Recruitment of the Protein-tyrosine Phosphatase SHP-2 to the C-terminal Tyrosine of the Prolactin Receptor and to the Adaptor Protein Gab2

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The protein-tyrosine phosphatase SHP-2 modulates signaling events through receptor tyrosine kinases and cytokine receptors including the receptor for prolactin (PRLR). Here we investigated mechanisms of SHP-2 recruitment within the PRLR signaling complex. Using SHP-2 and PRLR immunoprecipitation studies in 293 cells and in the mouse mammary epithelial cell line HC11, we found that SHP-2 co-immunoprecipitates with the PRLR and that the C-terminal tyrosine of the PRLR plays a regulatory role in both the tyrosine phosphorylation and the recruitment of SHP-2. Our results further indicate that SHP-2 association to the PRLR occurs via the C-terminal SH2 domain of the phosphatase. In addition, we determined that the newly identified adaptor protein Gab2, but not Gab1, is specifically tyrosine phosphorylated and is able to recruit SHP-2 and phosphatidylinositol 3-kinase in response to PRLR activation. Together, these studies suggest the presence of dual recruitment sites for SHP-2; the first is to the C-terminal tyrosine of the PRLR and the second is to the adaptor protein Gab2.

The prolactin receptor (PRLR) is a member of the large cytokine receptors superfamily. Ligand stimulation induces receptor dimerization, which leads to the activation of the constitutively associated Jak2 kinase as well as members of the Src family of cytoplasmic tyrosine kinases (1, 2). Activated Jak2 molecules phosphorylate themselves as well as receptor subunits on tyrosine residues creating docking sites for SH2 domain containing proteins belonging to various signaling pathways. Activated PRLR can induce the activation of the Stat5 (signal transducer and activator of transcription 5), mitogen-activated protein kinase, and PI3-K pathways and the activation of several signaling regulatory proteins including the SHP-2 (the SH2 domain containing protein-tyrosine phosphatase-2).

SHP-2 is a ubiquitously expressed protein that is characterized by having two N-terminally located SH2 domains and a C-terminal catalytic domain. The phosphatase plays a critical role in cell growth and differentiation as evidenced by the early embryonic lethality in SHP-2null null mice (3). SHP-2 plays an essential regulatory role in signaling from certain receptor tyrosine kinases like the epidermal growth factor receptor and insulin receptor (4) as well as cytokine receptors such as the αβ interferon receptors (5, 6) and the PRLR (7). We have previously shown that SHP-2 is tyrosine phosphorylated in response to PRL stimulation and physically associates with the PRLR-Jak2 complex. We also determined that SHP-2 is a positive mediator of PRLR signaling leading to the activation of β-casein gene promoter (7). However, the mechanism and site(s) of SHP-2 recruitment within the PRLR signaling complex remain unknown.

SHP-2 phosphatase can be directly recruited via its two SH2 domains to membrane receptors, cell surface proteins, and a number of intracellular signaling proteins. Specific tyrosine residues on the β chain of the granulocyte macrophage colony-stimulating factor receptor (8), the platelet-derived growth factor receptor (9), the gp130 subunit of the interleukin-6 receptor (IL-6R) (10), and the C-terminal portion of the growth hormone receptor (11) are sites for SHP-2 recruitment. Point mutations of these residues eliminated SHP-2 association and tyrosine phosphorylation. Furthermore, through the SH2 domains, SHP-2 interacts with a number of cellular proteins such as signal regulatory protein (12), SH2-containing inositol phosphatase (13), insulin receptor substrates 1 and 2 (14), and Jak2 (15) and, importantly, members of the Gab (Grb2 associate binder) family.

