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Recent advances in growth hormone signaling

Nathan J. Lanning · Christin Carter-Su

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Abstract Growth hormone (GH) is a major regulatory factor for overall body growth as evidenced by the height extremes in people with abnormal circulating GH levels or GH receptor (GHR) disruptions. GH also affects metabolism, cardiac and immune function, mental agility and aging. Currently, GH is being used therapeutically for a variety of clinical conditions including promotion of growth in short statured children, treatment of adults with GH deficiency and HIV-associated wasting. To help reveal previous unrecognized functions of GH, better understand the known functions of GH, and avoid adverse consequences that are often associated with exogenous GH administration, careful delineation of the molecular mechanisms whereby GH induces its diverse effects is needed. GH is a peptide hormone that is secreted into the circulation by the anterior pituitary and acts upon various target tissues expressing GHR. GH binding of GHR activates the tyrosine kinase Janus kinase 2 (JAK2), thus initiating a multitude of signaling cascades that result in a variety of biological responses including cellular proliferation, differentiation and migration, prevention of apoptosis, cytoskeletal reorganization and regulation of metabolic pathways. A number of signaling proteins and pathways activated by GH have been identified, including JAKs, signal transducers and activators of transcription (Stats), the mitogen activated protein kinase (MAPK) pathway, and the phosphatidylinositol 3′-kinase (PI3K) pathway. Although these signal transduction pathways have been well characterized, the manner by which GH activates these pathways, the downstream signals induced by these pathways, and the cross-talk with other pathways are not completely understood. Recent findings have added vital information to our understanding of these downstream signals induced by GH and mechanisms that terminate GH signaling, and identified new GH signaling proteins and pathways. This review will highlight some of these findings, many of which are unexpected and some of which challenge previously held beliefs about the mechanisms of GH signaling.

Keywords Growth hormone (GH) · Janus kinase 2 (JAK2) · Signal transducers and activators of transcription (Stats) · Mitogen activated protein kinase (MAPK) · Suppressor of cytokine signaling (SOCS) · Protein tyrosine phosphatase (PTP)

N. J. Lanning
Cellular and Molecular Biology Graduate Program,
University of Michigan Medical School,
1301 Catherine Street, Ann Arbor, MI 48109-0622, USA
e-mail: lanningn@umich.edu

C. Carter-Su (✉)
Department of Molecular and Integrative Physiology,
University of Michigan Medical School,
1301 Catherine Street, Ann Arbor, MI 48109-0622, USA
e-mail: cartersu@umich.edu

1 GH receptor dimerization and activation

The downstream signaling pathways mediated by the GHR are initiated upon the binding of GH to the extracellular domain of the GHR (Fig. 1a). Early analysis of the extracellular domain of GHR in association with GH indicated that one GH molecule binds sequentially to two GHR molecules [1]. Formation of this GH-GHR_2 trimer complex was thought to be necessary and sufficient for GH responses [1]. However, other studies indicated that dimerization of the GHR was insufficient for activation of GH-mediated signaling [2] and that preformed GHR dimers exist prior to GH binding [3]. These findings, along with

