A Catalytically Active Jak2 Is Required for the Angiotensin II-dependent Activation of Fyn*

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Recent work with interleukins has shown a convergence of tyrosine phosphorylation signal transduction cascades at the level of the Janus and Src families of tyrosine kinases. Here we demonstrate that activation of the seven-transmembrane AT1 receptor by angiotensin II induces a physical association between Jak2 and Fyn, in vivo. This association requires the catalytic activity of Jak2 but not Fyn. Deletion studies indicate that the region of Jak2 that binds Fyn is located between amino acids 1 and 240. Studies of the Fyn SH2 and SH3 domains demonstrate that the SH2 domain plays the primary role in Jak2/Fyn association. Not surprisingly, this domain shows a marked preference for tyrosine-phosphorylated Jak2. Surface plasmon resonance estimated the dissociation equilibrium constant (Kd) of this association to be 2.36 nM. Last, in vitro studies in vascular smooth muscle cells show that, in response to angiotensin II, Jak2 activation is required for Fyn activation and induction of the c-fos gene. The significance of these data is that Jak2, in addition to serving as a critical angiotensin II activated signal transduction kinase, also functions as a docking protein and participates in the activation of Fyn by providing phosphotyrosine residues that bind the SH2 domain of Fyn.

The Jak family of nonreceptor tyrosine kinases includes Jak1, Jak2, Jak3, and Tyk2. Each protein is approximately 130 kDa and contains seven conserved Jak homology domains (JH1 to JH7) (1, 2). The Jak kinases induce gene regulation through the signal transducers and activators of transcription. Unlike the Jaks, the other Src family bear no SH2 or SH3 domains. In contrast to the Jaks, members of the Src family of protein-tyrosine kinases are approximately 55–62 kDa in mass and do possess SH2 and SH3 domains. There are nine known members of the Src kinase family. While the expression of most members is restricted to hematopoietic cells, Fyn and Src are widely expressed by many cell types. There appears to be some functional redundancy of these two proteins, since knockout mice lacking either gene are born alive, while disruption of both the fyn and src alleles results in embryonic lethality (3–5). This redundancy is also seen in the activation of similar signaling pathways by Fyn and Src (6).

Recent studies have demonstrated various levels of cross-talk between Jak2 and other signaling pathways. For example, activation of gp130 by the hematopoietic cytokine, interleukin-11, induces protein complex formation between the Jak and Src family tyrosine kinases. Specifically, treatment of 3T3-L1 cells with interleukin-11 leads to a transient complex of Grb2, Jak2, and Fyn (7). Subsequent studies by Yang et al. demonstrated an interleukin-11-dependent association of Jak2 with other signaling molecules including protein phosphatase 2A, phosphatidylinositol-3-kinase, and the Src family kinase Yes (8). To date, the regions that mediate these interactions, as well as the hierarchy of the signal transduction cascades, have not been established.

Angiotensin II is the effector molecule of the renin angiotensin system. It is vital for maintaining a wide variety of physiological responses including salt and water balance, blood pressure, and vascular tone. These effects are transduced through a seven-transmembrane surface receptor called AT1 (9). We have previously demonstrated that treatment of vascular smooth muscle cells (VSMC) with angiotensin II results in Jak2 autophosphorylation and activation (10). The angiotensin II-dependent activation of Jak2 requires the intracellular amino acids 319–322 (YIPP) found in the AT1 receptor carboxyl terminus (11). Angiotensin II has also been shown to activate the kinase activity of Fyn and Src as measured by both autophosphorylation and phosphorylation of synthetic substrates (12, 13). However, neither the mechanism of activation nor the region of the AT1 receptor that mediates Fyn and Src activation is known.

Here we investigate whether angiotensin II, acting through the AT1 receptor, can cause convergence of the Jak and Src tyrosine kinase signal transduction cascades. This is an important question because angiotensin II acts through a seven-transmembrane receptor in contrast to the interleukins, which activate the cytokine superfamly of receptors. There are several differences between these receptor families. In the absence of ligand, an appreciable amount of Jak2 appears bound to the cytoplasmic tail of the cytokine receptor (14). Ligand binding to the extracellular surface of the cytokine receptor results in receptor aggregation and Jak2 activation. In contrast, little
Jak2 co-precipitates with the AT1 receptor in the absence of angiotensin II; ligand binding appears to trigger Jak2 autophosphorylation and concurrent binding with the AT1 receptor (10, 11). Once bound to the AT1 receptor, the catalytically active Jak2 activates the signal transducers and activators of transcription in a manner that is similar to the cytokine receptors and thus transduces signals to the nucleus (15–17).

In this report, we demonstrate that treatment of VSMC with angiotensin II leads to a physical association of Jak2 and Fyn. More importantly, we have characterized the molecular interactions that mediate this association. Specifically, the binding of Jak2 and Fyn requires a catalytically active Jak2 molecule and is mediated by the association of a Jak2 phosphotyrosine and the SH2 domain of Fyn. The functional consequence of this interaction is that, in response to angiotensin II, a tyrosine-phosphorylated Jak2 serves as docking site not only for Fyn binding but for Fyn activation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—All cells were cultured at 37 °C in a 5% CO2 humidified atmosphere. VSMC were grown in DMEM plus 10% fetal calf serum and 1% antibiotic/antimycotic and were cultured in serum-containing medium. BSC-40 cells were cultured in DMEM plus 10% newborn calf serum. 100-mm dishes of VSMC, at approximately 75% confluence, were growth-arrested by incubation in serum-free DMEM for 48 h before use. Cell culture reagents were obtained from Life Technologies, Inc. Tyrosine kinase inhibitors were purchased from Calbiochem. Other reagents were purchased from Sigma.

**Plasmid and GST Fusion Protein Constructs**—pBOSwtJk2 (Jak2 WT) and pBODJK2VIII (Jak2 DN) were a generous gift from Dr. D. M. Wojcieszynski and were previously described (18). Construction of the pRC-Jak2-WT and pRC-Jak2-ATD plasmids was described elsewhere (19). The vector expressing the wild type AT1 receptor cDNA (pgeo/WT) was previously reported (11). The c-fos/leucineresidue construct (p2FPL) was a generous gift from Dr. W. S. Chen and was described previously (20).

