Regulation of Angiotensin II-induced Phosphorylation of STAT3 in Vascular Smooth Muscle Cells*

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Ligand binding to the angiotensin II (Ang II) AT1 receptor on vascular smooth muscle cells (VSMCs) activates the Janus-activated kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. We have shown previously that the JAK2 tyrosine kinase and the Src family p59 Fyn tyrosine kinase are required for Ang II-induced STAT1 tyrosine phosphorylation in VSMCs. The mitogen-activated protein kinase phosphatase, MKP-1, is required for STAT1 tyrosine dephosphorylation. In the present study, using specific enzyme inhibitors and antisense oligonucleotides, we show that Ang II-induced tyrosine phosphorylation and nuclear translocation of STAT3 in VSMCs is mediated by p60 c-Src, whereas tyrosine dephosphorylation is mediated by calcineurin. Calcineurin is activated in response to Ang II stimulation of VSMCs and is translocated to the nucleus. In addition, we show that Ang II-induced serine phosphorylation of STAT3 in VSMCs is mediated by mitogen-activated protein kinase and that dephosphorylation is mediated by protein phosphatase 2A (PP2A). PP2A translocates to the nucleus in response to Ang II stimulation of VSMCs and forms a complex with STAT3 in an Ang II-dependent manner.

STAT (signal transducers and activators of transcription) proteins are latent cytoplasmic transcription factors that are activated by tyrosine phosphorylation by JAK (Janus-activated) kinases (1–3). Seven STAT proteins are known termed STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Four JAK enzymes are known termed JAK1, JAK2, JAK3, and Tyk2. Tyrosine phosphorylation of STAT proteins by the JAK kinases promotes STAT homo- and heterodimerization and translocation of activated STAT dimers to the nucleus. In the nucleus, the STATs regulate the expression of various genes important in cell proliferation. Angiotensin II (Ang II)-induced signal transduction in vascular smooth muscle cells (VSMCs), for example, proceeds in part through activation of the JAK/STAT pathway. We have shown previously that Ang II signaling through the G-protein-coupled AT1 receptor stimulates tyrosine phosphorylation and activation of JAK2 and Tyk2 in VSMCs followed by tyrosine phosphorylation of STAT1, STAT2, and STAT3 and translocation of STAT1 to the nucleus (4). In addition to the JAK kinases, the Src family of tyrosine kinases has also been implicated in phosphorylation and activation of STAT proteins (5–9). Therefore, we have recently investigated the role of specific Src family tyrosine kinases in Ang II-induced tyrosine phosphorylation and nuclear translocation of STAT1 in VSMCs (10). Using selective enzyme inhibitors and antisense oligonucleotides, we have shown that JAK2 and the Src family p59 Fyn tyrosine kinase (but not the p60 c-Src tyrosine kinase) are required for phosphorylation and nuclear translocation of STAT1. Neither of these two kinases, however, appears to function upstream from the other in a tyrosine phosphorylation cascade. Rather, p59 Fyn serves as an Ang II-activated docking protein for both JAK2 and STAT1. In this role, p59 Fyn brings JAK2 and STAT1 together in close physical proximity, thereby facilitating JAK2-mediated STAT1 phosphorylation. In addition, we also identified the dual specificity mitogen-activated protein kinase phosphatase, MKP-1, as the enzyme responsible for STAT1 tyrosine dephosphorylation in VSMCs.

Neutralizing antibodies against either STAT1 or STAT3 completely abolish the proliferative response of VSMCs to Ang II, suggesting that both transcription factors have an essential role in the response (11). Therefore, in the present study we have examined whether Ang II-induced tyrosine phosphorylation of STAT3 in VSMCs is regulated by the same tyrosine kinases and tyrosine phosphatases that regulate tyrosine phosphorylation of STAT1. Because maximal activation of transcription by STAT1 and STAT3 also requires serine phosphorylation of these proteins (12, 13), we have also identified the serine/threonine-specific protein kinases and serine/threonine-specific protein phosphatases that are responsible for Ang II-induced STAT3 serine phosphorylation and subsequent dephosphorylation.