The Gab family of proteins, p110 Gab1 (16), p97 Gab2 (17), and the Drosophila DOS protein (18), contain a number of structurally conserved regions. These proteins contain an N-terminal pleckstrin homology domain, proline-rich motifs, and multiple similarly situated tyrosine phosphorylation residues. In addition, these proteins have unique tissue distributions (17), suggesting that they serve nonredundant functions. These proteins appear to function primarily as adaptor proteins linking activated receptor tyrosine kinase and cytokine receptors to several signaling molecules. Epidermal growth factor, insulin (19), hepatocyte growth factor (20), IL-3, IL-6, interferon α/β (21), and erythropoietin (22, 23) have been reported to induce the tyrosine phosphorylation of Gab1 and its association with the SH2 domain containing protein SHP-2 (22–25), the p85 subunit of PI3-K (26, 27), SH2-containing inositol phosphatase (23), Shc (23), and Grb2 (16, 21). Other studies have shown that IL-2, colony stimulating factor-1 (17), IL-3, IL-6, and the activation of the T-cell receptor and B-cell receptor (28) can induce tyrosine phosphorylation of Gab2 and its association with

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**RESULTS**

**The C-terminal Tyrosine of the PRLR Regulates the State of SHP-2 Tyrosine Phosphorylation**—We have previously reported that the C-terminal tyrosine of the PRLR is critical for the induction of the prolactin responsive β-casein gene (29) and the activation of Stat5 (32). In addition, a positive role for SHP-2 in the activation of Stat5 and the induction of early genes has also been reported (7, 33). Therefore, we hypothesized a regulatory role for the C-terminal tyrosine of the PRLR in the activation of SHP-2. To test this hypothesis, we used various natural, mutant, and deletion forms of the PRLR for the purpose of focusing on the C-terminal tyrosine of the PRLR. The long form of the PRLR has nine intracellular tyrosine residues. The PRLR Nb2 form, found in Nb2 T-lymphoma cells, has an in-frame deletion mutation that removes six of the nine intracellular tyrosine residues found in the long form leaving tyrosine residues 237, 309, and 382 (34). The C-terminal tyrosine 382 of the PRLR Nb2 form corresponds to tyrosine 580 of the PRLR long form. Another deletion form that is capable of inducing β-casein gene expression is the PRLR Nb2 mutant form Δ296–322 (29). This receptor form has a 27-amino acid internal deletion and lacks tyrosine 309. Tyrosine to phenylalanine point mutants of the membrane proximal (LY237F, NY237F, and ΔY237F) and the C-terminal tyrosines (LY580F, NY382F, and ΔY382F) in the long form, Nb2 form, and the Δ296–322 mutant form were described previously (29, 32).

In addition, we determined that Gab2 but not Gab1 is tyrosine phosphorylated following PRL stimulation of mammary cells and that Gab2 presents a second site for SHP-2 recruitment to the PRLR signaling complex.

**EXPERIMENTAL PROCEDURES**

**Materials, Antibodies, and Plasmid Constructs**—Expression plasmids encoding the PRLR mutants LY237F, LY382F, NY237F, NY382F, Δ296–322, ΔY237F, ΔY382F, and Δ243–268 were described previously (29). Expression plasmids encoding SHP-2, the PRLR R322K and SHP-2 R135K, and the polyclonal antibody for p110 subunit of the PI3-K were obtained from Axel Ullrich (Max-Plank Institute, Germany). Monoclonal antibody to phosphotyrosine (4G10) was from Upstate Biotechnology, monoclonal antibody to SHP-2 was form Transduction Laboratories, and polyclonal antibody to SHP-2 was from Santa Cruz. Polyclonal Gab1 antibodies and HA-tagged Gab1 expression plasmid were kindly provided by Toshio Hirano (Osaka University, Osaka, Japan). Polyclonal Gab2 antibodies and HA-tagged Gab2 and Gab2DM expression plasmids were generously supplied by Benjamin Neel and Haihua Gu (Harvard Medical School, Boston, MA). The U6 monoclonal antibody to the PRLR was provided by Paul Kelly (Paris, France).

