Syp Associates with gp130 and Janus Kinase 2 in Response to Interleukin-11 in 3T3-L1 Mouse Preadipocytes*

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Protein tyrosine phosphorylation and thus dephosphorylation are part of the interleukin (IL)-11 response in mouse 3T3-L1 cells. We report here for the first time the involvement and interactions of the SH2-containing protein tyrosine phosphatase Syp in the IL-11 signal transduction pathway. Addition of IL-11 to 3T3-L1 cells resulted in an increase in the tyrosine phosphorylation of Syp. When cell lysates were precipitated with glutathione S-transferase fusion products of Syp, the C-terminal SH2 domain of Syp was shown to precipitate several proteins of 70, 130, 150, and 200 kDa that were tyrosine phosphorylated in response to IL-11. Reciprocal immunoprecipitation experiments showed that Syp was inducibly associated with both gp130 and J anus kinase 2 (J AK2). A phosphopeptide containing the sequence for a potential Syp binding site (YXXV) was used to compete with the associates of Syp with gp130 and J AK2. The phosphopeptide reduced the Syp association with both gp130 and J AK2. To summarize, Syp has multiple interactions in IL-11 signal transduction. In addition to the IL-11-induced tyrosine phosphorylation of Syp, Syp coprecipitated with gp130, J AK2, and other tyrosine-phosphorylated proteins in response to IL-11. These findings may have extensive significance to IL-11 and related cytokine signal transduction, suggesting new pathways and mechanisms.

IL-11 is a stromal fibroblast-derived cytokine involved in lymphopoiesis and hematopoiesis and is also important in proliferation and differentiation of a variety of cell types (1). IL-11 binds to a specific receptor, which requires the signal transducer gp130 for transmission of IL-11 signals into the cell (2). gp130 is the common signal transducer for IL-11 (3), ciliary neurotrophic factor (4), leukemia inhibitory factor (4), IL-6 (5), and oncostatin M (6). It has been shown that gp130 associates with J anus kinase 2 (J AK2) constitutively and that J AK2 is activated upon IL-11, IL-6, ciliary neurotrophic factor, oncostatin M, or leukemia inhibitory factor stimulation of cells (7, 8). J AK2 is a 130-kDa tyrosine-phosphorylated kinase whose response to IL-6 is blocked by point mutations or deletions of the membrane proximal portion of gp130 (9).

Tyrosine phosphorylation and dephosphorylation is a common means for cytokine-mediated intracellular signal transduction. Induction with IL-2 (10), IL-6 (11), IL-7 (12), or IL-11 (13) results in the activation of kinases leading to protein tyrosine phosphorylation. IL-6 also stimulates tyrosine dephosphorylation by unspecified phosphatases leading to the reduction of tyrosine phosphorylation (14). Recently, it was reported that ciliary neurotrophic factor can induce the tyrosine phosphatase of the protein-tyrosine phosphatase Syp (also named SH-PTP2, PTP1D, and PTP2C) (15). Syp is a recently discovered protein-tyrosine phosphatase containing two SH2 domains (16, 17). Syp is ubiquitously expressed and found to be involved in platelet-derived growth factor (PDGF) and epidermal growth factor signal transduction by direct association with the activated receptors (18-20). Syp is also involved in insulin receptor substrate 1 signal transduction (21-23). In addition, Syp functions as an adaptor protein for the signal transduction from PDGF through GRB2-Sos, leading to the activation of the Ras signaling pathway (24).

In this study, we identify and characterize a new member of the IL-11 signal transduction mechanism, Syp, and determine its interactions with established components of this pathway, gp130, and J AK2. Both gp130 and J AK2 have potential binding sites for Syp (25, 26). We suggest that the interactions of Syp and these components are integral parts of the IL-11 signaling pathway.

