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REVIEW ARTICLE

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Role of the tyrosine kinase JAK2 in signal transduction by growth hormone

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Abstract Chronic renal failure in children results in impaired body growth. This effect is so severe in some children that not only does it have a negative impact on their self-image, but it also affects their ability to carry out normal day-to-day functions. Yet the mechanism by which chronic renal failure causes short stature is not well understood. Growth hormone (GH) therapy increases body height in prepubertal children, suggesting that a better understanding of how GH promotes body growth may lead to better insight into the impaired body growth in chronic renal failure and therefore better therapies. This review discusses what is currently known about how GH acts at a cellular level. The review discusses how GH is known to bind to a membrane-bound receptor and activate a cytoplasmic tyrosine kinase called Janus kinase (JAK) 2. The activated JAK2 in turn phosphorylates tyrosines within itself and the associated GH receptor, forming high-affinity binding sites for a variety of signaling molecules. Examples of such signaling molecules include signal transducers and activators of transcription (Stats), which regulate the expression of a variety of GH-dependent genes, and the adapter protein Shc, which leads to activation of the Ras-Raf-MEK-MAP kinase pathway. In response to GH, JAK2 is also known to phosphorylate the insulin receptor substrates, leading to activation of phosphatidyl inositol 3' kinase and most likely other molecules that have been implicated in the regulation of metabolism. Finally, the ability of JAK2 to bind and activate the presumed adapter protein SH2-B is discussed. SH2-B has been shown to be a potent activator of GH-promoted JAK2 activity and downstream signaling events. Presumably these and other pathways initiated by GH combine to result in its ability to regulate body growth and metabolism.

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Introduction

As early as 1973 [1], growth hormone (GH) was recognized as binding to a membrane-bound receptor. Yet the mechanism by which GH binding to its receptor elicits the diverse responses to GH remained elusive for several more decades. Even cloning of the GH receptor [2] in 1987 did not shed light on the mechanism by which the GH receptor functioned, because the deduced amino acid sequence of the cloned rabbit and human liver GH receptors bore no homology to proteins with known function. Based upon our findings that GH stimulates tyrosyl phosphorylation of cellular proteins [3, 4] and GH-GH receptor co-purifies with a tyrosine kinase [5], we hypothesized that GH might act like a number of other growth factors, i.e., to activate a tyrosine kinase that is either intrinsic to the receptor or tightly associated with the receptor. After a prolonged search for our hypothesized GH receptor-associated tyrosine kinase, we identified Janus kinase 2 (JAK2) as a tyrosine kinase that binds to activated GH receptor. GH was found to promote the association of JAK2 with GH receptor, activate JAK2 and stimulate the phosphorylation of tyrosines within both the GH receptor and JAK2 [6]. These observations suggested the following mechanism by which GH binding to its receptor could initiate a variety of cellular responses (Fig. 1): binding of GH to the GH receptor increases the affinity of JAK2 for GH receptor and activates JAK2. JAK2 phosphorylates tyrosines within itself and the associated GH receptor. The phosphorylated tyrosines within JAK2 and GH receptor form high-affinity binding sites for a variety of signaling proteins containing Src homology (SH) 2 and other phosphotyrosine-binding domains. Recruitment of these signaling molecules to GH receptor/JAK2 complexes initiates a variety of signaling pathways that lead to the regulation