Hemagglutinin antibodies were from Santa Cruz. Protein A-Sepharose beads used for immunoprecipitations were from Amersham Pharmacia Biotechnology, monoclonal antibody to SHP-2 was form Transduction Laboratories, and polyclonal antibody to SHP-2 was from Santa Cruz. Polyclonal Gab1 antibodies and HA-tagged Gab1 expression plasmid were obtained from Nancy Hynes (Friedrich Miescher Institute, Basel, Switzerland) and Bernd Groner (Georg Speyer Haus, Frankfurt, Germany) who were grown to confluency in RPMI 1640 medium containing 10% fetal calf serum (Life Technologies, Inc.), insulin (5 μg/ml), and epidermal growth factor (10 ng/ml). Cells were then induced by incubating them for 3 days in RPMI medium containing 10% fetal calf serum, insulin (5 μg/ml), and hydrocortisone (1 μM) (30, 31). Depending on the specific experiment (see “Results”), cells were starved either in RPMI medium containing insulin (5 μg/ml) and hydrocortisone (1 μM) or in RPMI medium containing insulin and hydrocortisone. Cells were stimulated with ovine PRL (1.5 μg/ml) for 5 min at 4 °C. The lysates were then centrifuged at 12,000 × g for 10 min at 4 °C to remove insoluble material. Protein concentration was measured using the Bradford technique.

**Transient Transfection**—The human embryonic 293 cells were grown in Dulbecco’s modified Eagle’s medium (4.5 g/liter glucose) (Bio Media) containing 10% (v/v) fetal calf serum. Approximately 5 × 105 cells were plated then co-transfected with expression plasmids encoding the different forms of PRLR (1 μg each), SHP-2 (1 μg), and the kinase Jak2 (0.25 μg) by the calcium phosphate technique. After 24 h of expression, the cells were starved by serum deprivation overnight.

**Total Cell Lysis Immunoprecipitations and Western Blotting**—Transiently co-transfected 293 cells were stimulated with ovine PRL (1.5 μg/ml) for 5 min and then lysed in lysis buffer (10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 10% (v/v) glycerol, 0.5% Triton X-100) containing protease inhibitors (1 mM phenylmethylsulphonyl fluoride, 2 μg/ml leupeptin, 5 μg/ml aprotinin) for 5 min at 4 °C. The lysates were then centrifuged at 12,000 × g for 10 min at 4 °C to remove insoluble material. Protein concentration was measured using the Bradford technique. Equal amounts of protein obtained by total lysis were loaded and run on an 8% SDS-PAGE. Immunoprecipitations were performed on endogenous or overexpressed cell lysates. Extracts were immunoprecipitated for 2 h (or overnight for endogenous proteins) using specific antibodies (SHP-2, PRLR, HA, Gab1, and Gab2) and protein A-Sepharose beads. Precipitates were then washed with HNTG buffer (20 mM HEPEs, pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol) and separated on SDS-PAGE. Western blotting analysis was performed using the indicated antibodies. Proteins were revealed using chemiluminescence (Super Signal kit from Pierce) following the manufacturer’s instructions.
samples expressing the C-terminal mutant of the Nb2 form of the PRLR receptor NY382F (Fig. 1B). Similarly, tyrosine phosphorylation levels of SHP-2 were comparable in samples expressing the Δ296–322 form as well as its corresponding tyrosine to phenylalanine mutants ΔY237F (Fig. 1B). In agreement with the results obtained for the PRLR Nb2 form, SHP-2 tyrosine phosphorylation was lost in the sample expressing the C-terminal mutant of the Δ296–322 form, ΔY382F (Fig. 1B). The loss of SHP-2 tyrosine phosphorylation in samples expressing the C-terminal tyrosine to phenylalanine mutants of the PRLR suggests that this tyrosine plays a regulatory role in SHP-2 tyrosine phosphorylation. The sustenance of SHP-2 phosphorylation in the samples expressing the C-terminal tyrosine mutation in the long form of the PRLR suggests that in addition to the C-terminal tyrosine, one or more of the cytoplasmic tyrosine residues that are present in the long form of the PRLR but absent form the Nb2 form of the PRLR may play a role in SHP-2 phosphorylation. The membranes were stripped and reprobed with a monoclonal antibody to SHP-2 in a Western blot analysis to confirm equal expression of the phosphatase (Fig. 1, A and 1B, lower panels).

