Insulin Induces Suppressor of Cytokine Signaling-3 Tyrosine Phosphorylation through Janus-activated Kinase*

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Suppressor of cytokine signaling (SOCS) proteins were originally described as cytokine-induced molecules involved in negative feedback loops. We have shown that SOCS-3 is also a component of the insulin signaling network (1). Indeed, insulin leads to SOCS-3 expression in 3T3-L1 adipocytes. Once produced, SOCS-3 binds to phosphorylated tyrosine 960 of the insulin receptor and inhibits insulin signaling. Now we show that in 3T3-L1 adipocytes and in transfected COS-7 cells insulin leads to SOCS-3 tyrosine phosphorylation. This phosphorylation takes place on Tyr204 and is dependent upon a functional SOCS-3 SH2 domain. Purified insulin receptor directly phosphorylates SOCS-3. However, in intact cells, a mutant of the insulin receptor, IRY960F, unable to bind SOCS-3, was as efficient as the wild type insulin receptor to phosphorylate SOCS-3. Importantly, IRY960F is as potent as the wild type insulin receptor to activate janus-activated kinase (Jak) 1 and Jak2. Furthermore, expression of a dominant negative form of Jak2 inhibits insulin-induced SOCS-3 tyrosine phosphorylation. As transfected Jak2 have been shown to cause SOCS-3 phosphorylation, we propose that insulin induces SOCS-3 phosphorylation through Jak activation. Our data indicate that SOCS-3 belongs to a class of tyrosine-phosphorylated insulin signaling molecules, the phosphorylation of which is not dependent upon a direct coupling with the insulin receptor but relies on the Jak2s.

Although insulin and cytokines have clearly distinct physiological functions, some of the molecules they utilize to generate their cellular responses are shared. For instance, the insulin receptor substrates (IRSs) were originally described as direct and proximal substrates of the insulin receptor and thought to be specific for the insulin receptor. Later on, it appeared that these docking proteins are also phosphorylated in response to several cytokines (2). Conversely, the Stats were characterized as transcription factors involved specifically in interferon signaling (3). Several years later, we and others observed that insulin was capable of inducing the phosphorylation and the activation of Stat5B (4, 5). Finally, the Jak2s are cytosolic tyrosine kinases activated by cytokines (6), but they were also found to be stimulated in response to insulin (7, 8).

Recently we proposed that SOCS-3 could be considered as another example of these shared molecules (1). The SOCS define a family of proteins with homologous structure, i.e., an N-terminal region of variable length, a central SH2 domain, and a C-terminal SOCS box (9–12). Their expression is induced by various cytokines in a tissue-specific manner. Once expressed, they participate in negative feedback loops, by inhibiting cytokine-mediated Jak/Stat activation. Generally speaking, this inhibition can be mediated by the following mechanisms: (i) a direct association and inhibition of the Jak and/or (ii) the association of the SOCS with a tyrosine-phosphorylated cytokine receptor, leading to a competitive inhibition with SH2 domain-containing molecules, such as the Stats (13, 14). The physiological importance of this family of proteins was demonstrated by production of transgenic mice. SOCS-1 deletion causes perinatal lethality linked to excessive responses to interferon γ (15–17). Mice lacking SOCS-2 grow significantly larger than their wild type littermates (18). SOCS-3 knockout results in embryonic lethality associated with marked erythrocytosis (19).

Originally the SOCS were found to be induced by ligands acting through receptors belonging to the cytokine receptor family, such as LIF, IL-2, IL-3, IL-6, interferon γ, GH, and leptin (13, 14). We have shown that insulin induces SOCS-3 expression in 3T3-L1 adipocytes (1). Moreover, insulin leads to translocation of SOCS-3 from the cytoplasm to the cell membrane, where it colocalizes with the insulin receptor. The interaction with the receptor happens through the SH2 domain of SOCS-3 and the phosphorylated tyrosine 960 of the insulin receptor. Because Stat5B binds to a domain containing this residue (4), SOCS-3 was found to inhibit insulin-induced Stat5B activation. These data suggested to us that SOCS-3 was not only a partner of cytokine signaling but that it also interferes with insulin signal generation. To further characterize the functional links between the insulin receptor and SOCS-3 we investigated whether insulin leads to SOCS-3 tyrosine phosphorylation.