EXPERIMENTAL PROCEDURES

Materials—Phosphorothioate-modified antisense and sense oligonucleotides were synthesized in the Emory University Microchemical Facility. Antisense and sense sequences for JAK2, p60 c-Src, p59 Fyn, and MKP-1 were described previously (10). Phosphotyrosine-specific anti-STAT3 antibody was obtained from New England Biolabs. Phosphoserine-specific anti-STAT3 antibody was purchased from Upstate Biotechnology. Antibodies against calcineurin, PP2A, and STAT3 were obtained from Transduction Laboratories. AG-490, PP1, and PD98059 were purchased from Calbiochem. Ang II, okadaic acid, and p-nitrophenyl phosphate were purchased from Sigma. The sis-inducing element (SIE) gel shift oligonucleotide was obtained from Santa Cruz Biotech-
nology. Lipofectin came from Life Technologies, Inc. T4 polynucleotide kinase was obtained from Promega and [γ-32P]ATP was obtained from Amersham Pharmacia Biotech. PK506 was a generous gift of Dr. James A. Tumlin, Emory University.

Cell Culture—VSMCs were cultured from Harlan Sprague-Dawley rat aortas in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum supplemented with antibiotics (10). Cells were serum-starved overnight prior to Ang II treatments.

Immunoprecipitation and Immunoblotting—VSMCs were treated with Ang for various times, cells were lysed, and lysates were subjected to immunoprecipitation and immunoblotting as described previously (10).

Oligonucleotide Treatment—Antisense and sense oligonucleotide treatments were carried out using Lipofectin as described previously (10).

Isolation of VSMCs Nuclei—VSMCs cell nuclei were isolated as described previously (10).

Tyrosine Phosphatase Activity Assay—Calcineurin tyrosine phosphatase activity was determined as described previously by following the rate of formation of p-nitrophenol from p-nitrophenyl phosphate (14).

Electrophoretic Mobility Shift Assay—The SIE oligonucleotide was 32P-labeled with T4 polynucleotide kinase and incubated with nuclear extracts in the presence of 1 μg of poly(deoxyinosinic-deoxycytidylic acid), 10 mM Hepes, pH 7.8, 50 mM KCl, 1 mM EDTA, 5 mM MgCl2, 10% glycerol, 5 mM dithiothreitol, 1 mg/ml bovine serum albumin, 10 μg/ml leupeptin, 5 μg/ml pepstatin A, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM NaVO4. After a 15-min incubation at room temperature, the samples were electrophoresed on 4% polyacrylamide gels. Gels were analyzed by autoradiography.

RESULTS AND DISCUSSION

We have shown previously that Ang II-induced tyrosine phosphorylation of STAT1 in VSMCs is mediated by JAK2 and, in addition, requires activated p59 Fyn functioning as an Ang II-activated docking protein for both JAK2 and STAT1 (10). To determine whether Ang II-induced tyrosine phosphorylation of STAT3 in VSMCs is also mediated by JAK2, we utilized the JAK2-selective inhibitor, AG-490. This inhibitor inhibits JAK2 with an IC50 in the low micromolar range (<5 μM) but does not inhibit Src family tyrosine kinases even at concentrations as high as 50 μM (15). Cultured rat aortic VSMCs were preincubated in the presence or absence (control) of AG-490 (10 μM for 1 h) prior to stimulation of the cells for 0, 30, or 60 min with Ang II (100 nM). Cells were then lysed and lysates were immunoblotted with a STAT3 phosphotyrosine-specific antibody that recognizes the tyrosine residue 705-phosphorylated (but not the nonphosphorylated) form of STAT3. In the control, Ang II induced the tyrosine phosphorylation of STAT3 (92-kDa band) within 60 min (Fig. 1A). Phosphorylation, however, was unaffected by AG-490 treatment, indicating that, in contrast to the case of Ang II-induced tyrosine phosphorylation of STAT1, Ang II-induced tyrosine phosphorylation of STAT3 is not mediated by JAK2. To verify the lack of a role of JAK2 in Ang II-induced STAT3 phosphorylation, we also carried out experiments in which we utilized a specific JAK2 antisense oligonucleotide to suppress JAK2 protein expression. VSMCs were treated with the antisense oligonucleotide or a complementary sense oligonucleotide (negative control) for 12 h prior to stimulation of the cells for 0, 30, or 60 min with Ang II (100 nM). The antisense oligonucleotide (but not the sense oligonucleotide) completely abolished JAK2 expression in VSMCs after 12 h of treatment (data not shown) as reported previously (10). When Ang II-induced STAT3 tyrosine phosphorylation in oligonucleotide-treated cells was then assessed by immunoblotting with the phosphotyrosine-specific anti-STAT antibody, no effect of either sense or antisense oligonucleotides on Ang II-stimulated STAT3 tyrosine phosphorylation was observed (Fig. 1A), confirming that JAK2 is not required for Ang II-induced STAT3 phosphorylation in VSMCs.

