JNK-interacting Protein 1 Promotes Akt1 Activation*

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Members of the JNK pathway are organized together by virtue of interactions with JNK interacting protein 1 (JIP1), a scaffold protein. Here we have investigated the possibility that JIP1 may also affect the catalytic activity of Akt1, a serine/threonine kinase that has been implicated in multiple cellular processes, including survival and proliferation. JIP1 expression enhanced Akt1 kinase activity in a dose-dependent manner following serum starvation in 293 cells. Cellular activation of Akt1 following stimulation with low concentrations of insulin-like growth factor (IGF-1) was elevated in the presence of JIP1. JIP1 expression also prolonged Akt1 stimulation after a short IGF-1 pulse. The mechanism of JIP1-mediated Akt1 activation involved JIP1 protein binding to the Akt1 pleckstrin homology domain, which in turn promoted the phosphorylation of the activation T-loop of Akt1 by phosphoinositide-dependent kinase-1. These results suggest that, in certain cellular contexts, JIP1 may act as an Akt1 scaffold, which regulates the enzymatic activity of Akt1. This study also indicates that JIP1 expression can exert signaling effects independent of Akt1 activity.

The Akt family of serine/threonine-directed kinases regulates a diverse array of biological processes, including cellular survival, proliferation, glucose homeostasis, and vascular tone (1, 2). Akt activation requires the coordination of multiple molecular events (3). A critical initiating event is the activation of phosphoinositide-3 kinases (PI3K)1 (4, 5), which are stimulated by growth factor-induced Akt activation (11–13). Phosphoinositide kinase-1 (PDK1), a plasma membrane resident serine/threonine protein kinase, phosphorylates the Akt T-loop on Thr-308, an event that is minimally required for growth factor-induced Akt activation (11–13). An important positive modulatory phosphorylation occurs within a short C-terminal hydrophobic stretch on Akt Ser-473 (13). The identity of the one or more kinases, such as PDK2, that phosphorylate this site remains controversial (14).

Here we have recently found that JIP1 interacts directly with Akt1 (15). This association was found to inhibit the ability of JIP1 to form active JNK cascade complexes in the context of excitotoxic neuronal death. The JIP1 binding region of Akt1 was localized to amino acids 61–112 of Akt1, sequences within the PH domain of Akt1. Because the PH domain plays an important regulatory role in the activation of Akt family members, we hypothesized that JIP1 binding to Akt1 may affect the protein kinase activity of Akt1.

The Akt family has been shown to antagonize the activity of the JNK pathway in multiple systems, which accounts for some of the prosurvival effects of Akt (16–19). However, the possibility that JNK pathway members might regulate Akt activity has not been explored. JNK interacting protein 1 (JIP1) has been identified as a JNK scaffold protein, which can specify and insulate particular JNK modules (20). The JIP1 scaffold has been demonstrated to be required for stress-specific neuronal apoptosis in culture and in vivo (21).

Here we have studied the mechanism of JIP1-mediated Akt1 activation. The present study demonstrates that JIP1 enhances Akt1 activity following serum starvation and limited growth factor stimulation in 293 cells. Our findings indicate that JIP1 binding to Akt1 relieves the autoinhibitory constraint imposed by the PH domain and allows PDK1 to phosphorylate Akt1. The results provide insight into the regulation of Akt1 activity by a JNK scaffold protein and suggest that JIP1 may act as an Akt1 scaffold under conditions of limiting growth factor.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—Human embryonic kidney 293 were cultured at 37 °C in 5% CO2 in Dulbecco’s modified Eagle medium containing 10% fetal bovine serum, 2 mM glutamine, and penicillin/streptomycin (Invitrogen). For transient transfection, cells were cultured on 6-well plates and transfected with the indicated plasmids using FuGENE 6 (Roche Applied Science) for 18 h. For all experiments, total plasmid DNA amount was equalized by addition of vector pcDNA3.

