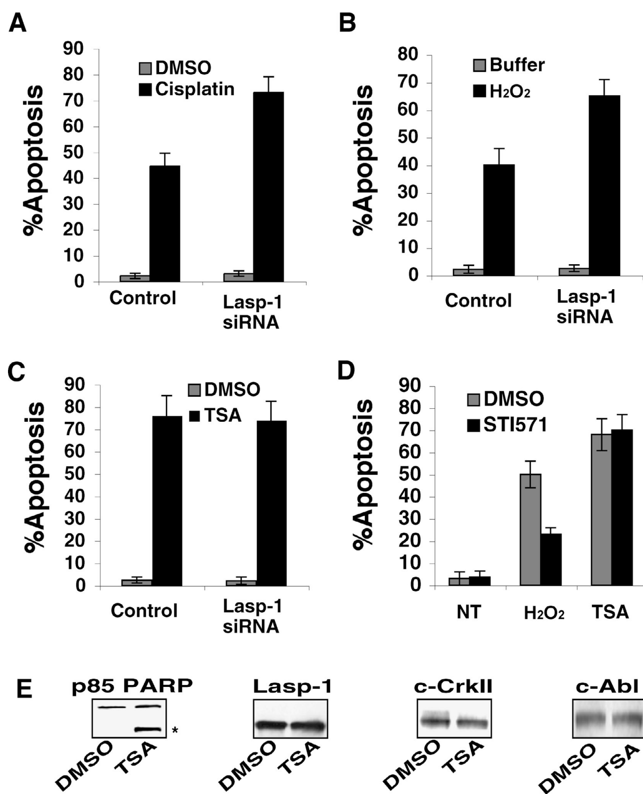

Figure 8. **Depletion of Lasp-1 protein increases cell apoptosis induced by H_2O_2 and cisplatin, but not TSA.** COS-7 cells were depleted of endogenous Lasp-1 protein using siRNA specific for Lasp-1 or control siRNA to GL2 luciferase as described in the Materials and methods. Cells were plated onto fibronectin-coated glass coverslips, and were treated with either (A) 25 μM cisplatin, (B) 1 mM H_2O_2, or (C) 300 ng/ml TSA for 24 h, or were treated with the indicated vehicle. (D) Cells were treated with or without (NT) H_2O_2 or TSA as described above in the presence or absence of 5 μM STI 571. Apoptotic cells were determined after 24 h by staining with propidium iodide and counting the number of cells per microscopic field with condensed nuclei as described previously (Kain et al., 2003). The bars represent the mean ± SEM of three independent experiments. (E) COS-7 cells were treated with 300 ng/ml TSA or with DMSO for 24 h, and were then lysed in detergent and Western blotted for the indicated proteins. Note that TSA does not induce a mobility shift of c-CrkII and Lasp-1 protein, indicating that these proteins are not phosphorylated and Abl kinase is not activated under these apoptotic conditions. Asterisk shows the cleaved form of PARP, indicating that TSA induced apoptosis in these cells. The top band is a nonspecific protein that served as a loading control.

demonstrate the tyrosine phosphorylation of Lasp-1 by endogenous Abl activation in response to apoptotic agents.