We show that insulin induces SOCS-3 tyrosine phosphorylation in 3T3-L1 adipocytes and in transfected COS-7 cells. This phosphorylation takes place on tyrosine 204 of SOCS-3 and necessitates a functional SOCS-3 SH2 domain. Although in vitro the insulin receptor directly phosphorylates SOCS-3, we found that in intact cells, insulin-induced tyrosine phosphorylation is mediated by the Jaks. Therefore, SOCS-3 belongs to a...
novel class of distal receptor substrates that are tyrosine-phosphorylated in response to insulin, independently of a direct coupling with the insulin receptor but dependent on cytosolic kinases such as the Jakks.

**EXPERIMENTAL PROCEDURES**

**Materials**—SOCS-3 antibodies were produced by Eurogentech (Hertford, Belgium) using a peptide corresponding to the N-terminal sequence of SOCS-3 (SKPPAGMSRPLDTSLR). This sequence is specific for SOCS-3 and is not found in the other known SOCS (9–12). The antibodies were tested for their ability to recognize specifically SOCS-3 by immunoprecipitation and by Western blotting (data not shown). Antibodies to Jak1, Jak2, and insulin receptor were from Santa Cruz Biotechnology (Santa Cruz, CA).

**Cells, Culture Conditions, and Transfection**—3T3-L1 fibroblasts were obtained from ATCC (Rockville, MD). They were grown and differentiated into adipocytes as described previously (20). COS-7 fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. 3T3-L1 and COS-7 cells were obtained from ATCC (Rockville, MD). They were grown and differentiated into adipocytes as described previously (20). COS-7 fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. 3T3-L1 and COS-7 cells were obtained from ATCC (Rockville, MD). They were grown and differentiated into adipocytes as described previously (20). COS-7 fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. 3T3-L1 and COS-7 cells were obtained from ATCC (Rockville, MD). They were grown and differentiated into adipocytes as described previously (20). COS-7 fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum.

**Constructs and GST-SOCS-3 Production**—The human insulin receptor cDNA was obtained from A. Ullrich (Martinsried, Germany) and subcloned in pCDNA3. SOCS-3 cDNA, subcloned in pEF, have been described previously (22). The dominant negative Jak2Δ2 cloned in pCDNA3 was described elsewhere (23). Blunt-ended SOCS-3 cDNA was inserted in-frame with the GST cDNA present in pGex-3X (Amersham Pharmacia Biotech) blunted after an EcoRI digestion and filled in with the Klenow fragment. GST and GST-SOCS-3 were produced according to the manufacturer’s instructions (Amersham Pharmacia Biotech). Point mutations (SOCS-3 Y204F and SOCS-3 R71K) were produced using the Quickchange site-directed mutagenesis kit from Stratagene (La Jolla, CA) following the manufacturer’s instructions. All constructions were verified by sequencing.

**In Vitro Reconstitution Experiments**—Partially purified insulin receptors (24) were incubated with insulin (10^{-7} M) for 45 min at room temperature in (30 mM Hepes, pH 7.2, 30 mM NaCl, 0.1% (v/v) Triton X-100) and incubated with GST-SOCS-3 or GST alone adsorbed on Sepharose beads. The phosphorylation reaction was initiated by adding 15 mM [γ-32P]ATP, 8 mM MnCl₂, and 4 mM MgCl₂. After 30 min incubation, the phosphorylation reaction was stopped by freezing on dry ice and the phosphorylated proteins were analyzed by SDS-PAGE followed by autoradiography.

