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Activation of JAK2 tyrosine kinase by prolactin receptors in Nb_2 cells and mouse mammary gland explants

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ABSTRACT **One of the earliest cellular responses to prolactin (PRL) binding in Nb_2 cells, a rat pre-T lymphoma cell line, is an increase in tyrosine phosphorylation of cellular proteins. In this work, immunologic techniques have been used to demonstrate that in Nb_2 cells and in mouse mammary gland explants, JAK2, a non-receptor tyrosine kinase, is activated following stimulation with PRL. PRL stimulated tyrosine phosphorylation of JAK2 at times as early as 30 sec and concentrations of PRL as low as 0.5 ng/ml (2.5 pM) in Nb_2 cells and 100 ng/ml (5 nM) in mammary gland explants. When JAK2 was immunoprecipitated from solubilized Nb_2 cells or mammary gland explants and incubated with [γ-^{32}P]ATP, ^{32}P was incorporated into a protein migrating with an apparent molecular weight appropriate for JAK2 only when cells had been incubated with PRL, indicating that JAK2 tyrosine kinase activity is exquisitely sensitive to PRL. In Nb_2 cells, JAK2 was found to associate with PRL receptor irrespective of whether or not the cells had been incubated with PRL. These results provide strong evidence that JAK2 is constitutively associated with the PRL receptor and that it is activated and tyrosine phosphorylated upon PRL binding to the PRL receptor. These results are consistent with JAK2 serving as an early, perhaps initial, signaling molecule for PRL.**

The binding of prolactin (PRL) to its cell surface receptor regulates diverse physiological processes, including mammary gland development, milk protein production, and immunomodulation presumably through T-lymphocyte mitogenesis (1, 2). However, little is known regarding early biochemical events in signal transduction through the PRL receptor. Three forms of the PRL receptor have been identified which differ primarily in the length of their cytoplasmic domains (see ref. 3 for review). Of the two predominant forms of the PRL receptor (termed long and short), only the long form confers PRL-dependent signaling when transfected into CHO cells along with a PRL-responsive reporter gene construct (4). Recently, an intermediate-length form of the PRL receptor was identified in Nb_2 cells, a PRL-dependent rat pre-T lymphoma cell line (5). Although this receptor lacks 198 amino acids (residues 323–520) present in the cytoplasmic domain of the long-form PRL receptor, it is capable of conferring PRL-dependent signaling when expressed in CHO cells (6). In Nb_2 cells, phosphorylation of tyrosine residues of cellular proteins is one of the most rapid responses known for PRL (6, 7). Protein-tyrosine kinase activity has been shown to copurify with the PRL receptor in these cells (6). This suggests that ligand-dependent activation of a tyrosine kinase is likely to be an important early event in signaling via the PRL receptor.

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PRL receptors are members of the cytokine/hematopoietin receptor family (3, 8). JAK-family tyrosine kinases (JAK1, JAK2, and Tyk2) have been identified as signal transducers for a number of members of this receptor family. JAK2, a 130-kDa protein, has been identified as a receptor-associated tyrosine kinase activated by binding of growth hormone (GH) (9), erythropoietin (10), and interleukin 3 (11) to their receptors. Other data indicate that JAK2 also serves as a signaling molecule for the receptors for granulocyte/macrophage-colony-stimulating factor (F. Quelle, B. Witthuhn, and J.N.I., unpublished data) and granulocyte-colony-stimulating factor (B. Witthuhn, F. Quelle, and J.N.I., unpublished data). Additionally, Tyk2 and JAK1 have been found to be required for interferon-α/β signaling and JAK2 and JAK1 for interferon-γ signaling (12–14). Of the proteins that are tyrosine-phosphorylated in response to PRL in Nb_2 cells, the one migrating with an apparent M_r of 121,000 is clearly the most prominent (6, 7) and appears to be associated with immunopurified PRL–PRL receptor complexes (6). Additionally, phosphate is incorporated into tyrosine residues of a 121-kDa protein when isolated PRL–PRL receptor complexes are incubated with ATP *in vitro* (6). These observations suggest that the 121-kDa protein is a PRL receptor-associated tyrosine kinase. Therefore, we asked whether JAK2 might function as the PRL receptor-associated tyrosine kinase. In this report, we provide evidence that PRL stimulates the tyrosine kinase activity and tyrosine phosphorylation of JAK2 in Nb_2 cells and in mouse mammary gland explants and that JAK2 is constitutively associated with the PRL receptor in Nb_2 cells.

