A Novel Interaction between the SH2 Domain of Signaling Adaptor Protein Nck-1 and the Upstream Regulator of the Rho Family GTPase Rac1 Engulfment and Cell Motility 1 (ELMO1) Promotes Rac1 Activation and Cell Motility*

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This article has been withdrawn at the request of the authors. The authors were recently made aware that the Coomassie Blue image in Fig. 1E was rotated 180°. The Myc immunoblot from lysate in Fig. 2B was reused in Figs. 2C and 6B. A portion of the GAPDH immunoblot in Fig. 2C was reused in Fig. 6B as GAPDH. The last lane of the Myc immunoblot from lysate in Fig. 3A was reused in the first lane of the Myc immunoblot from lysate in Fig. 3C. The first lane of the Myc immunoblot from lysate in Fig. 3E was reused in the last lane of the Myc immunoblot from lysate in Fig. 3G. The last lane of the Myc immunoblot from the IP in Fig. 3E was reused in the first lane of the Myc immunoblot from the IP in Fig. 4C. A portion of the Myc immunoblot from the lysate in Fig. 4A was reused as the HA immunoblot from lysate in Fig. 6C. A portion of the GAPDH immunoblot in Fig. 5A was reused in Fig. 6C as GAPDH. Although the authors believe that the biological conclusions outlined in the article are valid, the figures presented in the paper do not provide an accurate representation of the original data, and therefore the scientific integrity of the study has been compromised. The authors unanimously agree that the most appropriate course of action is to withdraw the article. All authors sincerely apologize to the scientific community for not detecting these errors prior to publication and any negative impact this may have caused.

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3 The abbreviations used are: N-WASP, neural Wiskott Aldrich syndrome protein; WIP, WASP-interactin protein; ELMO, engulfment and cell motility; PV, pervanadate.
ELMO1-Nck-1 Interaction Promotes Rac1 Activation

Previous studies have shown that ELMO1 binds directly to the SH3 domain of hematopoietic cell kinase (Hck), a Src family kinase, and is phosphorylated by Hck (19, 20). Tyrosine phosphorylation of ELMO1 is important for Rac1 activation. However, the mechanism by which tyrosine phosphorylation of ELMO1 influences the GEF activity is not clear. Here we reported that phosphotyrosine residues at position 18, 216, 395, and 511 of ELMO1 mediate the binding to the SH2 domain of Nck-1. The association of Nck-1 with ELMO1 facilitated the binding to the SH2 domain of Nck-1. The Nck-1-SH2 domain of Nck-1 was generated by PCR from human Nck-1 as the template and ligated into pGEX-4T-3 expression vector. Myc-ELMO1<sup>1–625</sup>, ELMO1<sup>1–495</sup>, ELMO1<sup>1–315</sup>, ELMO1<sup>531–551</sup>, ARM1, ARM2, and HA-RhoG<sup>V12A</sup> were generated by FulenGen, Guangzhou, China as described previously (17, 22, 23).

Recombinant Protein Purification—Escherichia coli (BL21) was transformed with pGEX-4T-3 or pGEX-Nck-1-SH2, pGEX-Nck-1-SH3<sup>1</sup>, pGEX-Nck-1-SH3<sup>2</sup>, or pGEX-Nck-1-SH3<sup>3</sup> and incubated with 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 h. The GST fusion proteins were purified from bacterial lysates with GST-Sepharose 4B beads according to the manufacturer’s instruction (Amersham Biosciences). The GST fusion protein was then washed with PBS and stored at −80 °C.

E. coli grown in LB medium supplemented with 100 μg/ml ampicillin was used to inoculate LB medium containing 1% glucose at 37 °C. The cultures were grown to an OD<sub>600</sub> of 0.6 at 30 °C. The fusion proteins were recovered by binding to GSH-Sepharose 4B beads according to the manufacturer’s recommendation. The beads were washed in PBS, then boiled in SDS sample buffer and fractionated by 10% SDS-PAGE. Immunoblot analysis was performed with His-ELMO1 and incubated with anti-Myc antibody.

Western Blot and Immunoprecipitation—For Western blot assay, cell lysates were prepared and spun at 15,000 × g for 15 min, and the supernatants were pre-cleared with GST-conjugated Sepharose beads and then incubated with GST or GST-Nck-1-SH2 that conjugated to Sepharose beads for 2 h at 4 °C. The proteins bound to Sepharose beads were eluted and then loaded on SDS-PAGE for Western blot analysis.

