The Identification of JAK2 Tyrosine Kinase as a Signaling Molecule for Growth Hormone (43744)

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Abstract. **The intracellular pathways by which the binding of growth hormone (GH) to its receptor elicits its diverse effects have eluded investigators for many years. Studies showing that GH rapidly stimulates tyrosyl phosphorylation of cellular proteins, and that tyrosine kinase activity co-purifies with GH-GH receptor complexes, led us to hypothesize that activation by GH of a receptor-associated tyrosine kinase is an important early, and perhaps, initiating step in signal transduction by GH. Here, we review the work identifying JAK2 as a GH receptor-associated tyrosine kinase that is rapidly activated by ligand binding.** [P.S.E.B.M. 1994, Vol 206]

The cellular mechanism(s) by which growth hormone (GH) elicits its well-known effects on body growth and metabolism (1, 2) have eluded investigators for many years. A number of years ago, we hypothesized that GH, like a variety of growth promoting factors, stimulates a tyrosine kinase (3). To establish the validity of this hypothesis, a variety of experiments were carried out using 3T3-F442A fibroblasts, cells which respond to GH by differentiating into adipocytes (4). Highly purified GH-GH receptor (GHR) complexes prepared from these cells were found to contain tyrosine kinase activity (5–7). In addition, GH was found to promote the tyrosyl phosphorylation of multiple proteins, including the GHR (3,5,8). These observations suggested that GHR might be a ligand-activated tyrosine kinase, like the receptors for such growth-promoting factors as epidermal growth factor and platelet-derived growth factor (9). However, during the course of these experiments, Leung and colleagues (10) cloned human and rabbit liver GHRs. The cloned liver GHR was found to be a single membrane spanning polypeptide having no homology to receptors with known signal transduction mechanisms, including receptors with intrinsic tyrosine kinase activity. GHR is now recognized as a member of the cytokine/hematopoietin receptor superfamily (11). Classification was based upon limited homology in the extracellular domain of these receptors.

The absence of homology between the cloned GHR and known tyrosine kinases suggested two possibilities. The first is that GH binds to two receptors, the cloned liver GHR and a second, not yet cloned GHR with intrinsic tyrosine kinase activity. The second possibility is that there is only one GHR, the one cloned by Leung and colleagues (10), which can associate with a cellular tyrosine kinase. A number of different approaches were used to try to differentiate between these possibilities. GHRs in different cell lines, including liver cell lines, were found to co-purify with tyrosine kinase activity (12). Antibodies against peptides corresponding to sequences in the cloned liver GHR were shown to precipitate tyrosine kinase activity (6). Polymerase chain reaction technology was unable to isolate an isoform of GHR containing tyrosine kinase activity (unpublished data). Finally, the cloned liver GHR expressed in four different cell lines co-purified with tyrosine kinase activity (13). These results led us to hypothesize that the cloned liver GHR associates with a cellular tyrosine kinase.

As a first approach to identifying the GHR-

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associated kinase, experiments were carried out to estimate the size of the kinase (5). Highly purified, kinase-active GH-GHR complexes were prepared from GH-treated 3T3-F442A cells which had been incubated overnight with [^{35}S]methionine and [^{35}S]cysteine and analyzed for the presence of two proteins—a ~121-kDa GHR and a protein of unknown size corresponding to the kinase. The presence of only one broad migrating with Mr ~121,000 suggested that the GHR-associated tyrosine kinase might be the same size as GHR.

Before embarking on the potentially difficult project of cloning what we believed to be a difficult-to-purify, low-abundant kinase, we decided to see if by any chance the kinase had already been cloned but just not recognized as a signaling molecule for GHR. Of particular interest was the Janus kinase (JAK) family, consisting of JAK1, JAK2, and tyk2. All three family members migrate with Mr (~130,000) appropriate for the GHR-associated kinase (14–16). They lack a membrane spanning domain and are expressed fairly ubiquitously. JAK1 and JAK2 were kinases of unknown function (15). Finally, tyk2 had recently been identified as a signaling molecule for the α/β-interferon receptor (17), a receptor distantly related to GHR (11).