It is intriguing that the exposures of cells to survival factors like serum and PDGF-BB cause Lasp-1 to translocate from the cell periphery to focal adhesions in an unphosphorylated state (Fig. 2). This suggests that translocation of unphosphorylated Lasp-1 to focal adhesions plays a role in mediating survival signals through the cytoskeleton. If this is the case, then phosphorylation of Lasp-1 by apoptotic agents may prevent Lasp-1 localization to focal adhesions and disrupt survival signals from these structures. To investigate this possibility, serum-starved cells expressing GFP Lasp were briefly treated with H_2O_2 to induce Lasp-1 tyrosine phosphorylation, and were then stimulated with growth factors to induce translocation of Lasp-1 to focal adhesions and ruffles, as shown before (Fig. 2 A). H_2O_2 strongly blocked GFP Lasp translocation to focal adhesions, but not membrane ruffles, in response to growth factors (Fig. 7). Importantly, the short-term exposure of cells to H_2O_2 only effected Lasp-1 translocation and did not generally impact vinculin-positive focal adhesions, which were similar to control cells (Fig. 7). Pretreatment of cells with pervanadate also led to increased Lasp-1 tyrosine phosphorylation and prevented Lasp-1 translocation to focal adhesions (unpublished data). Importantly, phosphorylation of tyrosine 171 and Abl kinase activity were required for the inhibitory response induced by H_2O_2 because cells expressing GFP LaspY171F or cells treated with STI 571 showed normal Lasp-1 translocation to focal adhesions (Fig. 7). As expected, vehicle-treated cells expressing GFP LaspY171F or cells treated with STI 571 showed normal translocation of Lasp-1 to focal adhesions in response to growth factors, as this process occurs independent of phosphorylation (unpublished data). It is noteworthy that GFP LaspY171F did not constitutively translocate to focal adhesions in the absence of growth factors (Fig. 7). This suggests that basal phosphorylation of Y171 is not a general mechanism used by the cell to regulate focal adhesion targeting of Lasp-1 in healthy cells, but rather is a specific mechanism that operates downstream of apoptotic stimuli and Abl tyrosine kinase activity. Importantly, although Abl-mediated tyrosine phosphorylation blocked focal adhesion targeting of Lasp-1 in apoptotic cells, it did not impact its translocation to membrane ruffles. Indeed, treatment of cells with H_2O_2 or pervanadate did not prevent Lasp-1 localization to actin-rich membrane ruffles in response to growth factors, indicating that translocation to this subcellular structure is not, per se, regulated by tyrosine phosphorylation (unpublished data). These findings also demonstrate that H_2O_2 does not globally block growth factor–induced signaling in these cells. It appears, then, that apoptotic stimuli that induce Abl activation promote Lasp-1 phosphorylation, which specifically prevents Lasp-1 localization to focal adhesions, but not ruffles. Conversely, under conditions that promote cell survival and motility, Lasp-1 is not phosphorylated and is strongly localized to focal adhesions as well as ruffles. Most importantly, Lasp-1 directly contributes to H_2O_2- and cisplatin-induced apoptosis because cells depleted of Lasp-1 protein by siRNA show significantly increased death in response to these apoptotic agents compared with control cells expressing Lasp-1 protein (Fig. 8). In contrast, apoptosis induced with the deacetylase inhibitor trichostatin A (TSA; Ruefli et al., 2001) occurred independent of Abl and Lasp-1 activity, and did not involve changes in tyrosine phosphorylation of Lasp-1 or c-Crk protein, a known endogenous substrate of Abl (Kain and Klemke, 2001; Fig. 8, C–E). Thus, phosphorylation of Lasp-1 is not a general response in cells undergoing apoptosis, but rather is a specific event related to Abl-dependent apoptosis. In support of this, cells expressing LaspY171F, which cannot be phosphorylated by Abl and readily transits to focal adhesions, show reduced apoptosis in response to cisplatin and H_2O_2, but not TSA (Fig. 9). Together, our findings indicate that phosphorylation of Lasp-1 at tyrosine

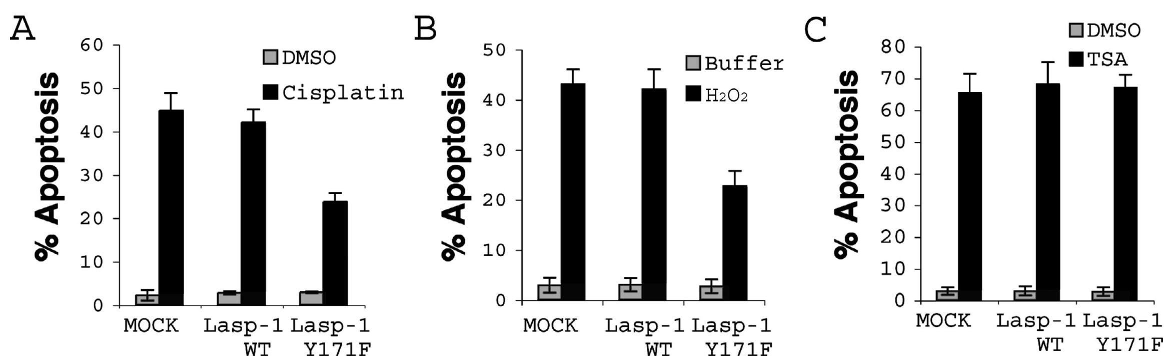

Figure 9. **Cells expressing LaspY171F show reduced apoptosis in response to cisplatin and H_2O_2, but not TSA.** COS-7 cells were transfected with the empty vector (Mock), wild-type Lasp-1, or Lasp-1 with tyrosine 171 mutated to phenylalanine (LaspY171F) along with GFP, which served as a reporter for transfected cells. Cells were then treated with either (A) cisplatin, (B) H_2O_2, or (C) TSA, and the number of GFP-positive apoptotic cells was determined as described in Fig. 8. Bars represent the mean ± SEM of three independent experiments.