For in vitro binding assay, purified His-ELMO1 were incubated with purified GST or GST-Nck-1-SH2 fusion protein that conjugated to Sepharose beads in 500 μl of reaction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 5 mM NaF, 1% Triton X-100, and protease inhibitor mixture) for 12 h at 4 °C. After centrifugation, the proteins bound to Sepharose beads were washed with ice-cold PBS, mixed with 2 × SDS sample buffer and then loaded on SDS-PAGE for Western blot analysis.

Plasmid Constructs—The full-length ELMO1 cDNA was amplified and cloned into the pReceiver M68 expression vector (FulenGen, Guangzhou, China). Full-length human Nck-1 (NCBI accession number BC006403) cDNA was amplified and cloned into the pReceiver M11 expression vector by FulenGen, Guangzhou, China. Flag-Dock180 was kindly provided by Dr. Michiyuki Matsuda (Kyoto University, Kyoto, Japan). The 4YF, 3YF, Y511F, Y395F, Y216F, and Y18F mutants of ELMO1 (20) and the R308K, SH3–1<sup>2</sup>, SH3–1<sup>3</sup>, SH3–1<sup>2</sup>, SH3–1<sup>3</sup>, SH3–1<sup>2</sup>, SH3–1<sup>3</sup>, SH3<sup>1</sup>, ARM1, ARM2, and HA-RhoG<sup>V12A</sup> were generated by FulenGen, Guangzhou, China as described previously (17, 22, 23).

EXPERIMENTAL PROCEDURES

Antibodies and Reagents—Rabbit polyclonal antibodies to rabbit polyclonal anti-Nck-1, mouse monoclonal anti-Nck-1, mouse monoclonal anti-MyC, mouse monoclonal anti-HA, mouse monoclonal anti-Nck-1, mouse monoclonal anti-Flag were purchased from Cell Signaling Technology (Beverly, MA); rabbit polyclonal anti-HA, rabbit monoclonal anti-Flag were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); mouse monoclonal anti-HA, rabbit polyclonal anti-Nck-1, mouse monoclonal anti-Nck were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); mouse monoclonal anti-HA, rabbit monoclonal anti-Flag were purchased from Cell Signaling Technology (Beverly, MA); monoclonal antibodies (Pelfreez), mouse monoclonal anti-Flag (AbBotMaster Mix were bought from Millipore, Billerica, MA).

Nck-1 siRNA was designed and synthesized by GenePharma, Shanghai, China. The sense targeting sequence was: GCA-GAAUAAUCCAUCUAACTT. An irrelevant dsRNA with the sense sequence UU-CUCGAACGUGUCAGTUTT was used as the control.

Cell Culture and Transfection—The HEK293T cells were obtained from the Cell Bank, Chinese Academy of Medical Sciences, Shanghai, China and maintained in high-glucose DMEM (Invitrogen, Gaithersburg, MD) supplemented with 10% FBS (Invitrogen). Pervanadate (100 μM) was added in the culture medium for 30 min at 37 °C after transfection.

The expression plasmids or siRNA were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. If necessary, carrier DNA or scramble siRNA was added to keep equal plasmid/siRNA concentration between different groups.

Plasmid Constructs—The full-length ELMO1 cDNA was amplified and cloned into the pReceiver M68 expression vector (FulenGen, Guangzhou, China). Full-length human Nck-1 (NCBI accession number BC006403) cDNA was amplified and cloned into the pReceiver M11 expression vector by FulenGen, Guangzhou, China. Flag-Dock180 was kindly provided by Dr.
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agarose and quantifying the protein concentrations, aliquots with equal amounts of proteins were incubated with Rac assay reagent (PAK-1 PBD, agarose) at 4 °C for 1 h. The precipitated GTP-bound Rac1 was then eluted in Laemmli reducing sample buffer, resolved in a SDS-PAGE, and immunoblotted with monoclonal anti-Rac1 antibody.

Immunofluorescence Staining—1 × 10^5 cells were plated on glass coverslips and transfected with various plasmids using Lipofectamine 2000. Cells were fixed with cold methanol (Sigma), permeabilized with 0.3% Triton X-100 min. Cells were incubated with anti-Myc and anti-Flag antibodies 4 °C overnight, followed by incubating with goat anti-mouse Alexa Fluor® 594 and goat anti-rabbit Alexa Fluor® 488 (Invitrogen) for 1 h. After washing, the chambers slides were mounted with Slow Fade®Gold antifade reagent (Invitrogen). All samples were observed and analyzed with a Olympus FV1000 confocal microscope (Japan).

