Interaction of c-Jun Amino-terminal Kinase Interacting Protein-1 with p190 rhoGEF and Its Localization in Differentiated Neurons*

(Received for publication, May 24, 1999, and in revised form, September 17, 1999)

Debra Meyer‡, Albert Liu§, and Ben Margolis‡§¶

From the ¶Howard Hughes Medical Institute and the Departments of §Internal Medicine and ¶Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109

c-Jun amino-terminal kinase (JNK) interacting protein-1 (JIP-1) was originally identified as a cytoplasmic inhibitor of JNK. More recently, JIP-1 was proposed to function as a scaffold protein by complexing specific components of the JNK signaling pathway, namely JNK, mitogen-activated protein kinase kinase 7, and mixed lineage kinase 3. We have identified the human homologue of JIP-1 that contains a phosphotyrosine binding (PTB) domain in addition to a JNK binding domain and an Src homology 3 domain. To identify binding targets for the hJIP-1 PTB domain, a mouse embryo cDNA library was screened using the yeast two-hybrid system. One clone encoded a 191-amino acid region of the neuronal protein rhoGEF, an exchange factor for rhoA. Overexpression of rhoGEF promotes cytoskeletal rearrangement and cell rounding in NIE-115 neuronal cells. The interaction of JIP-1 with rhoGEF was confirmed by coimmunoprecipitation of these proteins from lysates of transiently transfected HEK 293 cells. Using glutathione S-transferase rhoGEF fusion proteins containing deletion or point mutations, we identified a putative PTB binding site within rhoGEF. This binding site does not contain tyrosine, indicating that the JIP PTB domain, like that of XIa and Numb, binds independently of phosphotyrosine. Several forms of endogenous JIP-1 protein can be detected in neuronal cell lines. Indirect immunofluorescence analysis localized endogenous JIP-1 to the tip of the neurites in differentiated NIE-115 and PC12 cells. The interaction of JIP-1 with rhoGEF and its subcellular localization suggests that JIP-1 may function to specifically localize a signaling complex in neuronal cells.

Protein interaction domains are key components in the organization of cellular signaling complexes. Initially identified in the adaptor protein Shc (1–3), the phosphotyrosine interaction or phosphotyrosine binding (PTB) domain was found to interact with targets containing the motif Y-X-Asn-Pro-X-Tyr(P) (where Y is a hydrophobic residue). PTB domains have since been found in several functionally unrelated proteins, including XIa, Numb, and Disabled (4). Structurally, the PTB domain is similar to the pleckstrin homology domain forming a β-sandwich capped by an α-helix (5), and several amino acids within the domain have been found to be critical for interaction with the consensus binding motif (6–8).

The identification of additional PTB-containing proteins and their binding targets has revealed that phosphorylation of the tyrosine within this motif is not required by all PTB domains for their binding. For example, the PTB domain of the XIa family of proteins does not require phosphorylation of the tyrosine residue within this motif found on the amyloid precursor protein (8). Furthermore, the PTB domain of the mammalian Numb (mNumb) protein has been shown to interact with the protein LINX (ligand of Numb protein X) independent of tyrosyl phosphorylation (9), whereas Drosophila Numb (dNumb) interacts with Numb-associated kinase (NAK) through a motif that does not contain tyrosine (10). Therefore, it is apparent that the function of PTB domains is not restricted to interactions with tyrosine-phosphorylated partners but plays a more general role in the organization of protein complexes.

To further analyze the role of PTB domain proteins in cellular signaling, we have cloned and characterized the human homologue of the JNK interacting protein-1 (JIP-1). The murine JIP-1 (mJIP-1) was initially reported to act as a cytoplasmic inhibitor of the JNK family of kinases (11). More recently, JIP-1 was found to interact with mitogen-activated protein kinase 7, mixed lineage kinase 3, or DLK, as well as JNK1 and JNK2 (12). Coexpression of JIP-1 and JNK with mitogen-activated protein kinase 7 or mixed lineage kinase 3 increased JNK activation, presumably by facilitating formation of the JNK activating complex (12). An additional form of JIP-1 has been reported in mouse (JIP-1b) (12), rat (islet-brain 1) (13), and human (14), which contains a 47-amino acid insertion completing a carboxyl-terminal PTB domain. The rat JIP-1, referred to as islet-brain 1, was found to be nuclear as well as cytoplasmic and to function as a transactivator of the GLUT2 gene (13).