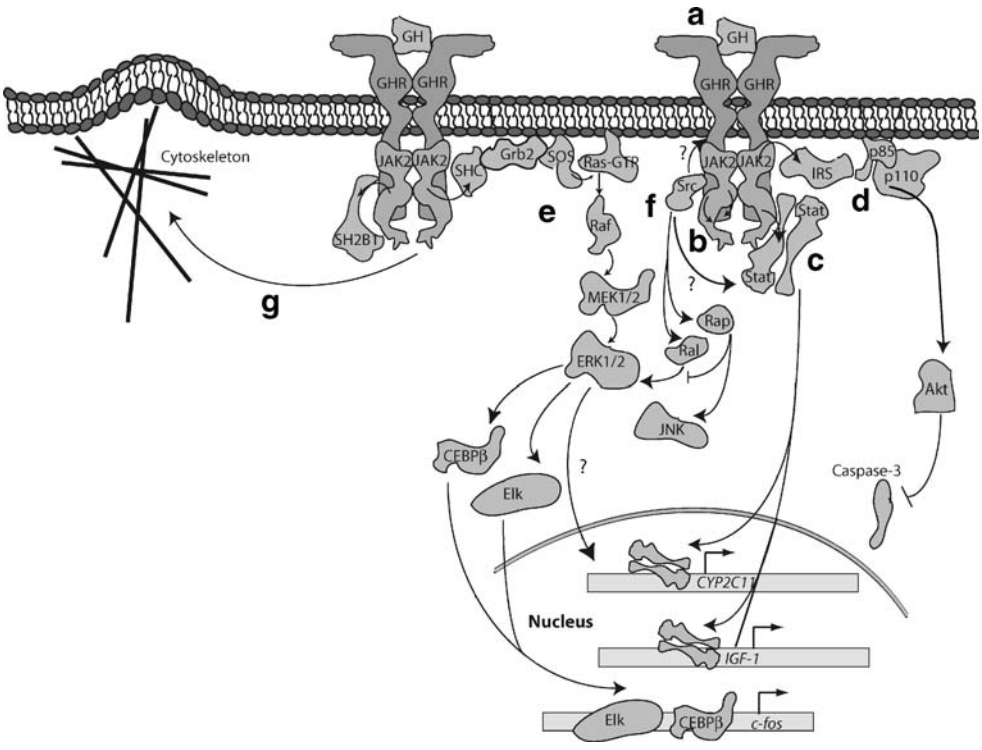

Fig. 1 Signal transduction pathways induced by growth hormone. **a** GH binds a GHR dimer, inducing a conformational change that activates two JAK2 molecules. **b** JAK2 activation induces JAK2 autophosphorylation and JAK2 phosphorylation on multiple GHR tyrosines. GH-activated JAK2 also phosphorylates and activates multiple signaling proteins and pathways including **c** Stats, **d** IRS and PI-3 kinase and **e** MAPK. **f** The binding of GH to GHR may also activate Src tyrosine kinase, initiating other signaling pathways. **g** GH induces changes in cytoskeletal dynamics

newer results from tests of GHR activation, lay the groundwork for a revised model of GHR activation. A recent study [4] confirmed that unliganded GHR exists as a dimer, using co-immunoprecipitation, fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET), and X-ray crystallography of the extracellular domain of GHR. Only minor differences were observed between the crystal structures of the liganded and unliganded GHR extracellular domain dimers. However, inducing a nominal 40° clockwise rotation in the lower α-helical transmembrane sequence by insertion of alanine residues or a nominal 100° clockwise rotation in the juxtamembrane helix of GHR just N-terminal of the Box 1 domain containing the JAK2 binding site resulted in constitutive activation of JAK2 and Stat5. From these and other findings, Brown et al. [4] suggested a model whereby GH binding asymmetrically at the receptor binding sites of preformed GHR dimers causes the intracellular domains of the GHR to undergo relative rotation. Because the cytoplasmic domain of each GHR molecule is thought to bind a single JAK2 molecule, this rotation is postulated to bring two JAK2 molecules into sufficient proximity to allow each JAK2 molecule to phosphorylate the activating tyrosine residue in the kinase domain on the other JAK2 molecule, thereby activating JAK2. Since GH binding has also been reported to increase the co-immunoprecipitation of JAK2 with GHR [5], the GH-induced conformational change in GHR may also increase the stability of the GHR-JAK2 interaction.

2 GH signal transduction via JAK2

Activation of JAK2 is thought to be the key step in initiating GH signaling [5]. The FERM domain of JAK2 is thought to mediate JAK2 binding to the cytoplasmic Box 1 region of GHR [6–8]. Following GH activation of JAK2, JAK2 autophosphorylates multiple tyrosines [9, 10] and subsequently phosphorylates multiple tyrosine residues in GHR [11] (Fig. 1a). Based upon JAK2 overexpression systems, some of the autophosphorylation sites in JAK2 appear to be regulatory sites since mutating them has been shown to either decrease (e.g. tyrosine 221) or stimulate (e.g. tyrosines 119, 570, 1,007) JAK2 activity [9, 12–14]. For some of those tyrosines, phosphorylation is thought to cause a conformational change in JAK2 that alters JAK2 activity. For example, phosphorylation of tyrosine 1007 is thought to expose the substrate and/or ATP binding sites [12] whereas phosphorylation of tyrosine 119 is thought to promote dissociation of JAK2 from its associated cytokine family receptor [14]. Autophosphorylation of some tyrosines are alternatively or additionally thought to regulate JAK2 activity indirectly by recruiting regulatory proteins to JAK2. For example, phosphorylated tyrosine 1007 has also been shown to bind the negative regulators of cytokine signaling SOCS1 [15], SOCS3 [16] and the phosphatase PTP1B [17] (discussed in more detail below). Autophosphorylation of tyrosine 813 appears to enhance JAK2 activity as a consequence of recruiting the adaptor protein, SH2B1 (also known as SH2-B or PSM-β) [10]. SH2B1 has

been hypothesized to either stabilize the active conformation of JAK2 [18] or promote the dimerization of JAK2 [19]. Some of the autophosphorylation sites in JAK2 (e.g. tyrosine 966 [20]) as well as the phosphorylated tyrosines in GHR, are thought to serve as docking sites for signaling molecules containing Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains. Based on mutational studies, seven different tyrosines within the cytoplasmic domain of the GHR have been implicated in at least one downstream GH response (reviewed in [21]). For example, five or six phosphorylated tyrosines in GHR have been hypothesized to bind Stat5a and Stat5b, based upon decreased GH-dependent Stat5 tyrosyl phosphorylation or Stat5-dependent responses in cells expressing mutated or truncated GHR [11, 22–24]. Recruitment of these signaling molecules to GHR-JAK2 complexes and their activation allows GH to elicit diverse biological and physiological effects. A number of signaling proteins and pathways are thought to be initiated at least in part as a consequence of binding to activated GHR-JAK2 complexes (reviewed in [21, 25]). Examples include Stats 1, 3, 5a and 5b, the MAPK pathway, and the phosphatidylinositol 3′-kinase (PI3K) pathway (Fig. 1b and c).