Constructs, Recombinant Proteins, and Antibodies—pcMV6-hemagglutinin (HA)- and myc-tagged wild-type murine Akt (herein referred to as Akt1)/HA-Akt1 and myc-Akt1, Akt1 mutant R25C (HA-Akt1 R25C) (gift of Dr. Ed Skolnik), Akt1 mutant deleted in amino acids 11–60 (HA-Akt11–60) (gift of Dr. Thomas Franke) (22), and p5K5-myc-tagged human JIP1 (myc-JIP1) (gift of Dr. Ben Margolis) (23) have been

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‡The abbreviations used are: PI3K, phosphoinositide 3-kinase; JNK, c-Jun N-terminal kinase; JIP, JNK interacting protein 1; IGF-1, insulin-like growth-1; PDK1, phosphoinositide-dependent kinase-1; PH, pleckstrin homology; PtdIns(3,4,5)P3, phosphatidylinositol(3,4,5)phosphate; PtdIns(3,4)P2, phosphatidylinositol(3,4)phosphate; PtdIns(3,4,5)P3, phosphatidylinositol(3,4,5)phosphate; CMV, cytomegalovirus; DTT, dithiothreitol; PVDF, polyvinylidene difluoride.

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described. pCDNA3-FLAG-tagged full-length JIP1 and JIP1 deletion mutants (depicted in Fig. 3 below) have also been described (15). Monoclonal anti-HA antibody 3F10 was from Roche Applied Science, and monoclonal anti-FLAG M2 antibody from Sigma. Anti-myc monoclonal 9E10, polyclonal anti-Akt1 C-20, monoclonal anti-Akt1 B-1, and monoclonal anti-phosphoryrosine PY99 were from Santa Cruz Biotechnologies. Polyclonal anti-phospho-Akt(Thr-308), anti-phospho-Akt(Ser-473), anti-BAD(Ser-136) were obtained from Cell Signaling Technologies. Polyclonal anti-gp85 antibody was purchased from Upstate Biotechnology, and a monoclonal antibody against the N-terminal SH2 domain of p85 was purchased from Seikagaku Corp.

**Immunoprecipitation, Akt1 Kinase Assay, and Immunoblotting—**
Transfected 293 cells were washed three times in serum-free medium (Dulbecco's modified Eagle's medium plus glutamine and penicillin/streptomycin, maintained in serum-free medium for 16 h). Where indicated, cells were exposed to insulin-like growth factor (IGF-1) (Sigma) or LY294002 (25 μM in Me2SO, BIOMOL). The Akt1 kinase assay has been described (22). Cells were washed with ice-cold phosphatase-buffered saline (pH 7.4) and lysed in 1% Nonidet P-40 lysis buffer (with 20 mM Tris, pH 8, 137 mM NaCl, 10% glycerol, 1 mM EDTA, 20 mM β-glycerophosphate, 10 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 1.5% aprotinin) plus 1 mM dithiothreitol (DTT). Clarified lysates were incubated with anti-HA antibody followed by protein G-agarose (Roche Applied Science). Immunoprecipitates were then washed three times in lysis buffer plus 1 mM DTT and twice in kinase assay buffer (20 mM HEPES, pH 7.4, 10 mM MgCl2, 10 mM MnCl2, and 1 mM DTT). Kinase reactions were initiated by the addition of 2 μM of [γ-32P]ATP, 10 μCi of [γ-32P]ATP (3000 Ci/mM, PerkinElmer Life Sciences), and 1 μg of GSK-3 fusion protein (Cell Signaling Technologies). Reactions were terminated by addition of 6× Laemmli buffer and boiling. Samples were then resolved by SDS-PAGE and transferred onto PVDF membrane. Extent of phosphorylation was quantitated with a Fuji BAS2000 image analyzer.

The PI3K activity assay—[γ-32P]ATP. Lipid products were visualized by thin layer chromatography, and [γ-32P]ATP incorporation was quantitated with a Fuji BAS2000 image analyzer. 

**Results**

**JIP1 Promotes Akt1 Kinase Activation—**It is well established that sustained, pharmacological concentrations of growth factors often elicit rapid induction of Akt kinase activity within 2–10 min (9, 25). However, the kinetics of Akt catalytic activity under more physiological scenarios, for example, in the presence of limiting growth factor or following a short growth factor pulse, have not been investigated. JIP1 has been found to bind Akt1 directly (15), raising the possibility that JIP1 may affect Akt1 catalytic activity. To test whether JIP1 affects Akt1 activity, we transiently transfected 293 cells with JIP1 and Akt1 constructs and assessed Akt1 activity in the cell lysates by both immunoprecipitation kinase assays and anti-Akt1 immunoprecipitation followed by immunoblotting with phosphospecific Akt antibodies (Fig. 1). Intriguingly, JIP1 enhanced Akt1 kinase activity 1.7-fold compared with Akt1 transfection alone following 18 h of serum starvation. However, JIP1 did not alter the maximal Akt1 activation achieved by 10 min of IGF-1 stimulation (100 ng/ml) (Fig. 1A). This JIP1-mediated increase in Akt1 activation required PI3K activity, because a 20-min incubation with LY294002 following serum starvation inhibited this enhancement (Fig. 1A).