**Preparation of Cell Extracts**—After appropriate treatments cells were washed twice in 30 mM Hepes, pH 7.2, 30 mM NaCl, 0.1% (v/v) Triton X-100 and incubated with GST-SOCS-3 or GST alone adsorbed on Sepharose beads. The phosphorylation reaction was initiated by adding 15 mM [γ-32P]ATP, 8 mM MnCl₂, and 4 mM MgCl₂. After 30 min incubation, the phosphorylation reaction was stopped by freezing on dry ice and the phosphorylated proteins were analyzed by SDS-PAGE followed by autoradiography.

**RESULTS**

**Insulin Induces SOCS-3 Tyrosine Phosphorylation in COS-7 Cells**—As we have previously shown that SOCS-3 participates in the insulin signaling network (1), we investigated whether SOCS-3 was phosphorylated in response to the hormone. COS-7 cells were transiently transfected with plasmids encoding SOCS-3 and the insulin receptor. Cells were treated for 5 min with 10^{-10} to 10^{-6} M insulin or for 0 to 30 min with 10^{-7} M insulin. Cells were lysed, and SOCS-3 was immunoprecipitated and analyzed by Western blot using antibody to phosphorylso tyrosine. In parallel, cell lysates were analyzed for SOCS-3 expression and insulin receptor tyrosine phosphorylation (Fig. 1). As observed, insulin induces an increase in SOCS-3 tyrosine phosphorylation. This phosphorylation was detectable after a treatment with 10^{-9} M of insulin and reached a maximum at 10^{-7} M. Thus, it appears that SOCS-3 phosphorylation occurs at physiologically relevant insulin concentration and with kinetics compatible with insulin action. Phosphorylation was observed after 5 min of insulin treatment and maintained a plateau of phosphorylation for at least 150 min. In all conditions tested SOCS-3 tyrosine phosphorylation paralleled that of the insulin receptor.

**Insulin Receptor Phosphorylates SOCS-3 in Vitro**—We then tested whether purified insulin receptor phosphorylates SOCS-3. GST or GST-SOCS-3 were incubated with various amounts of purified insulin receptors in the presence of a phosphorylation mixture containing [γ-32P]ATP. After 30 min, phosphorylation of GST and GST-SOCS-3 was analyzed by SDS-PAGE followed by autoradiography (Fig. 2). In the absence of insulin receptor, GST was phosphorylated by contaminating bacterial kinases. This contaminating phosphorylation was unaffected by addition of increasing doses of insulin receptors, indicating that GST is not a substrate of the insulin receptor. When GST-SOCS-3 was incubated in the presence of increasing amounts of insulin receptor, its phosphorylation intensified, indicating that SOCS-3 can be phosphorylated by purified insulin receptors.

**IRY960F Can Induce SOCS-3 Tyrosine Phosphorylation**—Using yeast two-hybrid experiments we have previously shown that SOCS-3 interacts directly with the phosphotyrosine 960 of the insulin receptor (1). This view was supported by confocal microscopy illustrating that, upon insulin stimulation, SOCS-3 and insulin receptor colocalize at the cell membrane. This interaction is strictly dependent on the phosphorylation of the insulin receptor tyrosine 960 both in the yeast two-hybrid system and in intact cells. Therefore, we compared the ability of a wild type insulin receptor (IRWT) and of the IRY960F mutant (unable to bind to SOCS-3) to induce SOCS-3 tyrosine
phosphorylation. As a control, we compared the phosphorylation of IRS-1 in cells expressing insulin receptor or IRY960F.

COS-7 cells were transfected with IRWT or IRY960F and with SOCS-3 or with IRS-1. Cells were treated with insulin and lysed. SOCS-3 or IRS-1 were immunoprecipitated from the cell lysates and analyzed by anti-phosphotyrosine Western blot (Fig. 3). Controls for the expression of the various transfected proteins and for the tyrosine phosphorylation of the insulin receptors were performed. As previously observed, insulin induces SOCS-3 tyrosine phosphorylation in cells expressing IRWT. Unexpectedly, the insulin-induced SOCS-3 phosphorylation was unaltered in cells expressing IRY960F. In contrast, IRS-1 was less phosphorylated in cells expressing IRY960F compared with cells expressing IRWT, indicating that IRY960F behaves as anticipated for IRS-1 (25).