MATERIALS AND METHODS

Materials. Stocks of Nb_2 cells (gift of P. Gout, University of British Columbia, Vancouver) were provided by C. T. Beer (Cancer Control Agency, Vancouver). Swiss Webster mice at 10–14 days of pregnancy were from Harlan Laboratories (Indianapolis). Human placental lactogen and human GH were gifts of J. Mills (Emory University, Atlanta) and J. Kostyo (University of Michigan, Ann Arbor), respectively. Ovine PRL (NIDDK-oPRL-19; 31 units/mg), bovine GH (NIH-GH-B16; 0.93 unit/mg), and anti-PRL antibody (αPRL) were provided by the National Institute of Diabetes and Digestive and Kidney Diseases/National Hormone and Pituitary Program, University of Maryland School of Medicine (Baltimore). The mouse monoclonal (U6) anti-PRL receptor antibody (αPRLR) was prepared as described (15).

Abbreviations: GH, growth hormone; PRL, prolactin; αPRL, anti-PRL antibody; αPRLR, anti-PRL receptor antibody; αPY, anti-phosphotyrosine antibody; αJAK1, anti-JAK1 antibody; αJAK2, antibody to JAK2 amino acids 758–776; $\alpha JAK2_{NT}$, antibody to JAK2 amino acids 19–31.

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The mouse monoclonal (4G10) anti-phosphotyrosine antibody (αPY) was obtained from Upstate Biotechnology (Lake Placid, NY). Anti-JAK2 antisera prepared against amino acids 758–776 (αJAK2) and 19–31 ($\alpha JAK2_{NT}$) and anti-JAK1 antiserum (αJAK1) were prepared as described (11). $\alpha JAK2_{NT}$ was a gift of O. Silvennoinen (New York University, New York). The ECL antibody detection system was purchased from Amersham.

Cell Culture and Tissue Preparation. Nb_2 cells were maintained as described (7). Twenty-four hours prior to treatment with PRL or other agents, the cells were placed into "stationary" medium (growth medium with 3% horse serum and no fetal bovine serum) at 1×10^6 cells per ml. Mouse mammary gland explants were prepared essentially as described (16). In brief, explants (3–6 mg each) from 12–15 animals were incubated with medium 199 (Earle's salts) with insulin at 1 mg/ml and 100 nM cortisol for 36 hr at 37°C under a humidified 95% air/5% CO_2 atmosphere prior to hormone treatment. These experiments were performed in compliance with the regulations of the Animal Care and Use Committee of Wayne State University.

Assays. After hormone treatment, Nb_2 cells (3×10^6) were washed twice with ice-cold PBSV (10 mM sodium phosphate/150 mM NaCl/1 mM Na_3VO_4, pH 7.4) and suspended in 300 μl of lysis buffer (50 mM Tris, pH 7.5/0.1% Triton X-100/137 mM NaCl/2 mM EGTA/1 mM Na_3VO_4/1 mM phenylmethylsulfonyl fluoride with aprotinin and leupeptin at 10 μg/ml) on ice. Mouse mammary explants were washed twice with PBSV, weighed, added to lysis buffer (1:5, wt/vol) and disrupted with a ground-glass homogenizer. Cell lysates were centrifuged at 12,000 × *g* for 10 min. The resulting supernatants were subjected to immunoprecipitation and immunoblot analysis (17). For the *in vitro* kinase assay, serum-deprived cells or tissue were incubated at 25°C in the absence or presence of the indicated concentrations of PRL for 60 min. Cells or tissue were washed with phosphate-buffered-saline, solubilized in 25 mM Hepes, pH 7.4/2 mM Na_3VO_4/0.1% Triton X-100/0.5 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride containing aprotinin and leupeptin at 10 μg/ml, and centrifuged at 100,000 × *g* for 1 hr at 4°C. *In vitro* kinase assay was then performed on proteins immobilized on αJAK2 or $\alpha JAK2_{NT}$ (1:1500 dilution) in the presence of 10 μM [γ-^{32}P]ATP and 5 mM $MnCl_2$ as described (9). The phospho amino acid content of phosphorylated proteins was determined by limited acid hydrolysis using a modification (18) of the procedure of Hunter and Sefton (19).