To examine JIP1-dependent activation further, Akt1 was immunoprecipitated followed by immunoblotting with antibodies directed against Akt1 phospho-Thr-308 (PT308) and Akt1 phosphoSer-473 (PS473). These results recapitulated the kinase assay findings (Fig. 1B). Phosphorylation of both Akt1 Thr-308 and Ser-473 was enhanced by JIP1 coexpression following serum starvation. As with the kinase assay, PI3K inhibition diminished the JIP1-mediated activation of Akt1. Due to the close correlation between the results from the kinase assay and the anti-PT308 immunoblot analysis, we used immunoblotting to characterize further JIP1 regulation of Akt1 activity. The effect of JIP1 upon Akt1 activity was JIP1-dose dependent (Fig. 1C), with higher JIP1 levels resulting in greater activation of Akt1.

To assess whether JIP1-mediated potentiation of Akt1 kinase activity has effects upon Akt1 downstream signaling in intact cells, we evaluated the phosphorylation of an Akt-specific substrate, BAD (26, 27), using antibodies directed against phosphorylated BAD (PS136) (Fig. 1D). When BAD was transfected alone, phosphorylation of BAD on Ser-136 was not detectable following serum starvation. However, in the presence of JIP1, BAD Ser136 was found to be phosphorylated. Because this serine residue lies within a consensus phosphorylation site for Akt, JIP1 likely led to productive BAD phosphorylation by increasing the kinase activity of endogenous Akt1 in cells. Similarly, the ability of transfected Akt1 to phosphorylate BAD in cells was greatly enhanced by coexpression of JIP1. These results indicate that the effect of JIP1 upon Akt1 kinase activity has observable biochemical consequences on an important downstream target in a cellular environment.

**JIP1 Enhances Akt1 Activity Stimulated by Low IGF-1 Concentrations and Prolongs the Duration of Akt1 Activation following an IGF-1 Pulse—**Under what circumstances does JIP1 augment Akt1 kinase activity? JIP1 can potentiate Akt1 kinase activity following serum starvation. This suggests that JIP1 may increase the growth factor responsiveness of Akt1, perhaps when activated by submaximal factor concentrations. We tested whether JIP1 expression could enhance Akt1 activity under more physiological growth factor concentrations by exposing transfected cells to a 10-min treatment of 10 ng/ml IGF-1. Following IGF-1 treatments, Akt1 activity was assessed by anti-PT308 immunoblotting following anti-Akt1 immunoblotting with the indicated antibodies.

Levels of protein in crude lysates and immunoprecipitates were detected by immunoblotting with the indicated antibodies.

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precipitation (Fig. 2A). As shown above, maximal activation of Akt1 after cellular treatment with 100 ng/ml IGF-1 did not differ between vector- and JIP1-transfected cells. However, JIP1 expression caused a marked increase in Akt1 activity following exposure to 10 ng/ml IGF-1 compared with vector transfection, suggesting that JIP1 can enhance Akt1 responsiveness under physiological growth factor concentrations.

Given the ability of JIP1 to enhance Akt1 activity following both serum starvation as well as treatment with low IGF-1 concentrations, we hypothesized that JIP1 may prolong Akt1 activation after cellular exposure to a discrete growth factor pulse (Fig. 2A). Transfected cells were treated with 100 ng/ml IGF-1 for 10 min and vigorously washed to remove the IGF-1. Four hours following the IGF-1 pulse, Akt1 activity remained substantially more elevated in JIP1-transfected cells compared with vector-transfected cells. Continuous incubation in IGF-1 (100 ng/ml) for 4 h led to sustained and maximal Akt1 activation regardless of JIP1 expression, indicating that the washes effectively removed IGF-1 from the culture. A detailed time course following a 10-min pulse of IGF-1 (100 ng/ml) demonstrated that, although the initial activation of Akt1 was comparable in JIP1-expressing and non-expressing cells, within 30–60 min, Akt1 activity in JIP1-expressing cells was considerably higher than in cells expressing Akt1 alone (Fig. 2B). Even after 1 h, the activity of Akt1 in JIP1-expressing cells consistently remained higher than in cells expressing Akt1 only for up to 10 h after growth factor stimulation. This striking response over the sustained time course indicates that JIP1 significantly extends the activation of Akt1 following a short growth factor exposure.