Migration Assay—Migration assay was performed as described previously (11). Briefly, cells were seeded onto the filter in the upper compartment of the chamber and incubated for 12 h. Cells in the upper surface of the transwell were removed using cotton swabs. Migrated cells attached on the undersurface were fixed with absolute methanol for 15 min and stained with crystal violet solution (0.5% in PBS) for 30 min. Cells were counted under microscope at 200×. Microphotographs of 9 random fields were taken and the average number of migrating cells was determined for each experimental condition.

Statistical Analysis—Statistical differences between two groups were determined by the Student’s t test. p < 0.05 was considered statistically significant. The results were expressed as mean ± S.D. from at least three experiments.

RESULTS

Characterization of a Direct Interaction between ELMO1 and the SH2 Domain of Nck-1—We previously identified a novel interaction between ELMO1 and the SH2 domain of Nck-1 by mass spectrometry (11). To confirm this interaction, Myc-tagged ELMO1 and Flag-tagged Nck-1 were co-transfected into HEK293T cells and exogenous ELMO1 was immunoprecipitated by anti-Myc antibody. As shown in Fig. 1A, exogenous ELMO1 was co-immunoprecipitated with exogenous Nck-1. We next analyzed the association of endogenous ELMO1 with endogenous Nck-1. In HEK293T cells, endogenous Nck-1 was detected in the immunoprecipitates in the presence of a specific
anti-ELMO1 antibody, but not in the presence of an isotype control antibody (Fig. 1B).

Previous study has shown that the C terminus (532–727aa) of ELMO1 mediates the interaction with Dock180 (24), and Dock180 interacts with Nck-2 (25). To exclude the possibility that the interaction between ELMO1 and Nck-2 is mediated by Dock180, we generated two Myc-tagged ELMO1 mutants, namely ELMO1<sub>H9004</sub>531 and ELMO1<sub>1–625</sub> (Fig. 1C), as described previously (24). The ELMO1<sub>H9004</sub>531 lacks the N-terminal 531 amino acid residues, whereas the ELMO1<sub>1–625</sub> represent truncations at residue 625. GST pull-down assay showed that ELMO1<sub>1–625</sub> bound to Nck-1-SH2 as well as that of wild-type ELMO1 (ELMO1<sub>WT</sub>). Whereas, no obvious interaction was detected between Nck-1-SH2 and ELMO1<sub>H9004</sub>531 (Fig. 1D), suggesting that Dock180 is not involved in the ELMO1-Nck-1 interaction. To further map the region of ELMO1 responsible for binding to Nck-1-SH2, we generated another two ELMO1 deletion mutants, namely ELMO1<sub>1–495</sub> and ELMO1<sub>1–315</sub> (Fig. 1C). As shown in Fig. 1D, ELMO1<sub>1–495</sub> maintained interaction with Nck-1-SH2, whereas, ELMO1<sub>1–315</sub> showed no binding, suggesting that the N-terminal 495 amino acid residues of ELMO1 are required for interacting with Nck-1-SH2.

To examine whether ELMO1 and Nck-1 interacts directly, we analyzed the ability of bacterially produced GST-Nck-1-SH2 and His-ELMO1 to associate in vitro. As shown in Fig. 1E, GST-Nck-1-SH2, but not GST, bound to His-tagged ELMO1, indicating that the Nck-1-SH2 fusion protein is capable of interacting with ELMO1 directly in solution.

To further confirm that the SH2 domain of Nck-1 mediates the binding with ELMO1, we generated GST fusion protein of individual SH2 or each of the three SH3 domains of Nck-1 (Fig. 2A). GST pull-down experiments were performed using lysates from Myc-tagged ELMO1-transfected HEK293T cells. As shown in Fig. 2B, the GST-Nck-1 SH2 domain bound to Myc-tagged ELMO1, whereas, none of the three Nck-SH3 domains could pull-down Myc-tagged ELMO1.