**Northern Blot Analysis**—Northern blot analysis. For these studies, VSMC/Neo and VSMC/Jak2 DN cell lines had a similar transfection efficiency (15%) by transfecting them with 1 mg of pRC-CMV (neomycin cassette) and 2 mg of pRC-Jak2-WT and pBODJK2 fusion protein (amino acids 1–294) was also reported (21). The Fyn fusion proteins GST/SH2, GST/SH3, and GST/SH2 + SH3 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

**Transient Cell Transfection**—COS-7 cells were transfected exactly as described (11). In order to maintain equal protein expression, we used 20% of the Jak2 DN plasmid when compared with Jak2 WT. For BSC-40 cell transfection, cells were seeded in 100-mm dishes and transfected by electroporation using the Biorad BM2000 (Biorad). Normalized lysates (±400 μg/ml) were immunoprecipitated with 1 μg of antibody and 20 μl of a 50% slurry of Protein A/O-agarse beads (Santa Cruz Biotechnology) for 6–16 h at 4 °C. The immunoprecipitating anti-Fyn (FN3) and anti-Jak2 (HR758) polyclonal antibodies and the anti-Fyn (15) monoclonal antibody were purchased from Santa Cruz Biotechnology. The anti-p130(ζ) mAb (P27820) and anti-Tyr(P) mAb (PY20) were purchased from Transduction Laboratories. Immune complexes were washed three times with wash buffer (25 mM Tris, pH 7.5, 0.9% NaCl, 0.05% Tween 20) and resuspended in SDS free buffer. For GST/Fyn pull down assays, COS-7 cell lysates were pre-cleared with 7.5 μg of Sepharose-bound GST for 1 h at 4 °C. To each sample, 0.2 μg of soluble GST or GST/Fyn fusion protein was added along with 20 μl of a glutathione-Sepharose 4B slurry and incubated for 15–20 min at 4 °C. Beads were washed four or five times with wash buffer containing 1 M NaCl and resuspended in sample buffer. All sample buffers containing proteins were separated by SDS-PAGE (National Diagnostics) and transferred onto nitrocellulose membranes (Schleicher and Schuell).

**Western Blotting**—After blocking for 1 h in 5% dry milk/TTBS (100 mM Tris, pH 7.5, 0.9% NaCl, and 0.05% Tween 20) at 23 °C, nitrocellulose membranes were probed with primary antibody for 2–24 h at 23 °C in 5% milk/TTBS. Blots were washed with TTBS, and proteins were visualized with ECL following the manufacturer’s instructions (Amersham Pharmacia Biotech). Blotting antibodies purchased from Santa Cruz Biotechnology were anti-Fyn mAb (15) and anti-GST mAb (B14). Antibodies purchased from Upstate Biotechnology, Inc. (Lake Placid, NY) were anti-Tyr(P) mAb (4G10) and anti-Jak2 pAb (758). Antibodies from Transduction Laboratories were anti-Fyn mAb (F19720), anti-Tyr(P) mAb (PY20), and anti-Stat1 mAb (S21120). All markers are kilodaltons.

**In Vitro Binding Assays**—Jak2 was synthesized in vitro using the TNT T7 Quick Coupled Transcription/Translation System (Promega) in the presence of [35S]methionine (Amersham Pharmacia Biotech). Yields were estimated to be 200 ng of Jak2/50-μl reaction as determined by internal controls provided by the manufacturer. For binding assays, 0.2 μg of [35S]methionine incorporation control protein (neomycin cassette) in 25 μl of Lysis Buffer. Samples were incubated at 4 °C for 2–3 h, washed three times with wash buffer, resuspended in sample buffer, and separated by SDS-PAGE. Gels were fixed for 0.5 h in isopropyl alcohol/H2O/acidic acid (25:65:10), washed twice with H2O, and soaked in Amplify (Amersham Pharmacia Biotech). Cells were dried under vacuum and exposed to film for 2–4 weeks at ~80 °C.

**Vaccinia Virus-mediated Jak2 Overexpression**—Jak2 was overexpressed using the vaccinia virus-mediated transfection/infection protocol (23). Briefly, 100-mm dishes of nearly confluent BSC-40 cells were transfected with 20 μg of pRC-Jak2-WT and 20 μl of Lipofectin. After 4 h, vTF7–3 (24) was added at a multiplicity of infection of 1.0 and incubated for 1 h. The medium was removed, and cells were incubated overnight in Dulbecco’s modified Eagle’s medium (DMEM) plus 10% NCS. At 18–20 h postinfection, lysates were prepared and loaded onto a Q Sepharose ion exchange column (Amersham Pharmacia Biotech). The column was washed extensively with 0.2 M NaCl, and Jak2 was eluted with 0.8 M NaCl. The sample was desalted, and proteins under 100 kDa in mass were removed by a Centricron 100 concentrator using a 100,000 Da exclusion filter. 15 μl of this fraction containing 25 μg of Tris, pH 7.4, 150 mM NaCl. Purity and quantity were determined by Ponceau staining and immunoblotting the sample.

**Surface Plasmon Resonance Measurements**—Kinetic parameters for the interaction between GST/Fyn (GST/SH2 + SH3) and partially purified Jak2 were estimated by surface plasmon resonance using a BIAcore instrument (Biacore AB). In this study, an anti-GST monoclonal antibody was immobilized on the chip surface. Then, 2 μl of a solution containing 25 mM Tris, pH 7.4, 150 mM NaCl, and 0.1% Tween 20) at 23 °C, nitrocellulose membranes were probed with primary antibody for 2–24 h at 23 °C in 5% milk/TTBS. Blots were washed with TTBS, and proteins were visualized with ECL following the manufacturer’s instructions (Amersham Pharmacia Biotech). Blotting antibodies purchased from Santa Cruz Biotechnology were anti-Fyn mAb (15) and anti-GST mAb (B14). Antibodies purchased from Upstate Biotechnology, Inc. (Lake Placid, NY) were anti-Tyr(P) mAb (4G10) and anti-Jak2 pAb (758). Antibodies from Transduction Laboratories were anti-Fyn mAb (F19720), anti-Tyr(P) mAb (PY20), and anti-Stat1 mAb (S21120). All markers are kilodaltons.