A variety of approaches were used to establish JAK2 as a GHR-associated tyrosine kinase (18). Since autophosphorylation is often the earliest manifestation of an activated kinase, we investigated whether GH stimulates the tyrosyl phosphorylation of JAK2. Figure 1 (Lane A and B) illustrates that in 3T3-F442A cells, GH stimulates tyrosyl phosphorylation of a protein that migrates with a size appropriate for JAK2. Increased tyrosyl phosphorylation was rapid (within 30 sec) and occurred at concentrations of GH as low as 5 ng/ml (18), well within the range of circulating GH in mice (19).

The ability of GH to activate JAK2 was examined more directly (18). 3T3-F442A cells were incubated with GH. JAK2 was precipitated using αJAK2 and assayed for kinase activity by addition of [^{32}P]ATP. Figure 2A illustrates that ^{32}P is incorporated into a protein migrating with Mr appropriate for JAK2 only when cells had been incubated with GH, indicating an exquisite sensitivity of JAK2 to GH. Phosphoamino acid analysis of the [^{32}P]labeled 130-kDa protein revealed that over 99% of the ^{32}P incorporated into amino acids is incorporated into tyrosines (Fig. 2B). We then examined whether phosphate is incorporated into tyrosyl residues in JAK2, GHR, or both proteins present in the αJAK2 immunoprecipitate. Proteins from GH-treated 3T3-F442A cells and a cell line (CHO4) that expresses a smaller (84-kDa) GHR (20), were precipitated using αJAK2. Precipitated proteins were analyzed for the presence of phosphorylated tyrosines by αPY immunoblot, before and after incuba-