Using the yeast two-hybrid system to identify binding partners for the hJIP-1 PTB, we identified the protein rhoGEF. RhoGEF is highly expressed in neuronal tissues and specifically interacts with rhoA (15). Activation of rhoA by stimulation with lysophosphatidic acid results in stress fiber formation and cytoskeletal contraction (16, 17). In N1E-115 cells, activation of rhoA or overexpression of rhoGEF results in neurite retraction and cell rounding (15). In this study, we have defined the JIP-1 PTB binding site on rhoGEF. Similar to the binding site on the dNumb PTB binding partner NAK, the rhoGEF binding site is related to the traditional PTB Y-X-Asn-Pro-X-Tyr(P) motif but lacks a tyrosine residue at the 0 position. Indirect immunofluorescence analysis demonstrates that endogenous JIP-1 is localized to the tip of the neurite extensions in differentiated N1E-115 and PC12 cells. These results suggest that in addition to its interaction with members of the
phosphate-buffered saline and lysed in lysis buffer (50 mM in cold 1 M NaCl, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EGTA) supplemented with 1 mM phenylmethylsulfonyl fluoride, 100 mM NaF, 200 mM sodium orthovanadate, 10 mM tetrasodium pyrophosphate, 10 μg of aprotinin/ml and 10 μg of leupeptin/ml. For immunoprecipitations, lysates (4.5 mg) were incubated with anti-Myc (9E10), anti-HA (12CA5), or anti-JIP-1 (177) and protein A-Sepharose overnight at 4 °C. Immunocomplexes were washed three times in HNTG (20 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, and 0.1% Triton X-100), boiled in 1× sample buffer, and separated by SDS-polyacrylamide gel electrophoresis. Transfer to nitrocellulose and immunoblotting were conducted using anti-Myc, anti-HA, and horseradish peroxidase-anti-mouse antibody as described elsewhere (22). Endogenous rhoGEF and immunoprecipitated endogenous JIP-1 were detected by immunoblotting with anti-rhoGEF (187, 1:1000) and anti-JIP-1 (176, 1:1000), respectively. Blots were visualized using chemiluminescence (Renaissance, NEN Life Science Products).

**GST Fusion Protein Pull-down Assay**—GST fusion proteins were expressed and purified as described previously (22). Lysates were incubated with GST fusion proteins bound to glutathione-agarose (Amersham Pharmacia Biotech). For immunoprecipitations, lysates (4.5 mg) were incubated with anti-Myc (9E10), anti-HA (12CA5), or anti-JIP-1 (177) and protein A-Sepharose overnight at 4 °C. Immunocomplexes were washed three times in HNTG, boiled in 1× sample buffer, and separated by SDS-polyacrylamide gel electrophoresis. Ponceau Red staining of the nitrocellulose membrane verified the presence of comparable amounts of GST fusion proteins.

**Immunofluorescence**—Differentiated and proliferating N1E-115 or PC12 cells were seeded on acid-washed coverslips. For PC12 cells, the coverslips were coated with poly-L-lysine (Sigma) 24-48 hours before plating (Collaborative Research). Cells were fixed to the coverslips with 4% paraformaldehyde for 30 min at room temperature and permeabilized with 0.1% Triton X-100 in 1× phosphate-buffered saline with 10 mM glycine. Coverslips were blocked in 100% goat serum for 1 h then incubated with affinity-purified anti-JIP-1 (152) in phosphate-buffered saline/glycine/2% goat serum /1:250 dilution) for 1 h in a humidified chamber. Coverslips were rinsed then incubated with goat anti-rabbit fluorescein isothiocyanate (1:500 dilution, Molecular Probes) in 1× phosphate-buffered saline/glycine/2% goat serum for 1 h in a humidified chamber. Coverslips were mounted to slides with Prolong (Molecular Probes) and analyzed with an Olympus BX60 fluorescent microscope. Digital images were taken with a SPOT CCD camera (Diagnostic Instruments Inc.).