We have shown previously that the Src family tyrosine kinases, p60 c-Src and p59 Fyn, are activated in VSMCs in response to Ang II stimulation (10, 16, 17). We have shown further that p59 Fyn (but not p60 c-Src) is required for Ang II-induced STAT1 phosphorylation in VSMCs by functioning as a Ang II-activated docking protein for both JAK2 and STAT1 (10). To determine whether Src family tyrosine kinases are required for Ang II-induced STAT3 phosphorylation in VSMCs, we utilized the Src family kinase-selective inhibitor, PP1. PP1 inhibits Src family kinases in the low nanomolar range but does not inhibit JAK2 even at 1000-fold higher concentrations (18). VSMCs were preincubated in the presence or absence (control) of PP1 (1 μM for 30 min) prior to stimulation of the cells for 0, 30, or 60 min with Ang II (100 nM). Tyrosine phosphorylation of STAT3 was then quantitated by immunoblotting of cell lysates with the phosphotyrosine-specific anti-STAT3 antibody. As shown in Fig. 1B, STAT3 was tyrosine-phosphorylated in the control condition within 60 min after Ang II exposure. PP1 pretreatment, however, completely blocked Ang II-induced phosphorylation of the factor. To confirm the requirement for Src family tyrosine kinases in phosphorylation of STAT3 in Ang II-stimulated VSMCs and to determine the specific roles of p59 Fyn and p60 c-Src in phosphorylation, experiments were also carried out using p59 Fyn and p60 c-Src antisense oligonucleotides. VSMCs were treated for 24 h with antisense or complementary sense (negative control) oligonucleotides to p59 Fyn or p60 c-Src prior to stimulation of the cells for 0, 30, or 60 min with Ang II (100 nM). As reported previously (10), the two antisense oligonucleotides completely abolished p59 Fyn and p60 c-Src protein expression in VSMCs within 24 h (data not shown). In contrast, the two sense oligonucleotides had no effect on expression of the two proteins. Additional experiments were then carried out to examine the effect of 24-h treatment with the antisense oligonucleotides on subsequent Ang II-stimulated STAT3 phosphorylation. The p59 Fyn antisense oligonucleotide had no effect on Ang II-induced STAT3 tyrosine phosphorylation (data not shown) indicating that, unlike the case of STAT1, Ang II-induced phosphorylation of STAT3 does not require p59 Fyn. However, when the effect of the p60 c-Src antisense oligonucleotide was examined, this...
VSMCs were treated with Ang II (100 nM) for the times shown following pretreatment of cells with either p60 c-Src sense or antisense oligonucleotides. Nuclei were isolated and STAT3 in nuclear fractions was quantitated by immunoblotting with anti-STAT3 antibody. Similar results were obtained in three experiments.

To determine whether calcineurin is activated in response to Ang II stimulation in VSMCs, cells were either pretreated or not pretreated (control) or pretreated with FK506. Cells were lysed, and lysates were immunoblotted with a phosphotyrosine-specific anti-STAT3 antibody. Similar results were obtained in three separate experiments.

Another protein phosphatase that is active in the cell nucleus following its translocation from the cytosol in response to Ca²⁺ signals (21) is the Ca²⁺- and calmodulin-dependent protein phosphatase known as calcineurin or protein phosphatase 2B (PP2B) (22). Calcineurin is generally considered a serine/threonine-specific protein phosphatase, based on its specificity in vitro assay for a synthetic peptide corresponding to residues 81–99 of the RII subunit of cAMP-dependent protein kinase (23). Calcineurin dephosphorylates both phosphoserine and phosphothreonine in this peptide but the kcat for phosphothreonine is 2 orders of magnitude lower than that for phosphoserine (22). Calcineurin, however, has also been shown to effectively dephosphorylate free phosphothreonine and other tyrosine-phosphorylated peptides and proteins in vitro (24–28).