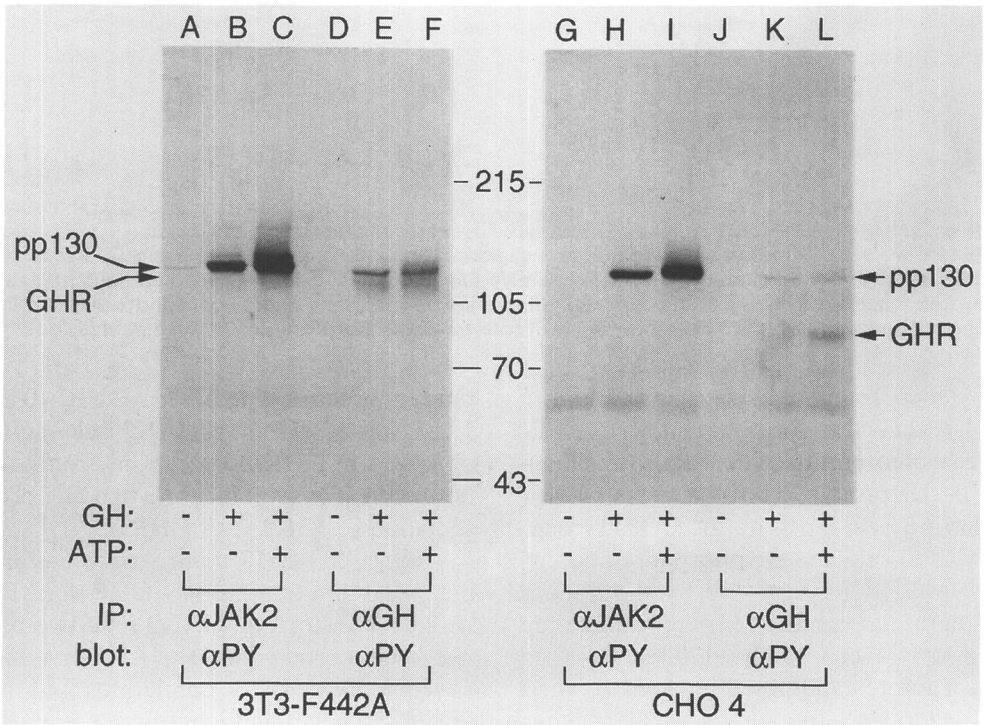

Figure 1. JAK2 phosphorylates tyrosyl residues in both JAK2 and GHR. 3T3-F442A cells (Lane A–F) or CHO4 cells (Lane G–L) were treated in the absence (Lane A, D, G, and J) or presence (Lane B, C, E, F, H, I, K, and L) of 30 ng/ml hGH. Cellular proteins were solubilized and immunoprecipitated with αJAK2 (Lane A–C and G–I) or αGH (Lane D–F and J–L). Immune complexes were treated with (Lane C, F, I, and L) or without (Lane A, B, D, E, G, H, J, and K) 10 μ*M* unlabeled ATP at 30°C for 10 min as described in Ref. 18. Proteins were analyzed by αPY-4G10 immunoblot. The mol wt ($\times 10^{-3}$) of protein standards and migration of pp130 and GHR are indicated.