**RESULTS**

**Identification of hJIP-1 Interacting Proteins**—We first identified the PTB domain of human JIP-1 as an expressed sequence tag following a data base search for sequences similar to the PTB domain of Shc (4). JIP proteins have been identified from several species (11, 13). In addition, we have identified a Caenorhabditis elegans JIP located on chromosome III, suggesting an evolutionarily conserved role for the JIP family of proteins. Fig. 1 shows an alignment of the JIP family of PTB domains. Based on alignment, several highly conserved sequences (indicated by boxes) represent predicted regions of secondary structure important for the formation of the β sandwich motif of the PTB domain (5). In order to further characterize the role of PTB-containing proteins in the organization of cellular signaling complexes, we sought to identify binding partners for the highly conserved PTB domain of JIP-1.

The hJIP-1 PTB-Gal4 fusion protein was expressed in yeast and selected for growth on media lacking tryptophan. Yeast positive for the expression of the hJIP-1 PTB were subsequently transformed with a mouse embryo (day 9 and 10) two-hybrid library (20). Positive interacting clones were selected for growth on plates lacking tryptophan, leucine, and histidine and subsequently tested for β-galactosidase activity. To confirm the specificity of the interaction, we coexpressed positive clones with the wild-type hJIP-1 PTB, hJIP-1 PTB

**JIP-1 PTB Interaction with p190 rhoGEF**

**Cell Culture and Transfection**—Human embryonic kidney (HEK) 293 and mouse N1E-115 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 100 units of penicillin/ml and 100 μg of streptomycin sulfate/ml and supplemented with 10% fetal calf serum. N1E-115 cells were morphologically differentiated by growth in serumfree DMEM containing 1% bovine serum albumin for 2–3 days. PC12 cells were maintained in DMEM plus antibiotics, supplemented with 7.5% fetal calf serum and 2.5% horse serum. PC12 cells were differentiated by growth in DMEM plus antibiotics, supplemented with 1% fetal calf serum and 50 ng/ml nerve growth factor. For transfections, 293 cells were seeded the day before at approximately 106 cells/10-cm plate. Cells were transfected with 5 μg of DNA using the calcium phosphate method (21).

**Immunoprecipitation and Immunoblotting**—Cells were washed twice in cold 1× phosphate-buffered saline and lysed in lysis buffer (50 mM HEPES, pH 7.5, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EGTA) supplemented with 1 mM phenylmethylsulfonyl fluoride, 100 mM NaF, 200 mM sodium orthovanadate, 10 mM tetra-

**DNA Constructs and Mutagenesis**—pAS2–1 JIP-1 PTB used in the two-hybrid screen was constructed by PCR amplification of the JIP-1 PTB domain (amino acids 554–711) and subsequent cloning into the Nco I site of pAS2–1 to create a Gal4 DNA-binding domain fusion protein. pAS2–1 JIP-1 PTB was generated by PCR-based site-directed mutagenesis from the wild-type pAS2–1 JIP-1 PTB. X11 was described in Ref. 8. Myc-JIP-1 was generated by PCR amplification and cloning this fragment into pGEX 4T1 (Amersham Pharmacia Biotech). GST-rhoGEF deletion mutants were constructed by PCR amplification of the regions indicated and cloned into pGEX 4T1 (Amerham Pharmacab Biotech). GST-rhoGEF deletion mutants were constructed by PCR amplification of the regions indicated and cloned into pGEX 4T1. All GST-rhoGEF point mutations were generated by PCR, ligated, and mutagenized. All constructs were sequenced using Sequenase, version 2.0 (United States Biological Corp.), or sequenced by the University of Michigan sequencing core.

**Yeast Two-hybrid Screening**—The yeast two-hybrid screen was carried out essentially as described in Refs. 18 and 19. Briefly, Y190 cells were transformed with pAS2–1 JIP-1 PTB, and yeast expressing the bait were selected for growth on medium lacking tryptophan. Y190 JIP-1 bait were subsequently transformed with a mouse embryo (day 9 and 10) two-hybrid library (kindly provided by Ann Vojtek, University of Michigan) (20). Positive interacting clones were selected for growth on plates lacking tryptophan, leucine, and histidine supplemented with 25 mM 3-aminoazonitrole. Histidine-positive colonies were subsequently tested for β-galactosidase activity. To confirm specificity of the interacting plasms, the β-galactosidase-positive colonies were reisolated and retransformed into Y190 expressing the wild-type JIP-1 PTB, JIP-1 PTB, or X11 a PTB. This yielded 152 clones conferring growth on plates lacking tryptophan, leucine, and histidine supplemented with 25 mM 3-aminoazonitrole. Histidine-positive colonies were subsequently tested for β-galactosidase activity. To confirm specificity of the interacting plasms, the β-galactosidase-positive colonies were reisolated and retransformed into Y190 expressing the wild-type JIP-1 PTB, JIP-1 PTB, or X11 a PTB.
PTB<sub>rhoGEF</sub> mutant, or X11a PTB domain. Phenylalanine 691 of JIP-1 represents a highly conserved amino acid in PTB domains that when mutated inhibits PTB domain function (5, 6, 23). Only clones that specifically interacted with the wild-type hJIP-1 PTB but not the mutant PTB domain were analyzed.