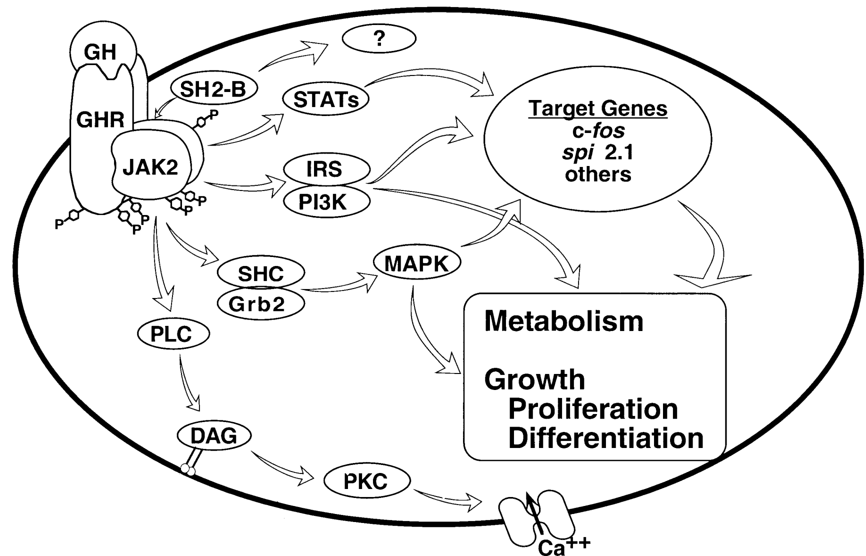

Fig. 1 Possible signaling pathways initiated by binding of growth hormone (GH) (*GHR* GH receptor, *P* phosphorylated tyrosines, *JAK* Janus kinase, *IRS* insulin receptor substrate *P13K* phosphatidylinositol-3-kinase, *PLC*, phospholipase C, *DAG* diacylglycerol, *PKC* protein kinase C, *MAPK* mitogen-activated protein kinase, *STAT* signal transducer and activator of transcription) [Adapted with permission from Carter-Su C, Smit LS (1998) Recent Prog Horm Res 53:61–83]

of transcription of specific genes, cellular metabolic enzymes, and the actin cytoskeleton, ultimately leading to GH stimulation of body growth and changes in protein, carbohydrate and fat metabolism. This review identifies a number of such signaling molecules that are recruited into GH-signaling pathways as a consequence of JAK2 activation by GH and discusses what is known about how those signaling pathways mediate the diverse responses to GH.

The GH receptor is a member of the family of cytokine receptors, all of which are known to activate members of the JAK family of tyrosine kinases

The GH receptor was the first receptor cloned of what is now known as the cytokine family of receptors. This family includes receptors for the hormones growth hormone, prolactin and leptin. It also includes the receptors for most of the interleukins (ILs) (ILs 2–7, 9–13), interferons α/β and γ, erythropoietin, thrombopoietin, granulocyte colony-stimulating factor, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor, granulocyte macrophage colony-stimulating factor, and cardiotropin (reviewed in [7]). Receptors in the cytokine receptor family were originally classified as a family based upon limited homology in the extracellular domain, including two pairs of cysteines and a WSXWS motif [8]. Eventually, it was recognized that members of this family also share some homology in the cytoplasmic domain, in particular, one or two proline-rich motifs [9, 10, 11]. At the time that our laboratory identified GH and prolactin receptors as binding to JAK2 [6, 12], Ihle and colleagues identified JAK2 as a signaling molecule for the receptors for erythropoietin and IL-3 [13, 14]. It is now known that all members of the cytokine family of receptors activate one or more members of the JAK family of tyrosine kinases, a family that presently consists of JAK1, JAK2, JAK3 and Tyk2 [7]. The recognition that all members of the cytokine family bind to and activate members of the JAK family of tyrosine kinases suggested that some of the signaling pathways utilized by the non-hormone receptors in the cytokine family may also be used by the GH receptor.

GH activates multiple members of the signal transducers and activators of the transcription (Stats) family

The pathway that immediately drew our attention was one that had been identified for the IFNs (interferons) and involves what are now referred to as signal transducers and activators of transcription (Stats). Presently, seven Stat proteins have been cloned: Stats 1, 2, 3, 4, 5a, 5b and 6. All Stats contain an SH2 domain. In response to ligand stimulation, they are recruited to phosphorylated tyrosines within hormone receptor/JAK complexes and are phosphorylated themselves on tyrosines. All of them homo- or heterodimerize, move to the nucleus, bind specific sequences of DNA and regulate the transcription of ligand-specific genes [15]. Our laboratory and others investigated the ability of GH to utilize Stat proteins to regulate gene transcription. As illustrated in Fig. 2, GH was found to stimulate the tyrosyl phosphorylation of Stats 1, 3 and 5. It did not stimulate the tyrosyl phosphorylation of Stat2 (data not shown). Consistent with these Stats serving as signaling molecules for GH, GH also stimulated their binding to Stat-specific sequences of DNA [16–20]. These results, and others investigating the ability of GH to regulate the expression of Stat-specific reporter gene constructs [21, 22], provide strong evidence that GH regulates gene transcription at least in part by recruiting and activating Stats 1, 3, 5a, and 5b (reviewed in [7]).