**Mapping of the Akt1 Activation Domain on JIP1**—To define the region of JIP1 responsible for the activation of Akt1, we examined a series of JIP1 deletion mutants (Fig. 3) by transfecting them into cells and assessing their ability to potentiate Akt1 activity following serum starvation as above. Coexpression of only the N-terminal third of JIP1, JIP1A (JIP1 amino acids 1–286), had no effect on Akt1 activity compared with Akt1 transfection alone. Likewise, expression of the C-terminal third of JIP1, JIP1C (JIP1 amino acids 287–487), had little effect on Akt1 activity. However, expression of the middle domain of JIP1, JIP1B (JIP1 amino acids 488–711), augmented the activity of Akt1 to as great an extent as full-length JIP1, indicating that this region of JIP1 is sufficient to mediate the Akt1 regulatory function. These data are largely in agreement with our previous mapping analysis of Akt1 binding, in that JIP1B can bind to Akt1 by coimmunoprecipitation (15). Although JIP1C could also interact with Akt1 in our analysis, this C-terminal region of JIP1 does not appear to be sufficient for enhancement of Akt1 kinase activity. The lack of Akt1 potentiation observed with JIP1A, which can act as a JNK suppressor (28, 29), suggests that Akt1 activation by full-length JIP1 does not involve cellular JNK inhibition.
JIP1 Expression Does Not Increase Activation of PI3K or Akt1 Homo-oligomerization

One possible mechanism for the effect of JIP1 on Akt1 activation by IGF-1 is activation of PI3K (13). We first assessed the extent of tyrosine phosphorylation of the p85 regulatory subunit of PI3K following anti-p85 immunoprecipitation, an assay that closely correlates with the level of PI3K activity (30). JIP1-transfected cells exhibited no significant difference in p85 tyrosine phosphorylation compared with vector-transfected cells immediately following 10 min of IGF-1 treatment or even 2 and 4 h after an IGF-1 pulse (Fig. 4A). Similar results were obtained with anti-phosphotyrosine immunoprecipitation followed by anti-p85 immunoblotting (data not shown). These results were confirmed with direct assessment of PI3K activity by immunoprecipitating p85 and measuring the associated 32P-phosphorylated lipid kinase activity in vitro (Fig. 4B). PI3K activity after 10 min of IGF-1 stimulation in JIP1-transfected cells was similar to that of vector-transfected cells; additionally, JIP1-expressing cells did not demonstrate elevated PI3K activity compared with control cells 3 h following a 10-min IGF-1 treatment or even 2 and 4 h after an IGF-1 pulse (Fig. 4A). Similar results were obtained with anti-phosphotyrosine immunoprecipitation followed by anti-p85 immunoblotting (data not shown). These results were confirmed with direct assessment of PI3K activity by immunoprecipitating p85 and measuring the associated 3’-phosphorylated lipid kinase activity in vitro (Fig. 4B). PI3K activity after 10 min of IGF-1 stimulation in JIP1-transfected cells was similar to that of vector-transfected cells; additionally, JIP1-expressing cells did not demonstrate elevated PI3K activity compared with control cells 3 h following a 10-min IGF-1 pulse. Together, these data suggest that IGF-1-mediated actions from the level of receptor recruitment to PI3K activation are not directly affected by JIP1.