3 GH signal transduction via Src tyrosine kinase

One of the more interesting recent developments in GH signaling is additional support for the hypothesis that not all GH signaling events lie downstream of JAK2. Zhu et al. [26] provide evidence using both pharmacological inhibitors and kinase inactive proteins in NIH3T3 cells that the tyrosine kinase Src is activated by GH independent of JAK2. Using the same reagents, the same group reported that full activation of the Ras-like small GTPases RalA, RalB, Rap1 and Rap2 by GH requires both c-Src and JAK2 [26, 27] (Fig. 1c) whereas activation of Stat5 requires only JAK2. Activation of RalA by GH was linked to increased phospholipase D activity and the formation of its metabolite, phosphatidic acid, which were in turn linked to GH-activation of extracellular regulated kinases (ERKs) 1 and 2 and subsequent Elk-1-mediated transcription [26], suggesting that GH activation of ERKs 1 and 2 is at least partially dependent upon GH activation of Src and independent of JAK2. GH-dependent Rap1 activity appears to be dependent on CrkII-C3G activation and capable of mediating CrkII enhancement of GH-stimulated JNK/SAPK activity. Rap1 was also implicated as an inhibitor of GH activation of RalA and its subsequent stimulation of ERKs 1 and 2 [27]. The latter suggests that the balance between Ral and Rap protein activation by GH would affect the relative levels of activation of ERKs 1/2 versus JNK/SAPK (Fig. 1c). Using a COS7 cell overexpression system, Manabe et al. [28] also found GH to modestly stimulate Src activity. They showed that Src can bind to and phosphorylate GHR and used Src inhibitor or anti-sense to implicate Src in GH-dependent tyrosyl phosphorylation of GHR and Stat5a/b but not JAK2 in F-36P human leukemia cells (Fig. 1c). Cell type specificity was one hypothesis put forward to explain the apparent discrepancy between these two groups regarding the role of Src in Stat5b phosphorylation [28]. Previous studies in IM-9 and CHO cells based upon truncated and mutated GHR and JAK2 inhibitors had suggested that regulation of cellular [Ca^{2+}] by GH may also be JAK2 independent [21], raising the possibility that this function might also be mediated by Src. Although recent inhibitor studies by Zhang et al. [29] found that human GH-induced increases in cytosolic free Ca^{2+} in and insulin secretion from BRIN-BD11 beta cells appeared to be dependent upon activation of both JAK2 and Src, these actions were not mediated via the GHR but rather the prolactin receptor which can also bind human GH. In contrast, the rise in cytosolic free Ca^{2+} elicited by bovine GH, which binds only to the GHR, was not blocked by inhibitors of either JAK2 or Src. Thus, these data support the GHR-mediated increase in cytosolic free Ca^{2+} being independent of JAK2. They also suggest that GH binding to the GHR activates an as yet unidentified, early signaling protein in addition to JAK2 and Src.

4 GH regulation of Stat transcription factors

A number of the responses to GH involve transcription factors and gene expression. Among these transcription factors, members of the SH2 domain-containing signal transducers and activators of transcription (Stat) family of proteins have been shown to be particularly important for JAK2-mediated GH signaling [30] and will be discussed below. The regulation of other transcription factors is outside the scope of this review, and is described elsewhere in detail [30, 31]. Activation of Stat proteins is known to require tyrosine phosphorylation-dependent homo or heterodimerization, a process that is facilitated by the GH-dependent creation of Stat binding sites on the activated GHR-JAK2 complex (Fig. 1b). Once bound to these sites on the activated GHR-JAK2 complex, Stats 1, 3, 5a and 5b are thought to be phosphorylated by JAK2 after which they dimerize, translocate to the nucleus and act as transcription factors for many important GH-regulated genes [30, 32, 33]. Many past and recent studies have shown that activation of Stat5a and Stat5b is critical for a variety of GH functions, including changes in metabolism, body growth and sex-dependent liver gene regulation (reviewed in [30, 33].

Although Stat5a and Stat5b have been implicated in body growth via mouse gene deletion studies, only recently

have Stat5b binding sites in the IGF-1 gene promoter elements been identified and shown to regulate IGF-1 transcription in a GH-dependent manner through Stat5b [34, 35]. In support of Stat5 being important for GH-dependent IGF-I levels in serum and body growth, a patient with severe growth retardation and immunodeficiency has been found to have a mutation in the *Stat5b* gene that results in the loss of GH-induced tyrosyl phosphorylation of Stat5b [36] (Fig. 2a).