The remaining level of IRS-1 phosphorylation could be because of endogenous IGF-I receptor or because of the phosphorylation of IRS-1 by Jak (see “Discussion”). To summarize, although insulin receptor phosphorylates SOCS-3 in vitro, and although SOCS-3 binds to the insulin receptor through phosphotyrosine 960, SOCS-3 does not have to associate with the insulin receptor to be phosphorylated on tyrosine.

**IRY960F Induces Jak Phosphorylation**—The Jak kinases appeared to be likely candidates for this insulin-induced SOCS-3 tyrosine phosphorylation. Indeed, it has been shown that (i) SOCS-3 interacts directly with Jak1 and Jak2 through its SH2 domain (26, 27), (ii) these two cytosolic tyrosine kinases induce SOCS-3 tyrosine phosphorylation (27), and (iii) insulin activates Jak1 and Jak2 (7, 8, 23). However, the ability of IRY960F to stimulate the Jak is not established. To address this issue, we transfected COS-7 cells with IRWT or IRY960F. Cells were treated or not with insulin, and endogenous Jaks were immunoprecipitated and analyzed by antiphosphotyrosine Western blot (Fig. 4). As shown, insulin treatment of cells expressing IRWT and IRY960F induces tyrosine phosphorylation of Jak1 and Jak2 to comparable levels. Because it has been reported that the autophosphorylation of the Jaks in response to insulin parallel their tyrosine kinase activity toward substrates (23), our observations indicate that Jak1 and Jak2 are activated by insulin independently of the phosphorylation of insulin receptor Tyr960.

**Expression of a Dominant Negative Jak Inhibits Insulin-induced SOCS-3 Phosphorylation**—We then tested the effect of the expression of a dominant negative form of Jak2 (Jak2Δ8) on insulin-induced SOCS-3 tyrosine phosphorylation. This mutant Jak2Δ8 is a construct mutated within the type VIII phosphotransferase motif of the C-terminal protein-tyrosine kinase domain and has been described previously (28). COS-7 cells were transfected with plasmids encoding insulin receptor, SOCS-3, and various amounts of Jak2Δ8. Cells were treated with insulin, and SOCS-3 was immunoprecipitated and analyzed by antiphosphotyrosine Western blot (Fig. 5). Whole cell lysates were used to verify insulin receptor tyrosine phosphorylation and SOCS-3 expression. As shown, insulin-induced tyrosine phosphorylation of SOCS-3 was inhibited by increasing amounts of Jak2Δ8. Jak2Δ8 did not modify insulin induced insulin receptor tyrosine phosphorylation or SOCS-3 expression. Together these results suggest that insulin causes SOCS-3 tyrosine phosphorylation through Jak activation.

**Insulin-induced SOCS-3 Phosphorylation on Tyrosine 204 Depends on a Functional SOCS-3 SH2 Domain**—Next we explored in more detail the insulin-produced SOCS-3 tyrosine phosphorylation. As SOCS-3 binds to Jak through its SH2 domain, we studied the involvement of SOCS-3 SH2 domain in the insulin-induced SOCS-3 tyrosine phosphorylation. We also investigated which tyrosine of SOCS-3 is phosphorylated. To do so, we produced an SH2-defective mutant of SOCS-3 (R71K) in which Arg71, crucial for the binding of the phosphorylated tyrosine residue, has been replaced by a Lys. We also prepared several tyrosine to phenylalanine mutants of SOCS-3, but we will discuss only Y204F for reasons that will become obvious later on. COS-7 cells were transfected with insulin receptor and with wild type SOCS-3, SOCS-3 Y204F, or SOCS-3 (R71K).