Figure 2 also illustrates the interesting and important observation that different ligands are more effective than

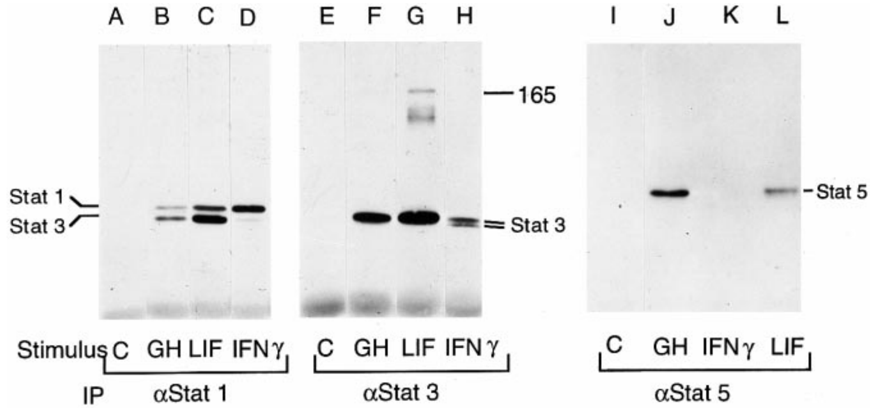

Fig. 2 GH, leukemia-inhibitoy factor (LIF), and interferon-γ (IFNγ) induce tyrosyl phosphorylation of Stat1, Stat3 and Stat5 in 3T3-F442A fibroblasts. 3T3-F442A fibroblasts were incubated for 15 min in the absence of hormone or with 500 ng/ml human GH, 25 ng/ml human LIF or 10 ng/ml murine IFNγ. Whole cell lysates were immunoprecipitated with antibodies to Stat1 (αStat1), Stat3 (αStat3) or Stat5 (αStat5), as indicated. Immunoprecipitated proteins were subjected to Western blot analysis using anti-phosphotyrosine antibody. Migrations of Stat1, Stat3 and Stat5 are indicated [Reprinted with permission from Carter-Su C, King APJ, Smit LS, VanderKuur JA, Argetsinger LS, Campbell GS, Huo W (1997) J Anim Sci 75 [Suppl 2]:1–10]

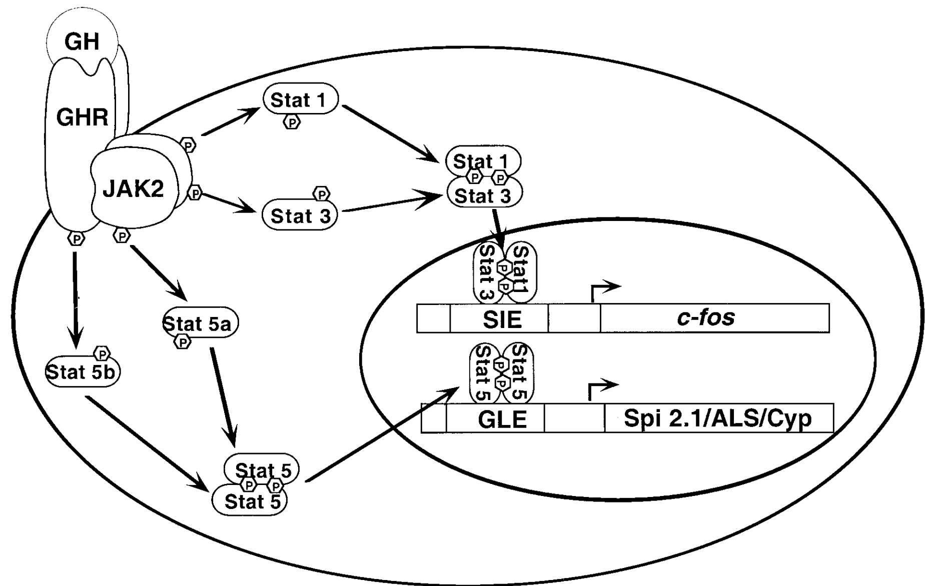

Fig. 3 GHR signaling via signal transducers and activators of transcription (Stat) proteins (*P* phosphotyrosines, *SIE* Sis-inducible element, *GLE* interferon-γ activated sequence-like element, *Spi* serine protease inhibitor, ALS acid labile subunit) (Adapted with permission from [7])