EXPERIMENTAL PROCEDURES

Materials—IL-11 (specific activity, 2.5 × 10⁶ units/mg) was generously provided by Genetics Institute (Cambridge, MA). Recombinant protein-A, anti-gp130, anti-J AK2, and antiphosphotyrosine (clone 4G10) were purchased from Upstate Biotechnology Inc. Rabbit polyclonal anti-Syp was generated as described (16). The enhanced chemiluminescence (ECL) material along with anti-rabbit IgG-horseradish peroxidase and anti-mouse IgG-horseradish peroxidase were acquired from Amersham. Polyvinylidene difluoride membranes were purchased from Millipore.

Preparation of GST-Syp Fusion Proteins—N/C-terminal GST-Syp-SH2 domains (amino acids 2–216), N-terminal GST-Syp-SH2 domain (amino acids 2–210), C-terminal GST-Syp-SH2 domain (amino acids 108–216), and GST alone as a control were prepared as described (16). For in vitro binding studies, glutathione-Sepharose beads (Pharmacia Biotech, Inc.) with bound fusion proteins (approximately 5 μg of fusion protein or 25 μM per binding reaction) were incubated with cell lysates made with digitonin lysis buffer (1% digitonin, Sigma), 150 mM NaCl, 2 mM EDTA, 50 mM Tris, pH 8.0, 2 mM sodium orthovanadate, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) at 4°C for 12-14 h. The beads were washed three times in digitonin lysis buffer before separation by SDS-polyacrylamide gel electrophoresis and immunoblot analysis. Experiments with 1% Triton X-100 replacing 1% digitonin were also performed.

Coimmunoprecipitations—3T3-L1 mouse preadipocytes were grown to confluence on 150 × 25-mm dishes in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM l-glutamine (pH 7.2) (complete Dulbecco’s modified Eagle’s medium). No IL-11 or 500 ng/ml IL-11 was added as indicated under “Results” at 37°C in the presence of 10% CO₂.
Reactions were stopped by placing the dish of attached cells in ice water and adding ice-cold phosphate-buffered saline containing 2 mM sodium orthovanadate to the cells. Cells were scrapped off the plate, washed in ice-cold phosphate-buffered saline, and then centrifuged at 500 × g for 5 min at 4 °C. Cells were resuspended in 0.5 ml of digitonin lysis buffer, vortexed, and incubated on ice for at least 10 min. Insoluble material was removed by centrifugation. The remaining lysate was used for immunoprecipitations with the appropriate antibodies for 12-14 h incubations. Immunoprecipitations of Syt to determine if IL-11 induced the tyrosine phosphorylation of Syt were performed as described above. Densitometry for quantitation of immunoblots was completed with a Bio-Rad GS-670 imaging densitometer coupled to a power Macintosh 8100/80.

Peptide Competition—The peptide YSTV and phosphopeptide VEY*STVVHS were prepared by a stepwise Fmoc (9-fluorenylmethoxy carbonyl) synthetic strategy as described (27), and their purity was verified by high performance liquid chromatography. The peptides were added immediately after addition of digitonin lysis buffer or to cells 5 min before IL-11 addition, in case any adaptation to the peptide was necessary, and freshly added after washes and with lysis buffer so that the peptide was continuously present at 1 μm until washed out at the end of the immunoprecipitation.

RESULTS

IL-11-induced Tyrosine Phosphorylation of Syt—Syt has not been previously shown to be involved in the signaling pathway of IL-11. An assay for the IL-11-induced tyrosine phosphorylation of Syt would suggest its involvement in the IL-11 signal transduction pathway. Therefore, we tested whether IL-11 induces the tyrosine phosphorylation of Syt. In this analysis, 3T3-L1 cells were stimulated with IL-11 for various amounts of time so that peak phosphorylation could be seen. Increased tyrosine phosphorylation of Syt with IL-11 addition was seen in as little as 30 s (data not shown) and peaked at 3 min (Fig. 1A). This suggests a relatively early involvement for Syt in the IL-11 signal transduction pathway. Dose response experiments showed tyrosine phosphorylation of Syt increasing in a dose-dependent manner peaking at 100 ng/ml IL-11 (Fig. 1B).