Cells were treated or not with insulin, and SOCS-3 was immunoprecipitated and analyzed by antiphosphotyrosine Western blot (Fig. 6). In parallel, the quantity of SOCS-3 and the tyrosine phosphorylation of the insulin receptor were verified using specific antibodies. As previously observed, insulin induces SOCS-3 tyrosine phosphorylation. When SOCS-3 was mutated on Y204, its tyrosine phosphorylation was nearly undetectable. With the SOCS-3 (R71K) mutant no phosphorylation was detected at all. These data indicate that, in response to insulin, SOCS-3 is phosphorylated on Y204 in a manner dependent on a functional SOCS-3 SH2 domain.

**SOCS-3 Is Phosphorylated on Tyrosine in Response to Insulin in 3T3-L1 Adipocytes**—We used 3T3-L1 adipocytes to determine whether the hormone induces tyrosine phosphorylation of endogenous SOCS-3. As SOCS-3 is not expressed in these cells under basal conditions, we studied its expression after LIF treatment. Differentiated 3T3-L1 adipocytes were treated for 1
to 4 h with LIF. SOCS-3 was immunoprecipitated and analyzed by Western blot using antibodies to SOCS-3 and IRS-1 (left panel) or IRS-1 (right panel). Cells were treated or not with insulin (10^{-7} M, 5 min). Whole cell lysates (WCL) were analyzed by antiphosphotyrosine (α-pTyr) or anti-SOCS-3 (WB). Transfected: IR WT IR960F Insulin: - + - + - + - + IP: Jak-1 Jak-2 Jak-1 Jak-2

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next investigated whether insulin-produced SOCS-3 tyrosine phosphorylation was specific for SOCS-3 induced by LIF. 3T3-L1 adipocytes were treated for 2, 4, and 6 h with insulin alone or pretreated for 1 h with LIF or GH and then stimulated with insulin. SOCS-3 was immunoprecipitated and analyzed by antiphosphotyrosine or by anti-SOCS-3 Western blot (Fig. 7C). Treatment with LIF and GH induced comparable levels of SOCS-3 expression. Insulin leads to SOCS-3 expression after 2 and 4 h of treatment, but after 6 h the expression becomes nearly undetectable. LIF and GH alone produce a weak increase in SOCS-3 tyrosine phosphorylation. In both conditions this basal phosphorylation is further increased after insulin

Fig. 3. IRY960F induces SOCS-3 tyrosine phosphorylation. A, COS-7 cells were transiently transfected with plasmids encoding IRWT or IRY960F and with SOCS-3 (left panel) or IRS-1 (right panel). Cells were treated or not with insulin (10^{-7} M, 5 min). Whole cell lysates (WCL) were analyzed by antiphosphotyrosine (α-pTyr) or anti-SOCS-3 (WB). SOCS-3 or IRS-1 were immunoprecipitated (IP) and analyzed by antiphosphotyrosine Western blot. IR, insulin receptor.

Fig. 4. IRY960 increases Jak tyrosine phosphorylation. COS-7 cells were transiently transfected with plasmids encoding IRWT or IRY960F. Cells were stimulated for 5 min with insulin (10^{-7} M). Jak1 and Jak2 were immunoprecipitated (IP) and analyzed by antiphosphotyrosine (α-pTyr) Western blot (WB).

Fig. 5. A dominant negative Jak2 inhibits insulin-induced SOCS-3 tyrosine phosphorylation. COS-7 cells were transiently transfected with plasmids encoding the insulin receptor (IR, 1 μg), SOCS-3 (1 μg), and Jak2Δ8 (0 to 4 μg) in 100-mm dishes. Cells were treated or not with insulin (10^{-7} M) for 5 min. SOCS-3 was immunoprecipitated (IP) and analyzed by antiphosphotyrosine (α-pTyr) Western blot (WB). Whole cell lysates (WCL) were analyzed by antiphosphotyrosine or anti-SOCS-3 (α-SOCS-3) Western blot.
treatment. Exposure of cells to insulin alone leads also to SOCS-3 tyrosine phosphorylation. This phosphorylation reached a maximum within 2 h of treatment and decreased after 4 h, although SOCS-3 expression was maintained at this time. We conclude that in 3T3-L1 adipocytes endogenous SOCS-3 is tyrosine-phosphorylated in response to insulin.