171 prevents localization to focal adhesions and the loss of survival signals and induction of cell death.

Discussion

Cell migration is characterized by morphological polarization with a dominant-leading pseudopodium and a tail region at the rear of the cell (Lauffenburger and Horwitz, 1996; Palecek et al., 1996). This process requires temporal and spatial organization of signal transduction processes that regulate actin polymerization and focal adhesion turnover. In this report, we used a novel method to isolate the leading pseudopodium from the cell body for biochemical analysis. The unique ability to fractionate the pseudopodium combined with recent advances in mass spectrometry that facilitate large-scale protein identification of complex protein samples allowed us to compare the relative protein profiles of these two subcellular compartments (Washburn et al., 2001; Cho and Klemke, 2002). We identified several previously associated pseudopodial proteins including filamin, tropomyosin, caldesmon, cofilin, Arp2/3, myosin 1B and C, PI3K, and α-actinin, demonstrating the feasibility of this approach (Conrad et al., 1989; Aizawa et al., 1997; Helfman et al., 1999; Bailly et al., 2001; Laukaitis et al., 2001; Cooper, 2002; Funamoto et al., 2002). Although numerous pseudopodial and cell body proteins were identified by MudPIT, they represent only a small portion of the complex proteome of these structures. Nevertheless, with the rapid advances in mass spectrometry to quantify and monitor complex post-translational modifications of proteins (Washburn et al., 2001), together with fractionation into cholesterol-rich membrane and detergent soluble/insoluble components, will provide more detailed information on the pseudopodial proteome, and will help unravel the complex signal transduction events that control this cellular structure.

We chose to characterize Lasp-1 because its biological function is not known. In this report, we provide several lines of evidence that demonstrate Lasp-1 is a dynamic protein that transits to focal adhesions and is necessary for proper cell migration. First, depletion of Lasp-1 protein from cells strongly inhibits cell migration in response to ECM proteins. The inhibitory effect is specific to migration, as cell attachment and spreading appear normal in cells without Lasp-1 protein. Second, exogenous amplification of Lasp-1 also inhibits cell migration, but not adhesion and spreading. We believe that exogenous expression of Lasp-1 disrupts its normal signaling polarity. Lasp-1 is strongly polarized in migrating cells, where it localizes to the leading edge of the pseudopodium and to nascent focal adhesions in this structure, but not the central body of the cell. That constitutive relocalization of Lasp-1 to the plasma membrane strongly induced membrane blebbing and detachment from the ECM supports this idea. Third, stimulation of nonmigratory cells with growth factors or ECM proteins that induce cell migration cause the rapid relocalization of Lasp-1 from the peripheral membrane to a subset of focal adhesions in the spreading edge of the pseudopodium. Because cells depleted of Lasp-1 still attach to the ECM and form focal adhesions, it would appear that Lasp-1 plays a supportive role in focal adhesion dynamics during cell migration rather than in the actual formation of these structures. That Lasp-1 localizes to focal adhesions at the tips of retracting tails of migrating cells also supports this notion, as these adhesive sites must turnover for proper cell body translocation and tail release (Palecek et al., 1996). Together, these findings demonstrate that Lasp-1 is a dynamic focal adhesion protein necessary for cell migration.

Although the cellular factors responsible for translocation of Lasp-1 to focal adhesions are not yet known, it appears that the COOH-terminal region of Lasp-1 is critical for this response. Our biochemical and time-lapse analyses (unpublished data) of Lasp-1 indicate that translocation occurs rapidly within 1–2 min of growth factor stimulation and is independent of tyrosine phosphorylation. Indeed, repeated attempts to demonstrate changes in tyrosine phosphorylation of Lasp-1, in response to ECM proteins or growth factors, failed to show this response, even though these factors activate several tyrosine kinases including src and Abl (Lewis et al., 1996; Plattner et al., 1999). Furthermore, we did not detect a mobility shift of Lasp-1 in SDS-PAGE in stimulated cells or purified pseudopodia, which is characteristic of phosphorylation by PKA (Chew et al., 1998) as well as tyrosine phosphorylation (Fig. 6, A and B). This suggests that phosphorylation does not regulate focal adhesion targeting of Lasp-1 during cell spreading or pseudopodial extension. However, in some cases PKA may regulate Lasp-1 in focal

adhesions as well as its association with actin in gastric parietal cells where it localizes to the actin-rich canalicular membrane (Chew et al., 2002; Butt et al., 2003). It is also possible that low levels of Abl and PKA activity regulate Lasp-1 dynamics in the microenvironment of the migrating cell, but this activity was below biochemical detection in our analyses.