Calcineurin is activated in response to Ang II stimulation in rat adrenal glomerulosa cells (29) and in rat cardiac myocytes (30). To determine whether calcineurin is activated in response to Ang II stimulation in VSMCs, cells were either pretreated or not pretreated (control) with the highly selective calcineurin-specific inhibitor, FK506 (100 µM for 1 h) (31, 32), prior to stimulation of the cells for various times with Ang II (100 nM).

**Fig. 2.** Effect of p60 c-Src sense and antisense oligonucleotides on Ang II-induced nuclear translocation of STAT3 in VSMCs. VSMCs were treated with Ang II (100 nM) for the times shown following pretreatment of cells with either p60 c-Src sense or antisense oligonucleotides. Nuclei were isolated and STAT3 in nuclear fractions was quantitated by immunoblotting with anti-STAT3 antibody. Similar results were obtained in three experiments.

**Fig. 3.** Ang II-induced activation of calcineurin in VSMCs. VSMCs were treated with Ang II (100 nM) for the times shown following either no pretreatment (○) or pretreatment (■) of cells with FK506. Cells were lysed and calcineurin was immunoprecipitated with anti-calcineurin antibody. Phosphatase activity of immunoprecipitates was estimated by measuring the rate of formation of p-nitrophenol from p-nitrophenyl phosphate. Results shown represent mean ± S.E. from three experiments.

**Fig. 4.** Ang II-induced nuclear translocation of calcineurin in VSMCs. VSMCs were treated with Ang II (100 nM) for the times shown following either no pretreatment (Control) or pretreatment with FK506. Cells were lysed, and lysates were immunoblotted with anti-calcineurin antibody. Similar results are representative of three experiments.

**Fig. 5.** Ang II-induced tyrosine phosphorylation of STAT3 in VSMCs. VSMCs were treated with Ang II (100 nM) for the times shown following either no pretreatment or pretreatment with FK506. Cells were lysed, and cell nuclei were isolated as described previously (4, 19). Relative amounts of STAT3 in nuclear fractions were then quantitated by immunoblotting with anti-STAT3 antibody. Results shown are representative of three experiments.
VSMCs. VSMCs were treated with Ang II (100 nM) for the times indicated following either no pretreatment (control) or pretreatment with FK506. Nuclear extracts were prepared and tested for their abilities to gel shift a 32P-labeled oligonucleotide containing a consensus SIF binding site. The results shown are representative of two separate experiments.

Cells were lysed, and calcineurin was immunoprecipitated from lysates with an anti-calcineurin antibody that recognizes the catalytic subunit of the enzyme. Immunoprecipitates were then assayed for tyrosine phosphatase activity by measuring the rate of formation of p-nitrophenol from p-nitrophenyl phosphate as described previously (14, 33). Ang II did indeed activate calcineurin in these experiments and activation was sustained out to at least 1 h. Furthermore, activation was completely blocked by FK506 (Fig. 3).

Calcineurin is translocated from the cytoplasm to the nucleus in baby hamster kidney fibroblasts in response to treatment with the Ca2+-ionophore A23187 (21). Whether the enzyme undergoes a similar nuclear translocation in response to Ca2+-mobilizing hormones, however, has not been described previously. To determine whether Ang II stimulation of VSMCs results in nuclear translocation of calcineurin, cells were treated with Ang II (100 nm) for 0, 30, and 60 min followed by isolation of cell nuclei. Amounts of calcineurin in nuclear fractions were then quantitated by immunoblotting with anti-calcineurin antibody. Similar results were obtained in three separate experiments.

To determine whether calcineurin is responsible for tyrosine dephosphorylation of STAT3 in VSMCs, cells were treated with Ang II (100 nm) for 0, 1, 2, 3, and 4 h following either no pretreatment (control) or pretreatment with FK506 (100 μM for 1 h). Cells were lysed, and lysates were immunoblotted with the phosphotyrosine-specific anti-STAT3 antibody. As shown in Fig. 5, STAT3 in the control condition was tyrosine-phosphorylated at 1 h and subsequently completely dephosphorylated within 2 h. In FK506-treated cells, however, STAT3 remained maximally tyrosine-phosphorylated out to at least 4 h, suggesting that calcineurin is the phosphatase responsible for STAT3 tyrosine dephosphorylation following its Ang II-induced tyrosine phosphorylation.