RESULTS

PRL Promotes Tyrosine Phosphorylation of JAK2. Because autophosphorylation is often the earliest manifestation of an activated kinase, solubilized proteins from Nb_2 cells incubated with or without PRL were immunoprecipitated with αJAK2 and analyzed for the presence of phosphorylated tyrosine residues by αPY immunoblot. PRL-dependent tyrosine phosphorylation of a protein with a M_r of ≈130,000, appropriate for JAK2, was clearly evident as early as 30 sec following PRL stimulation (Fig. 1*a*) and at PRL concentrations as low as 0.5 ng/ml (2.5 pM) (Fig. 1*b*). This protein comigrated with the PRL-dependent tyrosine phosphorylated protein previously referred to as "pp121" (7) observed in Nb_2 cell lysates (Fig. 1*a*, lanes K and L).

This 130-kDa phosphoprotein (pp130) was precipitated specifically by αJAK2 (Fig. 2*a*, lane F). Nonimmune serum (Fig. 2*a*, lane B), an unrelated immune serum (lane D), αJAK1 (lane L), and αJAK2 preadsorbed with the peptide antigen used to make the antibody (11) (lane H) failed to immunoprecipitate pp130. Preadsorption of αJAK2 with the analogous peptide from murine JAK1 (11) did not interfere with precipitation of pp130 by αJAK2 (Fig. 2*a*, lane J).