Although current dogma dictates that JAK2 phosphorylates Stat5 through direct interactions between GHR, JAK2 and Stat5, Adriani et al. [37] have recently demonstrated a requirement for the common cytokine receptor γ-chain (γ_c) for proper GH-mediated Stat5b activation in B cell lines. These investigators found that in EBV-transformed lymphocytes from γ_c negative X-SCID patients, GH was able to normally activate JAK2, but GH-dependent Stat5b phosphorylation and nuclear localization were significantly suppressed. These cells exhibited a total loss of GH-induced proliferation. Reconstitution of X-SCID patient BCLs with wild-type γ_c resulted in normal GH-induced phosphorylation of Stat5b and nuclear localization. This study suggests a novel dependence of GH signaling on the common cytokine receptor γ-chain in certain cell types, consistent with the presence of γ-chains in non-hematopoietic tissues and short stature of X-SCID patients (Fig. 2b). Whether this effect of γ-chain on GHR signaling is a direct or indirect effect remains to be determined.

Another clinical report in 2006 describes patients diagnosed with idiopathic short stature whose fibroblasts exhibit normal activation of Stats 5a and 5b but impaired activation of Stat3 [38] (Fig. 2c). Idiopathic short stature is characterized by a normal birth weight and no endocrine abnormalities but a retarded growth velocity and a height more than two standard deviations below the mean. In fibroblasts taken from these patients, GH-induced Stat3 activation was attenuated, cyclin levels were reduced and levels of $p21^{WAF/CIPI}$ (a negative regulator of the cell cycle) were elevated [38]. The idiopathic short stature phenotype and high $p21^{WAF/CIPI}$ levels in the human fibroblasts were reversed by treatment of patients with exogenous GH. Further studies are needed to know whether the elevated levels of $p21^{WAF/CIPI}$ and/or suppressed Stat3 activation are responsible for the short stature.

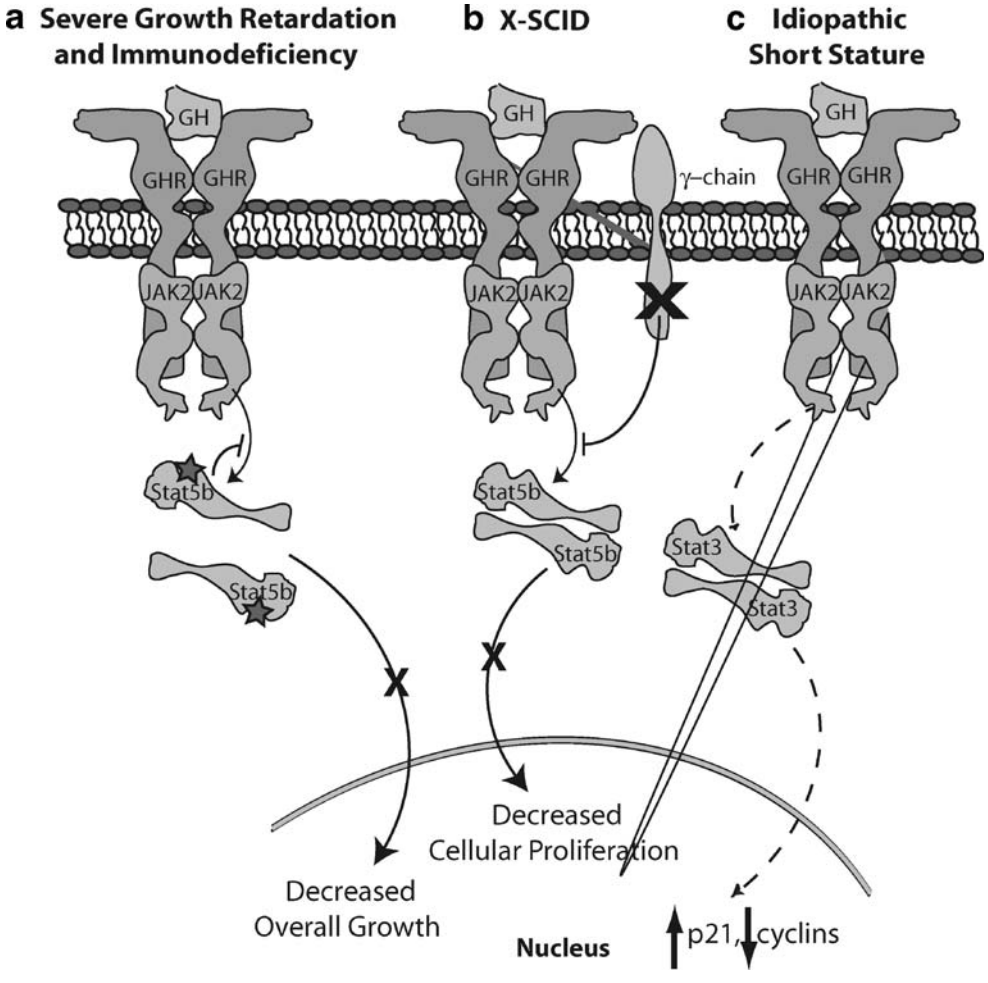

Fig. 2 Diseases and disorders associated with abnormal GH-induced JAK-Stat signaling. **a** A patient has recently been identified who has severe growth retardation and immunodeficiency as a result of a mutation in the Stat5b gene that abrogates JAK2-dependent phosphorylation and nuclear localization of Stat5b. **b** EVB-transformed lymphocytes from X-SCID patients lacking the common cytokine receptor γ-chain exhibit decreased GH-induced JAK2-dependent phosphorylation and nuclear localization of Stat5b. **c** Fibroblasts from several patients exhibiting idiopathic short stature exhibit attenuated GH-induced JAK2-dependent phosphorylation and nuclear translocation of Stat3, increases in the cell cycle inhibtor, p21, and decreases in cyclins