The C-terminal Tyrosine of the PRLR Is a Site for SHP-2 Recruitment—the loss of SHP-2 tyrosine phosphorylation in samples expressing the C-terminal tyrosine to phenylalanine mutant of the PRLR suggests that this tyrosine may act as a recruitment site for SHP-2. To determine whether SHP-2 associates to the PRLR, we initially examined this association in the PRL-responsive mammary epithelial cell line HC11. The PRLR was immunoprecipitated using the U6 monoclonal antibody against the PRLR. Immunoprecipitates were run on SDS-PAGE, transferred to nitrocellulose membranes, and blotted with monoclonal antibodies against the PRL receptor from the mouse mammary epithelial cell line HC11 that were induced and starved (see “Experimental Procedures”). Immunoprecipitates were run on SDS-PAGE, transferred to nitrocellulose membranes, and blotted with monoclonal antibody against SHP-2 in a Western blot analysis. PRL stimulation of HC11 cells induces a specific association between SHP-2 and the PRLR (Fig. 2A).

To confirm the association of SHP-2 to the PRLR and to determine whether the C-terminal tyrosine of the PRLR is a site for SHP-2 recruitment, the receptor was immunoprecipitated from 293 cells transiently transfected with expression plasmids encoding SHP-2, the Nb2 form of the PRLR, NY237F, NY382F, Δ296–322, ΔY237F, and ΔY382F and a limited amount of an expression vector encoding Jak2 kinase. Immunoprecipitates were run on SDS-PAGE, transferred to nitrocellulose membrane, and blotted with a monoclonal antibody against SHP-2 in Western blotting. No SHP-2 was detectable in samples expressing the control Δ243–268 deletion form of the PRLR (Fig. 2B). A clear SHP-2 co-immunoprecipitation with the PRLR was detectable in samples expressing the wild type Nb2 form, NY237F, Δ296–322, and ΔY237F (Fig. 2B). However, no co-immunoprecipitating SHP-2 was detectable with the PRLR complexes in the samples expressing the C-terminal tyrosine to phenylalanine mutants NY382F and ΔY382F (Fig. 2B). Altogether, these results indicate that the C-terminal tyrosine of the PRLR is a site for SHP-2 association.

No detectable decrease in the association between SHP-2 and the PRLR was observed in samples expressing the C-terminal tyrosine to phenylalanine mutant of the long form of the PRLR Δ1580F (data not shown). The sustenance of SHP-2 tyrosine phosphorylation and association to the PRLR with the C-terminal mutant of the long form of the PRLR suggests that there is redundancy in SHP-2 association to the PRLR long form where one or more of the tyrosine residues that are only present in the long form of the PRLR can provide additional association sites for SHP-2.

SHP-2 Utilizes the C-terminal SH2 Domain for Association to the PRLR—The protein-tyrosine phosphatase SHP-2 has two N-terminally located SH2 domains. To determine the mechanism of association between SHP-2 and the PRLR, the ability of the two SH2 domains to bind to phosphotyrosine motifs was interrupted by point mutations that substitute the critical arginine in the SH2 domain to lysine. 293 cells were co-transfected with expression plasmids for either the Δ243–268 deletion form of the PRLR along with SHP-2 wild type or the Nb2 form of the PRLR with SHP-2 wild type, the arginine to lysine mutant of the N-terminal SH2 domain, SHP-2 R32K mutant, or the C-terminal SH2 domain SHP-2 R138K mutant (7). In
SHP-2 Associates to the PRLR and to Gab2