Our findings indicate that the COOH-terminal portion of Lasp-1 is for targeting to focal adhesions. The SH3 domain of Lasp-1 may play a pivotal role in focal complex targeting, as these structures can direct proteins like p130CAS (Crk-associated substrate) to focal adhesions (Harte et al., 2000). However, a GFP fusion of the Lasp-1 SH3 domain failed to translocate by itself to focal complexes (unpublished data). It is possible that the GFP tag interfered with the normal targeting function of this domain. Additional truncations in the COOH terminus as well as point mutations that disrupt SH3 domain function will be necessary to pinpoint the region of Lasp-1 responsible for translocation to focal complexes and for identification of binding proteins that mediate this process. In any case, the Lim domain does not play a critical role in focal adhesion targeting, as truncation of this domain did not prevent Lasp-1 localization to these structures (unpublished data).

It is intriguing that Abl tyrosine kinase is activated by growth promoting as well as by apoptotic stimuli (Lewis et al., 1996; Gong et al., 1999; Plattner et al., 1999; Sun et al., 2000). This suggests that Abl activity is a complex process tightly regulated by temporal and spatial mechanisms that couple to specific effector molecules. Our findings indicate that growth factor/motility factors like serum, PDGF-BB, EGF, and ECM proteins do not mediate coupling of Abl to Lasp-1. Rather, Abl activation in response to apoptotic stimuli appears to selectively phosphorylate Lasp-1 on tyrosine 171, preventing its translocation into focal complexes. This event is specific to focal complex targeting, as Abl-induced phosphorylation of Lasp-1 does not inhibit its localization to actin-rich structures including membrane ruffles and actin cables. Thus, Abl-dependent tyrosine phosphorylation of Y171 is a distinct signaling event that specifically blocks Lasp-1 transiting to focal complexes in apoptotic cells. Although the mechanism of Lasp-1 translocation is not known, these findings have important implications, as Abl-mediated death has been reported to involve nuclear and mitochondrial events involving p53/p73 and cytochrome *c* release, respectively (Agami et al., 1999; Yuan et al., 1999; Kumar et al., 2001; Goldberg et al., 2002). Our findings suggest that Abl activation also targets cytoplasmic and focal complex substrates in response to these apoptotic agents. An early step in the apoptotic process involves remodeling of focal adhesions and detachment from the ECM (Mills et al., 1999). Interestingly, an intermediate adhesive strength is optimal for transmitting survival signals from focal adhesions, suggesting that this process is tightly coupled to focal adhesion dynamics and cell motility processes (Murphy-Ullrich, 2001; Truong et al., 2003). Prevention of Lasp-1 localization to focal complexes by Abl may disrupt critical survival signals and may contribute to the execution phase of the apoptotic response. Recent evidence indicates that cytoplasmic Abl inactivates the focal adhesion protein c-CrkII, which induces apoptosis of carcinoma cells (Kain et al., 2003). Interestingly, Abl coordinately regulates both the migration and apoptotic machineries of cells through c-CrkII phosphorylation (Kain and Klemke, 2001; Kain et al., 2003). However, we found that Abl only couples to Lasp-1 under conditions that induce cell apoptosis and not motility. The ability of Abl to differentially target cytoplasmic and focal adhesion substrates like Lasp-1 and c-CrkII under migratory or apoptotic conditions may be related to the duration of kinase activation. Apoptotic agents tend to induce strong and persistent Abl activity (>1 h), whereas survival stimuli like integrin and growth factor receptor activation typically promote only a transient response (<30 min; Lewis et al., 1996; Gong et al., 1999; Plattner et al., 1999; Sun et al., 2000). Interestingly, recent evidence indicates that integrins and cell adhesion to the ECM is necessary for Abl-mediated death in response to DNA-damaging agents like cisplatin (Lewis et al., 2002; Truong et al., 2003). This suggests that at least part of the Abl death signal requires integrins and focal adhesion structures. Although the kinetics of Abl activity was not addressed in these reports, it seems reasonable that part of the suicide program involves not only localization of Abl to the nucleus where it regulates p53/73 (Lewis et al., 2002; Truong et al., 2003), but also its localization to the cytoplasm where it inactivates specific focal adhesion proteins like Lasp-1 and c-CrkII. In this way, Abl activation would have widespread impact on the apoptotic machineries that operate at multiple compartments within the cell. This widespread insult is likely important in the cell's decision to commit suicide or to attempt a repair and rescue program. In any case, the current evidence indicates that Abl-mediated death involves a cooperative effort with integrins, cytoskeleton, focal adhesions, and mitochondrial and nuclear proteins. The challenge now is to understand how Abl coordinately regulates these diverse processes and whether these events contribute to cancer development and progression.