5 GH signal transduction via MAPK and phosphatidylinositol 3′-kinase pathways

The Ras/MAPK pathway has also been shown to be activated by GH (reviewed in [21, 25]). GH has been shown to stimulate the binding of the adapter protein Shc to GHR-JAK2 complexes; the tyrosyl phosphorylation of Shc and its binding to Grb2 and the guanine nucleotide exchange factor, SOS; and the activity of Ras, Raf, mitogen-activated protein kinase/extracellular-regulated protein kinase (MEK) and finally ERKs 1 and 2 [39, 40–42] (Fig. 1d). Although several groups have linked GH activation of ERKs 1 and 2 to JAK2, the Lobie laboratory have data suggesting that GH might also regulate ERKs 1 and 2 by a Src-dependent, JAK2-independent pathway that involves phospholipase D and RalA and RalB [26] or by a c-Src-FAK-Grb2 complex (reviewed in [25]). Alternatively, Yamauchi et al. [43] propose that GH activates the MAPK pathway by stimulating tyrosyl phosphorylation of a Grb2 binding site in the epidermal growth factor receptor. GH activation of the Ras/ERK pathway has been linked to GH activation of a variety of proteins (reviewed in [25]). Examples include phospholipase A2, which has been linked to GH-induced P450-catalyzed formation of an active arachidonic acid metabolite and expression of CYP2C12; and the transcription factor Elk1 whose phosphorylation by ERK1/2 is required for transcription via the *c-fos* serum response element (SRE). More recently, GH-induced ERK1/2 has been shown to phosphorylate the transcription factor CEBPβ, an event that has been implicated in CEBPβ nuclear translocation [44, 45] (Fig. 1c) and differentiation of 3T3-F442A preadipocytes into adipocytes [46]. Although Stat5b has been thought largely responsible for sex-dependent liver gene expression, including regulation of expression of the CYP2C11 gene [33], Verma et al. [47] raise the possibility that ERK1/2 may also regulate expression of CYP2C11 gene, based upon correlative data using different doses of pulsatile GH replacement therapy in mice (Fig 1c).

Recent results from Yang et al. [48] indicate that in 3T3-F442A cells, GHR is selectively enriched in caveolar and lipid raft domains of the plasma membrane. GH stimulation induced in this fraction accumulation and activation of Ras/MAPK, but not Stat signaling molecules. Disruption of these fractions with methyl-β-cycoldextrin inhibited GH-induced ERK1/2 activation, but had no effect on GH-stimulated Stat5 activation. These findings imply that GHR membrane localization may be important for the initiation of different GH-induced signal transduction pathways and that GH induction of ERKs 1/2 and Stat5 may require GHR in different cellular compartments.

In addition to activating the Ras/MAP kinase pathway, GH has also been shown to stimulate the PI-3 kinase pathway (reviewed in [25], Fig. 1b). One possible mechanism whereby GH activates PI-3 kinase is through tyrosyl phosphorylation of the large adaptor proteins designated insulin receptor substrate (IRS) proteins because of their known role in insulin signaling. GH stimulates the tyrosine phosphorylation of IRS-1, IRS-2 and IRS-3, and phosphorylation of these IRS proteins is known to lead to their association with multiple signaling molecules including the p85 subunit of PI-3 kinase. Other data suggest that GH might activate PI-3 kinase through a CrkII-IRS-1 interaction [49] or through binding of the p85α and p85β subunits of PI-3 kinase, as they have been shown to be capable of binding directly to phosphotyrosine residues in the carboxy-terminus of the GHR [50]. Activation of PI3 kinase has been linked by inhibitor studies to GH stimulation of glucose transport [51] and the anti-apoptotic serine kinase AKT [52]. This GH-dependent activation of Akt has been shown to be dependent on the presence of the JAK2 binding region of GHR, and implicated in GH promotion of cell survival, possibly through inhibition of the pro-apoptotic caspase-3 protein [53]. GH-induced activation of p70S6K, a kinase involved in the control of cell proliferation and differentiation, has also been shown to be activated in PI-3 kinase-dependent and PKC-dependent manners [54–56].