We next generated series full-length Nck-1 mutants containing specific amino acid substitutions W38K, W143K, and W229K in each of the SH3 domains predicted to inhibit interactions with proline-containing proteins, or R308K in the SH2 domain predicted to disrupt interaction with p-Y residues. As described previously (21), Nck-1 mutant proteins (SH3<sub>1–1</sub>, SH3<sub>2–2</sub>, and SH3<sub>3–3</sub>) in which two SH3 domains were inactive and therefore had only a...
single functional SH3 domain. Nck-1 mutant SH3–1\(^{-2},\)–3\(^{-3}\) with inactivating Trp-to-Lys substitutions in all three SH3 domains therefore has no functional SH3 domain. Nck-1 mutants or WT Nck-1 were co-transfected into HEK293T cells with Myc-tagged ELMO1, respectively. Co-immunoprecipitation assay showed that R308K interacted very weakly with Myc-tagged ELMO1 compared with that of WT Nck-1. However, SH3–1\(^{-2},\)–3\(^{-3}\) bound to ELMO1 as well as
that of WT Nck-1 (Fig. 2C). The SH2 domain of Nck-1 is sufficient to mediate the interaction with ELMO1.

Interaction of ELMO1 and Nck-1 Is Tyrosine Phosphorylation Dependent—SH2 domains are known to bind to phosphotyrosine residues in proteins (26). We thus examined whether the Nck-1-ELMO1 interaction is dependent on tyrosine phosphorylation of ELMO1. To address this issue, we transfected Myc-tagged ELMO1 into HEK293T cells and then treated cells with tyrosine phosphatase inhibitor pervanadate (PV). The tyrosine phosphorylation (p-Y) of ELMO1 was detected by immunoprecipitation using anti-Myc antibody followed by immunoblot using anti-4G10 antibody. As shown in Fig. 3A and B, the p-Y of ELMO1 was significantly enhanced by PV treatment. GST pull-down assay revealed that the interaction between GST-Nck-1-SH2 and Myc-tagged ELMO1 was enhanced by PV treatment, and the increase was statistically significant when compared with that of untreated control cells (Fig. 3C and D). Previous study reported that ELMO1 is a substrate of Hck and overexpression of Hck leads to tyrosine-phosphorylation of ELMO1 (19, 20). Consistent with previous reports, the p-Y of ELMO1 was enhanced in Hck overexpressing cells (Fig. 3E and F). GST pull-down assay demonstrated that the Nck-1-SH2-ELMO1 interaction was also significantly enhanced (Fig. 3G and H). These data provide evidence that the p-Y of ELMO1 is important in binding to Nck-1.

There are 5 tyrosine residues (Y18, Y216, Y395 or Y511, and Y720) on ELMO1 have been identified to be phosphorylated by Hck (20) and 4 of them are located in the N-terminal 531 amino acids of ELMO1. To determine the contribution of these tyrosine residues to Nck-1 binding, we generated Myc-tagged ELMO1 constructs with Y18, Y216, Y395, or Y511 substitutions, and then transiently transfected them into HEK293T cells, respectively. 24 h after transfection, the interaction between GST-ELMO1 and Nck-1-SH2 was analyzed by GST pull-down assay. As shown in Fig. 4A and B, mutation of individual Y18, Y216, Y395, or Y511 residue of ELMO1 attenuated the interaction with Nck-1-SH2 when compared with that of WT ELMO1. We next mutated three (18, 216, and 395), or four tyrosine residues (18, 216, 395, and 511) together; these mutants were designated 3YF and 4YF, respectively. GST pull-down assay revealed that binding of Nck-1-SH2 to 4YF mutant was markedly decreased (Fig. 4B). To further confirm this finding, WT ELMO1 or 4YF mutant was co-transfected into HEK293T cells with HA-tagged Hck and Flag-tagged Nck-1. As shown in Fig. 4C and D, p-Y of 4YF was dramatically lower than that of WT ELMO1. In line with the weak p-Y, the binding of 4YF to Nck-1 was substantially reduced compared with WT ELMO1. However, 4YF did not eliminate the binding to Nck-1. Collectively, these data indicate that p-Y of Y18, Y216, Y395, and Y511 is necessary, but not sufficient, for binding to Nck-1.

ELMO1-Nck-1 Interaction Promotes Rac1 Activation—ELMO1 and Dock180 act as a bipartite GEF to activate Rac1 (24). To explore the functional importance of the ELMO1-Nck-1 interaction, Myc-tagged ELMO1, and Flag-tagged Dock180 were co-transfected into HEK293T cells either with Nck-1 WT or Nck-1 R308K mutant. Consistent with previous report, co-expression of ELMO1 and Dock180 sig-
nificantly increased Rac1 GTP loading. Overexpressing WT Nck-1 further enhanced Rac1 GTP loading, whereas R308K mutant did not (Fig. 5, A and B). To further confirm this result, endogenous Nck-1 was silenced by siRNA (Fig. 5C). Compared with scramble siRNA, transfecting Nck-1 siRNA led to a significant decrease of Rac1 activity. Furthermore, transfecting siRNA-resistant-WT Nck-1, but not siRNA-resistant-R308K, rescued the reduced Rac1 GTP loading (Fig. 5D). These data indicate that Nck-1-ELMO1 interaction could enhance the ability of the Dock180/ELMO1 complex to promote Rac1 activity.