Materials and methods

Purification of pseudopodia and cell body fractions and MudPIT analysis

Purification of pseudopodial proteins was according to published procedures (Cho and Klemke, 2002). In brief, 1–1.5 × 10^6 NIH 3T3 cells were allowed to extend pseudopodia toward an LPA gradient (100 ng/ml) for 60 min. A pseudopodia isolation kit (ECM 660; CHEMICON International) or Costar chambers (tissue culture treated, 6.5-mm diam, 10-μm thickness, 8-μm pores, Transwell®; Costar Corp.) were used for isolation. For Western blotting, cells were rinsed in excess cold PBS and rapidly fixed in 100% ice-cold methanol. Cell bodies on the upper membrane surface were manually removed with a cotton swab and pseudopodia on the undersurface scraped into lysis buffer (100 mM Tris, pH 7.4, 5 mM EDTA, 150 mM NaCl, 1 mM sodium orthovanadate, and protease inhibitors [cocktail tablet; Roche]) containing the appropriate detergent for Western blotting whole-cell lysates (1% SDS). Cell bodies were purified in a similar manner, except pseudopodia on the undersurface were removed, and the cell body on the upper surface was scraped into lysis buffer and detergent. For MudPIT analysis, pseudopodia or cell bodies were scraped into PBS and the soluble and insoluble fractions were digested for mass spectroscopy analysis as described previously (Washburn et al., 2001).

Details of MudPIT and data analysis have been described elsewhere (Washburn et al., 2001; Tabb et al., 2002). Tandem mass spectroscopy spectra were searched against a combined database of human, mouse, and rat proteins using SEQUEST, and the combined database was constructed from the NCBI protein database (August 20, 2002). The results were further analyzed by DTASelect and Contrast software. Peptides with

nontryptic cleavage sites were not included. All the proteins identified with a minimum of a single peptide were used to make protein lists for the pseudopod and for the cell body. A total of 980 proteins was identified by SEQUEST search. For the proteins with only single peptide match, relatively high Xcorr values (+1, 1.8; +2, 2.5; +3, 3.5; Eng et al., 1994) and probability-based scoring system of peptide identification (Pep Probe) was used to filter peptide matches, and then every tandem mass spectroscopy spectra was manually evaluated for final verification purposes. The Xcorr values represent a cross-correlation function used to provide measurement of similarity between the mass-to-charge ratios for the fragment ions predicated from amino acid sequences obtained from the database and the fragment ions actually observed in the tandem mass spectrum (Eng et al., 1994). Unique proteins in each sample and proteins showing significant changes (>50%) in the number of peptides identified were combined to make a list of enriched proteins in the pseudopodium and in the cell body. Proteins identified via a single peptide were included only if identified in a single compartment. Only those proteins positively identified in each of the five independent experiments via MudPIT were included in the list.

Immunofluorescence analysis of Lasp-1 dynamics

NIH 3T3 cells were transfected with the appropriate GFP constructs on glass coverslips as described above. Cells were washed 2× with PBS, fixed with 4% PFA in PBS, permeabilized with 0.1% Triton X-100 in PBS for 1 min, and then blocked with 0.5% BSA for 1 h. Anti-vinculin antibodies or TRITC-conjugated phalloidin (Sigma-Aldrich) was diluted in blocking solution and incubated with the fixed cells for 60 min. Anti-vinculin–treated cells were then incubated with Alexa Fluor® 568–conjugated goat anti–mouse IgG antibodies and were washed with PBS and mounted on coverslips using a ProLong® anti-fade kit (Molecular Probes, Inc.). Immunofluorescence microscopy was performed using a microscope (model IX70; Olympus) and data acquisition by a liquid cooled CCD camera (500 KHz, 12 bit, 2MP, KAF1400GI, 1317 × 1035; model CH350L, Photometrics). Image data were deconvolved with DeltaVision softWoRx version 2.5 software (Applied Precision). To examine the role of Abl activation in Lasp-1 localization to focal adhesions, 2–5 μM STI 571 was added to serum-starved cells for 16 h. Cells were then stimulated for the indicated times with either 1 mM H_2O_2, 25 μM cisplatin, 2 mM sodium orthovanadate, 20 ng PDGF-BB, or 10% FBS, which was added for 15 min in addition to other chemicals. Vinculin-positive focal adhesions and the F-actin cytoskeleton were examined as described above.

