The Dbl-related Protein, Lfc, Localizes to Microtubules and Mediates the Activation of Rac Signaling Pathways in Cells*

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The possibility that the Dbl family member Lfc can activate Rac1 in cells is investigated in this study. Previously, we demonstrated that both Lfc and Lsc, like their closest relative Lbc, can act catalytically in stimulating the guanine nucleotide exchange activity of RhoA in vitro. Neither Lfc nor Lsc stimulated the in vitro exchange activity of Cdc42 or Rac1; however, Lfc was capable of forming a tight complex with Rac1 in vitro. We show here that Lfc stimulates c-Jun kinase (JNK) activity in COS-7 cells. This stimulation was blocked by a dominant negative mutant of Rac1 and somewhat less effectively by dominant negative RhoA, but not by dominant negative Cdc42. Overexpression of Lfc in NIH 3T3 cells induced the formation of actin stress fibers and membrane ruffles, consistent with the activation of both RhoA and Rac1 signaling pathways, whereas overexpression of Lsc led exclusively to well-developed stress fibers. Using a recently developed assay for measuring the cellular activation of Rac, we did not find that expression of Lfc increased the levels of GTP-bound Rac1. However, an examination of the cellular localization of Lfc showed that it was localized to microtubules, similar to what has been reported for activated Rac1, the mixed lineage kinase (MLK) and JNK. Moreover, we have found that the Pleckstrin homology (PH) domain of Lfc specifically associates with tubulin. Taken together, these findings suggest a model where the PH domain-mediated localization of Lfc to microtubules enables the recruitment of Rac to a site proximal to its signaling targets, resulting in JNK activation and actin cytoskeletal changes.

Lfc was initially identified based on its transforming activity when overexpressed in NIH 3T3 cells (1). The Lfc oncprotein is a member of the Dbl family of growth regulatory proteins. Many members of the Dbl family have been demonstrated to function upstream of the Rho-related GTP-binding proteins, acting as guanine nucleotide exchange factors (GEFs)1 by stimulating the exchange of GTP for GDP and thereby promoting G protein activation. This rapidly growing family of regulatory proteins includes greater than 20 members to date (reviewed in Ref. 2). The fact that there exist so many GEFs for the Rho subfamily suggests that there will be multiple pathways leading to the activation of an individual GTP-binding protein. All members of the Dbl family possess a Dbl homology (DH) domain in tandem with a Pleckstrin homology (PH) domain, and it has been demonstrated that both domains are required for the transforming activities of oncogenic members of the family. The DH domain typically represents the limit motif for binding the G protein and stimulating nucleotide exchange (3), whereas the PH domain appears to be essential for mediating the appropriate cellular localization of the protein (1, 4).

Additionally, members of the Dbl family contain a number of other structural motifs that indicate a role in signal transduction. These domains presumably function to mediate protein/protein and protein/lipid interactions and serve to link members of the Dbl family to upstream regulation (2). This has been most carefully worked out for the Dbl family member, Vav, which is a Rac-GEF that is regulated by tyrosine phosphorylation and by the phospholipids, phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3) (5–7). Lfc contains a cysteine-rich domain similar to the diacylglycerol binding domain found in protein kinase C which may function to couple Lfc with upstream generation of lipids (1).

Determining the regulation and cellular localization of the growing family of Rho-GEFs will be important for understanding how Rho-related GTP-binding proteins mediate multiple cellular activities. Activated RhoA, Rac1, and Cdc42 regulate both gene transcription and the actin cytoskeleton, contributing to the control of cell morphology, motility, and growth (reviewed in Refs. 8 and 9). Through their interactions with multiple targets, the Rho family of GTP-binding proteins is able to coordinate these diverse cellular functions. A number of targets have been identified including two related families of serine/threonine kinases, the p21-activated kinases (Paks) and the mixed lineage kinases (MLKs), which have been shown to play specific roles in regulating gene transcription and the actin cytoskeleton (10–15). It has previously been reported that Rac1 and Cdc42 stimulate the enzymatic activity of the mitogen-activated protein kinases (MAPKs), JNK and p38 (16). Additionally, both the Paks and MLKs have also been shown to stimulate JNK activity (11, 15, 17). The JNKs in turn phosphorylate and regulate the activity of proteins that control the expression of specific gene products involved in regulating growth and morphology (18, 19). The JNK family members have also been referred to as stress-activated protein kinases (SAPKs) because of the ability of ultraviolet radiation, osmotic shock, or inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α to stimulate their activity (20, 21).