C-terminal SH2 Domain of GST-Syp Fusion Proteins Associated with Tyrosine-phosphorylated Proteins in IL-11-stimulated Cells—Since Syt apparently responds to IL-11, GST-Sypt fusion constructs were prepared to study potential Syt-associated proteins. To determine if IL-11 induced association of specific SH2 domains of Syt with tyrosine-phosphorylated proteins in 3T3-L1 cells, the following procedures were performed. GST-Sypt fusion proteins containing the N/C-terminal SH2 domains and the separate N- or C-terminal SH2 domains were purified from Escherichia coli as described (16). Fig. 2 shows the purified GST-Sypt proteins of the expected molecular weights and a GST control. Upon precipitation of 3T3-L1 lysates with GST-Sypt fusion proteins, several tyrosine-phosphorylated protein bands were seen when the Western blot was probed with an antiphosphotyrosine antibody (Fig. 3). With IL-11 stimulation, tyrosine-phosphorylated protein bands of apparent molecular masses of 200 and 150 kDa associated with the N/C- and C-terminal SH2 constructs of GST-Sypt (Fig. 3, lanes 4 and 6). These protein bands were not seen or were seen only weakly with unstimulated cells, a GST control, the C-terminal SH2-GST-Sypt fusion protein (Fig. 2), and unrelated SH2 domains (data not shown). Other protein bands induced by IL-11 and associated with the C-terminal SH2 domain construct of GST-Sypt have molecular masses of approximately 70 and 130 kDa (Fig. 3, lane 4). Similar results were seen with equimolar GST-Sypt constructs or when 1% Triton-X 100 was used instead of 1% digitonin (data not shown).

Immunoprecipitations with Anti-gp130 and Anti-J AK2 Showed Coprecipitation of Syt—GST-Sypt precipitated proteins of molecular weights potentially corresponding to the IL-11 signal transduction proteins of gp130 and JAK2. To determine if these proteins interact with Syt, immunoprecipitations with anti-gp130 and anti-JAK2 were performed. Immunoprecipitations with anti-gp130 showed IL-11-induced tyrosine phosphorylation of gp130 (Fig. 4A). When this same immunoblot resulting from a gp130 precipitation was tested for the presence of Syt, gp130 was clearly seen with IL-11 induction (Fig. 4B). As a control for the gp130 concentration, the same blot was assayed with anti-gp130, which showed that equal amounts of immunoprecipitated gp130 were in each lane (Fig. 4C). The lack of tyrosine-phosphorylated Syt seen with gp130 in Fig. 4A is due to lower levels of Syt, which coprecipitated with gp130 as compared to a direct immunoprecipitation of Syt (Fig. 1).

To investigate JAK2 association with Syt, JAK2 immunoprecipitations were carried out. JAK2 immunoprecipitations coprecipitated Syt transiently in response to IL-11 with the highest level of association at 3 min (Fig. 5A), which corresponds to the peak level of Syt tyrosine phosphorylation (Fig. 1). JAK2 was shown to be present in equal amounts in each lane in the same experiment (Fig. 5B). The association between Syt and JAK2 appears to be constitutive in unstimulated cells but can be further induced by addition of IL-11.

The YXXV-containing Phosphopeptide Disrupted the IL-11-induced Binding of Syt to gp130 and JAK2—To further investigate protein interactions with Syt in response to IL-11, immunoprecipitations with anti-Syt were performed and analyzed by antiphosphotyrosine, anti-JAK2, anti-gp130, and anti-Sypt immunoblots (Fig. 6). An antiphosphotyrosine immunoblot showed the presence of tyrosine-phosphorylated proteins in response to IL-11 that may correspond to Syt, JAK2, and gp130 (Fig. 6A). The protein band that may represent...
gp130 (150 kDa) is less intense than the other protein bands. Proteins of approximately 90 and 200 kDa were also seen, but their identities were not determined. Protein bands of approximately 200, 150, and 130 kDa were also seen associated with GST-Syp (Fig. 3).