siRNA silencing

21-nucleotide double-stranded RNAs (Dharmacon Research) were synthesized by targeting human Lasp-1 (5′-AACUACAAGGGCUACGAGAAG-3′; corresponds to the coding region 127–147 relative to the first nucleotide of the start codon). Luciferase GL2 duplex (Dharmacon Research) was used as a negative control. The siRNAs were transfected into Cos-7 cells using Oligofectamine™ (Invitrogen) according to the manufacturer's instructions. Specific depletion of Lasp-1 was confirmed 48 h after transfection by Western blotting using anti-Lasp-1 and anti-actin antibodies.

Online supplemental material

A complete list of all pseudopodia and cell body–associated proteins is shown in Tables S1–S4 and Fig. S1. Materials and methods for cell lines, plasmids, constructs, antibodies, and cell-based assays, kinase assays, immunoprecipitation, and Western blotting are shown. Fig. S1 is a comparative schematic of the number of proteins identified in the each of the subcompartments of the cell body and pseudopodial fractions. Online supplemental material available at http://www.jcb.org/cgi/content/full/jcb.200311045/DC1.

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References

Agami, R., G. Blandino, M. Oren, and Y. Shaul. 1999. Interaction of c-Abl and p73α and their collaboration to induce apoptosis. *Nature.* 399:809–813.

Aizawa, H., Y. Fukui, and I. Yahara. 1997. Live dynamics of *Dictyostelium* cofilin suggests a role in remodeling actin latticework into bundles. *J. Cell Sci.* 110:2333–2344.

Allen, W.E., D. Zicha, A.J. Ridley, and G.E. Jones. 1998. A role for Cdc42 in macrophage chemotaxis. *J. Cell Biol.* 141:1147–1157.

Bailly, M., L. Yan, G.M. Whitesides, J.S. Condeelis, and J.E. Segall. 1998. Regulation of protrusion shape and adhesion to the substratum during chemotactic responses of mammalian carcinoma cells. *Exp. Cell Res.* 241:285–299.

Bailly, M., I. Ichetovkin, W. Grant, N. Zebda, L.M. Machesky, J.E. Segall, and J. Condeelis. 2001. The F-actin side binding activity of the Arp2/3 complex is essential for actin nucleation and lamellipod extension. *Curr. Biol.* 11:620–625.

Barila, D., R. Mangano, S. Gonfloni, J. Kretzschmar, M. Moro, D. Bohmann, and F. Superti. 2000. A nuclear tyrosine phosphorylation circuit: c-Jun as an activator and substrate of c-Abl and JNK. *EMBO J.* 19:273–281.

Brahmbhatt, A.A., and R.L. Klemke. 2003. ERK and RhoA differentially regulate pseudopodia growth and retraction during chemotaxis. *J. Biol. Chem.* 278:13016–13025.

Buchdunger, E., J. Zimmermann, H. Mett, T. Meyer, M. Muller, B.J. Druker, and N.B. Lydon. 1996. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Res.* 56:100–104.

Butt, E., S. Gambaryan, N. Gottfert, A. Galler, K. Marcus, and H.E. Meyer. 2003. Actin binding of human LIM and SH3 protein is regulated by cGMP- and cAMP-dependent protein kinase phosphorylation on serine 146. *J. Biol. Chem.* 278:15601–15607.

Chew, C.S., J.A. Parente, C. Zhou, E. Baranco, and X. Chen. 1998. Lasp-1 is a regulated phosphoprotein within the cAMP signaling pathway in the gastric parietal cell. *Am. J. Physiol.* 275:C56–C67.

Chew, C.S., X. Chen, J.A. Parente, S. Tarrer, C. Okamoto, and H.Y. Qin. 2002. Lasp-1 binds to non-muscle F-actin in vitro and is localized within multiple sites of dynamic actin assembly in vivo. *J. Cell Sci.* 115:4787–4799.

Cho, S.Y., and R.L. Klemke. 2002. Purification of pseudopodia from polarized cells reveals redistribution and activation of Rac through assembly of a CAS/Crk scaffold. *J. Cell Biol.* 156:725–736.