others in activating specific Stat proteins, even in a single cell type. Thus, in 3T3-F442A cells, GH is better than IFNγ or leukemic inhibitory factor (LIF) at stimulating tyrosyl phosphorylation of Stat5, whereas IFN-γ is the most effective at stimulating tyrosyl phosphorylation of Stat1 and LIF is the most effective of the three ligands at stimulating tyrosyl phosphorylation of Stat3. Differences in the level of JAK activation cannot explain this ligand preference for specific Stats. In 3T3-F442A cells, GH is more than an order of magnitude better than LIF or IFNγ at activating JAK2 (judged by JAK2 tyrosyl phosphorylation), and JAK1 is activated to a similar extent by all three ligands [18]. It is currently thought that ligand specificity of Stat activation is due largely to the presence of high-affinity binding sites for specific Stat proteins in the receptor. Thus, Stat3 has been shown to bind with high affinity to the motif pYXXQ [23]; the receptor for LIF contains seven such motifs, whereas the receptors for IFNγ and GH contain no such motifs. Similarly, the receptor subunit for IFNγ contains a high-affinity binding site for Stat1 [24] that is absent from the receptors for LIF and GH. Based upon these observations, we and others hypothesized that the receptor for GH may contain multiple high-affinity binding sites for Stats 5a and 5b. Subsequent studies suggest that GH receptors do contain multiple binding sites for Stat5a and 5b [25, 26], explaining at least in part why GH is such a potent activator of Stat5a and 5b. In terms of their physiological role in GH action, Stats 1 and 3 have been implicated in the regulation of c-*fos* gene expression by GH [16, 17, 22, 27], whereas Stats 5a and 5b have been shown to bind to gamma-activated sequence (GAS)-like sequences in the promoters of the genes encoding serine protease inhibitor 2.1, insulin, the acid labile subunit (ALS) of

IGF-binding proteins, and a number of male-specific cytochrome P450 enzymes (Fig. 3) [21, 25, 28–30]. Studies using mice lacking Stat5a, Stat5b or both have also implicated Stat5a and/or Stat5b in the regulation of some of these genes and in the growth-promoting actions of GH [31–33].

While the phosphorylation, dimerization and DNA-binding properties of Stats have been well studied, little is known about how Stat proteins move from cytokine receptor-JAK complexes, presumably at the plasma membrane, to the nucleus. Our laboratory has started to investigate this process by using green fluorescent protein (GFP)-tagged Stat5b [34]. When expressed in monkey kidney derived COS-7 cells, murine NIH-3T3 fibroblasts or human fibrosarcoma 2C4 cells, GFP-tagged Stat5b was observed to be distributed within the cytoplasm and the nucleus, as revealed by laser scanning confocal microscopy of live cells. GH stimulation caused the GFP-Stat5b to accumulate in the nucleus of all three cell types within 30 min. When alanine substitutions were introduced into several regions of Stat5b and GH-dependent nuclear localization was assessed, only the mutation that prevented binding to DNA (466VVVI469) abrogated GH-stimulated nuclear localization. This mutant fusion protein is tyrosyl phosphorylated and dimerizes in response to GH. These results suggest that either high-affinity binding to DNA contributes to nuclear accumulation of Stat5b or that amino acid residues 466–469 are crucial for two functions, accumulation of Stat5b into the nucleus and DNA binding.

GH stimulates the Ras-MAP kinase and insulin receptor substrate-phosphatidylinositol 3' kinase pathways

GH binding to GH receptors initiates a variety of pathways in addition to pathways involving Stat proteins. Two of these include the Ras-MAP kinase pathway and the pathway involving the insulin receptor substrate (IRS) proteins (Fig. 1). We have shown that GH binding to its receptor recruits the SH2 domain containing adapter protein Shc, to GHR-JAK2 complexes, at least in part via the SH2 domain of Shc (Fig. 4). Shc becomes phosphorylated on tyrosines, presumably by the GH receptor-associated JAK2. The phosphorylated Shc then binds grb2-SOS complexes, presumably via the SH2 domain of grb2. This results in the activation of the small GTP-binding protein Ras, which results in the activation of the serine/threonine kinase Raf, which activates the mixed function tyrosine/serine/threonine kinase MEK, which activates the extracellular signal regulated kinases ERKs 1 and 2 [35, 36]. Whereas MEK appears to be quite specific for ERKs 1 and 2, ERKs themselves are thought to phosphorylate many different proteins, including cytoskeletal targets (e.g., MAPs 1, 2, 4, Tau and caldesomon), cytosolic proteins (e.g., p90rsk, cytoplasmic phospholipase A2, phospholipase (γ) and nuclear proteins, including Elk-1, c-*fos*, c-Jun, ATF-2, c-myc and p53 [37, 38]. Of these potential