We have previously shown that Lfc, which functions specif-

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1 The abbreviations used are: GEF, guanine nucleotide exchange factor; DH, Dbl homology; PH, Pleckstrin homology; Pak, p21-activated kinase; MLK, mixed lineage kinase; JNK, c-Jun kinase; MBP, myelin basic protein; PBD, p21 (Cdc42/Rac1) binding domain; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; GST, glutathione S-transferase.
Cellular Activity of Lfc

ically as a GEF for RhoA in vitro, binds tightly to Rac1 in a nucleotide-independent manner (22). Additionally, Lfc strongly activates JNK in COS-7 cells. These findings suggest that Lfc may have a broader specificity in cells that would include promoting a Rac1-mediated pathway which leads to JNK activation. This led us to examine the cellular activity of Lfc. Thus far, Vav, Tiam-1, and SOS (7, 23, 24) are Dbl family members that with the help of cellular co-factors (e.g., lipid second messenger) have been shown to stimulate Rac activation in cells. In the present studies, we show that Lfc is another Dbl family member that has a positive effect on Rac signaling. However, in this case, we do not detect a direct Lfc-stimulated activation of Rac1. Rather Lfc, through direct interactions with microtubules as mediated by its PH domain, may serve to mark the cellular site for a Rac-signaling complex that leads to JNK activation and/or actin cytoskeletal changes.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—Plasmids pAX142 HAD7-Lfc and HAD6-Lfc (1) and D7-Lsc (30) have been described previously. The GST-Lfc (PH) was generated by PCR from the D7-Lfc cDNA. Primers for PCR encoded the restriction sites HindIII and XbaI to subclone into the Escherichia coli expression vector pEXKG. The PCR generated fragment encompassed the entire PH domain plus a small amount of flanking sequence (amino acid sequence 450–573). Generation of all constructs encoding Rac1, Cdc42, and their dominant negative mutants have been described previously (10, 22, 26). These were subcloned into the expression vector pCDNA3, which has been engineered to contain either the HA- or Myc-tag for expression in mammalian cells. The generation of J33Pak3 has been described (17); plasmid J33Pak3 (K297R) expresses Myc-tagged kinase-defective Pak3. Kinase-defective Pak3 was made by mutating Lys at position 297 in the ATP-binding site to Arg using PCR overlap method. The pCMV6 Dbl was generated by ligating a BamHI fragment encoding oncogenic Dbl from plasmid pc11dbl (a gift from Dr. Sandra Eva, Institute Giannina Hullinger Mannheim) and 5 mM MgCl2. The lysates were cleared by centrifugation at 12,000 × g for 25 min at 4 °C, frozen in liquid nitrogen, and stored at −80 °C. The expression of proteins was confirmed by Western blotting of an aliquot prior to affinity precipitation, and lysates used for affinity precipitation were normalized for HA-Rac1 levels. Affinity precipitations were carried out for 1 h at 4 °C, washed three times in lysis buffer, and analyzed by Western blotting.

**Rac1 Activation Assay**—The p21 (Cdc42/Rac1) binding domain (PBD) of Pak3 was expressed in E. coli as a GST fusion protein and immobilized by binding to glutathione-Sepharose beads. The immobilized GST-PBD was used to precipitate activated Rac1 from transfected COS-7 cell lysates. Cells were washed in cold phosphate-buffered saline and lysed in 20 mM Hepes, pH 7.4, 100 mM NaCl, 0.5% Nonidet P-40, 10 mM MgCl2, 10 mM β-glycerophosphate, 10% glycerol, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. Lysates were cleared by centrifugation at 12,000 × g for 25 min at 4 °C, frozen in liquid nitrogen, and stored at −80 °C. The expression of proteins was confirmed by Western blotting of an aliquot prior to affinity precipitation, and lysates used for affinity precipitation were normalized for HA-Rac1 levels. Affinity precipitations were carried out for 1 h at 4 °C, washed three times in lysis buffer, and analyzed by Western blotting. HA-tagged Rac1 was detected with monoclonal antibody 12CA5 (Berkley Antibody Co). The primary antibody was detected with horseradish peroxidase-coupled sheep anti-mouse antibody using the enhanced chemiluminescence detection reagent ECL (Amersham Pharmacia Biotech).