Conrad, P.A., M.A. Nederlof, I.M. Herman, and D.L. Taylor. 1989. Correlated distribution of actin, myosin, and microtubules at the leading edge of migrating Swiss 3T3 fibroblasts. *Cell Motil. Cytoskeleton.* 14:527–543.

Cooper, J.A. 2002. Actin dynamics: tropomyosin provides stability. *Curr. Biol.* 12:R523–R525.

Daley, G.Q., E. Van, and D. Baltimore. 1990. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science.* 247:824–830.

Elbashir, S.M., J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, and T. Tuschl. 2001. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature.* 411:494–498.

Eng, J.K., A.L. McCormack, and J.R. Yates III. 1994. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* 5:976–989.

Funamoto, S., R. Meili, S. Lee, L. Parry, and R.A. Firtel. 2002. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell.* 109:611–623.

Goldberg, Z., S. Vogt, M. Berger, Y. Zwang, R. Perets, E. Van, M. Oren, Y. Taya, and Y. Haupt. 2002. Tyrosine phosphorylation of Mdm2 by c-Abl: implications for p53 regulation. *EMBO J.* 21:3715–3727.

Gong, J.G., A. Costanzo, H.Q. Yang, G. Melino, W.G. Kaelin, M. Levrero, and J.Y. Wang. 1999. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature.* 399:806–809.

Grosveld, G., T. Verwoerd, T. van Agthoven, A. de Klein, K.L. Ramachandran, N. Heisterkamp, K. Stam, and J. Groffen. 1986. The chronic myelocytic cell line K562 contains a breakpoint in bcr and produces a chimeric bcr/c-abl transcript. *Mol. Cell. Biol.* 6:607–616.

Harte, M.T., M. Macklem, C.L. Weidow, J.T. Parsons, and A.H. Bouton. 2000. Identification of two focal adhesion targeting sequences in the adapter molecule p130(Cas). *Biochim. Biophys. Acta.* 1499:34–48.

Helfman, D.M., E.T. Levy, C. Berthier, M. Shtutman, D. Riveline, I. Grosheva, Z. Lachish, M. Elbaum, and A.D. Bershadsky. 1999. Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions. *Mol. Biol. Cell.* 10:3097–3112.

Houle, F., S. Rousseau, N. Morrice, M. Luc, S. Mongrain, C.E. Turner, S.

Tanaka, P. Moreau, and J. Huot. 2003. Extracellular signal-regulated kinase mediates phosphorylation of tropomyosin-1 to promote cytoskeleton remodeling in response to oxidative stress: impact on membrane blebbing. *Mol. Biol. Cell.* 14:1418–1432.

Huot, J., F. Houle, S. Rousseau, R.G. Deschesnes, G.M. Shah, and J. Landry. 1998. SAPK2/p38-dependent F-actin reorganization regulates early membrane blebbing during stress-induced apoptosis. *J. Cell Biol.* 143:1361–1373.

Kain, K.H., and R.L. Klemke. 2001. Inhibition of cell migration by Abl family tyrosine kinases through uncoupling of Crk-CAS complexes. *J. Biol. Chem.* 276:16185–16192.

Kain, K.H., S. Gooch, and R.L. Klemke. 2003. Cytoplasmic c-Abl provides a molecular rheostat controlling carcinoma cell survival and invasion. *Oncogene.* 22:6071–6080.

Kumar, S., A. Bharti, N.C. Mishra, D. Raina, S. Kharbanda, S. Saxena, and D. Kufe. 2001. Targeting of the c-Abl tyrosine kinase to mitochondria in the necrotic cell death response to oxidative stress. *J. Biol. Chem.* 276:17281–17285.

Lauffenburger, D.A., and A.F. Horwitz. 1996. Cell migration: a physically integrated molecular process. *Cell.* 84:359–369.

Laukaitis, C.M., D.J. Webb, K. Donais, and A.F. Horwitz. 2001. Differential dynamics of α5 integrin, paxillin, and α-actinin during formation and disassembly of adhesions in migrating cells. *J. Cell Biol.* 153:1427–1440.

Lewis, J.M., R. Baskaran, S. Taagepera, M.A. Schwartz, and J.Y. Wang. 1996. Integrin regulation of c-Abl tyrosine kinase activity and cytoplasmic-nuclear transport. *Proc. Natl. Acad. Sci. USA.* 93:15174–15179.

Lewis, J.M., T. Truong, and M.A. Schwartz. 2002. Integrins regulate the apoptotic response to DNA damage through modulation of p53. *Proc. Natl. Acad. Sci. USA.* 99:3627–3632.