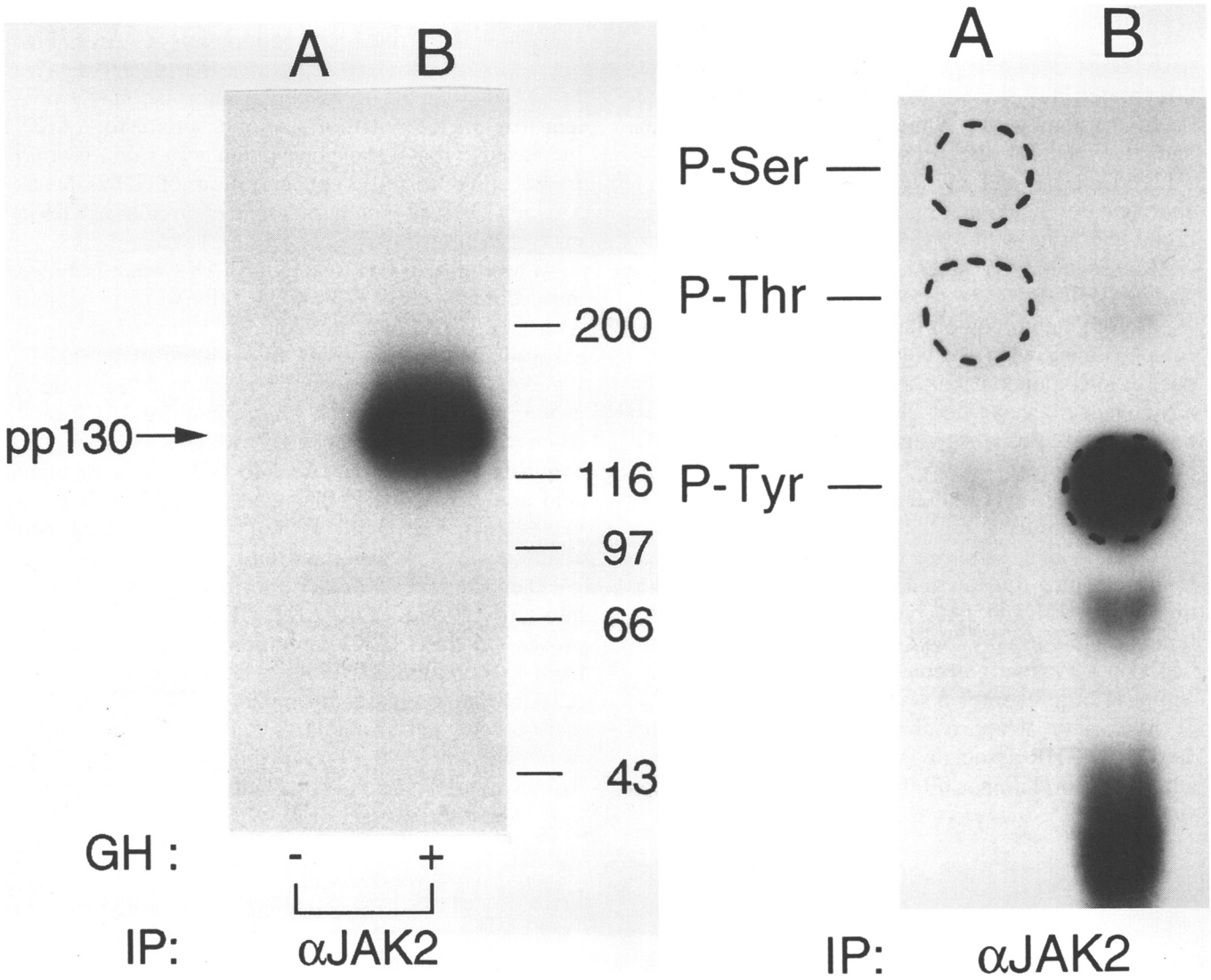

Figure 2. Purified JAK2 possesses tyrosine kinase activity. A. 3T3-F442A cells were incubated at 25°C in the absence (Lane A) or presence (Lane B) of 30 ng/ml hGH for 1 hr. Solubilized proteins were immunoprecipitated using αJAK2 and incubated with [γ-^{32}P]ATP (10 μM). Mol wt ($\times 10^{-3}$) of protein standards and migration of pp130 are indicated. B. pp130 was excised from the gel visualized in Panel A, Lane B and subjected to limited acid hydrolysis at 109°C for 1.25 hr. Following 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) as previously described (5, 12, 13) (from Ref. 20 with permission).

tion with ATP. A comparison of lanes B and C, and H and I in Figure 1 reveals that for both cell types additional phosphate is incorporated into tyrosyl residues primarily in a 130-kDa protein. This is consistent with JAK2 protein serving as a substrate for JAK2 kinase. The ability of JAK2 to catalyze the phosphorylation of tyrosyl residues in GHR is suggested by the finding that a diffusely migrating protein of Mr appropriate for GHR (84,000 in CHO4 cells and ~120,000 in 3T3-F442A cells) incorporates additional phosphate when αGH immunoprecipitates containing JAK2 are incubated with ATP (compare Lane E and F, K and L in Fig. 1).

To establish that JAK2 forms a complex with GHR, we examined whether JAK2 co-purifies with GHR (18). GHR was purified from 3T3-F442A cells by immunoprecipitating with αGHR. The presence of JAK2 was assessed by αJAK2 immunoblot. JAK2 was observed in GHR preparations from GH-treated, but not control, cells, indicating that GH promotes the association of JAK2 with GHR.

When GH-GHR complexes were prepared by immunoprecipitation using αGHR and immunoblotted with αPY instead of αJAK2 (18), two bands were visible. One corresponds to JAK2 and the other is believed to be GHR. It comigrates with a similarly diffuse ~120-kDa band identified in αGHR immunoblots of αGH immunoprecipitates (18). These results indicated that GH promotes tyrosyl phosphorylation of GHR. Furthermore, the JAK2 proteins that are bound to GHR are tyrosyl phosphorylated. JAK2 is the first signaling molecule identified that interacts with GHR.