**Immunofluorescence**—For morphological studies, cells were serum starved for 24 h and fixed for 1–2 min with 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100 for 5 min. Actin cytoskeleton was stained by incubation with 3.3 μM Texas Red-conjugated phalloidin (Molecular Probes) for 1 h followed by three washes with phosphate-buffered saline. For immunolocalization studies, cells were fixed and permeabilized as above. HA-D7 Lfc was detected using anti-HA11 IgG monoclonal antibody (a gift from Dr. G. Bloom, Southwest Medical Center, Dallas, TX) for 1 h, followed by rhodamine-conjugated anti-IgG-specific secondary antibody (Southern Biotechnology Associates, Inc., Birmingham, AL) for 1 h. The secondary antibodies did not show nonspecific labeling, or cross-reactivity. Cells were observed and photographed under a Zeiss fluorescence microscope.

**Affinity Precipitation Assay for Tubulin Binding**—The expression and purification of GST-DPH Lfc (GST-D10 Lfc) from SF21 insect cells has been previously described (22). The GST-PH fusion protein was expressed in E. coli and an overnight culture that was grown from a single colony was used to inoculate a 1-liter culture that was grown at 37 °C while shaking, to an OD of 0.6–0.8. Protein expression was then induced by the addition of 200 μM isopropyl-β-D-thiogalactoside for 1 h. Bacteria were harvested by centrifugation and frozen at −80 °C. The pellets were thawed in 20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 2 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfon fluoride. Bacteria were lysed on ice by adding 0.5 mg/ml lysozyme followed by 10 mg/ml of DNase I (Boehringer Mannheim) or 5 mg/ml of RNase A and 5 mg/ml of RNase T1 for 30 min at 30,000 rpm. Proteins were purified by glutathione-agarose affinity chromatography. The glutathione-agarose bound GST fusion proteins were used in affinity precipitations from Jurkat T-cell lysates (20 mM Hepes, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1 mM sodium orthovanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). Precipitates were washed out at 4 °C for 2 h and washed in cell lysis buffer three times, and then the resuspended samples were subjected to Western blot analysis. Precipitated tubulin was detected with monoclonal anti-tubulin primary antibody (Amersham Pharmacia Biotech), and the primary antibody was detected with horseradish peroxidase-coupled sheep anti-mouse antibody using the chemiluminescence detection reagent ECL (Amersham Pharmacia Biotech).

**RESULTS AND DISCUSSION**

It has been reported previously that both Rac1 and Cdc42 can activate the JNK mitogen-activated protein kinase cascade in COS-7 cells, whereas RhoA does not (16). We have observed that the overexpression of Lfc in COS-7 cells leads to JNK activation. In Fig. 1, we show a schematic representation of full-length Lfc and Lsc, and the different forms of the recombinant proteins used in this study. Using an in vitro kinase assay to determine the activity of immunoprecipitated Flag-tagged JNK from lysates, we find that Lfc and Dbl can activate (MBP) (Sigma), 10 μCi [γ-32P]ATP, and 20 μM ATP in a final volume of 30 μl for 10 min at 22 °C. The kinase reactions were terminated by the addition of EDTA containing Laemmli sample buffer and resolved by SDS-PAGE. The incorporation of [32P] was visualized by autoradiography.
JNK in COS-7 cells to an extent similar to the activation induced by exposing cells to ultraviolet radiation, whereas Lsc shows no detectable stimulation (Fig. 2A). The Lfc-mediated stimulation of JNK activity was not dependent on the diacylglycerol binding domain since the D6-Lfc and D7-Lfc constructs both yielded strong JNK activation (Fig. 2B). The lack of stimulation by Lsc indicates that although RhoA can activate JNK in some cell types, as reported by Teramoto et al. (25), it does not activate JNK in COS-7 cells. Thus, we set out to determine whether Rac1 mediates the Lfc-stimulated activation of JNK in COS-7 cells.