6 GH regulation of the actin cytoskeleton

Because GH is able to induce cell motility, and proliferation and cellular differentiation, it is not surprising that GH has also been implicated in actin reorganization [57] (Fig. 1e). The Lobie group initially presented evidence that treating GH-responsive cells with GH results in a rapid depolymerization of actin stress fibers, followed by the formation of focal, filamentous, actin containing complexes [57], as well as alterations in cellular microtubule physiology [58]. They have implicated the GH-stimulated formation of a p130cas-CrkII complex that also contains the p85 subunit of PI-3 kinase in the control of cytoskeletal dynamics [59], as their data indicate that PI-3 kinase regulates GH-stimulated reorganization of the actin cytoskeleton [57] and others have shown that formation of the p130cas-CrkII complex is sufficient for cell migration [60]. They also provide evidence for an interaction between JAK2 and FAK (focal adhesion kinase, an important regulator of cytoskeletal rearrangement) that results in tyrosyl phosphorylation of FAK and two of its substrates (paxillin and tensin) [61]. More recently, this group has implicated p38 MAPK as being important for GH-induced cytoskeletal rearrangements [62]. Emerging evidence indicates that GH-induced phosphorylation of the JAK2 adaptor protein, SH2B1β, is involved in GH regulation of the actin cytoskeleton, possibly by facilitating recruitment of Rac and other actin

regulating proteins to GHR-JAK2 complexes at the plasma membrane [63, 64].

7 Negative regulation of GH receptors

GH is secreted episodically and GH responses are transient. In contrast, prolonged activation of JAK2 has been associated with cell transformation and cancer. Thus, precise regulation of GH signaling is vitally important for the proper maintenance of body growth and metabolism, with down-regulation of GH signaling being an important aspect of proper GH signaling. Current knowledge of down-regulation of the GH signal reviewed here includes blockage or removal of SH2 and PTB binding sites by inhibitory molecules or dephosphorylation, and ubiquitin-dependent GHR endocytosis.

The suppressor of cytokine signaling (SOCS) family of proteins plays an important role in the negative regulation of GH signaling. There are eight members of the SOCS family, of which GH has been reported to induce the expression of four, namely, SOCS-1, -2, -3 and CIS (cytokine-inducible SH2-containing protein) (reviewed in [65]). SOCS proteins share a centrally located SH2 domain and a motif termed the SOCS box, which resides in the carboxy-terminus. Although it has been known for some time that the SOCS family is involved in negatively regulating GH signaling, more recent studies continue to shed light on the mechanisms of this regulation (Fig. 3). SOCS-1 is thought to bind the activating tyrosine in the kinase domain of JAK2 and inhibit JAK2 activity [66] (Fig. 3a). SOCS-3 has been shown to bind this residue in JAK2 as well as to phosphorylated residues in GHR [16, 67, 68]. SOCS-3's mechanism of GH signaling inhibition is thought to be by inhibition of JAK2 kinase activity through a mechanism dependent on SOCS-3 binding GHR (Fig. 3a). SOCS-2 has been shown to bind phosphorylated GHR GST fusion proteins and peptides [67–69]. CIS has also been shown to bind phosphorylated GST-GHR fusion proteins [67, 68]. The mechanism of SOCS-2 and CIS inhibition of GH signaling may be through inhibiting Stat5b binding of GHR, however, SOCS-2 and CIS inhibition of GH signaling seems to be less effective than SOCS-1 and SOCS-3 (Fig 3a). SOCS-1, and possibly SOCS-2 and SOCS-3, also appear to be involved in the ubiquitination of the GHR-JAK2 complex as each have been shown to be associated with ubiquitin ligase activity (reviewed in [65], Fig. 3b). Results using pharmacological inhibitors of proteasomes and dominant negative forms of CIS indicate that CIS negatively regulates GHR signaling at least in part by stimulating GHR