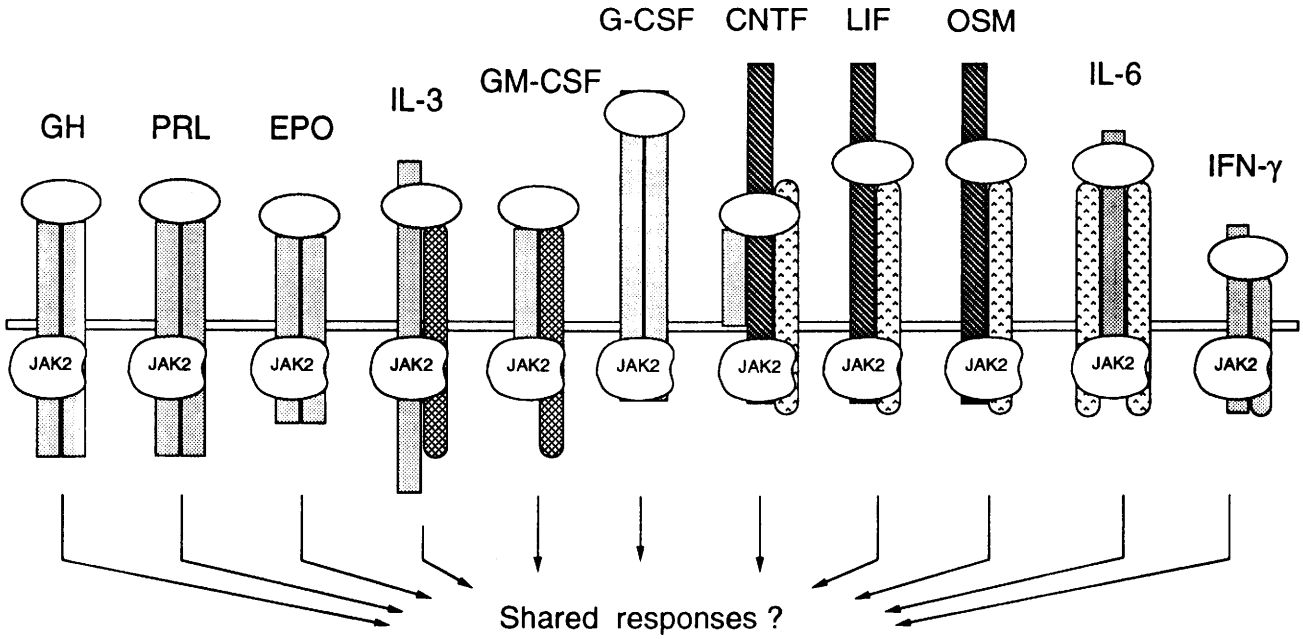

Figure 3. JAK2 serves as a signaling molecule for multiple members of the cytokine/hematopoietin receptor family. PRL, prolactin; EPO, erythropoietin; IL-3, interleukin-3; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; CNTF, ciliary neurotrophic factor; LIF, leukemia inhibitory factor; OSM, oncostatin M; IL-6, interleukin-6; IFN-γ, γ-interferon.

Its identification has already allowed us to determine that GH promotes the association of kinase with GHR, activates the kinase, and promotes phosphorylation of tyrosyl residues on both GHR and the kinase. The use of JAK2 as a signaling molecule by GHR is even more exciting when taken in the context that JAK2 is also