**Ability of Dominant Negative Mutants of the Rho Subfamily to Inhibit Lfc-stimulated JNK Activity**—We next examined whether the activation of JNK in COS-7 cells could be inhibited by dominant negative mutants of the Rho family of small GTP-binding proteins. Based on in vitro binding studies, which indicated that Lfc could form stable complexes with both RhoA and Rac1, we would anticipate that dominant negative versions of RhoA (RhoA(T19N)) and Rac1 (Rac(S17N)) would be capable of binding Lfc and inhibiting its activation of JNK. As shown in Fig. 3, Rac(S17N) blocked Lfc-stimulated JNK activity, whereas comparable levels of RhoA(T19N) showed a significantly weaker inhibition. At higher levels of expression, RhoA(T19N) was then able to provide an inhibitory effect. Dominant negative Cdc42 (Cdc42(T17N)) was not able to inhibit Lfc-stimulated JNK activity under any condition.

**Co-expression of Dominant Negative Forms of Pak3 and MLK3 Interfere with Lfc-stimulated JNK Activity**—The Pak family, which serve as targets for Cdc42 and Rac but not RhoA, have been shown to mediate the activation of JNK in certain cell types (10, 11, 17). Thus, we examined whether a kinase-defective mutant of Pak3 (Pak3(K297R)), by interfering with Rac1-stimulated signaling, would inhibit Lfc-mediated JNK activation. Fig. 4A (where KDP represents the Pak3 mutant) shows that this was the case. However, it was interesting that we did not find that Lfc stimulated Pak3 activity in these cells. We examined this by transiently co-expressing Myc-tagged Pak3 with either Lfc, Lsc, or Dbl in COS-7 cells. These studies showed that Lfc did not activate Pak3, under conditions where Dbl stimulated Pak3 activity (Fig. 4B). This suggests that the direct stimulation of Rac (and Pak3) activity by Lfc is not the...
underlying mechanism by which Lfc mediates JNK activation (also, see below). MLK3 is another putative protein kinase target for Cdc42 and Rac that has recently been shown to be a strong activator of JNK (15). Fig. 5 shows that like the kinase-defective Pak3, a kinase-defective form of MLK3 (MLK3 K114R) (Fig. 5, designated KDKMLK), inhibits Lfc-stimulated JNK activity. Thus far, we have not been able to determine whether Lfc stimulates MLK3 activity because this protein kinase is constitutively active when expressed in cells, and so it has not been possible to conclude whether MLK is an essential participant in the Lfc-mediated signaling pathway leading to JNK activation.

**Detection of Rac1 Activation in Vivo**—Our initial guanine nucleotide exchange measurements performed in vitro indicated that Lfc stimulated GDP-GTP exchange on RhoA but not on Rac1 (22), suggesting that Lfc did not act as a GEF for Rac1. However, similar in vitro results have been obtained for Vav, Tiam-1, and Sos, but it was subsequently shown that cellular co-factors were necessary to enable each of these Dbl-related molecules to act as GEFs for Rac1 (23). Thus, we took advantage of a recently developed assay for Rac activation (26) to determine whether Lfc could increase the levels of GTP-bound Rac1 in cells. Specifically, the p21-binding domain (PBD) of Pak3 was expressed as a GST-fusion protein and immobilized on glutathione-Sepharose beads and then was used as an affinity reagent to precipitate GTP-bound (activated) HA-Rac1 from transfected COS-7 cell lysates. As shown in Fig. 6, when using this assay, we were unable to detect an increase in the levels of GTP-bound Rac1 in cells that were co-transfected with Lfc and HA-Rac1. However, we were able to detect an increase in GTP-bound Rac1 when Dbl was co-expressed with HA-Rac1, consistent with prior results indicating that Dbl can stimulate the activation of Rac in cells (26).