Fig. 3. SHP-2 utilizes the C-terminal SH2 domain for association to the PRLR. A, 293 cells were transfected with expression plasmids encoding either the Δ243–268 mutant form along with wild type SHP-2 or the N2b form along with either the wild type SHP-2, the N-terminal, or the C-terminal SH2 domains arginine to lysine point mutants R32K and R138K, respectively. Total cellular lysates were run on SDS-PAGE, transferred to nitrocellulose membranes, and blotted with monoclonal antibodies against SHP-2 and PI3-K in Response to PRL Stimulation in HC11 Cells—

addition, cells were co-transfected with an expression plasmid for the kinase Jak2. Cellular lysates were prepared for immunoprecipitation of the PRLR using the U6 monoclonal antibody. The immune complexes were separated on SDS-PAGE, transferred to a nitrocellulose membrane, and then blotted with antibodies to phosphotyrosine. As shown in Fig. 4, SHP-2 was tyrosine phosphorylated in the basal condition in HC11 cells. However, there is a clear increase in its phosphorylation state following PRL stimulation. Furthermore, these data indicate that SHP-2 interacts with multiple tyrosine phosphorylated proteins in mammary epithelial cells.

Gab2 Is Specifically Tyrosine Phosphorylated and Recruits SHP-2 and PI3-K in Response to PRL Stimulation in HC11 Cells—Recently a family of proteins that have molecular sizes in the range of 95–110 kDa, co-precipitating with SHP-2. Two of these proteins having molecular masses of 180 and 110 kDa are constitutively tyrosine phosphorylated. However, a protein of 95 kDa undergoes tyrosine phosphorylation in response to PRL stimulation. Together, these data indicate that SHP-2 interacts with multiple tyrosine phosphorylated proteins in mammary epithelial cells.

95–110-kDa Tyrosine Phosphorylated Proteins Associate to SHP-2 in HC11 Cells—The protein-tyrosine phosphatase SHP-2 is known to associate with multiple tyrosine phosphorylated proteins. To examine the different possible associations of SHP-2 with tyrosine phosphorylated proteins in response to PRLR activation, we performed SHP-2 immunoprecipitations in the mouse mammary epithelial cell line HC11. These cells were grown to confluency, induced to differentiate, and starved for 48 h. Then the cells were either unstimulated or stimulated with PRL for 5, 10, and 20 min. Cellular lysates were prepared for immunoprecipitation with monoclonal antibody to SHP-2. The complexes were separated on SDS-PAGE, transferred to a nitrocellulose membrane, and then Western blotted with a monoclonal antibody to phosphotyrosine. The membrane was stripped and reprobed with monoclonal antibodies against SHP-2. Control sample contains antibody and beads but no lysates. IP, immunoprecipitation; Wb, Western blot.
SHP-2 Associates to the PRLR and to Gab2

Gab family members were also reported to associate with the p85 subunit of the PI3-K. To determine whether PRL stimulation can induce the association of PI3-K to Gab members, the membranes used were stripped and reprobed with polyclonal antibodies to the p110 subunit of the PI3-K. Gab1 associated to p110 irrespective of PRL stimulation (Fig. 5A), in contrast, Gab2 showed selective recruitment of p110 following PRLR activation (Fig. 5B). Altogether, our results indicate that Gab2, but not Gab1, is a specific target for PRLR activation and that the adaptor protein is capable of selectively recruiting SHP-2 and PI3-K in response to PRL stimulation.