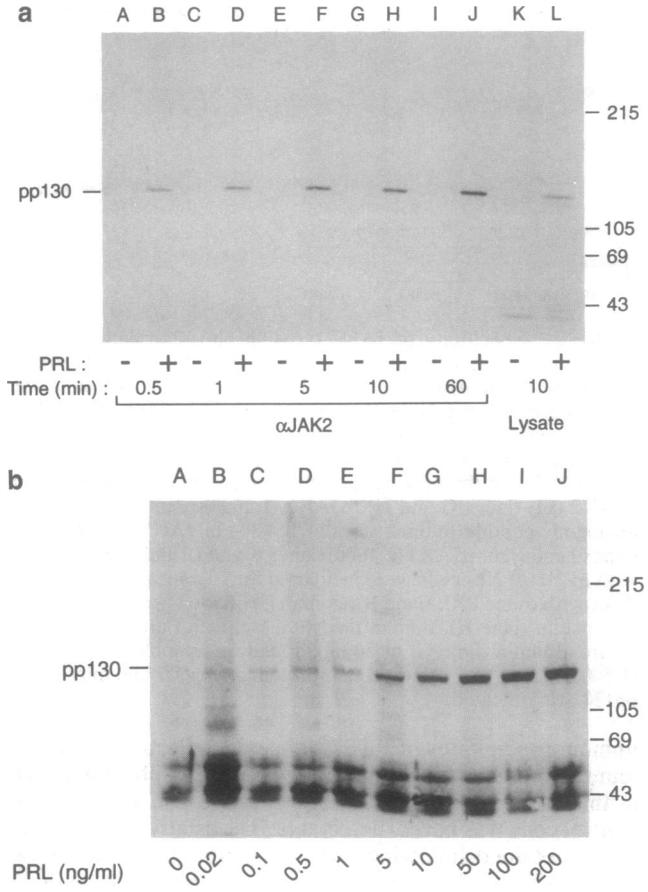

FIG. 1. PRL promotes tyrosine phosphorylation of JAK2. (*a*) Nb_2 cells were incubated without (lanes A, C, E, G, I, and K) or with (lanes B, D, F, H, J, and L) PRL (20 ng/ml) for the indicated times. Whole cell lysates were immunoprecipitated with αJAK2 (1:1000 dilution). Immunoprecipitated proteins (lanes A–J) and unfractionated lysates (lanes K and L) were subjected to αPY (1:5000) immunoblot analysis. The small apparent increase in tyrosine phosphorylation at 60 min compared with 10 min was not reproducible. (*b*) Nb_2 cells were incubated with the indicated concentrations of PRL for 10 min and subjected to immunoprecipitation and immunoblot analysis as above. $M_r \times 10^{-3}$ of prestained protein standards and migration of pp130 are indicated.

Consistent with pp130 being JAK2, sequential probing of immunoblots of αJAK2 immunoprecipitates with αPY followed by αJAK2 revealed comigration of pp130 and JAK2 (data not shown).

Tyrosine phosphorylation of the 130-kDa protein precipitated from Nb_2 cells by αJAK2 was stimulated only by factors that bind and activate the PRL receptor (Fig. 2*b*). Both ovine PRL and human GH stimulated tyrosine phosphorylation of pp130, but ovine PRL was more potent, consistent with the relative affinities of these hormones for PRL receptor (21) and the absence of GH receptor expression in Nb_2 cells (7, 22). With longer exposure, pp130 was also detected in the immunoprecipitate from cells stimulated with human placental lactogen (data not shown), consistent with the lower biological potency of human placental lactogen compared with ovine PRL (22). Bovine GH—which, unlike human GH, does not bind PRL receptors (22, 23)—insulin, and bovine serum albumin failed to promote detectable tyrosine phosphorylation of pp130.

Stimulation by PRL of JAK2 Kinase Activity. To determine whether PRL stimulated JAK2 tyrosine kinase activity, JAK2 was immunoprecipitated from Nb_2 cells with either of two antisera against distinct regions of the JAK2 molecule and subjected to an *in vitro* kinase assay (Fig. 3*a*). In αJAK2

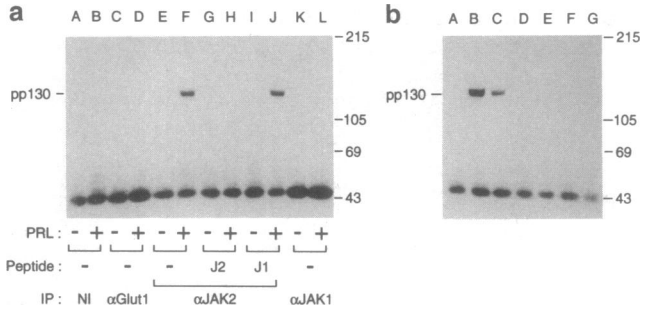

FIG. 2. Specificity of JAK2 tyrosine phosphorylation. (*a*) Lysates from unstimulated (lanes A, C, E, G, I, and K) and PRL-treated (20 ng/ml, 10 min) (lanes B, D, F, H, J, and L) Nb_2 cells were immunoprecipitated (IP) with nonimmune rabbit serum (NI) (1:1000) (lanes A and B), an irrelevant antiserum (αGlut-1) (1:1000) (20) (lanes C and D), αJAK2 (lanes E and F), αJAK2 (1:1000) preincubated for 1 hr at 0°C with the peptide (J2, 30 μg/ml) used as antigen to make αJAK2 (11) (lanes G and H), αJAK2 (1:1000) preincubated with an analogous peptide to amino acids 785–804 of JAK1 (J1, 30 μg/ml) (lanes I and J), or αJAK1 (1:1000) (lanes K and L) and immunoblotted with αPY. (*b*) Nb_2 cells were incubated for 10 min with vehicle (lane A) or with ovine PRL (lane B), human GH (lane C), bovine GH (lane D), insulin (lane E), human placental lactogen (lane F), or bovine serum albumin (lane G) at 50 ng/ml and immunoblotted with αPY (1:5000). $M_r \times 10^{-3}$ of prestained protein standards and migration of pp130 are indicated.