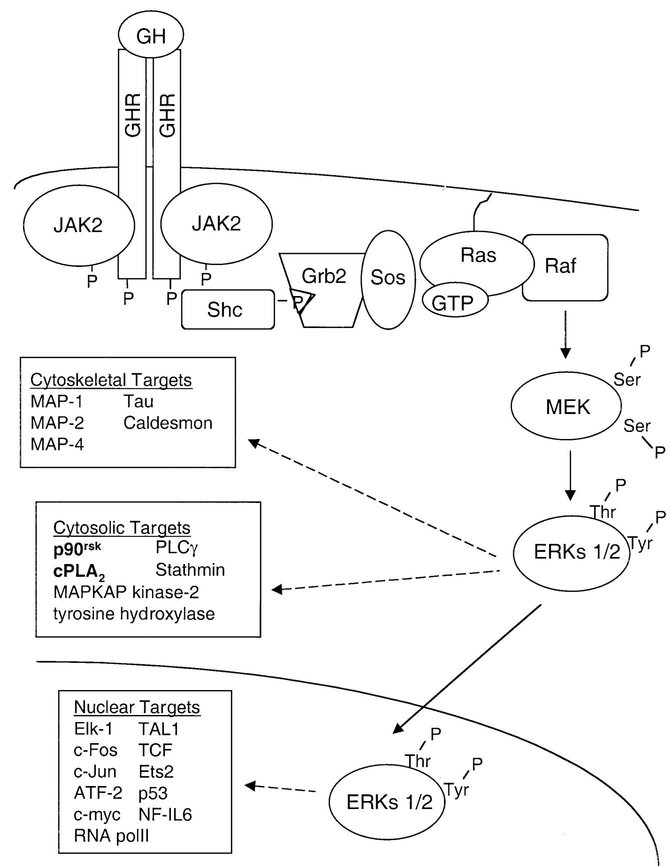

Fig. 4 GHR signaling via the Ras-MAP Kinase pathway. *Solid arrows* and *signaling molecules* and *bold targets* indicate pathways and proteins regulated by GH. *Dotted arrows* and *targets in medium type* indicate pathways and targets utilized in cells treated with other cytokines and growth factors that activate MAP kinase or are detected in vitro. These have not yet been shown to be involved in GH signal transduction (*PLA2* phospholipase A2, *TCF* ternary complex factor, *GTP* guanosine triphosphate, *Sos* son of sevenless, *MEK* MAP kinase/ERK kinase, *TAL* T-cell acute lymphoblastic leukemia gene, *NF* nuclear factor IL-6, *ATF* activating transcription factor-2, *MAPKAP* MAP kinase activated protein kinase-2) (Reprinted with permission from [7])

substrates, GH has been shown to stimulate the phosphorylation of p90rsk [39], phospholipase A2 [40], and Elk-1 [41], suggesting that GH regulation of at least some of these proteins is mediated by this MAP kinase pathway. Interestingly, the c-*fos* gene, which is regulated by GH, contains both a Sis-inducible element, which is known to bind Stats 1 and 3, and a serum response element (SRE), which binds SRF and ternary factor, such as Elk-1. Both the Sis-inducible element (SIE) and SRE have been shown to be required for maximal activation of the c-*fos* promoter [42], suggesting that the different pathways initiated by GH may act in concert to regulate specific cellular functions, such as c-*fos* gene expression.

Insulin receptor substrates represent another pathway by which GH may elicit some of its cellular effects (Fig. 5). We and others have now shown that GH stimulates the tyrosyl phosphorylation of IRS1 and IRS2, presumably by JAK2 although the exact mechanism by