Nck-1 Enhances ELMO1-RhoG Interaction—We next explored the underlying mechanism of Nck-1-promoted Rac1 activity. Previous study demonstrated that the GTP-bound form of RhoG binds to the N-terminal 115 residues of ELMO1 comprising the Armadillo repeats 1 (9-49 amino acid residues) and 2 (65-110 amino acid residues) and thus promotes Rac1 activity (23). Since Nck-1 also binds to the N terminus of ELMO1, we thus examined whether ELMO1 binds to active RhoG and Nck-1 simultaneously. To this end, we generated ARM1 and ARM2 ELMO1 mutants by mutating 2 residues in each repeat based upon their high degree of conservation among CED12/ELMO proteins as well as other ARM repeat-containing proteins (Fig. 6A) (23). As shown in Fig. 6B, Nck-1-SH2 bound to ARM1 and ARM2 ELMO1 mutants as well as WT-ELMO1, indicating that ELMO1 associates with Nck-1 through a region distinct from that of active RhoG.

We next evaluated the impact of Nck-1 on the interaction between ELMO1 and active RhoG. Flag-tagged WT Nck-1, R308K, or SH3−1,2,3− (in which all three SH3 domains of Nck-1 are inactivated) was co-transfected with Myc-tagged ELMO1 and HA-tagged RhoG(V12A) (constitutively active RhoG) into HEK293T cells. As shown in Fig. 6C, the interaction between ELMO1 and RhoG(V12A) was dramatically enhanced by overexpressing WT Nck-1 when compared with empty vector transfected cells. However, R308K mutant of Nck-1 had no significant effect on ELMO1-RhoG(V12A) interaction. On the other hand, SH3−1,2,3− (in which all three SH3 domains are inactivated) showed comparable promoting effect as that of WT Nck-1, indicating that the SH2 domain of Nck-1 is sufficient to promote ELMO1-RhoG interaction. Since 4YF mutant of ELMO1 did not interact with Nck-1-SH2, we assume that its binding to active RhoG might be reduced. To test this issue, WT ELMO1 or 4YF was transfected into HEK293T cells together with RhoG(V12A). Co-immunoprecipitation assay revealed that the intensity of 4YF mutant co-precipitated with RhoG(V12A) was dramatically decreased compared with that of

![FIGURE 5. Nck-1 promotes ELMO1/Dock180-induced Rac1 activity.](image-url)
WT ELMO1 (Fig. 6D), providing further evidence that Nck-1 promotes ELMO1-RhoG interaction.

ELMO1-Nck-1 Interaction Promotes Relocation of ELMO1 and Cell Migration—To assess the biological relevance of Nck-1-ELMO1 interaction, we first investigated whether Nck-1 affects the localization of ELMO1 by immunofluorescence staining. As previously found (24), Myc-tagged ELMO1 was observed in the cytoplasm when expressed alone. When co-expressing Myc-tagged ELMO1 with WT Nck-1, ELMO1 was observed in plasma membrane and co-stained with Nck-1. In contrast, Myc-tagged ELMO1 remained cytosolic when co-transfected with R308K Nck (Fig. 7A). These data indicate a role of Nck in recruiting ELMO1 to plasma membrane.

Because Rac1 lies downstream of the ELMO1/Dock180 complex in signaling pathways leading to cell migration, we next carried out cell migration assays with wild-type and R308K mutant forms of Nck-1. As reported previously (24), co-expression of Dock180 and ELMO1 enhanced cell migration. Co-expressing WT Nck-1 with Dock180 and ELMO1 further enhanced ELMO1/Dock180-promoted cell migration. However, R308K mutant forms of Nck-1 failed to augment ELMO1/Dock180-promoted cell migration (Fig. 7B). In reciprocal experiments, we examined whether knockdown endogenous Nck-1 would inhibit cell migration. As expected, silencing endogenous Nck-1 by siRNA resulted in a ~60% reduction on cell migration compared with scramble siRNA transfected cells. Re-expression of siRNA-resistant WT Nck-1, but not R308K mutant, rescued the cell mobility (Fig. 7C). Together, these data indicate that Nck-1 functions synergistically with ELMO1/Dock180 complex to promote cell motility.