immunoprecipitates of PRL-stimulated cells, a 130-kDa protein that comigrated with JAK2 was readily phosphorylated in this assay; incorporation of ^{32}P into JAK2 isolated from control cells was barely detectable. The relative intensities of signal obtained following immunoprecipitation with αJAK2 and $\alpha JAK2_{NT}$ (lanes B and D) were consistent with the relative affinities of these antibodies for JAK2. The fact that both JAK2 antibodies immunoprecipitated a 130-kDa protein capable of *in vitro* phosphorylation provides evidence that the ^{32}P-labeled 130-kDa protein visualized is JAK2 itself rather than an antigenically related protein. Phospho amino acid analysis of the 130-kDa protein recovered from PRL-stimulated cells (Fig. 3*a*, lane B) revealed that ^{32}P was exclusively (>99%) associated with tyrosine residues (Fig. 3*b*). While it is possible that JAK2 was phosphorylated in this

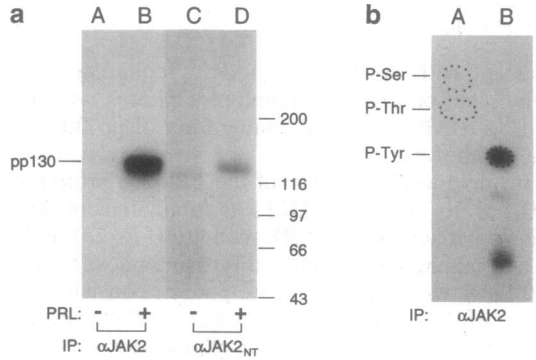

FIG. 3. PRL stimulates JAK2 tyrosine kinase activity in Nb_2 cells. (*a*) Nb_2 cells were incubated at 25°C in the absence (lanes A and C) or presence (lanes B and D) of PRL (30 ng/ml) for 60 min. Solubilized cellular proteins were immunoprecipitated (IP) with either αJAK2 (lanes A and B) or $\alpha JAK2_{NT}$ and then incubated with [γ-^{32}P]ATP and separated by SDS/PAGE. $M_r \times 10^{-3}$ of protein standards and migration of pp130 are indicated. (*b*) pp130 was excised from the gel visualized in *a*, lane B, and subjected to limited acid hydrolysis at 109°C for 1.25 hr. After partial purification on Dowex-50, fractions containing *O*-phosphoserine (P-Ser) and *O*-phosphothreonine (P-Thr) (lane A) or *O*-phosphotyrosine (P-Tyr) (lane B) were resolved by thin-layer electrophoresis (pH 3.5). Migration of phospho amino acid standards is indicated by the areas enclosed by dots.

assay by a coprecipitating kinase rather than by JAK2 itself, we favor the interpretation that JAK2 was the kinase activated by PRL binding to its receptor. Consistent with this, JAK2 has been shown to be an activated kinase when immunoprecipitated from COS-7 cells transfected with JAK2 cDNA (W.-H. Huo, L.S.A., and C.C.-S., unpublished work). In addition, when 3T3-F442A cells are stimulated with GH, another ligand that signals through JAK2, highly purified JAK2, isolated by sequential immunoprecipitation using αPY followed by αJAK2, exhibits ligand-dependent kinase activation (9).

JAK2 Forms a Complex with the PRL Receptor. To determine whether JAK2 was present in a complex with PRL receptor, PRL–PRL receptor complexes and associated proteins were immunoprecipitated from PRL-treated solubilized Nb_2 cells with αPRL. In immunoblots of these immunoprecipitates, αJAK2 identified a 130-kDa protein (Fig. 4, lane B) which comigrated with the 130-kDa protein recognized by αPY (lane F). Neither αJAK2 nor αPY detected the 130-kDa protein when Nb_2 cells were not stimulated with PRL (Fig. 4, lanes A and E), indicating that precipitation of JAK2 resulted from its association with PRL–PRL receptor complexes. When an antibody to the PRL receptor (αPRLR) was used instead of αPRL in the initial immunoprecipitation, immunoblotting with αJAK2 revealed JAK2 in precipitates of both control and PRL-stimulated Nb_2 cells (Fig. 4, lanes C and D). Thus, PRL binding is not necessary for the formation of a complex between JAK2 and the PRL receptor in Nb_2 cells. However, consistent with PRL stimulating JAK2 kinase activity, tyrosine phosphorylation of a protein which comigrated with JAK2 was detected only after PRL stimulation (Fig. 4, lanes G and H). In the experiment shown, tyrosine-phosphorylated JAK2 migrated just above a protein exhibiting PRL-independent tyrosine phosphorylation.