The Three Natural Forms of the PRLR, Long, Intermediate Nb2m, and Short Forms, Can Induce Gab2 Tyrosine Phosphorylation and Association to SHP-2 in 293 Cells—The rat PRLR has three forms: the long, the intermediate Nb2, and the short form. Only the long and Nb2 forms are tyrosine phosphorylated following PRL stimulation, and in biological assays testing β-casein gene promoter induction, only the long and Nb2 forms were able to activate expression of the PRLR responsive milk protein (34, 36). To determine whether stimulation of these three forms leads to tyrosine phosphorylation of Gab2 and its association to SHP-2, 293 cells were transfected with plasmids expressing HA-tagged Gab2 along with SHP-2 and the long, Nb2, or the short forms of the PRLR. In addition, the deletion mutant form of the PRLR, 2A43–268, was used as a control. The cells were also transfected with a limited amount of an expression plasmid for the kinase Jak2. Cellular lysates were separated into two parts. The first part was used for immunoprecipitation with polyclonal antibodies to the HA tag. Immunoprecipitates were run on SDS-PAGE, transferred to nitrocellulose membranes, and probed with monoclonal antibodies to phosphotyrosine. The membranes were stripped and then reprobed with monoclonal antibodies against SHP-2, p110 subunit of the PI3-K, and with polyclonal antibodies to Gab1 or Gab2. IP, immunoprecipitation; Wb, Western blot.

A

Fig. 5. Gab2 is specifically tyrosine phosphorylated and recruits SHP-2 and PI3-K in response to PRL stimulation of HC11 cells. HC11 cells were either unstimulated or stimulated with PRL for 5 or 20 min. Cells were lysed, and lysates were immunoprecipitated with polyclonal antibodies to either Gab1 or Gab2. Sample (Ctrl) contains antibodies and beads, but no lysate was used as a control. Immune complexes were run on SDS-PAGE, transferred to nitrocellulose membranes, and blotted with monoclonal antibodies to phosphotyrosine. The membranes were stripped and then reprobed with monoclonal antibodies against SHP-2, p110 subunit of the PI3-K, and with polyclonal antibodies to Gab1 or Gab2. IP, immunoprecipitation; Wb, Western blot.

B

To determine whether SHP-2 recruitment to Gab1 or Gab2 might be regulated by PRLR activation in HC11 cells, the membranes were stripped and reprobed with monoclonal antibodies to SHP-2. Similar to the tyrosine phosphorylation pattern of Gab1, SHP-2 recruitment to Gab1 was constitutive and was not affected by PRL stimulation (Fig. 5A). On the other hand, SHP-2 recruitment to Gab2 showed clear responsiveness to PRLR activation. Stimulation of HC11 cells for 5 or 20 min led to a significant increase in SHP-2-Gab2 complex formation (Fig. 5B). Therefore, SHP-2 association to Gab2 is regulated by PRL stimulation in the mammary epithelial cell line HC11.

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SHP-2 Association to Gab2 Requires Tyrosines 604 and 633 on the C-terminal Part of Gab2—SHP-2 binding to Gab2 in the IL-3 receptor signaling system requires tyrosines 604 and 633 located in the C-terminal portion of Gab2 (17). We intended to determine the mechanism of association between SHP-2 and Gab2 in the PRLR signaling system. 293 cells were co-trans-
SHP-2 Associates to the PRLR and to Gab2