A total of 9 × 10<sup>5</sup> clones were screened. Four clones that specifically interacted with the wild-type JIP-1 PTB were identified, the strongest of which encoded a 191-amino acid region of the neuronal protein rhoGEF, an exchange factor for rhoA (15). Yeast expressing the rhoGEF plasmid and each of the PTB plasmids grew on media lacking leucine and tryptophan (Fig. 2, top panel), whereas positive interaction resulted in growth on −Leu−/−Trp−/−His plates (middle panel) and β-galactosidase activity (bottom panel). Interaction was only observed between the wild-type JIP-1 PTB and rhoGEF as demonstrated by expression of the histidine and β-galactosidase reporters. This indicates that the interaction of rhoGEF is specific for a functional JIP-1 PTB domain.

**JIP-1 and rhoGEF Interaction in Vivo**—We next evaluated the ability of JIP-1 and rhoGEF to interact when expressed together in the context of a eukaryotic cell. HEK 293 cells were transiently transfected with pcDNA3 (vector control), rhoGEF containing an HA epitope tag, and/or a Myc-tagged form of JIP-1. Lysates were collected 48 h after transfection and analyzed by immunoblotting with anti-HA (Fig. 3, top panel) or anti-Myc (bottom panel). Immunoprecipitation of the Myc-tagged JIP-1 with anti-Myc antibodies coimmunoprecipitated the HA-tagged rhoGEF (Fig. 3, top panel, right lane). Conversely, immunoprecipitation of HA-tagged rhoGEF resulted in the coimmunoprecipitation of this Myc-JIP-1 (bottom panel, right lane).

**Localization of the JIP-1 PTB Binding Site in rhoGEF**—Initial characterization of the PTB domain found that this domain targeted sequences containing a Ψ-X-Asn-Pro-X-Tyr(P) motif (where Ψ is a hydrophobic residue) motif. This consensus motif was not present in the 191 amino acids of rhoGEF encoded by the yeast two-hybrid clone. RhoGEF contains several domains, including a leucine-rich region, a cysteine-rich zinc finger-like motif, and a Dbl-homologous domain next to a pleckstrin homology domain, all of which are commonly found in guanine nucleotide exchange factors (15, 24). The two-hybrid clone represents amino acids 1424–1615 of rhoGEF; this region contains the carboxyl-terminal end of a potential α-helical coiled-coil structure (15). In order to define the location of the JIP-1 PTB binding site, deletions of this 191-amino acid region were generated and expressed as GST fusion proteins. The ability of these deletion and point mutants to interact with JIP-1 was analyzed.