PRL Activates JAK2 in Mouse Mammary Gland Explants. The PRL receptor expressed by Nb_2 cells is of intermediate length. We were interested in determining whether PRL addition to cells known to express the long form of the PRL receptor would stimulate JAK2. In αJAK2 immunoprecipitates from mouse mammary gland explants, tyrosine phosphorylation of a 130-kDa protein was detected with PRL concentrations as low as 100 ng/ml (Fig. 5*a*). Increased tyrosine phosphorylation of this protein was detectable within 30 sec of PRL addition (data not shown). Although the smaller JAK2 signal superimposed on a background phos-

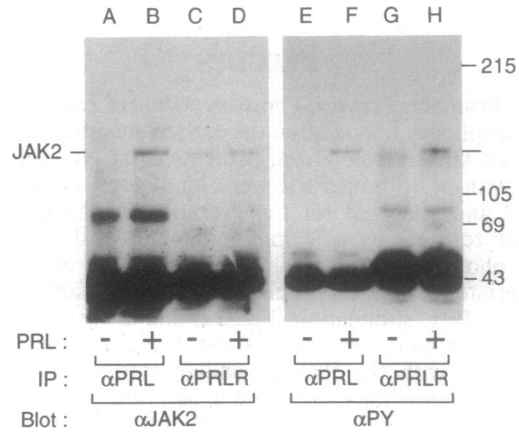

FIG. 4. JAK2 associates with the PRL receptor in Nb_2 cells. Nb_2 cells were incubated with vehicle (lanes A, C, E, and G) or PRL (lanes B, D, F, and H) at 50 ng/ml for 10 min. Solubilized proteins were immunoprecipitated (IP) with αPRL (1:200) (lanes A, B, E, and F) or αPRLR (5 μg/ml) (lanes C, D, G, and H) and subjected to immunoblot analysis with αJAK2 (1:5000) (lanes A–D) or αPY (1:5000) (lanes E–H). $M_r \times 10^{-3}$ of prestained protein standards and migration of JAK2 (left) and pp130 (right) are indicated.