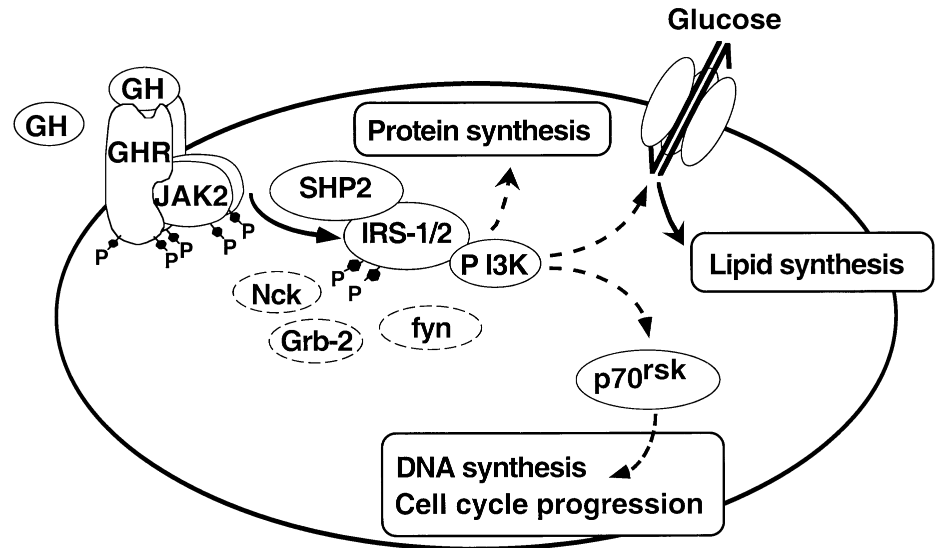

Fig. 5 GHR signaling via insulin receptor substrate-1 (IRS-1) and IRS-2. *Solid molecules* and *arrows* indicate signaling molecules and pathways regulated by GH. *Dashed molecules* and *arrows* indicate signaling proteins known to bind IRS-1 and/or IRS-2 via SH2 domain-mediated interactions (Nck, Grb-2, fyn) in response to insulin, proteins with enzymatic activities that are stimulated by the action of phosphatidylinositol-3'-kinase (P13K), or signaling pathways utilized in cells treated with insulin. These have not yet been shown to be involved in GH signal transduction (*SHP2* SH2 domain containing tyrosine phosphatase) (Reprinted with permission from [7])

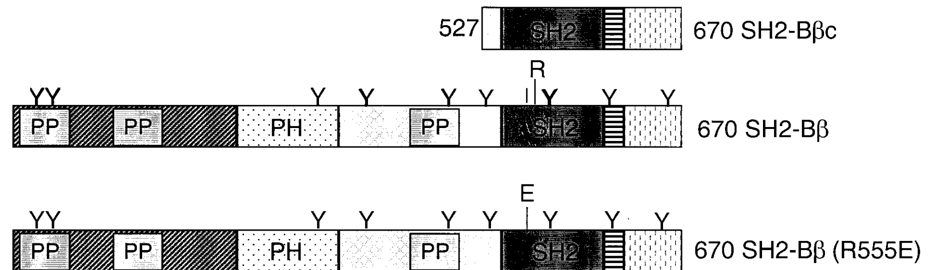

Fig. 6 Schematic representation of SH2-B isoforms, truncations and mutations. The PH and SH2 domains, proline-rich regions (PP), and all tyrosines (Y) are noted

which this occurs is unknown. The phosphorylated IRS proteins have been shown to recruit both the p85 regulatory subunit of phosphatidylinositol 3 (PI 3') kinase and the SH2-domain containing tyrosine phosphatase SHP2 [43–46]. Binding of both of these proteins to phosphorylated tyrosines within IRS proteins has been shown to activate their enzymatic activity [47–49]. Based upon what is known about the actions of PI 3-kinase and the actions of GH, pathways that may lie downstream of IRS and PI 3-kinase proteins include the following: (1) the rapid and transient stimulation of glucose transport and lipid synthesis seen in adipocytes following a period of GH deprivation; (2) regulation of MAP kinase activity and the downstream regulation of c-*fos* gene expression; (3) regulation of certain isoforms of protein kinase C; and (4) the regulation of p70rsk and the regulation of cell cycle progression [7].

GH stimulates the binding of SH2-Bβ to JAK2 and the tyrosyl phosphorylation of SH2-Bβ

In addition to the pathways just described, other laboratories have provided evidence for the activation of phospholipase C, increased diacylglycerol, increased activity of certain isoforms of PKC, and regulation of L-type calcium channels [7] (Fig. 1). Whether or not these responses to GH are dependent upon JAK2 is not yet known, although in at least one cell type the ability of GH to stimulate L-type calcium channels appears to be independent of JAK2 activation [50]. All of the signaling pathways just described were identified because they involve signaling molecules activated by other tyrosine kinases. In an effort to identify signaling molecules unique to GH or to JAK2-coupled receptors, we decided to pursue strategies that would identify new signaling molecules. To this end, we used the yeast two-hybrid system to try to identify signaling molecules that bound to activated JAK2. A truncated form of JAK2 containing the kinase domain was fused to the DNA-binding portion of Lex A and used as the bait to screen a rat fat tissue-derived cDNA library. By this technique, we identified an SH2 domain containing fragment of SH2-Bβ (SH2-Bβc) (Fig. 6) as a binding partner of activated, but not kinase-inactive, JAK2 [51]. SH2-B has the properties of an adapter or scaffolding protein (Fig. 6), in that it contains a number of potential protein-protein interaction domains and no homology to proteins with other known functions. Thus, it contains an SH2 and PH domain, multiple proline-rich domains, and multiple serines/threonines and tyrosines, which if phosphorylated could form binding sites for additional signaling proteins. We carried out multiple assays to verify that SH2-B and JAK2 form a complex [51]. Firstly, we showed in a yeast two-hybrid system that SH2-Bβ binds to constitutively activated and tyrosyl-phosphorylated wild-type JAK2 but not to unphos-