DISCUSSION

The protein-tyrosine phosphatase SHP-2 plays a critical role in signaling downstream from several receptor tyrosine kinases and cytokine receptors including the PRLR (7). We have reported earlier that tyrosine phosphorylated SHP-2 was found in a complex containing the PRLR and Jak2 (7). In this study we investigated the mechanisms of SHP-2 recruitment and activation within the PRLR signaling complex. We identified SHP-2 in PRLR immunoprecipitates from PRL-stimulated mouse mammary epithelial HC11 cells, suggesting that the PRLR is able to recruit SHP-2 leading to its tyrosine phosphorylation, an event that is known to regulate the activation of the phosphatase. In addition, we determined that the C-terminal tyrosine of the PRLR has a regulatory role in the recruitment and tyrosine phosphorylation of SHP-2. SHP-2 tyrosine phosphorylation as well as association to the PRLR was lost in samples expressing the C-terminal tyrosine mutant of the Nb2 form of the PRLR. However, we did not observe a loss of SHP-2 association to the PRLR with the C-terminal tyrosine mutant of the long form of the PRLR, suggesting that possibly one or more of the other tyrosine residues present in the long form of the PRLR but absent from the Nb2 form may act as sites for the association between SHP-2 and Gab2. The tyrosine phosphorylation level of SHP-2 was not significantly enhanced in the presence of Gab2 overexpression. The association between SHP-2 and Gab2 was eliminated in samples expressing the Gab2DM mutant, suggesting that the association between SHP-2 and Gab2 is mediated through either one or two of the tyrosines in the C-terminal portion of Gab2. The tyrosine phosphorylation level of SHP-2 was not affected by the lack of association between SHP-2 and Gab2, suggesting that SHP-2 phosphorylation can take place through the interaction of SHP-2 with the C-terminal tyrosine of the PRLR (7). The membranes were stripped and then reprobed with a monoclonal antibody to SHP-2 and then with monoclonal antibody to the HA tag (Fig. 7, middle and lower panels, respectively).
C-terminal tyrosine might be two sequential events during the activation process. Alternatively, both molecules can associate to the C-terminal tyrosine through a third intermediate adaptor protein. Therefore, it is possible to speculate that the C-terminal tyrosine residue of the PRLR may coordinate multiple pathways downstream from the PRLR.

The association between cytokine receptors and SHP-2 has been previously reported for various systems. Two specific tyrosine residues in the intracellular domains of the platelet-derived growth factor receptor were shown to be sites for SHP-2 direct recruitment (9, 39). Similarly, three tyrosine residues in granulocyte macrophage colony-stimulating factor receptor were also shown to be sites for SHP-2 association (8). In addition, SHP-2 was found in a complex containing growth hormone receptor, Jak2, and signal regulatory protein α, and the interaction between SHP-2 and growth hormone receptor was mapped to the C-terminal tail of the receptor (11). Besides SHP-2 recruitment to phosphorylated receptors, receptor tyrosine phosphorylation-independent recruitment of SHP-2 has also been suggested. The association of SHP-2 to activated interferon α/β receptors was found to be independent of receptor tyrosine phosphorylation (40).

The association between SHP-2 and the platelet-derived growth factor receptor was mapped to the N-terminal SH2 domain of the phosphatase (41, 42). Similarly, SHP-2 associates to the insulin receptor substrate 1 subunit through the domain of the phosphatase (41, 42). Similarly, SHP-2 associator tyrosine phosphorylation (40).

In immunoprecipitates of SHP-2 from HC11 cells, we normally find multiple tyrosine phosphorylated proteins in the range of 95–100 kDa that co-immunoprecipitate with SHP-2 in response to PRL stimulation. SHP-2 has been reported to interact with members of the Gab family of adaptor proteins that range of 95–100 kDa that co-immunoprecipitate with SHP-2 in the C-terminal SH2 domain of SHP-2. The sequence of the tyrosine of the PRLR that binds to SHP-2 is DYLDP. To our knowledge, an optimal phosphorysine motif for the C-terminal SH2 domain of SHP-2 has not been determined.

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We identified that SHP-2 associates to the PRLR through its C-terminal SH2 domain leaving the N-terminal SH2 domain to possibly bind to Gab2. This finding favors that one SHP-2 molecule is binding simultaneously to the two sites. On the other hand, we found SHP-2 to be tyrosine phosphorylated in samples expressing the short form of the PRLR that itself does not become tyrosine phosphorylated (35). This suggests that the interaction of SHP-2 with Gab2 is independent of PRLR tyrosine phosphorylation state. Therefore, possibly two different molecules of SHP-2 are binding independently to the PRLR and to Gab2. Further investigation is required to elucidate the effects of SHP-2 recruitment to the PRLR and to Gab2 on the modulation of its functions in signaling downstream of the PRLR.

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