In the present study, we characterized a novel interaction between ELMO1 and the SH2 domain of Nck-1. SH2 domains are known to bind to phosphotyrosine residues in proteins (26). In the present study, we demonstrated that four Hck-dependent pTyr sites (Y18, Y216, Y395, and Y511) on ELMO1 are necessary for binding to Nck-SH2. Mutation of all four tyrosine residues, but not single residue, substantially reduced the binding to Nck-SH2. Moreover, we noted that mutation of all 4 tyrosines does not completely eliminate the binding. It is possible that other non-Hck-dependent pTyr sites on ELMO1 also bind to Nck-SH2. This type of multisite binding has been documented for many protein-protein interactions. For example, multisite phosphorylation of cyclin-dependent kinase inhibitor Sic1 is required for its degradation by SCF ubiquitin ligase pathway (27). In addition, three pYDxV binding sites on Nephrin have been found and mutation of all three tyrosine residues in YDxV motifs abolished the binding to the SH2 domain of Nck (28). These data suggest that multisite phosphorylation may be a more general mechanism to set thresholds in regulated protein-protein interactions.

Conventional SH2 domains normally bind to their cognate ligands in a two-pronged mode mediated primarily by the pTyr residue. However, it has been reported that the SH2 domain of SAP interacts with its ligand at residues positions −2 and +3 relative to pTyr and the pTyr residue itself. The SAP/SH2D1A...
SH2 domain can recognize a peptide ligand using either all three residues for maximal affinity or a combination of any two (29, 30). Whether the low level binding observed between 4YF mutant and GST-Nck-1-SH2 is caused by the binding of Nck-1-SH2 to non pTyr residue of ELMO1 needs further investigation.

Previous study reported that tyrosine phosphorylation of ELMO1 is important for the GEF activity of ELMO1/Dock180 complex (18, 20). However, the underlying mechanism remains unknown. The present study revealed that the interaction between ELMO1 and active RhoG was substantially reduced when all four tyrosine residues are mutated. In addition, 4YF mutant could not recruit ELMO1 to the plasma membrane. In line with our finding, previous studies have shown that phosphorylation of a tyrosine residue in the conserved acidic region of Vav1, Vav2, or Vav3 is required for their GEF activity (31).

The present study adds ELMO1/Dock180 complex to the list of Src-activated GEFs targeting Rac1 and/or Cdc42, which includes Vav proteins, β-PIX, RGRF1 (also known as CDC25 or GRF1) and FRG (also known as FARP2) (32–35). It will be inter-
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 resting to examine whether phosphorylation of tyrosine residues would cause conformational change of these Src-activated GEFs.

A number of studies have shown a role of Nck in Rac1 activation. For example, Pak serves as a common effector protein of Rac/cdc42 (36). Nck recruits Pak1 to the plasma membrane to bind and to be activated by Rac/cdc42 (37). On the other hand, Yoshii et al. showed that PDGF stimulation causes association of the αPIX, a GEF for Rac, with the p85 subunit of PI-3K and Nck. This association results in activation of the αPIX (38). In this case, Nck appears to act upstream of Rac by relocating the Pak1-bound αPIX to the membrane. The present study showed a novel function of Nck-1 acting upstream of Rac1: Nck-1 promotes ELMO1 binding to active RhoG and thus recruits ELMO1/Dock180 complex to plasma membrane. Previous study demonstrated that engagement of RhoG to ELMO causes conformational changes of ELMO and disrupts autoinhibition of ELMO (39, 40). It is possible that binding to Nck-1 promotes conformational changes of ELMO1, or stabilizes the open conformation of ELMO induced by active RhoG.

It has been shown that GTP-loaded Rac1 activates the Arp2/3 complex via WAVE proteins to induce actin-based plasma membrane projection (41, 42). Our study showed that ELMO1 directly interacts with the SH2 domain of Nck-1. Since Nck binds with N-WASP through its SH3 domains to stimulate Arp2/3 complex and induces actin nucleation (43), these data suggested that Nck-1-ELMO1-Dock180 complex might function as another link between GTP-Rac and Arp2/3 complex. Future detail experiments may help to understand how Nck-1-ELMO1-Dock180 complex activation of Arp2/3 complex.

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