**Morphological Changes Induced by Lfc in NIH 3T3 Cells**—Rearrangements in the actin cytoskeleton have been shown to be regulated by members of the Rho subfamily of GTP-binding proteins (reviewed in Ref. 9). Moreover, it has been shown that each of the Rho family members induces distinct morphological phenotypes. By taking advantage of these morphological changes, we set out to determine whether Lfc promotes a Rac1-like morphology. Cells were stained with Texas Red-conjugated phalloidin (Molecular Probes) to visualize the actin cytoskeleton in NIH 3T3 cells co-transfected with an expression vector encoding HA-D7 Lfc or HA-D7 Lsc and pEGFP (an expression vector containing green fluorescent protein) (Fig. 7). Similar types of experiments were performed using indirect immunofluorescence with an anti-HA monoclonal antibody (HA11, Babco) to identify cells expressing HA-D7 Lfc or HA-D7 Lsc. We also examined the actin rearrangements induced by transient expression of GTPase-defective RhoA (RhoA G14V) and Rac1 (Rac Q61L). As shown in Fig. 7, cells expressing the GTPase-defective RhoA mutant have enhanced actin stress fibers (Fig. 7C) as compared with control NIH 3T3 cells (Fig. 7A). NIH 3T3 cells expressing GTPase-defective Rac1 (Fig. 7B) have an enhanced actin meshwork at their periphery, resulting in membrane ruffles. We show in Fig. 7E that Lsc strongly induces actin stress fibers and a morphology similar to that observed when over-expressing the activated RhoA mutant. Lfc, however, appears to not only promote the formation of actin stress fibers but also induces lamellipodia and membrane ruffling, indicative of signaling through both activated RhoA and Rac1 (Fig. 7D).

**Cellular Localization of Lfc to the Microtubules**—We used HA-D7 Lfc in immunofluorescence experiments to examine the cellular localization of Lfc. Using a monoclonal anti-HA antibody (HA11, Babco) as the primary antibody, and Bodipy-
labeled secondary antibody, we detected a distinct cellular distribution for Lfc, as reflected by a filamentous pattern (data not shown). This pattern of staining closely resembled the microtubule network. We next double stained cells expressing Lfc and measured by immune complex kinase assays using \([\alpha-\text{32P}])\) ATP and c-Jun as substrates. Proteins were separated by SDS-PAGE and analyzed by autoradiography to show phosphorylation of substrate (top panel) and Western blot analysis to show relative amounts of Flag-JNK in the kinase reactions (bottom panel).

**FIG. 5.** Dominant negative mutant of MLK3 blocks Lfc-stimulated JNK activity. COS-7 cells were transiently co-transfected with Flag-JNK (0.4 \(\mu g\)) together with an empty expression (−) vector or with an expression vector encoding HA-D7 Lfc (+) (1 \(\mu g\)) and 2 \(\mu g\) of MLK3 KDMLK, kinase dead (−) or 2 \(\mu g\) of MLK3 WTMLK, wild type). Cells were lysed at 36–48 h post-transfection, and JNK activity was measured by immune complex kinase assays using (10% TCL) and Western blot analysis to show relative amounts of Flag-JNK in the kinase reactions (bottom panel).

**FIG. 6.** Lfc does not detectably activate Rac1 in vivo. Shown is an assay for Rac1 activation in vivo. COS-7 cells were transiently co-transfected with HA-Rac1 (1 \(\mu g\)) together with an empty expression vector (−) or with an expression vector encoding HA-D7 Lfc, HA-D7 Lsc, or Dbl (1 or 2 \(\mu g\)). Cells were lysed at 36–48 h after transfection and affinity precipitated (AP) with immobilized GST-PBD. Proteins were separated by SDS-PAGE and Western blotted and probed with anti-HA antibody (top panel). Bottom panel represents 10% of the total cell lysate (TCL) used in the binding assay.

**FIG. 7.** Lfc induces the formation of lamellipodia and actin stress fibers. Serum-starved NIH 3T3 cells transiently expressing GTPase-defective Rac(Q61L), GTPase-defective RhoA(Q63L), Lfc, or Lsc were analyzed by indirect immunofluorescence analysis for actin cytoskeletal organization with Texas Red phalloidin. Vector control transfected cells (A) show very few organized actin filaments. Expression of activated Rac (B) induces lamellipodia, whereas activated Rho (C) strongly induces stress fiber formation. Expression of Lfc (D) leads to the formation of lamellipodia and stress fibers while Lsc (E) induces stress fiber formation, similar to that seen with activated RhoA.
RhoA also inhibited the Lfc-stimulated activation of JNK. Given that RhoA(T19N) was less effective than Rac1(S17N) in blocking JNK activation suggests that Lfc may show a preference for binding Rac1 compared with RhoA in COS-7 cells. The ability of Lfc to stimulate a Rac1-mediated JNK pathway may be favored by a specific cellular localization. It is possible that the demonstrated localization of Lfc to microtubules favors interactions with Rac1, which has also been reported to localize to microtubules (27). A recent report has also demonstrated that both MLK and JNK are localized to microtubules (28), again supporting the concept of a specific localization and proximity of a full Rac1 signaling pathway. The biochemical data presented here indicate that the localization of Lfc to microtubules may be mediated by the binding of its PH domain to tubulin.