The Syp immunoprecipitation was assayed for the coprecipitation of JAK2 and showed that IL-11 induced the increased association of JAK2 to Syp (Fig. 6B). Further analysis of the Syp immunoprecipitation involved testing for the coprecipitation of gp130. gp130 was seen coprecipitating with Syp when the cells were induced with IL-11 (Fig. 6C). In addition, peptides corresponding to the putative Syp binding site (YSTV) on gp130 were used in a peptide competition experiment to study this association. Immunoprecipitation of Syp in the presence of the unphosphorylated YSTV-containing peptide reduced the association of Syp to gp130 (Fig. 6C, lane 3) but did not decrease the induced association of JAK2 to Syp significantly (Fig. 6B, lane 3). The phosphorylated YSTV-containing peptide blocked significant association of JAK2 and gp130 to Syp. A control assay with anti-Syp was done on the same membrane to test for the amount of Syp with each variable and demonstrated equal quantities of Syp immunoprecipitated (Fig. 6D). Peptide addition before or after lysis coupled with the continued peptide presence throughout the immunoprecipitation gave similar results (data not shown).

**DISCUSSION**

Our current understanding of IL-11 signal transduction is that after IL-11 joins with its cognate receptor, it then complexes with the signal transducer gp130. gp130 increases its association with JAK2, and JAK2 shows an increase in activity and tyrosine phosphorylation (7) along with an increase in the tyrosine phosphorylation of gp130 (3). IL-6, which has a similar mechanism to IL-11, causes dimerization of gp130 (5), and it has been shown with IL-6 that gp130 tyrosine phosphorylation occurs after JAK2 activation (28). JAK2 is a relatively large (130 kDa) tyrosine-phosphorylated cytoplasmic signaling protein involved in many signaling pathways (29). JAK2 is known to activate signal transducers and activators of transcription proteins (30) and associate with receptors (31, 32). In the present study, we have identified a component of the IL-11 pathway that may be equally pervasive. We have shown that the ubiquitously expressed phosphotyrosine phosphatase Syp associates with gp130 and JAK2 and that IL-11 stimulation...
increases these associations. The association of Syp with gp130 was reduced with the peptide YSTV and blocked with the YSTV-containing phosphopeptide. The YSTV peptide sequence was derived from gp130 and is similar to sequences known to bind Syp (11, 33). This finding suggests that Syp is involved in the interaction with the YSTV site on gp130. The unphosphorylated YSTV peptide, however, does not compete away JAK2 association with Syp as much, suggesting that the Syp-JAK2 association is unique and may not require the gp130-Syp association. The induced association of JAK2 and Syp has not been previously presented, although JAK2 has several potential Syp binding sites (26, 33, 34). The association of Syp in all pathways that activate JAK2 such as erythropoietin, growth hormone, prolactin, granulocyte-colony-stimulating factor, IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor will require further investigation (35).

Experiments with GST-Syp fusion proteins indicate specific IL-11-induced interactions between the C-terminal SH2 of Syp and tyrosine-phosphorylated proteins. There are reports of the C-terminal SH2 domain having a higher affinity to the PDGF receptor (36) and the N-terminal SH2 domain binding preferably to the erythropoietin receptor (37). The differences seen in the tyrosine-phosphorylated protein bands precipitated between the N/C- and C-terminal GST-SH2-Syp precipitations may indicate a role for the N-terminal GST-SH2 domain of Syp of increasing the specificity and affinity of binding of Syp toward associated proteins. This synergistic role for the two Syp SH2 domains has been reported recently in response to PDGF (36). Tyrosine-phosphorylated protein bands of the size of JAK2 (130 kDa) and gp130 (150 kDa) were seen precipitated with GST-Syp in addition to 90- and 200-kDa proteins of undetermined origin. The large protein may be the insulin receptor substrate-1 docking protein, which binds with Syp (21).