Discussion

We have previously presented evidence for the idea that SOCS-3 is a component of the insulin signaling network (1). Indeed, insulin induces SOCS-3 mRNA expression in 3T3-L1 adipocytes. In addition, insulin leads to the translocation of SOCS-3 from the cytoplasm to the plasma membrane where it colocalizes with the insulin receptor. This interaction with the receptor could be a mean for desensitization of insulin signaling, because we have shown that SOCS-3 inhibits insulin-dependent Stat5B activation. Here we show that insulin leads to SOCS-3 tyrosine phosphorylation in transfected COS-7 cells and in 3T3-L1 adipocytes after induction of endogenous SOCS-3. Interestingly, it would appear that in intact cells SOCS-3 does not have to be coupled directly to the insulin receptor to mediate insulin signaling.
receptor to be phosphorylated on tyrosine. This is in apparent contradiction with our observation that purified insulin receptor phosphorylates SOCS-3 and that SOCS-3 binds to the insulin receptor phosphorytosrine 960. Indeed, using the yeast two-hybrid system, we have shown that SOCS-3 binds through its SH2 domain to the phosphorytosrine 960 of the insulin receptor (1). Further, in cells expressing a mutant of the receptor (IRY960F), which does not bind SOCS-3, insulin does not modify the cytoplasmic localization of SOCS-3. However, insulin-induced SOCS-3 tyrosine phosphorylation in cells expressing IRY960F is comparable with that seen in cells expressing wild type insulin receptor (Fig. 3). This observation is intriguing, because most recognized substrates of the insulin receptor, such as the IRS, Shc, and Gab, have to be coupled to the insulin receptor to be phosphorylated on tyrosine (29, 30). This interaction can be direct, through the phosphotyrosine binding domain of the IRS and Shc to tyrosine 960 of the insulin receptor or through the IRS-2 kinase regulatory binding domain to the so-called regulatory loop domain of insulin receptor (31). The coupling can also be indirect through the plekstrin homology domain of IRS and Gab-1 (32, 33). These plekstrin homology domains may bind acidic peptide motifs in membrane proteins or phospholipids that link substrates to activated cell surface insulin receptors (34). Differently from these proximal substrates, SOCS-3 does not require to be localized to the cell membrane and coupled with the insulin receptor to be phosphorylated on tyrosine in response to insulin. Therefore, it is likely that in intact cells, the insulin receptor does not directly phosphorylate SOCS-3 but utilizes an intermediary tyrosine kinase for this to be achieved. Several experimental observations indicate that the Jaks are the kinases involved. First, it has already been reported that the Jaks are activated in response to insulin. This was observed originally in cells overexpressing insulin receptors (7) and later confirmed in intact rats where injection of insulin stimulates Jak2 in liver, muscle, and adipose tissue (8). This activation is probably linked to a coupling of the Jak to the insulin receptor. Indeed, using GST fusion proteins and communoprecipitation experiments, our laboratory showed that the Jaks interact directly with the insulin receptor (23). This interaction involves two domains of Jak1, one located in its N terminus and the other located in its C terminus, and necessitates a phosphorylated insulin receptor. Second, it has also been already observed that ectopic expression of Jak1 or Jak2 induces SOCS-3 tyrosine phosphorylation (27). This can be correlated with the direct association between the Jak and SOCS-3 (26, 27). Interestingly, we show that a mutant of SOCS-3 defective in its SH2 domain is not phosphorylated in response to insulin, probably because (as previously reported) it cannot bind to Jak (26). Third, both wild type insulin receptor and IRY960F cause a comparable phosphorylation of Jak1 and Jak2, which is in agreement with the ability of these two receptors to induce SOCS-3 tyrosine phosphorylation. Fourth, the use of a dominant negative Jak2 mutant showed that the Jaks are necessary to mediate insulin-induced tyrosine phosphorylation of SOCS-3 (Fig. 5). Such kinase-dead Jak2 mutants have previously been reported to inhibit the effect of both Jak1 and Jak2 on Stat3 activation by IGF-I, for example (35). Therefore, we would like to suggest that insulin stimulates the Jaks in a manner independent of the phosphorylation of insulin receptor tyrosine 960. Once activated, some of the Jak molecules would translocate to the cytoplasm, capture the SOCS-3 through binding to their SH2 domain, and then phosphorylate the SOCS-3.