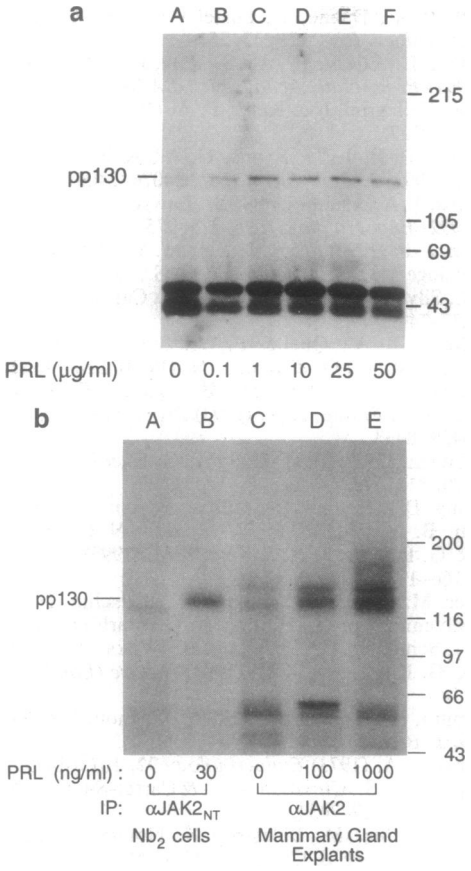

FIG. 5. PRL promotes the tyrosine phosphorylation and tyrosine kinase activity of JAK2 in mouse mammary gland explants. (*a*) Mouse mammary gland explants were incubated with the indicated concentrations of PRL for 10 min and then subjected to immunoprecipitation with αJAK2 followed by immunoblot analysis with αPY as in Fig. 1. (*b*) Lanes A and B correspond to lanes C and D of Fig. 3*a* and were included to facilitate identification of JAK2. For lanes C and D, mouse mammary gland explants were incubated at 25°C for 60 min with vehicle (lane C) or with PRL at 100 ng/ml (lane D) or 1000 ng/ml (lane E). Proteins were immunoprecipitated with αJAK2 and subjected to *in vitro* kinase assay (see Fig. 3). $M_r \times 10^{-3}$ of protein standards and migration of pp130 are indicated.

phorylation of proteins migrating just above and below JAK2 makes phosphorylation of JAK2 more difficult to visualize in mammary gland explants than in Nb_2 cells, PRL-dependent ^{32}P incorporation into a 130-kDa protein that comigrated with the 130-kDa protein detected from Nb_2 cells was observed when αJAK2 immunoprecipitates from mammary gland explants were subjected to an *in vitro* kinase assay (Fig. 5*b*). The data shown in Fig. 5 suggest that, as in the Nb_2 cells, PRL promotes rapid activation of JAK2 in mouse mammary tissue. The identities of the additional proteins exhibiting PRL-dependent tyrosine phosphorylation in this assay are unknown but of great interest, since they could represent JAK2 substrates.

The PRL concentrations required to activate JAK2 in mammary gland explants are relatively high. To confirm that JAK2 activation was caused by PRL binding to its receptor and not by a trace contaminant of ovine GH stimulating GH receptors in the mouse mammary gland explants, the dose–response experiment shown in Fig. 5*a* was repeated with recombinant bovine PRL produced in *Escherichia coli*. Recombinant PRL and ovine PRL were equally potent for stimulating JAK2 tyrosyl phosphorylation in the mammary gland explants (data not shown). The relatively higher concentrations of PRL required to activate JAK2 in mammary gland explants compared with Nb_2 cells may reflect decreased accessibility of PRL to its receptors in the organ culture. Alternatively, they may represent true differences in sensitivity to PRL. Nb_2 cells are known to be very sensitive to PRL. The concentrations of PRL used with the mammary gland explants are consistent with serum PRL levels observed in lactating mice (24) and rats (25) during suckling.

DISCUSSION

This study provides strong evidence that JAK2 is a PRL receptor-associated tyrosine kinase that is activated upon ligand binding. Its sensitivity to PRL and rapid onset following PRL addition make tyrosine phosphorylation of JAK2 among the most sensitive and rapid responses to PRL yet identified. Previous studies have demonstrated that JAK2 is also activated following ligand engagement of a number of other members of the cytokine/hematopoietin receptor family, including the receptors for GH, interleukin 3, erythropoietin, granulocyte/macrophage-colony-stimulating factor, granulocyte-colony-stimulating factor, and the distantly related receptor for interferon γ (9–11, 13). These findings suggest that activation of JAK2 is likely to be a critical early event in signal transduction through the PRL receptor and other members of the cytokine/hematopoietin receptor family.

The mechanism by which PRL and other factors that work through JAK2-coupled receptors stimulate JAK2 is not known. In our work with the GH receptor–JAK2 interaction in 3T3-F442A cells (9), JAK2 association with the GH receptor was highly GH dependent. Since GH binding is thought to cause dimerization of the GH receptor (26), these observations led us to propose a model in which GH–GH receptor dimers bound and presumably activated JAK2. In the present study, we found that in Nb_2 cells, JAK2 was constitutively associated with the PRL receptor. Because JAK2 activity was detected only in the presence of PRL, these data suggest that JAK2 binding to a cognate receptor is not sufficient to activate JAK2. This conclusion is supported by the observation that the cytoplasmic domain of a mutant erythropoietin receptor which lacks the ability to activate JAK2 still binds JAK2 (10). Therefore, in addition to the formation of a receptor–JAK2 complex, a ligand-dependent event appears to be necessary for JAK2 activation. Whether this event is receptor oligomerization, ligand-induced conformational changes, association with additional proteins, or a combination of these remains to be determined.