The time course of tyrosine phosphorylation for Syp differs from the results found for A31 mouse fibroblasts induced by PDGF (16). Tyrosine phosphorylation of Syp may correspond directly to activation (19) or protein targeting (38), and either may be the case here. Since activated Syp has not been found to be phosphorylated during insulin receptor substrate-1 activation (21), tyrosine phosphorylation may not be required for activation. Syp-gp130 or Syp-JAK2 interactions may lead to activation of Syp as does a portion of the PDGF receptor and Syp (39).

The IL-11-inducible binding of Syp to both gp130 and JAK2 was shown by reciprocal immunoprecipitations. It was shown previously that the 61 amino acids proximal to the transmembrane region on gp130 are required for the mitogenic signal through gp130 (9). The suggested Syp binding site on gp130 (15) is not included in this region (25) and therefore not required for the mitogenic signal. A possible function of Syp-gp130 binding is the localization of Syp to the membrane to allow easier association with JAK2 since IL-11 induces association of gp130 and JAK2 (38). The necessary site for gp130 and JAK2 association with each other is within the membrane proximal region of gp130 that is required for the mitogenic response (9). Further evidence of Syp being involved in the mitogenic response is a series of microinjection experiments by Xiao and others (41), which showed that the functional inhibition of Syp also inhibited the mitogenic signal. Another potential mitogenic pathway for Syp is as an adaptor protein for the Grb2-Sos-Ras pathway in response to PDGF (24). The induced tyrosine phosphorylation of Shc and other members of the Ras pathway has been shown previously in the gp130 signal transduction pathway (15).

Incubation of cells and lysates with the unphosphorylated YSTV peptide appeared to significantly reduce the IL-11-induced association of Syp with gp130 but not reduce the JAK2 association with Syp. Phosphorytose-independent peptide binding has been reported previously at a lower affinity than the same tyrosine-phosphorylated peptide at sites of SH2 domain interactions (42, 43). A similar interaction may be occurring here. The affinities of the activated gp130 and YSTV peptide may be similar enough for them to compete and reduce gp130-Syp association. The activated JAK2 affinity for Syp may be stronger and thus less sensitive to competition with the unphosphorylated YSTV peptide. An alternative explanation is that the YSTV peptide bound to gp130 and altered the gp130 configuration or blocked gp130 dimerization and thus reduced its association with Syp.

Binding to Syp is strongly enhanced in phosphopeptides containing the protein motif Y**X**V (33), of which JAK2 has two possible sites (Y**T**NSV and Y**1007**YKV) (26). A similar interaction may be occurring here. The affinities of the activated gp130 and YSTV peptide may be similar enough for them to compete and reduce gp130-Syp association. The activated JAK2 affinity for Syp may be stronger and thus less sensitive to competition with the unphosphorylated YSTV peptide. An alternative explanation is that the YSTV peptide bound to gp130 and altered the gp130 configuration or blocked gp130 dimerization and thus reduced its association with Syp.

In summary, the ubiquitous tyrosine phosphatase Syp associates with the signal transducer gp130 and cytoplasmic kinase JAK2. This association increases with IL-11 stimulation and is decreased by a tyrosine-phosphorylated peptide containing the sequence YSTV. A non-phosphopeptide, YSTV, affects gp130-Syp but does not affect JAK2-Syp-induced association. These results support a model in which Syp interacts with gp130 and JAK2 independently. Further experiments must be conducted to determine the nature of these interactions.