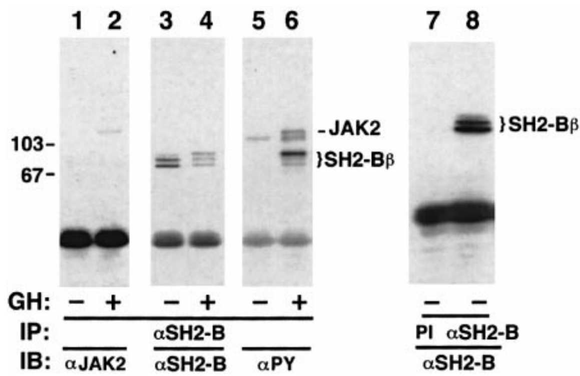

Fig. 7 GH-dependent association of SH2-Bβ with tyrosyl-phosphorylated JAK2 and tyrosyl phosphorylation of SH2-Bβ. 3T3-F442A cells were treated for 15 min with 500 ng/ml GH (lanes 2, 4 and 6) or with vehicle (lanes 1, 3, and 5). Whole cell lysates were immunoprecipitated with αSH2-B and subsequently immunoblotted with αPY (lanes 5 and 6). The blot corresponding to lanes 5 and 6 was stripped and reprobed with αSH2-B (lanes 3 and 4) and then stripped again and reprobed with αJAK2 (lanes 1 and 2). The migration of molecular weight standards ($x10^{-3}$), JAK2 and SH2-Bβ is indicated (Reprinted in modified form with permission form [51])

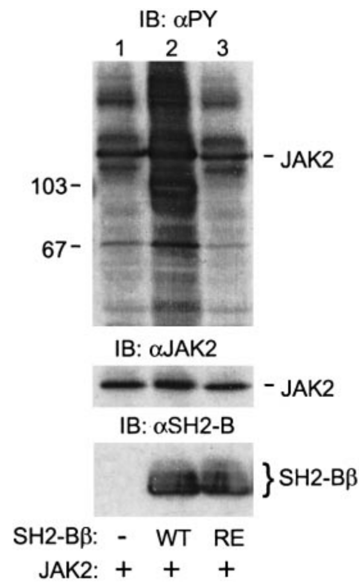

Fig. 8 The SH2 domain of SH2-Bβ is required for SH2-Bβ-induced tyrosyl phosphorylation of JAK2 and multiple other cellular proteins. COS cells were cotransfected with plasmid (4 μg) encoding JAK2 and either control plasmid (10 μg) or plasmid (10 μg) encoding SH2-Bβ (WT) or SH2-Bβ(R555E) (RE). After 48 h, cells were lysed, and proteins in the lysates were resolved by SDS/PAGE, immunoblotted with αPY (top panel), and reprobed with αJAK2 (middle panel). In a parallel experiment proteins in the lysates were immunoblotted with αSH2-B (bottom panel) (Reprinted in modified form with permission from [52])