Translocation of activated STAT homo- and heterodimers to the nucleus results in formation of a complex of STAT proteins termed six-inducing factor (SIF). SIF binds to gene promoter elements known as SIEs. To determine whether calcineurin-mediated tyrosine dephosphorylation of STAT3 reverses SIF binding to SIE, we carried out electrophoretic mobility shift assays with nuclear extracts from Ang II-stimulated VSMCs following preincubation of the cells in the absence (control) or presence of FK506. VSMCs were preincubated with or without FK506 (100 μM for 1 h) and then treated for 0, 1, 2, 3, and 4 h with Ang II (100 nm). Nuclear extracts were prepared and tested for their abilities to gel shift a 32P-labeled double-stranded oligonucleotide probe containing the SIF consensus binding site. With nuclear extracts from both control and FK506-treated cells, Ang II treatment induced a clear gel shift within 1 h, presumably due to binding of SIF to the SIE (Fig. 6). In the control condition, binding was maximal at 1 h and subsequently declined in a time-dependent manner so
that, after 4 h, binding was <10% of that observed at 1 h. In contrast, nuclear extracts from the FK506-pretreated cells sustained a maximal level of binding out to at least 4 h. When nuclear extracts were incubated with both the $^{32}$P-labeled probe and with an anti-STAT3 antibody, a gel supershift was observed (Fig. 6, far right lane), demonstrating that the SIF was comprised of STAT3 homo- and/or heterodimers. Taken together, the results of the electrophoretic mobility shift assays suggest that calcineurin-mediated tyrosine dephosphorylation of STAT3 is responsible for SIF dissociation from the SIE and thus termination of Ang-II-induced JAK/STAT signaling through the SIE promoter element.

Based on the results of the present study and those of our previous studies (4, 10), it is clear that both STAT1 and STAT3 are phosphorylated on tyrosine residues in response to Ang II stimulation of VSMCs. These two proteins have also been shown to be phosphorylated on serine residues in other cell types in response to both growth factor and cytokine receptor signaling. Phosphorylation of a single serine residue (residue 727) in each protein is required for maximal transcriptional activity and appears to be mediated by MAP kinase, because it occurs within a PMSP MAP kinase recognition sequence (12, 13). Whether STAT1 and/or STAT3 are serine-phosphorylated as a result of ligand binding to the Ang II AT1 receptor or other G-protein-coupled receptor has not been reported. To examine whether Ang II induces the serine phosphorylation of STAT3 in VSMCs, cells were treated for 0, 1, 2, 3, and 4 h with Ang II (100 nM) and then lysed. Experiments were also performed in which cells were pretreated with PD98059 (30 μM for 1 h), a highly selective MAP kinase kinase (and hence MAP kinase) inhibitor (34) to determine whether Ang II-induced STAT3 serine phosphorylation in VSMCs is mediated by MAP kinase. Lysates were immunoblotted with a STAT3 phosphoserine 727-specific antibody that recognizes the serine-phosphorylated (but not the nonphosphorylated) form of STAT3. As shown in Fig. 7, Ang II induced the serine phosphorylation of STAT3 within 1 h. Phosphorylation was sustained out to 3 h after which the factor became dephosphorylated by 4 h. Phosphorylation was almost completely blocked by PD98059 confirming that the modification was catalyzed by MAP kinase.

As indicated above, calcineurin is generally thought to be a serine/threonine-specific protein phosphatase. Our results, however, showing that STAT3 tyrosine dephosphorylation is blocked by FK506 suggest that it may in fact be a dual specificity protein phosphatase that dephosphorylates both serine/threonine and tyrosine residues. In order to determine whether calcineurin dephosphorylates STAT3 at phosphoserine residue 727, we examined the time course of Ang II-induced serine phosphorylation/dephosphorylation of STAT3 in VSMCs in the presence and absence (control) of FK506. Cells were treated with Ang II (100 ng) for 0, 1, 2, 3, and 4 h and then lysed. Lysates were immunoblotted with the phosphoserine-specific anti-STAT3 antibody to quantitate the relative levels of phosphoserine in STAT3 at each time point. In contrast to STAT3 tyrosine dephosphorylation, which is completely blocked by FK506, STAT3 serine dephosphorylation was unaffected by the inhibitor (data not shown), suggesting that calcineurin does not dephosphorylate STAT3 at serine 727.