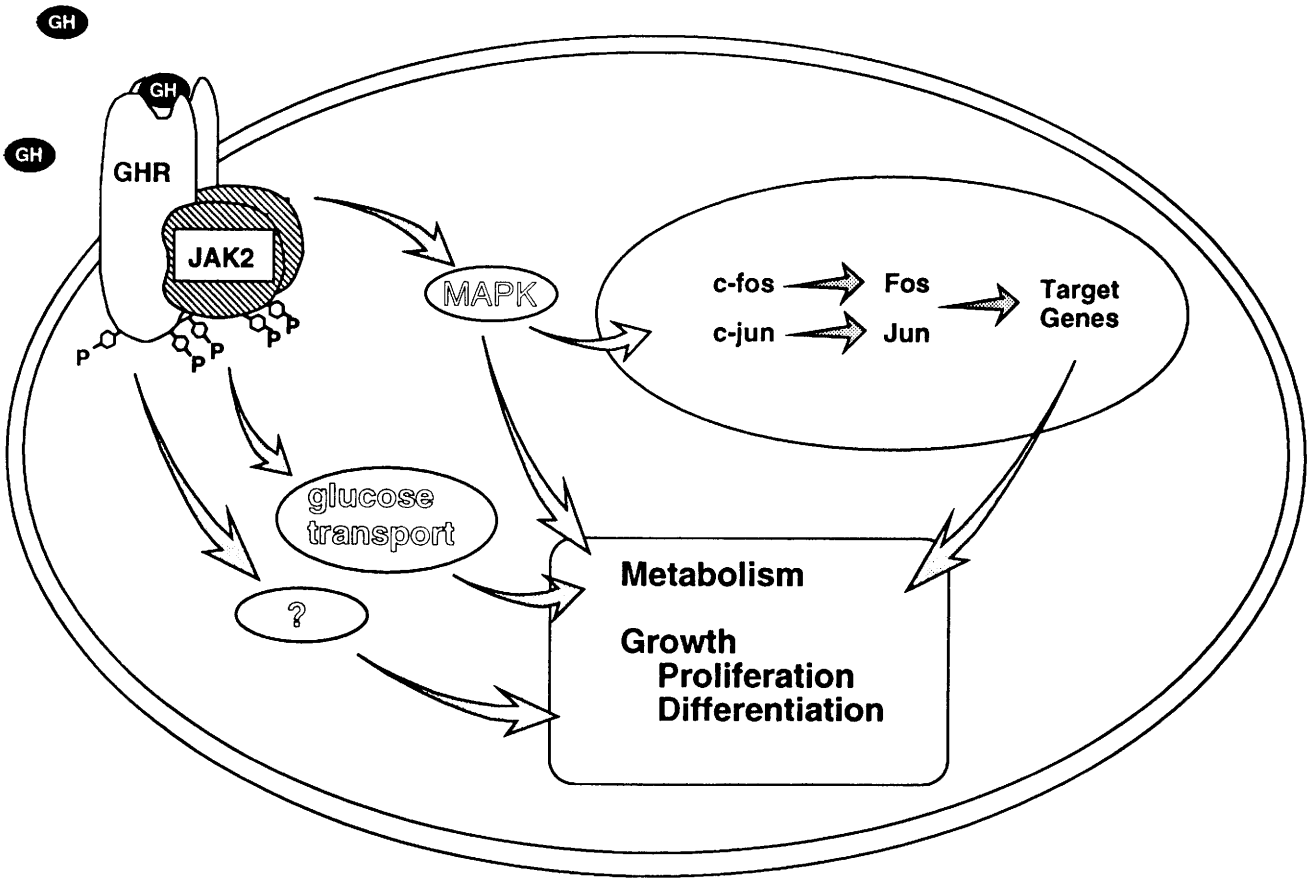

Figure 4. Hypothetical model of the molecular mechanism of action of GH.

utilized as a signaling molecule by other members of the cytokine/hematopoietin receptor family. We have shown that JAK2 serves as a signaling molecule for prolactin (21). In addition, Ihle and collaborators have found that JAK2 serves as a signaling molecule for a number of other members of the cytokine receptor family (14, 22, 23) (Fig. 3). The fact that all of these receptors can activate JAK2 makes it seem quite likely that multiple receptors in the cytokine/hematopoietin receptor family will share signaling pathways and elicit at least some of the same responses.

Figure 4 summarizes the results presented above and presents a working hypothesis about how GH binding to its receptor could lead to the diverse actions of GH. GH binds to its receptor which either exists naturally as a dimer in the membrane or forms a dimer as a consequence of one molecule of GH binding to two receptors (24). Binding of GH causes JAK2 to bind to GHR and become activated. Once bound, JAK2 phosphorylates both itself and GHR, and presumably other proteins. The phosphorylated tyrosine residues on both JAK2 and GHR are likely to serve as docking sites for SH2-containing proteins in various signaling pathways (25) that in turn lead to several responses to GH. We and others have already identified a variety of functions that appear to depend upon activation of this kinase, including stimulation of MAP kinases, tyrosyl phosphorylation of a variety of cellular proteins, c-*fos* gene expression, and glucose transport (8, 26). Thus, identification of JAK2 as a GH-activated, GHR-associated kinase provides a significant advance in understanding how GH acts in the cell. The next few years should be a very exciting, productive period of GH research during which a new vision will be obtained of how GH acts and interacts with other growth factors and cytokines to regulate body growth and metabolism.

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