Addendum—While this work was in review, a paper supporting the importance of the gp130 YSTV sequence for Syp tyrosine phosphorylation was published by Stahl et al. (40). In this paper, a point mutation (Y to F, of YSTV) of an over-expressing gp130-neurotrophin-3 fusion protein blocked the induced antiphosphotyrosine immunoprecipitation of Syp.
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19. Kazlauskas, A., Feng, G.-S., Pawson, T., and Valius, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6939–6942
20. Vogel, W., Lammers, R., Huang, J., and Ullrich, A. (1993) Science 259, 1611–1614
21. Lechleider, R. J., Sugimoto, S., Bennett, A. M., Kashishian, A. S., Cooper, J. A., Shoelson, S. E., Walsh, C. T., and Neel, B. G. (1993) J. Biol. Chem. 268, 21478–21481
22. Kuhne, M. R., Pawson, T., Lienhard, G. E., and Feng, G.-S. (1993) J. Biol. Chem. 268, 11479–11481
23. Sun, X. J., Crimmons, D. L., Myers, M. G., Malpeix, M., and White, M. F. (1993) Mol. Cell. Biol. 13, 7418–7428
24. Li, W., Nishimura, R., Kashishian, A., Batzer, A., Kim, W., Cooper, J. A., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 509–517
25. Hibi, M., Murakami, M., Salto, M., Hirano, T., Taga, T., and Kishimoto, T. (1990) Cell 63, 1149–1157
26. Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Cleveland, J. L., Yi, T., and Ihle, J. N. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8429–8433
27. Dornhege, S., Auger, K., Chatterjee, S., Burke, T., and Shoelson, S. (1992) Biochemistry 31, 9865–9870
28. Wang, Y., and Fuller, G. (1994) Mol. Biol. Cell 5, 619–628
29. Darnell, J. E., Jr., Kerr, I. M., and Stark, G. R. (1994) Science 264, 1415–1421
30. Silvennoinen, O., Ihle, J. N., Schlessinger, J., and Levy, D. E. (1993) Nature 366, 583–585
31. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Ihle, J. N. (1993) Cell 74, 227–236
32. Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N., and Carter-Su, C. (1993) Cell 74, 237–244
33. Songyang, Z., Shoelson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Roberts, T., Ratnofsky, S., Lechleider, R. J., Neel, B. G., Birge, R. B., Fajardo, J. E., Chou, M. M., Hanafusa, H., Schaffhausen, B., and Cantley, L. C. (1993) Cell 72, 767–778
34. Harpur, A. G., Andres, A.-C., Ziemiecki, A., Aston, R. R., and Wilks, A. F. (1992) Oncogene 7, 1347–1353
35. Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B., and Silvennoinen, O. (1994) Trends Biochem. Sci. 19, 222–227
36. Feng G.-S., Shen, R., Heng, H. Q., Tsui, L.-C., Kazlauskas, A., and Pawson, T. (1994) Oncogene 9, 1345–1350
37. Tauchi, T., Feng, G.-S., Shen, R., Haatlin, M., Bagby, G. C., Kabat, D., Lu, L., and Bromeyer, H. E. (1995) J. Biol. Chem. 270, 5631–5635
38. Feng, G.-S., and Pawson, T. (1994) Trends Genet. 10, 54–58
39. Sugimoto, S., Lechleider, R., Shoelson, S., Neel, B., and Walsh, C. (1993) J. Biol. Chem. 268, 22771–22776
40. Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., and Yancopoulos, G. D. (1995) Science 267, 1349–1353
41. Xiao, S., Rose, D. W., Sasakta, T., Maegawa, H., Burke, T. R., Roller, P., Shoelson, S. E., and Olefsky, J. M. (1994) J. Biol. Chem. 269, 21244–21248
42. Malek, S. N., and Desiderio, S. (1994) J. Biol. Chem. 269, 33009–33020
43. Pendergast, A. M., Muller, A. J., Havlik, M. H., Maru, Y., and Whitte, O. N. (1991) Cell 66, 161–171
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