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antibody (Biacore) was covalently linked to CM5 research grade sensor surfaces using an amine coupling kit (Biacore). Briefly, carboxylate moieties on CM5 dextran surfaces were activated with a 30-µl injection of 1:1 mixture of N-hydroxysuccinimide and N-ethyl-N’-(3-dimethylamino)propylcarbodiimide hydrochloride at a flow rate of 5 µl/min. Immediately following activation, 45 µl of 30 µg/ml of anti-GST in 10 mM sodium acetate, pH 5.5, was injected across each surface at a flow rate of 5 µl/min. Unreacted N-hydroxysuccinimide esters were blocked with a 35-µl injection of 1 M ethanolamine, pH 8.5, at a flow rate of 5 µl/min. This procedure typically yielded 4,000–5,000 resonance units of immobilized anti-GST. Either the fusion protein, GST/Fyn, or GST alone (as a control) was captured on anti-GST surfaces by injecting 5 µg/ml of protein in TBS buffer (25 mM Tris-HCl, 150 mM NaCl, 3.4 mM EDTA, 0.005% P2O, pH 7.4) at a flow rate of 5 µl/min, yielding an increase of 830–550 resonance units. Captured GST/Fyn and GST dissociated from immobilized anti-GST at a rate of <3.0 resonance units/min. Binding interactions were conducted by injecting either 60 µl of partially purified Jak2 (~45 nM) in TBS buffer or TBS alone (as a control) over captured GST/Fyn or GST surfaces at a flow rate of 20 µl/min. Following each Jak2 injection, anti-GST surfaces were regenerated with 10 µl of 10 mM glycine, pH 2.2, followed by 10 µl of 0.05% SDS at a flow rate of 5 µl/min. Segrersons representing the interaction between captured GST/Fyn and partially purified Jak2 were corrected by subtracting the response yielded by injecting partially purified Jak2 over GST alone as a control surface. Association (kA) and dissociation (kd) rate constants were determined from corrected sensograms using a global fitting routine provided by Biacore (BIAevaluation 3.0).

Jak2 Transphosphorylation Kinase Assays—COS-7 cells were transfected as described above to express either no Jak2, wild type Jak2, or dominant negative Jak2. Two days later, normalized lysates were prepared. To each lysate, 0.1 µg of Sepharose-bound GST/Jak2 (amino acids 1–284) or GST alone was added, and samples were incubated at 23 °C for 25–30 min. Samples were pelleted by centrifugation, and beads were washed four or five times with radioimmune precipitation buffer (23). Samples were placed in sample buffer, separated on SDS-PAGE, transferred onto nitrocellulose, and Western blotted with anti-Tyr(P) mAbs.

In Vivo Kinase Assays—To determine if Jak2 was sufficient for Fyn activation, COS-7 cells were transfected to overexpress either no Jak2, wild type Jak2, or dominant negative Jak2. To determine if Jak2 was required for Fyn activation, COS-7 cells were transfected with 2 µg of the wild type AT1 receptor cDNA (pZeo/WT) and either 2.0 µg of wild type Jak2 or 2.4 µg of dominant negative Jak2. Two days later, cells were treated as described in the figure legends, and normalized lysates were immunoprecipitated with anti-Fyn Ab. The immunoprecipitates were washed twice with wash buffer and twice with kinase buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 10 mM MgCl2, and 0.5 mM dithiothreitol). The precipitates were resuspended in 40 µl of the same kinase buffer containing 50 µM ATP, 2.5 µCi of [γ-32P]ATP (Amersham Pharmacia Biotech), and 4 µg of GAP p62 tyrosine kinase substrate (Santa Cruz Biotechnology). Samples were incubated for 25 min at 30 °C, and reactions were terminated by adding SDS sample buffer. Radiolabeled proteins were separated by SDS-PAGE, transferred onto nitrocellulose, and exposed to film.

RESULTS

Angiotensin II-dependent Association of Jak2 and Fyn—Angiotensin II, acting through the seven-transmembrane AT1 receptor, has been shown to activate Jak2 and Fyn as measured by autophosphorylation or phosphorylation of synthetic substrates (10, 11, 19). We hypothesized that the AT1 receptor might act mechanistically similar to the cytokine superfamily of receptors and cause association of the Jak and Src family kinases. To investigate this possibility, VSMC were stimulated with angiotensin II, and the resulting lysates were immunoprecipitated with an anti-Fyn pAb (FYX3) that does not cross-react with other Src tyrosine kinases. The immunoprecipitates were then blotted with anti-phosphotyrosine antibody. As shown in Fig. 1A, angiotensin II induced the association of several phosphorylating proteins with Fyn. Maximal protein association was seen at about 5 min after the addition of angiotensin II. Even at 1 h after the ligand addition, the level of associated proteins containing phosphotyrosine was substantially above base line. Jak2 is 130 kDa in mass and is tyrosine-phosphorylated in response to angiotensin II (10, 11).

Fig. 1A demonstrated that several phosphotyrosine-containing proteins in the 130-kDa size range, as well as the 68–72-kDa range, associated with Fyn in an angiotensin II-dependent manner. Equal loading of all lanes was verified by reprobing the blot with anti-Fyn mAbs (Fig. 1A).

To specifically investigate if Jak2 associates with Fyn in an angiotensin II-dependent manner, we now blotted the anti-Fyn pAb immunoprecipitates with anti-Jak2 pAb (Fig. 1B, lanes 1–4). This study showed an increased association of Jak2 and Fyn as early as 1 min after the addition of angiotensin II. In multiple time course studies, the Jak2 signal returned to near basal levels 2 h after angiotensin II treatment (data not shown). The specificity of Jak2/Fyn association was confirmed by five separate approaches. First, Fig. 1B, lanes 5–7, shows the result of preabsorbing the anti-Fyn pAb with the FYN3-immunizing peptide. As expected, the peptide competes with and prevents the immunoprecipitation of Fyn and the associated Jak2 protein. Second, Fig. 1B, lanes 8–10, shows that if pooled rabbit IgG is substituted for the immunoprecipitating anti-Fyn antibody, neither Fyn nor Jak2 is immunoprecipitated. Third, bloting anti-Fyn pAb immunoprecipitates with an anti-Jak1 mAb did not produce a ligand-dependent association, indicating that the association between Jak2 and Fyn is specific for these two molecules (data not shown). Fourth, we switched our immunoprecipitating anti-Fyn pAb to an anti-Fyn mAb that was raised against a different region of Fyn. Like the polyclonal antibody, it does not cross-react with other Src family kinases. Western blotting of the anti-Fyn mAb immunoprecipitates with Jak2 gave results that were virtually identical to those seen with the polyclonal antibody (Fig. 1C). Finally, reciprocal immunoprecipitation experiments (immunoprecipitating VSMC with anti-Jak2 pAb and blotting with anti-Fyn mAbs) produced results that were similar to those obtained using the inverse protocol (Fig. 1D). Collectively, the data show that in VSMC, activation of the seven-transmembrane AT1 receptor by angiotensin II causes a specific in vivo association of Jak2 and Fyn.