In addition to calcineurin (PP2B), there are three other major serine protein phosphatases in mammalian cells that are referred to as PP1, PP2A, and PP2C (35). PP1 and PP2A are potently inhibited by okadaic acid. In contrast, calcineurin and...
PP2C are unaffected by okadaic acid (36, 37). To determine whether STAT3 serine dephosphorylation in VSMCs is mediated by either PP1 or PP2A, we examined whether it could be blocked by okadaic acid. VSMCs were preincubated with okadaic acid (100 nM) for 0, 1, 2, 3, 4, or 6 h. STAT3 serine phosphorylation at each time point was then quantitated by immunoblotting with the phosphoserine-specific anti-STAT3 antibody. In contrast to the case where no inhibitor was present (Fig. 7, Control), when okadaic acid was present, STAT3 serine phosphate was not removed from the protein even after 6 h (Fig. 8), suggesting that PP1 and/or PP2A are responsible for STAT3 serine dephosphorylation following its serine phosphorylation in response to Ang II.

Consistent with our results in VSMCs, studies in other cell types have implicated PP2A as the phosphatase responsible for STAT3 serine dephosphorylation (12). To determine whether PP2A is the phosphatase responsible for STAT3 serine dephosphorylation in VSMCs following its Ang II-induced serine phosphorylation, we investigated the time course of STAT3-PP2A complex formation. Cells were treated with Ang II (100 nM) for 0, 1, 2, 3, and 4 h and then lysed. Lysates were immunoprecipitated with anti-STAT3 and anti-PP2A antibodies. Anti-STAT3 immunoprecipitates were then immunoblotted with anti-PP2A antibody, and anti-PP2A immunoprecipitates were immunoblotted with anti-STAT3 antibody. As shown in Fig. 9, STAT3 was coimmunoprecipitated by the anti-PP2A antibody, and PP2A was coimmunoprecipitated by the anti-STAT3 antibody in an Ang II-dependent manner, demonstrating an Ang II induction of complex formation of STAT3 with PP2A. Complex formation was maximal at 3 h, just prior to the time when STAT3 became serine dephosphorylated (Fig. 7). These data suggest that PP2A does indeed catalyze STAT3 serine dephosphorylation. Because dephosphorylation likely occurs in the nucleus, we also tested the possibility that Ang II might induce a translocation of PP2A from the cytoplasm to the nucleus. VSMCs were treated with Ang II (100 nM) for 0, 0.5, 1, 2, and 3 h before isolation of cell nuclei as described previously (4, 19). Relative amounts of PP2A in nuclear fractions were then quantitated by immunoblotting with an antibody that recognizes the catalytic subunit of PP2A (36-kDa band). A clear Ang II-stimulated nuclear translocation of PP2A was observed in these experiments (Fig. 10) again consistent with the conclusion that PP2A is the phosphatase that dephosphorylates serine 727 in STAT3 following its Ang II-induced phosphorylation.

In summary, the results of the present study demonstrate that, in contrast to the case of Ang II-induced tyrosine phosphorylation of STAT1 (10), Ang II-induced tyrosine phosphorylation of STAT3 in VSMCs requires neither JAK2 nor p59 Fyn, but is instead mediated by p60 c-Src (Fig. 11). Also unlike the case of STAT1 (10), tyrosine dephosphorylation of STAT3 does not require the activity of the dual specificity MKP-1 protein kinase phosphatase but rather that of the phosphatase known as calcineurin. Calcineurin is generally thought to be a serine/threonine-specific protein phosphatase, although our results suggest that it also functions as a protein-tyrosine phosphatase. Calcineurin is activated in response to Ang II stimulation of VSMCs and is translocated to the nucleus. Ang II-induced serine phosphorylation of STAT3 in VSMCs appears to be mediated by MAP kinase. MAP kinase activation likely occurs downstream from c-Src activation as we have shown previously that Ang II controls Ras activation and Ras-Raf-1 complex formation via p60 c-Src (38), events that are upstream from MAP kinase activation in the Ras-Raf-1-MAP kinase cascade. Finally, serine dephosphorylation of STAT3 in VSMCs appears to be mediated by PP2A. PP2A translocates to the nucleus in response to Ang II stimulation of VSMCs and transiently forms a complex with STAT3 just prior to the time during which STAT3 becomes serine-dephosphorylated.

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