Fig. 4A displays a schematic diagram of the GST-rhoGEF fusion proteins used in binding site localization analysis. Lysates from 293 cells transiently transfected with pRK5 (vector alone) or Myc-JIP-1 (amino acids 307−711) were incubated with GST, GST-rhoGEF 1424−1615, or deletion mutants 1424−1554, 1424−1489, or 1543−1615. Binding was observed by immunoblotting with anti-Myc. As expected, GST-rhoGEF 1424−1615 was able to bind Myc-tagged JIP-1 (Fig. 4B); however, a deletion of the carboxyl-terminal one-third of this region (1424−1554) resulted in a loss of binding. A GST fusion protein of this carboxyl-terminal portion (1543−1615) was able to interact with JIP-1. To identify the exact binding site, we targeted asparagine residues within this carboxyl-terminal portion (1543−1615). The presence of an asparagine residue in the binding site has been shown to be critical for the binding of other PTB domains due to direct interaction of a conserved phenylalanine in the PTB domain with the asparagine residue (5, 8). All of the residues subjected to site-directed mutagenesis are indicated in Fig. 4A. These mutations were made in the context of the GST fusion protein rhoGEF 1424−1615. Fig. 4C indicates that mutation of asparagine 1590 to alanine (N1580A) did not inhibit the binding of GST-rhoGEF to Myc-JIP-1; however, mutation of asparagine 1565 to alanine severely impaired the ability of rhoGEF to interact with JIP-1. Additional residues around asparagine 1565 were targeted for site-directed mutagenesis based on their position in respect to the previously defined Ψ-X-Asn-Pro-X-Tyr(P) motif or their conservation among other JIP-1 PTB interacting clones identified in the yeast two-hybrid screen. Fig. 4D demonstrates that phenylalanines 1563 and 1568 were both critical for the binding of rhoGEF to JIP-1. Phenylalanine 1563 corresponds to the conserved hydrophobic residue at the −5 position in the Ψ-X-Asn-Pro-X-Tyr(P) motif, whereas phenylalanine 1568 corresponds to the tyrosine residue at the 0 position. Therefore, the JIP-1 PTB binding site on rhoGEF does not conform to the classical Ψ-X-Asn-Pro-X-Tyr(P) PTB binding site.

Fig. 5 represents a list of PTB domains and some of their target binding sites. It is clear that several recently characterized PTB domains have binding sites that do not fit the original consensus Ψ-X-Asn-Pro-X-Tyr(P) motif yet are similar to this sequence. The binding site on rhoGEF is most closely related to the dNumb PTB binding site on NAK. In the case of the NAK and rhoGEF binding sites, a hydrophobic residue at the −5 position is critical for interaction with JIP-1.
position and the asparagine residue at the −3 position are still required; however, a phenylalanine residue can substitute for the tyrosine residue at the 0 position. Interestingly, the JIP-1 PTB domain is most closely related to the PTB domain of dNumb, suggesting that related PTB domains may fall into groups that target similar binding sites.

Expression of JIP-1 and rhoGEF in Neurons—When overexpressed in 293 cells, JIP-1 is cytoplasmic, consistent with previous findings (11). We were interested in investigating the subcellular localization of endogenous JIP-1. Although detected in several tissues, rhoGEF and JIP-1 are both predominately expressed in the brain (11, 15). Several forms of JIP-1 are detectable in neuronal cells (Fig. 6A). In the human neuroblastoma cell line SK-N-F1, endogenous JIP-1 can be detected migrating 115 kDa, consistent with the reported size for the pancreatic form of JIP-1, islet-brain 1 (13). No JIP-1 proteins were detected in undifferentiated PC12 cells; however, in differentiated PC12 cells two proteins were found to be immunoreactive with our anti-JIP-1 antibodies, including the previously reported 115-kDa form and an additional protein migrating at 180 kDa. In mouse neuroblastoma N1E-115 cells JIP-1 proteins are detected in both differentiated and undifferentiated cells with differentiated cells displaying a new form, JIP-1*, which we have determined by phosphatase treatment to be a phosphorylated form of the 115-kDa JIP-1 protein (data not shown). These JIP proteins are specifically detected with two independent antibodies (152 and 177), and in the case of the peptide antibody (177), they can be specifically competed with the antigenic peptide (data not shown). The 200-kDa rhoGEF protein is expressed in both PC12 and N1E-115 cells, with a slight increase in protein expression in differentiated cells (Fig. 6B). However, due to the relatively low level of expression of JIP-1 and rhoGEF in neurons, it is not possible to determine the subcellular localization of endogenous JIP-1 and rhoGEF in neuronal cells.