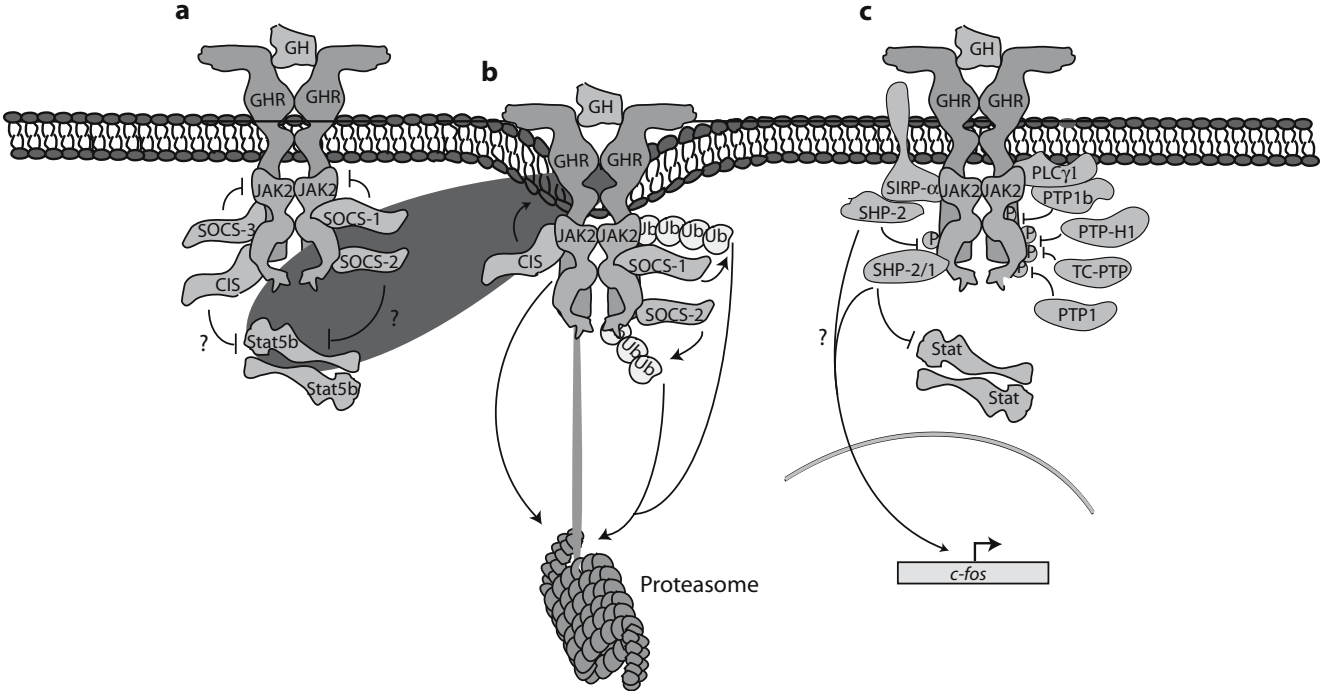

Fig. 3 Negative regulation of GH signaling. **a** SOCS-1 binds JAK2 and inhibits JAK2 kinase activity. SOCS-3 is thought to bind phosphorylated GHR and inhibit JAK2 kinase activity. SOCS-2 and CIS bind phosphorylated GHR and may compete with Stat5b for GHR binding sites. **b** Internalization and degradation of activated GHR may be facilitated by CIS and the ubiquitin ligase activity associated with SOCS proteins. **c** Protein tyrosine phosphatases negatively regulate GH signaling by binding the activated receptor complex and presumably dephosphorylating phosphotyrosines on JAK2, GHR or associated signaling proteins. SHP2 has been hypothesized to be both a positive and negative regulator of GH signaling

internalization and proteasomal degradation [70] (Fig. 3b). New evidence indicates that SOCS-2 may also inhibit GH responses in the animal indirectly by antagonizing IGF-1 signaling [71]. Mice lacking SOCS-2 are large [72], suggesting physiological relevance of SOCS-2 as a negative regulator of GHR. Although at high concentrations, SOCS-3 is able to down-regulate GH-induced JAK2 activity and has been shown to be associated with ubiquitin ligase activity, the physiological significance of SOCS-3 action on GH signaling is currently unclear because liver-specific SOCS-3$^{-/-}$ mice do not differ in size from wild-type littermates [73]. Similarly, CIS$^{-/-}$ and SOCS-1$^{-/-}$ mice are not bigger than normal [74, 75]. It is always possible that these SOCS proteins share some redundancy in function, and that increased body size would be observed if they were deleted in combination. Because SOCS proteins are synthesized in response to other ligands, a number of recent studies have investigated whether GH insensitivity is a consequence of elevated levels SOCS protein. In that regard, Leung et al. [76] have implicated SOCS-2 upregulation in the known ability of estrogen to inhibit GH signaling. Similarly, increases in SOCS 1 and 3 have been implicated in the ability of sepsis to inhibit GH signaling in liver [77, 78], and increases in SOCS 2 and 3 in the negative effect of uremia on hepatic GH signaling and growth [79].

Another important mechanism whereby GH signaling is thought to be negatively regulated is through protein tyrosine phosphatases (PTPs). A number of phosphatases have been reported to down-regulate GH signaling, including SH2 domain-containing protein-tyrosine phosphatase (SHP-1), SHP-2, protein-tyrosine phosphatase (PTP)-H1, PTP1, TC-PTP and PTP1b (reviewed in [65], Fig. 3c). SHP-1 has been implicated as a negative regulator of GH signaling based upon the observation that GH-dependent tyrosyl phosphorylation of JAK2 and DNA binding of Stat5b are prolonged in liver extracts from motheaten mice deficient in SHP-1 [80]. SHP-2 is reported to both positively and negatively regulate GH signaling. Based upon phosphatase inactive forms of SHP-2, Frank et al. [81] observed that overexpression of a catalytically inactive form of SHP-2 inhibited GH stimulation of *c-fos* enhancer-driven luciferase reporter, leading them to conclude that SHP-2 is a positive regulator of GH signaling. In contrast, Stofega et al. [82] reported that mutating the SHP-2 binding sites in GHR enhanced and prolonged GH-dependent tyrosyl phosphorylation of JAK2, GHR, and Stat5b, leading them to propose that SHP-2 is a negative inhibitor of GHR signaling. Because the dominant negative forms of SHP-2 could affect multiple steps in GH signaling, not just JAK2 activity, and mutation of GHR binding sites for SHP-2 could also affect binding of other proteins to GHR, definitive resolution of the role of SHP-2 in GH signaling awaits further studies. Interestingly, Stofega et al [83] have also shown that GH stimulates the tyrosyl phosphorylation of the JAK2-associated membrane protein SIRP-α (signal regulatory protein alpha). The phosphorylated tyrosines in SIRP-α recruit SHP-2 (Fig. 3c). Mutation of those tyrosines enhances GH signaling, suggesting that recruitment of SHP-2 to SIRPα in response to GH may also contribute to the attenuation of GH signaling [83]. Pasquali et al. [84] have recently shown that PTP-H1, PTP1, TC-PTP, and PTP1b are all able to dephosphorylate GHR. PTP1b knock-out mice also display increased JAK2, Stat5 and Stat3 phosphorylation in response to GH compared to wild type mice [85]. Interestingly, Choi et al. [86] have recently demonstrated that phospholipase Cγ1 provides a physical link between JAK2 and PTP1b in a GH-dependent manner, leading to attenuation of GH-induced signaling. Why phospholipase Cγ1 would serve as an adapter protein for PTP1b is unclear.