Schematically speaking, it would appear that SOCS-3 can interfere at different levels in insulin signaling. Indeed, we found earlier that SOCS-3 inhibits insulin-induced Stat5 activation probably by competing for the same phosphotyrosine 960 on the receptor (1). Here we show that insulin leads to SOCS-3 tyrosine phosphorylation. It is likely that this phosphorylation has an impact on insulin signaling. We did not detect a striking difference in the subcellular localization of SOCS-3 and SOCS-3 Y204F, before or after insulin treatment, and both molecules inhibit insulin-induced Stat5B activation in a similar manner (data not shown). However, SOCS-3 Tyr204 is located in close vicinity to the consensus elonginBC (the complex of elongin B and elongin C) binding site (36, 37). Because the function of the association between SOCS-3 and elonginBC (the complex of elongin B and elongin C) is still a matter of debate, it is difficult to predict the effect of SOCS-3 tyrosine phosphorylation on this event (36, 37). It can also be envisioned that SOCS-3 tyrosine phosphorylation may create binding sites for SH2-containing molecules. SOCS-3 would be then able to create a bridge between the insulin receptor and a SH2-containing molecules. This would be reminiscent of Shp-2, which binds to the platelet-derived growth factor receptor through its SH2 domain and to Grb2 through its phosphorylated tyrosine (38). This docking function of SOCS-3 could be occurring when SOCS-3 is in the cytosol. After its translocation to the cell surface SOCS-3 could function as a cargo bringing proteins in vicinity to the insulin receptor. SOCS-3, located at the cell surface, could modify the functioning of the insulin receptor by docking proteins.

Our observations add weight to the role of the Jaks in insulin signaling. Indeed, although the Jaks have been shown to induce IRS-1 and IRS-2 tyrosine phosphorylation, their relative contribution to this process, compared with the direct phosphorylation of IRS by the insulin receptor, is thought to be small. This view comes from the finding that mutation of the insulin receptor on tyrosine 960 leads to a decrease in insulin-induced IRS-1 tyrosine phosphorylation (25). However, it can be noted that as shown in Fig. 3 (and as observed by other authors) some insulin-induced IRS-1 phosphorylation is still detectable in cells expressing IRY960F (39, 40). This remaining phosphorylation could be because of the Jaks. Because we have shown earlier that the phosphopeptide maps of IRS-1 phosphorylated in vitro by insulin receptor versus Jak1 are different (23), it is tempting to think that the docking function of IRS-1 will also differ and consequently so will its signaling potential.

In summary, to the best of our knowledge, SOCS-3 appears as the first example of a protein tyrosine phosphorylated in intact cells in response to insulin in a fashion that is entirelyJak-dependent. However, it is possible that other proteins share this property. Several substrates of the insulin receptor (such as Tub; see Ref. 41) have been proposed to be direct substrates of the insulin receptor based on their phosphorylation by purified insulin receptors. Considering our present work the possible implication of the Jaks should be evaluated. Finally, it is tempting to suggest that insulin uses the Jak activity not only to modulate its action through SOCS-3 but also to generate specific biological responses.

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