![FIG. 4. Localization of the JIP-1 PTB binding site in rhoGEF. A, schematic of GST-rhoGEF fusion proteins used in binding site localization analysis. B, HEK 293 cells were transiently transfected with pRK5 (vector alone) or pRK5-Myc-JIP-1 (amino acids 307–711). Lysates were prepared and incubated with GST, GST-rhoGEF 1424–1615, or deletion mutant 1424–1489, 1424–1554, or 1543–1615 fusion proteins bound to beads. Following incubation, the beads were washed, and proteins were separated by SDS-polyacrylamide gel electrophoresis. Binding was observed by immunoblotting with anti-Myc. C, GST-rhoGEF 1424–1615 and point mutations GST-rhoGEF N1565A and N1580A. D, GST, GST-rhoGEF 1424–1615, and point mutations GST-rhoGEF F1562G, F1563G, F1568A, M1571A, and S1572A.](http://www.jbc.org/content/35116/1/35116/F4)

![FIG. 5. Comparison of PTB binding sequences. Study of the binding sites for the first PTB domains identified, Shc and insulin receptor substrate-1 (IRS-1), generated the consensus PTB binding site $\Psi^X\text{Asn-Pro-X-Tyr(P)}$ (where $\Psi$ is a hydrophobic residue) (1–3). EGFR, epidermal growth factor receptor; IR, insulin receptor. A circled p above the sequence denotes phosphotyrosine. Analysis of the X11a PTB with its binding partner $\beta$ amyloid precursor protein (BAPP) revealed that phosphorylation of the tyrosine is not required for binding (8). The LNX (9) and NAK (10), the binding partners of mNumb and dNumb, respectively, also lack phosphorylated tyrosine residues within their binding sites. The JIP-1 binding site on rhoGEF is most similar to that of NAK, representing a modified consensus site in which a hydrophobic residue at the −5 position and an asparagine at the −3 position are still required, but a phenylalanine can substitute for the tyrosine at the 0 position, and a proline at −2 is not necessary.](http://www.jbc.org/content/35116/1/35116/F5)
expression of JIP-1 and rhoGEF in these cell lines, we have been unable to detect coimmunoprecipitation of endogenously expressed rhoGEF with JIP-1.

Subcellular Localization of JIP-1—In order to gain insight into the possible role that JIP-1 plays in neuronal cells, we investigated the localization of endogenous JIP-1 in N1E-115 and PC12 cells by indirect immunofluorescence. Endogenous JIP-1 was detected in N1E-115 and PC12 cells with an affinity-purified antibody raised against the Src homology 3 domain of JIP-1 and visualized by the use of a fluorescein isothiocyanate-labeled secondary antibody. Fig. 7 shows that in proliferating N1E-115 and PC12 cells anti-JIP-1 displays diffuse cytoplasmic staining (A and D); however, upon differentiation, JIP-1 becomes concentrated at the tip of the extending neurites (B, C, E, and F). This staining is specific and can be competed using the antigen used to generate the antibody (152). In addition, a second antibody raised against a carboxyl-terminal peptide from JIP yielded similar results (data not shown). JIP-1 was not detected in the nucleus.

DISCUSSION

We have cloned the human homologue of JIP-1 and have identified rhoGEF as a binding target for the PTB domain. Using point mutants, we have identified the amino acids on rhoGEF involved in binding to the JIP-1 PTB. This binding site is similar to the consensus PTB binding site but does not require tyrosyl phosphorylation for binding. An evaluation of currently identified PTB-containing proteins reveals that PTB binding to phosphorylated targets may be a specialized function of a subset of PTB domains. The PTB domains of Shc and insulin receptor substrate-1 have been found to require phosphorylation of their binding targets (1, 2). The binding of the PTB domains of X11 α, Numb, and now JIP-1 all occur independently of tyrosyl phosphorylation (8–10). Of the PTB domains characterized to date, the PTB domain of JIP-1 is most similar to that of Numb (28% identical), suggesting that similar PTB domains may bind similar sequences.

Northern blot analysis reveals that, like rhoGEF, JIP-1 is expressed in the brain (11, 15), and we have shown that endogenous JIP-1 is detected in both PC12 and N1E-115 neuronal cell lines. Sequence analysis of JIP-1 indicates that it lacks any known catalytic domains. Based on its subcellular localization in differentiated neurons, it is likely that one function of JIP-1 may be to localize rhoGEF either away from or into position to interact with its target rhoA. In N1E 115 cells, rhoA facilitates neurite retraction following cellular stimulation with lysophosphatidic acid or other G protein-coupled receptor agonists (25–27). When overexpressed in N1E 115 cells, rhoA translocates to the plasma membrane upon activation (28). JIP-1 may localize a rhoA activation complex to the tip of the extending neurite so that it is poised to initiate neurite retraction upon the proper signaling cue. The small GTPases rac1 and cdc42, as well as rhoA, have all been implicated in the dynamic process of dendritic growth and remodeling, which requires neurite outgrowth and growth cone collapse in response to inhibitory or promoting...
cues (29, 30). When JIP-1 alone or JIP-1 and rhoGEF, are overexpressed with rhoA in 293 cells, no effect on rhoA activation is observed. It is possible that JIP-1 plays solely a localization function that cannot be addressed in an overexpression system. Alternatively, a yet to be identified activation signal may be required for activation of the complex.