An Active Jak2 Kinase Is Required for Jak2/Fyn Association—We wanted to determine whether the kinase activity of Jak2 and/or Fyn was required for this angiotensin II-mediated event. To determine this, quiescent VSMC were either pretreated with Me2SO (control), pretreated with the Jak2 kinase inhibitor AG-490 (25), or pretreated with the Src family kinase inhibitor PP1 (26). For this study, we used 100 µM of the Jak inhibitor AG-490, the lowest dose that in our hands fully inhibits Jak2 in cultured cells (27). After inhibitor treatment, cells were then left untreated or stimulated with 10−7 M angiotensin II for either 5 min (Fig. 2A) or 60 min (Fig. 2B). The resulting lysates were immunoprecipitated with anti-Fyn pAb and blotted with anti-Jak2 pAb. Association of Jak2 and Fyn was observed in the control cells and the PP1-treated cells. However, angiotensin II-dependent association was not seen after treatment with the Jak2 inhibitor AG-490. To rule out the possibility that the PP1 was not biologically active, in parallel experiments, we pretreated VSMC with PP1 and demonstrated that the dose and time of PP1 preincubation used in Fig. 2, A and B, could completely block the angiotensin II-dependent tyrosine phosphorylation of p130cas, a known substrate of c-Src (28) (Fig. 2C). Thus, the data in Fig. 2 suggest that Jak2 kinase activity, but not Fyn kinase activity, is required for the angiotensin II-dependent association of Jak2 and Fyn.

The data in Fig. 2 made use of pharmacologic enzyme inhibitors. As a complementary approach to investigate this question, we used a protocol employing a dominant negative Jak2 construct. Specifically, we transiently transfected COS-7 cells (a cell line with very little endogenous Jak2) with either a wild
Fig. 1. **Angiotensin II-dependent association of Jak2 and Fyn.** A, quiescent VSMC were stimulated with $10^{-7}$ M angiotensin II as indicated, and the anti-Fyn pAb immunoprecipitates were blotted with anti-Tyr(P) mAbs. The membrane was then stripped and blotted with anti-Fyn mAbs. Shown is one of two representative blots. B, quiescent VSMC were stimulated with $10^{-7}$ M angiotensin II as indicated, and the resulting lysates were immunoprecipitated with anti-Fyn pAb (lanes 1–7) or with rabbit IgG (lanes 8–10). In lanes 6–7, the immunoprecipitating anti-Fyn pAb was preabsorbed with 5 μg of FYN3-immunizing peptide. The immunoprecipitates were blotted with anti-Jak2 pAb. The membrane was then blotted with anti-Fyn mAbs to demonstrate equivalent loading. Similar results were seen in six separate experiments. 

C, quiescent VSMC were stimulated with $10^{-7}$ M angiotensin II as indicated, and the anti-Fyn mAb immunoprecipitates were blotted with anti-Jak2 pAb. The membrane was then blotted with anti-Fyn pAb. Similar results were seen in two separate experiments. 

D, quiescent VSMC were stimulated with $10^{-7}$ M angiotensin II as indicated, and the anti-Jak2 pAb immunoprecipitates were blotted with anti-Fyn mAb. The membrane was then blotted with anti-Jak2 pAb. Similar results were seen in four separate experiments.

Fig. 2. **Inhibition of angiotensin II-dependent association of Jak2 and Fyn by a Jak2 tyrosine kinase inhibitor.** Quiescent VSMC were treated with either 2% Me$_2$SO (v/v) for 16 h (control), 100 μM AG-490 for 16 h, or 20 μM PP1 for 1 h. Cells were then stimulated with $10^{-7}$ M angiotensin II for either 5 min (A) or 60 min (B), and the anti-Fyn pAb immunoprecipitates were blotted with anti-Jak2 pAb. The same membranes were blotted with anti-Fyn mAbs to demonstrate equivalent loading. The results were observed in three separate experiments. 

C, quiescent VSMC were treated with Me$_2$SO or with 20 μM PP1 for 1 h and then stimulated with $10^{-7}$ M angiotensin II as indicated. The anti-p130Cas mAb immunoprecipitates were blotted with anti-Tyr(P) mAbs. The same membrane was blotted with anti-p130Cas mAb.
high endogenous levels of Jak2 present in VSMC; the only difference between each lysate is the catalytic activity of Jak2. We have previously used this system to delineate other Jak2 tyrosine phosphorylation-dependent cellular events (27). Using this system, COS-7 cells were transfected to express empty vector, Jak2 WT, or Jak2 DN. The resulting lysates were immunoprecipitated with anti-Jak2 pAb and subsequently blotted as indicated. These results were observed in three separate experiments. B, the anti-Fyn mAb Western blots from A (bottom) were scanned and plotted graphically as increased Jak2/Fyn association as a function of Jak2 status. All conditions are normalized to empty vector control. Fold increases are expressed as the mean ± S.E.; n = 3. *, p < 0.0025 (Student’s t test distribution).

**FIG. 3.** A catalytically active Jak2 is required for efficient association with Fyn. A, COS-7 cells were transfected with an empty vector control, a wild type Jak2 (Jak2 WT), or a dominant negative Jak2 (Jak2 DN). Jak2 activation (tyrosine autophosphorylation) was attained via oligomerization of the highly expressed Jak2 protein rather than by ligand-dependent activation. The resulting lysates were immunoprecipitated with anti-Jak2 pAb and subsequently blotted as indicated. These results were observed in three separate experiments. B, the anti-Fyn mAb Western blots from A (bottom) were scanned and plotted graphically as increased Jak2/Fyn association as a function of Jak2 status. All conditions are normalized to empty vector control. Fold increases are expressed as the mean ± S.E.; n = 3. *, p < 0.0025 (Student’s t test distribution).
significant amounts of Jak2 in a manner similar to the GST/SH2 construct. After each in vitro synthesis of $^{35}$S-labeled Jak2, an aliquot of the reaction was separated by SDS-PAGE to confirm translation of the protein. The corresponding starting material for these data is also shown for comparison (Fig. 5A).