phorylated, inactive JAK2 K882E in which the critical lysine in the kinase domain is replaced with a glutamine. Secondly, we showed that when SH2-Bβ and JAK2 are coexpressed in COS cells, SH2-Bβ and JAK2 form a complex that can be precipitated with antibody to SH2-Bβ (αSH2-Bβ). The SH2-Bβ that is coexpressed with wild-type but not kinase-dead JAK2 is tyrosyl phosphorylated. Thirdly, activated and tyrosyl-phosphorylated JAK2 from GH-treated 3T3-F442A cells binds to SH2-Bβ expressed as a glutathione-*S*-transferase fusion protein to a much greater extent than inactive, untyrosyl-phosphorylated JAK2 from control cells. The SH2 domain of SH2-B is sufficient for this interaction. Finally, αSH2-Bβ immunoprecipitates JAK2 from GH-treated 3T3-F442A fibroblasts to a much greater extent than from control 3T3-F442A cells (Fig. 7). GH also promotes the tyrosyl phosphorylation of SH2-B, suggesting that SH2-B not only binds to JAK2 in response to GH, but also is tyrosyl phosphorylated by the activated JAK2. Interestingly, when the αSH2-Bβ immunoprecipitate is immunoblotted with αSH2-Bβ, multiple bands migrating with a molecular weight appropriate for SH2-Bβ are visible, both in the presence and absence of GH. Based upon studies using the serine/threonine-specific phosphatase protein phosphatase 2A (PP2A), the multiple bands are believed to represent SH2-Bβ that is phosphorylated on serines/threonines to different extents. Taken together, these results suggest that, in response to GH, JAK2 is recruited to the GH receptor, is activated and autophosphorylates thereby forming one or more binding sites for the SH2 domain of SH2-Bβ. Upon binding to JAK2, SH2-Bβ itself becomes phosphorylated on tyrosines, resulting in additional binding sites for signaling molecules.

SH2-Bβ is a potent activator of JAK2 activity

Because SH2-Bβ binds directly to JAK2, we examined whether SH2-B might regulate the activity of JAK2. To facilitate these experiments, we introduced a point mutation in the SH2 domain of SH2-Bβ [SH2-Bβ (R555E)], which is known to prevent binding of SH2 domains to phosphorylated tyrosines (Fig. 6). This mutated SH2-Bβ (R555E) would be expected to show greatly diminished binding to JAK2. We coexpressed wild-type SH2-Bβ and SH2-Bβ (R555E) with JAK2 in COS-7 cells, prepared cell lysates, and blotted the lysates with antibody to phosphotyrosine. Figure 8 illustrates that cells overexpressing wild-type SH2-Bβ exhibit greatly enhanced levels of tyrosyl-phosphorylated proteins including JAK2. In contrast, tyrosyl phosphorylation of cellular proteins was not enhanced by SH2-Bβ (R555E). The increased tyrosyl phosphorylation required the presence of JAK2; overexpression of SH2-Bβ in the absence of JAK2 did not result in enhanced phosphorylation of cellular proteins. This result suggests that one function of SH2-Bβ is to stimulate the activity of JAK2. Subsequent studies support this initial finding [52]. Thus, overexpression of SH2-Bβ, but not of SH2-Bβ (R555E), stimulates the tyrosyl phosphorylation of JAK2 and the enzymatic activity of JAK2, the latter assessed by the ability of immunoprecipitated JAK2 to autophosphorylate in an in vitro assay. In addition, when GH receptor and SH2-B are coexpressed in COS-7 cells, the presence of SH2-B enhances

the ability of GH to stimulate the tyrosyl phosphorylation of endogenous JAK2. Consistent with SH2-B activating JAK2, overexpression of SH2-B with GH receptor and with Stat3 or Stat5b enhances the ability of GH to stimulate the tyrosyl phosphorylation of the respective Stat. Thus, one of the actions of SH2-B in GH signaling appears to be the enhancement of JAK2 activation by GH.

Summary and conclusions

All of these results contribute to our current understanding of how GH elicits its diverse responses in a variety of cell types. To summarize (Fig. 1), GH is thought to bind to two GH receptors. The resulting change in receptor structure and/or dimerization increases the affinity of the receptor for JAK2 and activates JAK2. The activated JAK2 tyrosyl phosphorylates itself as well as the GH receptor, forming high-affinity binding sites for a number of signaling proteins. Many of these proteins become phosphorylated on tyrosines, presumably by JAK2. These signaling proteins include the: (1) Stat proteins that are thought to regulate the transcription of a variety of GH-dependent genes; (2) IRS proteins that recruit PI 3-kinase and SHP2 and most likely other proteins, some of which are believed to regulate cellular metabolism and perhaps the transcription of some GH-dependent genes; (3) Shc proteins, which are thought to be the first step in the Ras-MAP kinase pathway, which has been shown to regulate a variety of cellular proteins; and (4) SH2-Bβ, a protein that activates JAK2 and may link other more specific signaling pathways to GH receptor/JAK2 complexes. It is remarkable to think that all of these pathways have been delineated largely within the past 6 years. Presumably, the next 6 years will reveal additional signaling pathways required for the known actions of GH, including the regulation of insulin-like growth factor-I, as well as for new actions of GH. Hopefully this new information will explain why children suffering from chronic renal failure are short and suggest therapies to make them grow taller.

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