JIP-1 has been reported to serve as a substrate for JNK (11) and has recently been reported to serve as a scaffolding protein for the organization of an activating JNK complex (12). It has been reported that treatment of PC12 cells with nerve growth factor results in an increase in JNK activity in addition to the well documented increase in mitogen-activated protein kinase activity (31, 32). Differentiation of PC12 by alternative methods, such as expression of constitutively active forms of the heterotrimic Gq family members, microinjection of activated phosphatidylinositol 3 kinase, or staurosporine treatment, has also been reported to involve the JNK pathway (33–35). We have observed the phosphorylation of JIP-1 expressed in 293 cells upon activation of the JNK pathway by either coexpression of activated DLK or treatment of the cells with anisomycin.2 This phosphorylation does not influence the ability of JIP-1 to interact with DLK, or rhoGEF. It is possible that the phosphorylation of JIP-1 may serve as a signal for its translocation to extending neurites. In NIE 115 cells, we consistently see a phosphorylated form of JIP-1 upon differentiation. Interestingly, DLK, which has been reported to directly interact with JIP-1 (12), is also localized to the neurite extensions, as well as the nucleus, in differentiated NIE-115 cells.3 The localization of endogenous rhoGEF in differentiated neurons could not be determined due to the lack of a rhoGEF antibody suitable for immunofluorescence. Further investigation is required to determine which form of JIP-1 is present at the neurite tips and what other JIP-1 interacting proteins may colocalize to this region.

What might be the possible reason for the inclusion of rhoGEF in the JIP/JNK complex? Recently, two rhoA-specific exchange factors, mNET1 and Lfc, have been reported to activate GEF in the JIP/JNK complex4 Recently, two rhoA-specific ex-