This same experimental question was investigated using a second protocol. Here, Jak2 WT or Jak2 DN was transiently overexpressed in COS-7 cells, and Jak2 was activated via oligomerization and autophosphorylation of the highly expressed protein. Cell lysates were then prepared, and Jak2/Fyn association was assessed by pull down experiments using GST/Fyn fusion proteins composed of the SH2, SH3, or SH2 plus SH3 domains of Fyn. The fusion proteins were collected by centrifugation, separated by SDS-PAGE, and transferred onto nitrocellulose. The membrane was then blotted with anti-Jak2 pAb (Fig. 5B). GST did not bind Jak2. GST/SH2 bound theJak2 WT and, to a substantially lesser degree, the Jak2 DN. GST/SH3 bound small amounts of both Jak2 WT and Jak2 DN equally. The GST/SH2 + SH3 construct bound Jak2 WT and Jak2 DN with a pattern similar to the GST/SH2 protein. Thus, the two experimental approaches presented in Fig. 5, A and B, suggest an important role of the Fyn SH2 domain in its association with Jak2. Given the affinity of SH2 domains for phosphotyrosine, it is not surprising that this domain associates more efficiently with the catalytically active Jak2 WT as compared with Jak2 DN.

\section*{Dissociation Equilibrium Constant of Jak2/Fyn Association—Having demonstrated an interaction between Jak2 and Fyn, we wanted to quantitate this interaction. This was accomplished by surface plasmon resonance analysis using a Biacore$^\text{TM}$ 2000 instrument. This instrument permits a quantitative and sensitive analysis of protein-protein interaction. The Biacore machine provides four separate flow cells (surfaces), which may be used independently or in series. In the current study, an equivalent amount of anti-GST monoclonal antibody was covalently immobilized in flow cell 1 (fc1) and flow cell 2 (fc2). The anti-GST surfaces were used to capture GST/Fyn (SH2 plus SH3) on fc1 and GST on fc2. We chose to use the SH2 plus SH3 fusion protein because it more closely approximated full-length Fyn. Partially purified Jak2 (~45 nM) was then passed over the GST/Fyn (fc1) and GST (fc2) surfaces in series, thereby generating a binding sensogram and a background sensogram with a single injection. The anti-GST surfaces were restored by treatment with low pH and SDS (see “Experimental Procedures”). Thus, each binding cycle consisted of three steps: 1) fusion protein capture; 2) Jak2 binding interaction (association followed by disassociation); and 3) regeneration. A total of five such cycles were conducted. For data analysis, background sensograms (fc2) were subtracted from binding sensograms (fc1), yielding five corrected binding sensograms (two are shown in Fig. 5C). Global analysis, fitting all five corrected sensograms to a single equation, was performed. This analysis gave an association rate constant ($k_a$) of $4.20 \times 10^7 \pm 9.95 \times 10^6$ M$^{-1}$ s$^{-1}$ and a dissociation rate constant ($k_d$) of $9.91 \times 10^{-4} \pm 5.15 \times 10^{-3}$ s$^{-1}$, resulting in a dissociation equilibrium constant ($K_d$) of 2.36 nM. It must be noted that the kinetic parameters reported here were derived using an estimated value (~45 nM) for Jak2 concentration. Hence, these constants are estimates of the kinetic values and are probably within 3-fold of the true values, assuming a 3-fold error in the approximated Jak2 concentration. Thus, our data not only demonstrate an interaction between Jak2 and Fyn; they also demonstrate an interaction of high affinity.

\section*{Transphosphorylation of the N-terminal Region of Jak2 in Vitro—The data presented to this point indicate that Fyn binds tyrosine-phosphorylated Jak2 far better than unphosphorylated Jak2 and that the amino-terminal portion of Jak2 is critical for the efficient physical association with Fyn. We next wanted to investigate the obvious hypothesis that Jak2 activation leads to the Jak2-mediated tyrosine phosphorylation of its amino terminus. If Jak2 autophosphorylation is able to generate an N-terminal phosphotyrosine capable of binding the SH2 domain of Fyn, we predicted that incubation of a GST/Jak2 fusion protein (encoding Jak2 amino acids 1–294) with an active Jak2 molecule would also result in transphosphorylation of the fusion protein. To test this hypothesis, we transfected COS-7 cells to produce a cell lysate with either no Jak2 (empty vector), catalytically active wild type Jak2 (Jak2 WT), or kinase-deficient Jak2 (Jak2 DN). The GST/Jak2 fusion protein was added to each lysate and incubated. After collection by centrifugation and separation by SDS-PAGE, the nitrocellulose membrane was blotted with anti-Tyr(P) mAbs. We found that the GST/Jak2 fusion protein was transphosphorylated by wild type Jak2 but not by the dominant negative-Jak2 (Fig. 6, top). To demonstrate that equivalent amounts of GST/Jak2 were loaded into each lane, the membrane was blotted with anti-GST mAb. Although, in this experiment, there was poor cap-
ture of GST/Jak2 from the lysate expressing no Jak2 (Fig. 6, bottom, lane 1), there was no significant difference in the amount of GST/Jak2 between wild type Jak2 and the dominant negative Jak2 (Fig. 6, bottom, lanes 2 and 3). In experiments where GST alone was added to the lysates, absolutely no increased tyrosine phosphorylation was observed (data not

Fig. 5. The SH2 domain of Fyn binds Jak2 in a tyrosine phosphorylation-dependent manner. A, 35S-labeled Jak2 was incubated with the indicated GST/Fyn fusion proteins and captured by centrifugation. After separation by SDS-PAGE, the gel was dried and exposed to film in order to detect bound 35S-labeled Jak2. Also shown is a sample of the 35S-labeled Jak2 starting material. This result was observed in four independent experiments. B, COS-7 cells were transfected to express no Jak2, a wild type Jak2, or a dominant negative Jak2. The resulting lysates were incubated with GST/Fyn fusion proteins as indicated. Bound proteins were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blotted with an anti-Jak2 pAb to measure Jak2/Fyn association. The figure represents one of three separate experiments. C, partially purified Jak2 (~45 ms) was injected over GST/Fyn (GST/SH2 + SH3) and GST surfaces to generate binding and background sensograms, respectively. Corrected binding sensograms were derived by subtracting background from binding sensograms. Five corrected sensograms were generated (two are shown). The five corrected binding sensograms were fit to a single equation using BIAevaluation 3.0 (global analysis). The smooth solid lines depict the fit to corrected response versus time data. The kinetic fit parameters were $k_a = 4.20 \times 10^5 \pm 9.95 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$ and $k_d = 9.91 \times 10^{-4} \pm 5.15 \times 10^{-5} \text{ s}^{-1}$. The S.D. value for the relative residual plot was 0.15, and $\chi^2 = 0.303$. 