Because of the ability of JIP1 to homo-oligomerize (31), we also examined the possibility that JIP1 may exert an effect upon Akt1 homo-oligomerization, an event that has been suggested to be involved in Akt1 activation (32). The ability of JIP1 to influence Akt1 oligomerization was assessed by coimmunoprecipitation (Fig. 4C). Using two differentially tagged Akt1 constructs (HA-Akt1 and myc-Akt1), JIP1 was in fact found to decrease the homo-oligomerization of Akt1, implying that JIP1 does not promote Akt1 kinase activity by augmenting Akt1 oligomerization.
Fig. 4. JIP1-dependent activation of Akt1 does not alter PI3K activity or Akt1 oligomerization. A, cells transiently transfected with pcDNA3 or myc-JIP1 plasmid were serum starved for 24 h and then not treated (C, control) or treated with 100 ng/ml IGF-1 for 10 min. Times indicate incubation in serum-free medium following washout of the ligand. Cell lysates were then immunoprecipitated with anti-p85 (IP). Immunoprecipitates and crude lysates (Lys) were subjected to immunoblot analysis with the indicated antibodies (W). h, hours; PY, phosphotyrosine. B, cells transiently transfected with the indicated constructs were serum-starved for 24 h, treated with IGF-1 as in A, washed, and incubated in serum-free medium for the indicated times. Lysates were immunoprecipitated with anti-p85 antibody and then subjected to an in vitro PI3K assay (upper panel). In the lower panel, lysates were subjected to immunoblot analysis with the indicated antibody. Data are representative of two independent experiments. C, cells transiently transfected with the indicated constructs were serum-starved for 24 h, treated with IGF-1 as in A, washed vigorously, and incubated in serum-free medium for 4 h. Lysates were immunoprecipitated with anti-HA. Immunoprecipitates and crude lysates were subjected to immunoblot analysis with the indicated antibodies. Data are representative of three independent experiments.

**JIP1 Increases the Access of Akt1 to PDK1-mediated Phosphorylation**—Binding of phospholipids to the PH domain of Akt1 is necessary for subsequent catalytic activation. To determine the relationship between JIP1 binding and phospholipid binding, we first assessed the PtdIns(3,4,5)P$_3$ dependence of JIP1-mediated Akt1 activation by testing an Akt1 mutant deficient in PtdIns(3,4,5)P$_3$ binding (Fig. 5A). The Akt1A111–60 mutant has been shown to be unresponsive to growth factor stimulation in mammalian cells because of the absence of critical phosphoinositide-binding residues (22) but retains its ability to bind JIP1 (15). Similar to above, wild-type Akt1 activity was enhanced by JIP1 expression 6 h following an IGF-1 pulse. Consistent with the PI3K inhibitor results, the Akt1A111–60 mutant could not be activated by JIP1 coexpression in cells 6 h after an IGF-1 pulse or by 10 min of maximal IGF-1 stimulation. Similar results were obtained with the Akt1R25C mutant, which harbors a point mutation that eliminates both growth factor-induced kinase activation in cells and PtdIns(3,4,5)P$_3$ binding (data not shown) (8, 12). Therefore, the mechanism of JIP1-mediated Akt1 enhancement in cells still depends upon PIP$_3$ binding to the Akt1 PH domain.

To investigate further the effects of JIP1 on the PtdIns(3,4,5)P$_3$ dependence of Akt1 activity, we assessed the time-dependent effects of LY294002 treatment on Akt1 activity following 10 min of IGF-1 stimulation in cells expressing wild-type Akt1 with or without JIP1 (Fig. 5B). These experiments were conducted at room temperature to reduce the speed of PI3K inhibition. As before, incubation with LY294002 prior to ligand stimulation obliterated the JIP1-mediated activation of Akt1. However, treatment with PI3K inhibitor after IGF-1 exposure demonstrated that JIP1-expressing cells exhibited a higher degree of Akt1 activity for a longer duration than cells expressing Akt1 alone. Similar results were obtained using 10 ng/ml IGF-1, in that JIP1 expression extended the duration of IGF-1-induced Akt1 activation when LY294002 was added to the media after IGF-1 treatment was begun (data not shown). These results raise the possibility that, in the presence of JIP1, Akt1 may be more efficiently activated by the available cellular 3-phosphoinositides.

Phosphoinositides play a dual role in the activation of Akt kinases in cells, promoting translocation to PDK1-resident membranes as well as relieving the conformational constraint imposed by the Akt1 PH domain to allow effective phosphorylation by PDK1 (3). Due to the interaction between the Akt1 PH domain and JIP1, we hypothesized that JIP1 may bind Akt1 and thereby induce an Akt1 conformation that is more accessible to PDK1. In vitro Akt1 phosphorylation experiments were performed using recombinant active PDK1 and either wild-type Akt1 or Akt1A111–60 immunoprecipitated from transfected cells (Fig. 6). In agreement with previous observations, PDK1-mediated phosphorylation on wild-type Akt1 or Akt1A111–60 occurred only in the presence of PtdIns(3,4,5)P$_3$ (11, 12, 33, 34). Due to the lack of affinity for 3-phosphoinositides, Akt1A111–60 could not be phosphorylated by PDK1 even in the presence of PtdIns(3,4,5)P$_3$.