REFERENCES
1. Blaikie, P., Immanuel, D., Wu, J., Li, N., Yajnik, V., and Margolis, B. (1994) J. Biol. Chem. 269, 32031–32034
2. Gustafson, T. A., He, W., Craparo, A., Schaub, C. D., and O’Neill, T. J. (1995) Mol. Cell. Biol. 15, 2500–2508
3. Kavanau, W. M., and Williams, L. T. (1994) Science 266, 1862–1865
4. Bork, P., and Margolis, B. (1995) Cell 80, 693–694
5. Zhou, M. M., Ravid, D., Kääriäinen-Kottos, E. P., Petsas, A. M., Moodie, T. G., Sattler, M., Harlan, J. E., Wade, W. S., Burakoff, S. J., and Fesik, S. W. (1995) Nature 378, 584–592
6. Yajnik, V., Blaikie, P., Bork, P., and Margolis, B. (1996) J. Biol. Chem. 271, 1813–1816
7. van der Geer, P., Wiley, S., Gish, D. G., Lai, V. K., Stephens, R., White, M. F., Kaplan, D., and Pownson, T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 963–968
8. Borg, J. P., Ooi, J., Levy, E., and Margolis, B. (1996) Mol. Cell. Biol. 16, 6229–6241
9. Dho, S. E., Jacob, S., Wolting, C. D., French, M. B., Rohsreider, L. R., and McGlade, C. J. (1998) J. Biol. Chem. 273, 9179–9187
10. Chien, C. T., Wang, S., Rothenberg, M., Jan, L. Y., and Jan, Y. N. (1998) J. Biol. Chem. 273, 598–607
11. Dickens, M., Rogers, J., S. C. Vannagh, J., Raitano, A., Xia, Z., Halpern, J. R., Greenberg, M. E., Sawyer, C. L., and Davis, R. J. (1997) Science 277, 693–696
12. Whitmarsh, A. J., Cavanagh, J., Tournier, C., Yasuda, J., and Davis, R. J. (1998) Science 281, 1671–1674
13. Bonny, C., Nicod, P., and Waebber, G. (1998) J. Biol. Chem. 273, 1843–1846
14. Mosser, V., Maillard, A., Bonny, C., Steimann, M., Shaw, P., Yarnall, D. P., Burns, D. K., Schorderet, D. F., Nicod, P., and Waebber, G. (1999) Genomics 55, 202–208
15. Gebhink, M. F., Kraneburg, H., Poland, M., van Horek, P. F., Houssa, B., and Moolenaar, W. H. (1997) J. Biol. Chem. 272, 1605–1613
16. Symons, M. (1996) Trends Biochem. Sci. 21, 178–181
17. Machesky, L. M., and Hall, A. (1997) J. Cell Biol. 138, 913–926
18. Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilburn, A. E., Lee, W. H., and Elledge, S. J. (1993) Genes Dev. 7, 553–569
19. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) Cell 75, 805–816
20. Vojtek, A. B., and Heilgenberg, S. M. (1995) Methods Enzymol. 255, 331–342
21. Chen, C., and Okawanya, H. (1987) Mol. Cell. Biol. 7, 2745–2752
22. Batzer, A. G., Blaikie, P., Nelson, K., Schlessinger, J., and Margolis, B. (1995) Mol. Cell. Biol. 15, 4405–4409
23. Yaich, L., Oei, J., Park, M., Bork, P., Landry, C., Bodmer, R., and Margolis, B. (1998) J. Biol. Chem. 273, 10381–10388
24. Van Aelst, L., and D’Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
25. Jalink, K., Eichholtz, T., Postma, F. R., van Corven, E. J., and Moolenaar, W. H. (1993) Cell Growth Differ. 4, 247–255
26. Jalink, K., van Corven, E. J., Hengeveld, T., Morii, N., Narumiya, S., and Moolenaar, W. H. (1994) J. Biol. Chem. 269, 801–810
27. Postma, F. R., Jalink, K., Hengeveld, T., and Moolenaar, W. H. (1996) EMBO J. 15, 2388–2392
28. Kraneburg, O., Poland, M., Gebhink, M., Oomen, L., and Moolenaar, W. H. (1997) J. Cell Sci. 110, 247–2527
29. Threadgill, R., Bobb, R., and Ghosh, A. (1997) Neuron 19, 625–634
30. Kuhn, T. B., Brown, M. D., Wilcox, C. L., Raper, J. A., and Bamburg, J. R. (1999) J. Neurosci. 19, 1665–1675
31. Kobayashi, M., Ogata, S., Kita, Y., Nakatsu, N., Ibara, S., Kaibuchi, K., Kuroda, S., Uy, M., Iba, H., Konishi, H., Kikawa, U., Saitoh, I., and Fuki, Y. (1997) J. Biol. Chem. 272, 16089–16092
32. Lepca, S., Saffrich, R., Ansorge, W., and Bohmann, D. (1998) EMBO J. 17, 4404–4413
33. Heasly, L. E., Storey, B., Fanger, G. R., Butterfield, L., Zamarripa, J., Blumberg, D., and Maue, R. A. (1996) Mol. Cell. Biol. 16, 648–656
34. Kita, Y., Kinuura, R. D., Kobayashi, M., Ibara, S., Kaibuchi, K., Kuroda, S., Uy, M., Iba, H., Konishi, H., Kikawa, U., Nagata, S., and Fuki, Y. (1998) J. Cell Sci. 111, 907–915
35. Yao, R., Yoshiihara, M., and Osada, H. (1997) J. Biol. Chem. 272, 18261–18266
36. Alberts, A. S., and Treisman, R. (1998) EMBO J. 17, 4075–4085
37. Glaven, J. A., Whitehead, I., Bagrodia, S., Kay, R., and Cerione, R. A. (1999) J. Biol. Chem. 274, 2279–2285
Interaction of c-Jun Amino-terminal Kinase Interacting Protein-1 with p190 rhoGEF and Its Localization in Differentiated Neurons
Debra Meyer, Albert Liu and Ben Margolis

J. Biol. Chem. 1999, 274:35113-35118.
doi: 10.1074/jbc.274.49.35113

Access the most updated version of this article at http://www.jbc.org/content/274/49/35113

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 28 of which can be accessed free at http://www.jbc.org/content/274/49/35113.full.html#ref-list-1