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shown). Thus, the transphosphorylation of the GST/Jak2 fusion protein by wild type Jak2 is specific for the sequence containing the N-terminal 294 amino acids of Jak2 and not GST.

Tyrosine-phosphorylated Jak2 Is Required, but Is Not Sufficient, for Fyn Activation—The data from Figs. 2 and 3 demonstrated that catalytically active Jak2 is required for association with Fyn. To investigate whether an active Jak2, per se, can activate Fyn, COS-7 cells were transfected to overexpress either no Jak2, wild type Jak2, or kinase-deficient Jak2. Again, Jak2 was activated by overexpression. The cell lysates were immunoprecipitated with anti-Fyn pAb and, after extensive washing, the Fyn precipitates were resuspended in kinase buffer containing \( \gamma^{32}P \)ATP and the specific Src family kinase substrate, GAP p62 (32–34). Any change in Fyn kinase activity as a function of Jak2 activity would be detected by increased \( ^{32}P \) incorporation into the substrate. After separation by SDS-PAGE and transfer onto nitrocellulose, the radiolabeled proteins were exposed to film. Analysis of the film demonstrated that there was no increased \( ^{32}P \) incorporation into GAP p62 (Fig. 7A, top). The membrane was later blotted with anti-Fyn mAbs to demonstrate equal Fyn precipitation. Similar results were seen in three separate experiments.

Although an active Jak2 kinase was in of itself insufficient for Fyn activation, we wanted to determine if an active Jak2 was required for Fyn activation. To test this possibility, COS-7 cells were transfected with plasmids encoding either the wild type AT\(_1\) receptor and Jak2 WT or with the wild type AT\(_1\) receptor and Jak2 DN. This system is different from that described for Fig. 7A, because in the previous experiment, neither the AT\(_1\) receptor nor angiotensin II were used. Here, treatment of cells with angiotensin II allows for activation of the AT\(_1\) receptor.
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receptor and all of the downstream signaling events that contribute to the activation of Fyn. What is different about the two arms of this experiment is that cells are transiently transfected with either Jak2 WT or Jak2 DN. Two days after transfection, the cells were treated with angiotensin II for the indicated times, and normalized lysates were immunoprecipitated with anti-Fyn pAb. Fyn kinase activity was measured by resuspending the precipitates in kinase buffer containing [γ-32P]ATP and GAP p62. Cells transfected with Jak2 WT showed an angiotensin II-dependent increased phosphorylation of GAP p62, indicating that Fyn kinase activity was increased as a function of angiotensin II treatment (Fig. 7B, top). However, in three separate experiments, cells transfected with Jak2 DN did not show increased 32P incorporation into GAP p62. To demonstrate equal loading, the membrane was subsequently blotted with anti-Fyn mAbs (Fig. 7B, bottom). GAP p62 is not thought to be a Jak2 substrate. However, to eliminate the possibility that we were measuring the catalytic activity of Jak2 and not Fyn, we performed an equivalent protocol in the presence of the Src family kinase inhibitor PP1. Cells were transfected with the wild-type AT1 receptor and Jak2 WT constructs. The cells were then treated with either Me6SO or PP1 and subsequently stimulated with angiotensin II. After immunoprecipitation with anti-Fyn pAb, kinase reactions were performed. Treatment with Me6SO had no effect on the 32P incorporation into GAP p62, whereas PP1 blocked the angiotensin II-dependent increase (Fig. 7C, top). To demonstrate equal loading, the membrane was blotted with anti-Fyn mAbs (Fig. 7C, bottom). The combination of 1) using Fyn immunoprecipitates, 2) using a Src family kinase substrate, and 3) observing that the phosphorylation of GAP p62 is blocked with PP1 strongly indicates that the kinase that is acting on GAP p62 is Fyn and not Jak2. Collectively, these data indicate that an active Jak2 molecule, capable of tyrosine autophosphorylation, is required for the angiotensin II-dependent activation of Fyn.

Catalytically Active Jak2 Is Required for Angiotensin II-dependent Fyn Kinase Activation in VSMC—Thus far our data indicate that in response to angiotensin II, Jak2 and Fyn physically associate. The consequence of this association is that a tyrosine-phosphorylated Jak2 serves as a docking site for Fyn activation. We wanted to determine whether these findings are seen in physiologically relevant VSMC. Therefore, quiescent VSMC were pretreated with either Me6SO or AG-490 and then stimulated with angiotensin II. The resulting lysates were immunoprecipitated with anti-Fyn pAb, and kinase assays were performed using the substrate GAP p62 (Fig. 8A). AG-490 completely blocked the angiotensin II-induced activation of Fyn, indicating that Jak2 kinase activity is required for Fyn activation in VSMC. Equal loading of Fyn was confirmed by blotting the same membrane with anti-Fyn mAbs (Fig. 8A).

To investigate the role of Jak2 in Fyn activation using an approach different from pharmacologic inhibition, we generated a stable VSMC cell line overexpressing the Jak2 DN construct (VSMC/Jak2 DN). Control cells were VSMC stably transfected with a vector containing only the neomycin cassette (VSMC/Neo). Jak2 protein expression was studied by Western blotting equal amounts of whole cell lysate from both cell lines with anti-Jak2 pAb (Fig. 8B). As expected, the VSMC/Jak2 DN cell line contained significantly more Jak2 protein than the VSMC/Neo cell line. Because the Jak2 DN protein migrates at a position similar to endogenous Jak2, we confirmed the expression of the Jak2 DN mRNA transcript in VSMC/Jak2 DN cell lines by Northern blot analysis (data not shown). To test the functional inhibition of the Jak2 DN protein in VSMC, quiescent VSMC/Neo and VSMC/Jak2 DN cell lines were stimulated with angiotensin II, and the resulting anti-Tyr(P) mAb immunoprecipitates were Western blotted with anti-Jak2 pAb (Fig. 8C). We observed that the angiotensin II-dependent tyrosine phosphorylation of Jak2, seen in the VSMC/Neo cell line, was lacking in the VSMC/Jak2 DN cell line. Thus, the Jak2 dominant negative protein blocks angiotensin II-mediated Jak2 activation in the VSMC/Jak2 DN cell line.