The Strous laboratory has elucidated mechanisms whereby GHR is internalized in both ubiquitin-dependent and independent manners. They have identified a motif in the cytosolic domain of GHR that recruits the ubiquitin conjugation system to GHR [87]. The recruitment of the ubiquitin conjugation system, as well as the activity of the proteasome, seem to be necessary for GHR internalization [88, 89]. Although both an intact ubiquitin conjugation system and full proteasome activity seem to be required for subsequent proteasome-specific degradation of GHR, actual conjugation of ubiquitin to GHR does not seem to be necessary. The ubiquitination, internalization and degradation of the GHR/JAK2 complex have also recently been reported by Rico-Bautista et al. [90] to depend on an intact actin cytoskeleton.

8 Receptor processing & subcellular localization

Like amyloid precursor protein, Notch, and ErbB4, GHR appears to undergo "regulated intramembrane processing," or RIP [91]. Following the same RIP program as the three receptors above, the extracellular domain of GHR is initially cleaved by the metalloprotease, tumor necrosis factor-α converting enzyme (TACE or ADAM-17), which results in shedding of the GHR extracellular domain [92]. The remaining membrane-bound GHR is clipped within the lipid bi-layer, releasing the intracellular portion of GHR into the cytosol [93]. The exact functional significance of the releasing of this domain of GHR into the cytosol is currently unknown. However, studies of other receptors undergoing RIP and those classically thought to be membrane-bound that are now being found in the cytosol may give hints as to the function of cytosolic GHR domain. Following RIP, ErbB4 has been shown to translocate to the nucleus where it plays a role in regulating transcription [94].

It is interesting to note that full-length GHR has been reported in the nucleus of various cell types. Using monoclonal antibodies specific for GHR, Lincoln et al. [95] identified GHR in the nucleus of a variety of normal and neoplastic cell types. Gevers et al. [96] found GHR present in the nucleus of both germinal and proliferating chondrocytes in the rat growth plate and Vespasiani Gentilucci et al. [89] found GHR in the nucleus of hepatocytes from patients in the later stages of chronic liver disease. It will be interesting to see if the γ-secretase-processed intracellular domain of GHR also translocates to the nucleus and to determine the function of nuclear GHR.

Along with being targeted to the nucleus, GH and GHR have been reported in the mitochondria [97, 98]. Perret-Vivancos et al. [99] recently reported that GH and GHR internalization through the caveolar pathway was essential for their targeting to the mitochondria, and hypothesized that mitochondrial targeting was required for the observed GH stimulation of cellular oxygen consumption.

9 Conclusions

Due to the ongoing vibrant research being invested in GH signaling by many laboratories around the world, key pieces to the puzzle of GH's mechanism of action are continually being elucidated. Concomitant with these findings is the realization that the signaling events initiated by the binding of GH to its receptor are diverse, multi-faceted and intricately related. There appear to be multiple ways to activate specific downstream responses to GH and multiple responses appear to share some of the same signaling proteins. Furthermore, differential levels and regulation of specific signaling proteins seem likely to contribute to the variations in GH signaling pathways and responses observed in different cell types and tissues while mutation or levels of multiple signaling proteins appear to contribute to the GH insensitivity observed in various disease states.

10 Key unanswered questions

Despite the wealth of knowledge accumulated on GH signaling, there are still many questions that need to be addressed. To truly understand how GH binding to GHR activates JAK2 requires a crystal structure of GHR bound to both GH and JAK2. Due to GH's diverse biological effects, there are most likely additional molecules and pathways as yet unidentified. Which molecules and pathways these are and how they interact need to be described. Determination of JAK2-independent pathways relevant for GH-mediated effects and further exploration of the role of Src in mediating GH effects are warranted. Defining the contributions to down regulation of GH signals of the many inhibitors of GHR-JAK2 action also needs to be addressed along with the biological significance of GHR internalization. What happens to the γ-secretase-processed GHR intracellular domain, and what role does GHR play in the nucleus? Laboratories around the world are currently investigating these questions and their findings will hopefully bring about the development of better treatments for patients.

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