To test the effect of JIP1 binding on Akt1 Thr-308 phosphorylation, similar in vitro experiments were performed using Akt1 or Akt1A111–60 bound to JIP1 immunoprecipitates (Fig. 6). In this case, both wild-type and mutant Akt1 were phosphorylated by PDK1 in the absence of PtdIns(3,4,5)P$_3$. Interest-

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**Fig. 4. JIP1-dependent activation of Akt1 does not alter PI3K activity or Akt1 oligomerization.** A, cells transiently transfected with pcDNA3 or myc-JIP1 plasmid were serum starved for 24 h and then not treated (C, control) or treated with 100 ng/ml IGF-1 for 10 min. Times indicate incubation in serum-free medium following washout of the ligand. Cell lysates were then immunoprecipitated with anti-p85 (IP). Immunoprecipitates and crude lysates (Lys) were subjected to immunoblot analysis with the indicated antibodies (W). h, hours; PY, phosphotyrosine. B, cells transiently transfected with the indicated constructs were serum-starved for 24 h, treated with IGF-1 as in A, washed, and incubated in serum-free medium for the indicated times. Lysates were immunoprecipitated with anti-p85 antibody and then subjected to an in vitro PI3K assay (upper panel). In the lower panel, lysates were subjected to immunoblot analysis with the indicated antibody. Data are representative of two independent experiments. C, cells transiently transfected with the indicated constructs were serum-starved for 24 h, treated with IGF-1 as in A, washed vigorously, and incubated in serum-free medium for 4 h. Lysates were immunoprecipitated with anti-HA. Immunoprecipitates and crude lysates were subjected to immunoblot analysis with the indicated antibodies. Data are representative of three independent experiments.
PDK1 to phosphorylate and activate Akt1. and conformational alteration of the Akt1 PH domain, allowing the Akt1 activity appears to occur through JIP1 binding to the Akt1 PH domain. Taken together, JIP1-mediated enhancement of Akt1 activation involves a novel role for JIP1 binding in altering the Akt1 PH domain, enabling PDK1 access to Akt1. These results indicate that JIP1 can functionally substitute for PtdIns(3,4,5)P3 in derepressing Akt (11). The Akt1 PH domain has been reported to have at least two important regulatory functions in the activation of Akt family kinases. Increases in 3-phosphoinositide levels at the plasma membrane cause PH domain-dependent membrane translocation function in vitro. These in vitro studies indicate that JIP1 can functionally substitute for PtdIns(3,4,5)P3 in derepressing the Akt1 PH domain. Taken together, JIP1-mediated enhancement of Akt1 activity appears to occur through JIP1 binding and conformational alteration of the Akt1 PH domain, allowing PDK1 to phosphorylate and activate Akt1.

**DISCUSSION**

The results from the current study indicate that JNK interacting protein 1 also regulates Akt1. The kinase activity of Akt1 was potentiated by coexpressed JIP1 in a dose-dependent manner following serum starvation. JIP1 enhanced Akt1 activation in cells exposed to submaximal concentrations of IGF-1, suggesting a left shift in the growth factor dose-response curve of Akt1. Additionally, expression of JIP1 prolonged Akt1 activation following a short IGF-1 pulse, suggesting that JIP1 may function to extend Akt1-mediated biological effects. The mechanism of JIP1-mediated Akt1 activation involves a novel role for JIP1 binding in altering the Akt1 PH domain, enabling PDK1 to phosphorylate Akt1 on the T-loop Thr-308 residue.

Although positive regulation of Akt kinase activity by protein-protein interactions has been described to occur with other Akt interactors, such as Tcl-1 family members and Hsp90, the presumptive mechanisms of these binding partners differ considerably from the current study (35–38). Tcl-1 augments oligomerization of Akt, whereas Hsp90 both prevents protein phosphatase 2A-mediated dephosphorylation of Akt and promotes PDK1 stability. An important negative regulator of Akt kinase activity, C-terminal modulator protein, has also been described to occur via protein-protein interactions with Akt kinases, although the precise mechanism for Akt inhibition is unknown (39).