To test the hypothesis that a catalytically active Jak2 is required for the co-association and activation of Fyn, we now repeated several of our earlier studies using the VSMC/Jak2 DN cell line. Fig. 1A demonstrated in VSMC that, in response to angiotensin II, Fyn bound several tyrosine-phosphorylated proteins. When this study was repeated using VSMC/Jak2 DN cells, we observed that the phosphorylation pattern of Fyn-associated proteins was significantly different (Fig. 8D). The bands in the 130-kDa range were virtually undetectable, and a prominent band of about 60–62 kDa was now observed. The IgG heavy chain was faintly visible at the 50-kDa position. When we repeated the specific Jak2/Fyn co-association assays, we observed that angiotensin II elicited a ligand-dependent association of Jak2 and Fyn in the VSMC/Neo control cells but not in the VSMC/Jak2 DN cells, again indicating that a catalytically active Jak2 is required for this ligand-dependent association (Fig. 8E). To demonstrate equal loading, the same membrane was blotted with anti-Jak2 pAb (Fig. 8E). To directly measure angiotensin II-mediated Fyn kinase activity, we stimulated VSMC/Neo and VSMC/Jak2 DN cells with angiotensin II and conducted kinase assays on the resulting anti-Fyn pAb immunoprecipitates using the substrate GAP p62 (Fig. 8F). Treatment of the VSMC/Neo cells resulted in an angiotensin II-dependent activation of Fyn. However, this was blocked by expression of the Jak2 DN construct, again indicating that Jak2 activation is a prerequisite for Fyn activation in VSMC. We confirmed equal precipitation by blotting the membrane with anti-Fyn mAbs (Fig. 8F). Collectively, the data in Fig. 8 strongly argue that in VSMC, a catalytically active Jak2 is required for the angiotensin II-dependent association with and activation of Fyn kinase.

Functional Consequences of Jak2 Activation in Angiotensin II Signaling—We now wanted to identify a putative downstream signaling substrate of Jak2 in order to determine whether Jak2 is in fact important for angiotensin II signaling. A well established downstream signaling molecule of Jak2 in cytokine signaling is Stat1 (1, 2). Recent study has also demonstrated that angiotensin II stimulation leads to Stat1 phosphorylation and activation (10, 15–17). To determine whether Stat1 is a physiological substrate of Jak2 in angiotensin II signaling, we stimulated quiescent VSMC/Neo and VSMC/Jak2 DN cells with angiotensin II. The resulting lysates were immunoprecipitated with anti-Tyr(P) mAb and blotted with anti-Stat1 mAb (Fig. 9A). We observed an angiotensin II-dependent tyrosine phosphorylation of Stat1 in the VSMC/Neo cell line but not in the VSMC/Jak2 DN cell line. To rule out the possibility that the VSMC/Jak2 DN cell line was somehow nonspecifically altered by the dominant negative Jak2 protein, we tested whether these cells could mediate the tyrosine phosphorylation of p130Cas, a protein whose phosphorylation is dependent on Ca2+/c-Src/Pyk2 but independent of Jak2 (28, 35, 36). Analysis of p130Cas tyrosine phosphorylation indicated that the VSMC/Jak2 DN cells phosphorylated p130Cas in a fashion identical to the VSMC/Neo control cells (Fig. 9B).

To determine whether the inhibition of Stat1 tyrosine phosphorylation by Jak2 DN had a functional consequence in angiotensin II signaling, we examined the angiotensin II-dependent activation of the c-fos promoter. Angiotensin II induces expression of several early response genes including c-fos (37, 38). Therefore, we transiently transfected both the VSMC/Neo...
and VSMC/Jak2 DN cell lines with a luciferase reporter under the control of the c-fos promoter (20). This plasmid contains two copies of the c-fos 5'-regulated enhancer element (-357 to -276), a core thymidine kinase TATA-containing promoter (-200 to +70), and the firefly luciferase cDNA. Each copy of the c-fos enhancer contains the Stat1-binding sis-inducible element, the serum response element, and an AP-1 binding site. After transfection, both cell lines were made quiescent and then treated with angiotensin II. Fig. 9C shows the quantitation of luciferase activity as a function of time after angiotensin II addition. Expression of the Jak2 DN construct significantly reduced the angiotensin II-dependent induction of the c-fos promoter when compared with the VSMC/Neo control cells, suggesting that the angiotensin II-dependent tyrosine phosphorylation of Stat1 by Jak2 is important for maximal induction of the c-fos promoter. To ascertain whether the Jak2 DN-expressing allele had a similar effect on endogenous c-fos mRNA, Northern blot analysis was performed. The VSMC/Neo and VSMC/Jak2 DN cell lines were treated with angiotensin II for periods ranging from 0 to 60 min (Fig. 9D). Treatment with angiotensin II resulted in the ligand-dependent accumulation of c-fos mRNA in the VSMC/Neo control cells but not in the VSMC/Jak2 DN cells. Prolonged exposures of the blot indicated some ligand-dependent increase in c-fos mRNA in the VSMC/Jak2 DN cells, but it was vastly less than that seen from similarly treated VSMC/Neo cells (data not shown). To demonstrate that the difference in c-fos mRNA levels was not a loading artifact, the membrane was subsequently probed with GAPDH (Fig. 9D).

While these data come short of proving a physiologic role of Jak2 in whole animals, they do suggest that in physiologically relevant vascular smooth muscle cells, Jak2 activation is required for the angiotensin II-dependent association with and activation of Fyn kinase. One functional consequence of this appears to be that a catalytically active Jak2 is critical for the angiotensin II-dependent tyrosine phosphorylation of Stat1 and maximal induction of the c-fos promoter.