The ability of JAK2 to serve as a signaling molecule for several receptors implies that these JAK2-coupled receptors will share some signaling mechanisms. Presumably, JAK2 serves to propagate the signal for PRL and other activating cytokines by phosphorylating additional proteins. Consistent with this notion, PRL-dependent increases in tyrosine phosphorylation of several other cellular proteins have been observed in Nb_2 cells (6, 7). While substrates of JAK2, other than JAK2 itself, are largely unknown, one pathway, first identified as a component of signaling through the interferon receptors, is emerging as a pathway likely to be shared by receptors coupled to JAK family kinases. In response to interferon γ, the 91-kDa component of the ISGF-3 (interferon-stimulated gene factor 3) complex undergoes tyrosine phosphorylation and then translocates to the nucleus, where it binds to DNA at the γ-activated site (27). Evidence now suggests that this pathway, at least in part, is shared by other receptors that utilize JAK2, including the receptor for GH (28). PRL-dependent tyrosine phosphorylation of an ≈91-kDa protein has been observed in Nb_2 cells (6, 7), and preliminary experiments indicate that a protein antigenically related to the 91-kDa component of the ISGF-3 complex is phosphorylated on tyrosine in PRL-stimulated Nb_2 cells

(G.S.C., L.S.A., A. Larner, and C.C.-S., unpublished data). Thus, this pathway may be involved in regulation of gene transcription by PRL.

Since each ligand that stimulates JAK2 elicits a distinct set of responses, JAK2 activation alone cannot account for specificity. Specificity could be obtained by a limited number of JAK2-coupled receptors in any one cell type or by interaction with other ligand-specific signaling pathways. Additionally, phosphorylation of the activating receptor could provide a mechanism by which specificity could be achieved. In this scheme, phosphorylated residues of both the activating receptor and JAK2 act as binding sites for specific proteins containing Src homology 2 (SH2) domains that would link the receptor and/or JAK2 to various intracellular signaling pathways (29). Signaling pathways stemming from interactions with phosphorylated tyrosine residues on JAK2 would presumably be shared. However, each receptor could initiate its unique responses by binding to distinct subsets of SH2-containing proteins. Consistent with this, several of the cytokine/hematopoietin receptors that activate JAK2 are themselves tyrosine phosphorylated following ligand engagement [e.g., receptors for GH (9, 30), erythropoietin (31–33), and interleukin 3 (11, 34)]. In the case of the GH receptor–JAK2 interaction, JAK2 was found to phosphorylate the GH receptor *in vitro* (9). Whether JAK2 also phosphorylates PRL receptors is not clear. When PRL–PRL receptor complexes were immunopurified from Nb_2 cells, Rui *et al.* (6) observed increased tyrosine phosphorylation of a protein with an apparent molecular mass appropriate for the PRL receptor. In the present study we did not detect increased tyrosine phosphorylation of a protein of a size appropriate for the PRL receptor, possibly due to differences in PRL concentrations and incubation times used. However, the PRL receptor in Nb_2 cells lacks 198 of the 357 amino acids present in the cytoplasmic domain of the long-form receptor, including six of the nine tyrosine residues (5). Thus, even if PRL does not stimulate tyrosine phosphorylation of the PRL receptor in Nb_2 cells, PRL-dependent tyrosine phosphorylation of the long-form PRL receptor by JAK2 could still be involved in PRL signaling in other cells.

In summary, identification of JAK2 as the PRL receptor-associated tyrosine kinase should lead to greater understanding of the regulation of physiological function by PRL. The rapidity and sensitivity of JAK2 activation by PRL strongly suggest that this is a critical early event in PRL signaling. That JAK2 also serves as a signal mediator for other cytokine/hematopoietin receptors suggests that these receptors share some signaling pathways. Insight gained from the study of PRL signaling will therefore most likely have significance in those other systems and vice versa.

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