An obvious question is how does Lfc promote Rac1-signaling? Typically, a GEF binds with the highest affinity to the nucleotide-depleted state of a GTP-binding protein (31). Such a preference is consistent with the fact that GEFs, by stabilizing the nucleotide-depleted state, stimulate GDP dissociation from the G protein and enable GDP-GTP exchange to occur. As we previously reported, this is the case for the interaction of Lfc with RhoA, but not with Rac1 (22). The in vitro interaction of Lfc with Rac1 shows no nucleotide specificity. Thus far, we have not been able to detect an Lfc-stimulated increase in the amount of GTP-bound Rac1 in cells, as read-out by the ability of GTP-bound Rac1 to form a complex with its binding domain on Pak3 (Fig. 6). We also have not been able to directly detect Lfc-mediated Rac1 activation when co-expressing Lfc with potential upstream activators, such as an activated Src mutant (Src(Y527F)), GTPase-defective Ras (Ras(G12V)), or a constitutively active form of the PI3-kinase (data not shown). In addition, we have been unable to detect Lfc-stimulated nucleotide exchange activity on Rac1 in vitro using an insect cell-expressed GST fusion protein of Lfc that contains the DH and PH domains (22), nor have we been able to detect nucleotide exchange activity using immunoprecipitated Lfc from lysates of COS-7 cells overexpressing the protein (data not shown). Thus, our data raise the possibilities that Lfc regulates Rac1-mediated signaling and stimulates JNK activation and/or actin cytoskeletal changes by an alternative mechanism, for example by recruiting activated Rac-1 to microtubules. This could bring GTP-bound Rac1 to a site proximal to MLK or another appropriate Rac1 target(s) that initiates a pathway leading to JNK activation and/or changes in the actin cytoskeleton and cell morphology. In this model, dominant negative Rac, by either directly preventing the activation of endogenous Rac molecules or by binding Lfc and competitively inhibiting the binding of activated (GTP-bound) Rac, would prevent the Lfc-mediated activation of Rac-signaling. Such a mechanism would also explain why we cannot detect an Lfc-stimulated increase in the cellular levels of activated Rac1 and would be consistent with the reported GTP-dependent association of Rac1 with microtubules (27). Thus, rather than acting as a GEF, Lfc may serve to recruit Rac to the appropriate cellular location such that it can

**Fig. 8. Colocalization of Lfc and microtubules.** NIH 3T3 cells transiently expressing HA-Lfc were fixed, and cells expressing Lfc were visualized by indirect immunofluorescence using anti-HA (HA11) and BODIPY-labeled secondary antibody to detect HA-Lfc (A) and anti-tubulin antibody and a rhodamine-labeled secondary antibody to visualize microtubules (B).

**Fig. 9. Lfc binds tubulin in vitro.** A, a schematic representation of the GST-fusion proteins used for affinity precipitation of tubulin from Jurkat T-cell lysates. GST-Lfc (DH–PH) was purified from SF-21 insect cells, and GST-Lfc (PH) was purified from E. coli. B, GST-fusion proteins of Lfc (DH/PH) and Lfc (PH) (~5 µg) were immobilized on glutathione-agarose beads and used in affinity precipitations from Jurkat cell lysates. Proteins were separated by SDS-PAGE and Western blotted using an anti-tubulin antibody. GST (~15 µg) bound to glutathione-agarose was used as a negative control.
engage the necessary signaling partners for efficiently activating JNK and/or for triggering the necessary actin cytoskeletal changes that lead to lamellipodia formation.

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