**DISCUSSION**

The studies in this report were done to better understand the relationship between the Jak2 and Fyn tyrosine kinase signaling pathways. To this end, we showed that activation of the...
seven-transmembrane \( \text{AT}_1 \) receptor by angiotensin II results in a rapid physical association of Jak2 and Fyn. This ligand-dependent association requires an active Jak2 kinase, since association can be blocked either by pharmacological means or by a kinase-deficient Jak2 molecule. We demonstrated that the portion of Jak2 that mediates this interaction is contained in the first 240 amino acids of the protein. While both the SH2 and SH3 domains of Fyn participate in Jak2/Fyn association, the data indicate that the SH2 domain is the dominant interaction and binds Jak2 in a phosphotyrosine-dependent manner. Surface plasmon resonance analysis estimated the dissociation equilibrium constant \( (K_d) \) for this interaction to be 2.36 nM, indicating that the interaction is of high affinity. Finally, our in vitro and in vivo analysis collectively suggest that an active Jak2 molecule, which is capable of autophosphorylation, is required for Fyn activation in response to angiotensin II in both COS-7 cells and in VSMC.

Based on the observations described in previous work and the results obtained from this study, we propose a model for Fyn activation by the \( \text{AT}_1 \) receptor (Fig. 10). Because the \( \text{AT}_1 \) receptor lacks intrinsic kinase activity, activation of cellular tyrosine kinase signaling cascades must occur in a manner that is different from the classical tyrosine kinase growth factor receptors. Activation of the \( \text{AT}_1 \) receptor by angiotensin II results in Jak2 autophosphorylation, including phosphorylation of the amino-terminal tyrosines (10). A tyrosine-phosphorylated Jak2 binds to the \( \text{AT}_1 \) receptor at amino acids 319–322 (YIPP) of the \( \text{AT}_1 \) receptor. The tyrosine-phosphorylated \( N \) terminus of Jak2 acts as a scaffold and directly binds the SH2 domain of Fyn, allowing for Fyn activation.

![Figure 9](image_url): Functional consequence of Jak2 DN expression in angiotensin II-mediated signaling. Quiescent VSMC/Neo and VSMC/Jak2 DN cells were treated with 10^{-7} M angiotensin II, and the resulting anti-Tyr(P) mAb immunoprecipitates were Western blotted with either anti-Stat1 mAb (A) or anti-p130^{Cas} mAb (B). Shown is one of three representative results for each. C, VSMC/Neo (●) and VSMC/Jak2 DN (▲) cells were transiently transfected with a plasmid encoding firefly luciferase under the control of the c-fos promoter. The cells were subsequently treated with 10^{-7} M angiotensin II for the indicated times, and luciferase activity was measured. Values are expressed as the mean ± S.E.; \( n = 6 \) for each time point. *, \( p < 0.025; **, p < 0.0005 \) (Student’s t test distribution). Shown is one of two representative results. D, Northern blot analysis of RNA from VSMC/Neo and VSMC/Jak2 DN cells treated with 10^{-7} M angiotensin II. The blot was probed with the mouse c-fos cDNA, stripped, and then probed with the human GAPDH cDNA. Shown is one of two representative results.

![Figure 10](image_url): Model of Jak2-dependent activation of Fyn by the \( \text{AT}_1 \) receptor. Top, in the absence of ligand, Jak2 is unphosphorylated and not bound to the \( \text{AT}_1 \) receptor. Bottom, binding of angiotensin II to the \( \text{AT}_1 \) receptor results in Jak2 tyrosine phosphorylation and its coincident binding to amino acids 319–322 (YIPP) of the \( \text{AT}_1 \) receptor. The tyrosine-phosphorylated N terminus of Jak2 acts as a scaffold and directly binds the SH2 domain of Fyn, allowing for Fyn activation.
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pathways in a manner that is similar to the cytokine superfamily of receptors is a novel observation. However, the significance of this report is that we have defined a role for Jak2 that, to date, has not been described. Specifically, our data indicate that in addition to serving as a signal transduction kinase, Jak2 also functions as a scaffold protein providing phosphotyrosine residues that participate in activating other signaling pathways. It appears that Jak2 itself is serving as a docking site for Fyn activation. A recent report described the crystal structure of pp60src (39). Resolution of pp60src in the inactive state revealed a conformation in which the tyrosine phosphorylated tail of Src binds its SH2 domain. This conformation simultaneously blocks the kinase domain and sequesters the SH2 and SH3 domains. When a phosphotyrosine with higher affinity binds the SH2 domain, the result is a conformational change in the protein that frees the kinase domain, the SH2 domain, and the SH3 domain. Previous studies using Biacore have measured the affinity of the Src SH2 domain for synthetic phosphotyrosine-containing peptides and reported Keq values in the range of 600 nm (40). Our data demonstrate that Jak2 binds the SH2 domain of Fyn and that this is tyrosine phosphorylation-dependent. The estimated Keq for this interaction is 2.36 nm. Thus, assuming a 3-fold error in our Biacore data (see “Results”), the affinity of a Jak2 phosphotyrosine/Fyn SH2 interaction is at least 1 order of magnitude higher than those reported for peptides. This strong interaction could presumably displace the Fyn phosphotyrosine from its SH2 domain and therefore lead to Fyn activation. Our observation indicating a high affinity interaction between a Jak2 phosphotyrosine and the SH2 domain of Fyn resembles a report describing an interaction between Jak2 and the SH2-containing protein termed SH2-B. Carter-Su and co-workers (41) report that the carboxyl terminus of SH2-B, which contains the SH2 domain, specifically interacts with kinase-active, tyrosine-phosphorylated Jak2 but not the kinase-inactive, unphosphorylated Jak2 in the yeast two-hybrid system. Recruitment of SH2-B to signaling complexes in PC12 cells appears to mediate nerve growth factor-induced neuronal differentiation (42).

Hand-in-hand with defining a new role for Jak2 in cellular signal transduction is the observation that we have defined a pathway of Fyn activation by a seven-transmembrane receptor. The Src family tyrosine kinases known to be activated by a broad range of seven-transmembrane receptor ligands including angiogenin II, platelet-activating factor, lysophosphatidic acid, bombesin, and thrombin (12, 43, 44). To date, the precise mechanism of this activation remains unclear. In the case of angiogenin II, our data suggests that the activation of Fyn is a Jak2-dependent event. Indeed, the data in this paper give some functional insight into the role of Jak2 in angiogenin II signaling. Not only does a dominant negative Jak2 block Fyn activation, but it also interrupts the signaling by which angiogenin II stimulates the early response gene c-fos. In summary, this work provides insight into the fundamental mechanism of how the seven-transmembrane AT1 receptor initiates tyrosine phosphorylation signal transduction cascades and helps define the cross-talk that is observed between the Jak and Src tyrosine kinase pathways.

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