Liu, F., D.E. Hill, and J. Chernoff. 1996. Direct binding of the proline-rich region of protein tyrosine phosphatase 1B to the Src homology 3 domain of p130(Cas). *J. Biol. Chem.* 271:31290–31295.

Mills, J.C., N.L. Stone, and R.N. Pittman. 1999. Extranuclear apoptosis. The role of the cytoplasm in the execution phase. *J. Cell Biol.* 146:703–708.

Murphy-Ullrich, J.E. 2001. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? *J. Clin. Invest.* 107:785–790.

Palecek, S.P., C.E. Schmidt, D.A. Lauffenburger, and A.F. Horwitz. 1996. Integrin dynamics on the tail region of migrating fibroblasts. *J. Cell Sci.* 109: 941–952.

Parent, C.A., and P.N. Devreotes. 1999. A cell's sense of direction. *Science.* 284: 765–770.

Plattner, R., L. Kadlec, K.A. DeMali, A. Kazlauskas, and A.M. Pendergast. 1999. c-Abl is activated by growth factors and Src family kinases and has a role in the cellular response to PDGF. *Genes Dev.* 13:2400–2411.

Ruefli, A., M. Ausserlechner, D. Bernard, V. Sutton, K. Tainton, R. Kofler, M. Smith, and R. Johnstone. 2001. The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell death pathway characterized by cleavage of Bid and production of reactive oxygen species. *Proc. Natl. Acad. Sci. USA.* 98:10833–10838.

Schreiber, V., L. Moog, C.H. Regnier, M.P. Chenard, H. Boeuf, J.L. Vonesch, C. Tomasetto, and M.C. Rio. 1998. Lasp-1, a novel type of actin-binding protein accumulating in cell membrane extensions. *Mol. Med.* 4:675–687.

Smith, J.M., S. Katz, and B.J. Mayer. 1999. Activation of the Abl tyrosine kinase in vivo by Src homology 3 domains from the Src homology 2/Src homology 3 adaptor Nck. *J. Biol. Chem.* 274:27956–27962.

Sun, X., P. Majumder, H. Shioya, F. Wu, S. Kumar, R. Weichselbaum, S. Kharbanda, and D. Kufe. 2000. Activation of the cytoplasmic c-Abl tyrosine kinase by reactive oxygen species. *J. Biol. Chem.* 275:17237–17240.

Tabb, D.L., W.H. McDonald, and J.R. Yates. 2002. DTASelect and Contrast: tools for assembling and comparing protein identifications from shotgun proteomics. *J. Proteome Res.* 1:21–26.

Tomasetto, C., L. Moog, C.H. Regnier, V. Schreiber, P. Basset, and M.C. Rio. 1995a. Lasp-1 (MLN 50) defines a new LIM protein subfamily characterized by the association of LIM and SH3 domains. *FEBS Lett.* 373:245–249.

Tomasetto, C., C. Regnier, L. Moog, M.G. Mattei, M.P. Chenard, R. Lidereau, P. Basset, and M.C. Rio. 1995b. Identification of four novel human genes amplified and overexpressed in breast carcinoma and localized to the q11-q21.3 region of chromosome 17. *Genomics.* 28:367–376.

Truong, T., G. Sun, M. Doorly, J.Y. Wang, and M.A. Schwartz. 2003. Modulation of DNA damage-induced apoptosis by cell adhesion is independently mediated by p53 and c-Abl. *Proc. Natl. Acad. Sci. USA.* 100:10281–10286.

Wang, B., E.A. Golemis, and G.D. Kruh. 1997. ArgBP2, a multiple Src homology 3 domain-containing, Arg/Abl-interacting protein, is phosphorylated in v-Abl-transformed cells and localized in stress fibers and cardiocyte Z-disks. *J. Biol. Chem.* 272:17542–17550.

Washburn, M.P., D. Wolters, and J.R. Yates. 2001. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat. Biotechnol.* 19:242–247.

Woodring, P.J., T. Hunter, and J.Y. Wang. 2003. Regulation of F-actin-dependent processes by the Abl family of tyrosine kinases. *J. Cell Sci.* 116:2613–2626.

Wyckoff, J.B., J.G. Jones, J.S. Condeelis, and J.E. Segall. 2000. A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. *Cancer Res.* 60:2504–2511.

Yuan, Z.M., H. Shioya, T. Ishiko, X. Sun, J. Gu, Y.Y. Huang, H. Lu, S. Kharbanda, R. Weichselbaum, and D. Kufe. 1999. p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. *Nature.* 399:814–817.