The Akt PH domain has been reported to have at least two important regulatory functions in the activation of Akt family kinases. Increases in 3-phosphoinositide levels at the plasma membrane cause PH domain-dependent membrane translocation of Akt kinases (9, 10). Simultaneously, 3-phosphoinositide binding alters the conformation of the Akt PH domain, allowing PDK1 to the plasma membrane to phosphorylate and activate Akt (11, 12). The Akt1Δ11–60 mutant has previously been shown to be unresponsive to platelet-derived growth factor stimulation in mammalian cells (22), although in insect cells, this mutant exhibits constitutive activity (40). These discrepancies in activity have been attributed to a higher level of expression in the insect cell system (3). We show here that PDK1-mediated phosphorylation of the Akt1Δ11–60 mutant does not occur in vitro, even in the presence of PtdIns(3,4,5)P3. However, this mutant could be phosphorylated by PDK1 in the presence of JIP1. Therefore, this mutant PH domain can still be conformationally inhibited by protein-protein interactions. The lack of cellular activation with this Akt1 mutant may reflect an inability of JIP1 to compensate for the membrane translocation function of 3-phosphoinositides.

The structural determinants for Akt1 PH domain recognition

![Figure 5](image)

**Figure 5.** Examination of the phosphoinositide dependence of JIP1-mediated Akt1 activation. A, cells transfected with the indicated constructs were serum-starved for 24 h and treated with 100 ng/ml IGF-1 for 10 min. B, cells transfected with the indicated constructs were serum-starved for 24 h and then not treated (C, control) or treated with 100 ng/ml IGF-1 for the indicated minutes at room temperature. 25 μM of LY294002 was added to the media either before IGF-1 (pre, minutes prior to IGF-1) or after exposure to IGF-1 (post, minutes after IGF-1 begun), at room temperature. Cell lysates were then analyzed as in A. Data are representative of three independent experiments.

![Figure 6](image)

**Figure 6.** JIP1 increases the access of Akt1 to PDK1-mediated phosphorylation. 293 cells were transiently transfected with the indicated constructs and immunoprecipitated with anti-HA (IP) to isolate Akt1 proteins or anti-myc to isolate JIP1-associated Akt1 proteins. Immunoprecipitated Akt1 proteins were then used as substrates for an in vitro PDK1 kinase assay using recombinant active PDK1 and PtdIns3,4,5P3-containing vesicles. Akt1 phosphorylation was monitored by anti-phospho-Akt1 (Thr-308) immunoblotting. The levels of Akt1 and JIP1 proteins in immunoprecipitates were assessed by immunoblot analysis with the indicated antibodies. W, Western blot. Data are representative of three independent experiments.
of 3-phosphoinositides has been inferred based upon the crystal structures of related PH domains, such as Grp1 and DAPP1. PH domains in general share a common fold, a seven-stranded β-sandwich (β1-7) with a C-terminal α-helix (α1) at one end. Unlike the Grp1 and Btk PH domains, which exhibit exquisite specificity for PtdIns(3,4,5)P3, Akt1 binds both PtdIns(3,4,5)P3 and PtdIns(3,4)P2 with similar affinities (7), a characteristic that can be accounted for by a lack of additional binding sequences in Akt1 (41, 42). JIP1 binding to Akt1 was mapped to Akt1 amino acids 61–112 (15), which spans PH domain sequences after the β5 strand and up to the α1 helix terminus. The JIP1 interaction with Akt1 could therefore potentially disrupt phosphoinositide contacts with the 4'-phosphate. However, this does not appear to be the case as cellular mediated stimulation (9, 10).

The potentiation effect of JIP1 upon Akt1 kinase activity appears to be independent of the ability of Akt1 to disrupt JIP1-mediated JNK scaffolding (15). JIP1 has been shown to play an important role in stress-specific JNK activation and subsequent apoptosis in neuronal culture and in vivo (15). In our previous study, both wild-type and kinase-dead Akt1 were able to inhibit JIP1-mediated enhancement of JNK activity and kainate-induced neuronal apoptosis (15). Taken together, these results suggest that, in the particular context of excitotoxicity and JNK scaffolding, Akt1 kinase activation by JIP1 does not play a significant role. We speculate that the ability of Akt1 to act as a scaffold for Akt1 may contribute to the maintenance of cellular survival under conditions of limited growth factor supply.

The current results also raise a significant caveat when interpreting the biological effects of high levels of JIP1, whether endogenous or ectopic. Although introduction of short N-terminal regions of JIP1 can act as effective and specific JNK inhibitors, which have been generally assumed to occur through JNK inhibition. JIP1-mediated JNK scaffolding (15). JIP1 has been shown to play an important role in stress-specific JNK activation and Hemmings, B. A. (1998) Science 280, 1040–1043.

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