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REVIEW ARTICLE

The molecular basis of growth hormone action

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Summary Recent studies have begun to elucidate the molecular actions of growth hormone, a major regulator of somatic growth and metabolic functions. The cell surface growth hormone receptor, a member of the cytokine receptor superfamily, binds as a dimer to a single growth hormone molecule. Receptor dimerization precedes signal transduction, which is predominantly mediated by the non-receptor tyrosine kinase, Jak2. Activation of Jak2 leads to mitogenic proliferation, phosphorylation of intracellular proteins, MAP kinase activation, activation of Stats 1, 3, and 5, and induction of target gene expression. Specific cytoplasmic domains of the growth hormone receptor mediate Jak2 activation, metabolic actions of growth hormone, Stat activation, and calcium influx.

GROWTH HORMONE

Human growth hormone (GH), a 191 amino acid polypeptide secreted by the anterior pituitary, has a molecular weight of 22 kDa, although a 20 kDa variant arises through alternative mRNA splicing that deletes residues 32–46. The sequence and structure of GH are evolutionarily conserved, and GH binds to its cognate GH receptor (GH-R) and/or GH binding protein (GH-BP) with K_d of ~0.3 nM, with slight variation according to cell type and species. GH is a member of the helix-bundle peptide hormone family, which possess a common tertiary structure comprised of four antiparallel α helices.[1] In GH, a long non-helical loop connects helices 1 and 2, a short region connects helices 2 and 3, and a long loop connects helices 3 and 4, resulting in an 'up-up-down-down' configuration. Two disulfide linkages are present: one between C^{53} of the interhelical loop between helix 1 and 2 and C^{165} of helix 4, and the other linkage near C^{182} and C^{189} of the carboxy-terminus.

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GH interacts with the GH-R/GH-BP through two separate interfaces on the surface of the GH molecule.[1–3] SITE 1 has a slightly concave interface of ~1230 Å^2, and is composed of residues on the exposed surface of helix 4, helix 1, and the interhelical region between helix 1 and 2. Alanine substitution has revealed a 'functional epitope' at the center of contact in SITE 1, a region where 8 out of 31 residues possess ~85% of the binding activity within this domain.[4] SITE 2 has a slightly smaller, flat interface of ~900 Å^2 composed of residues located on helix 1, helix 3, and the amino-terminus, and comprises only 8% of the total binding activity. The side chains of six residues (P^{61}, R^{64}, K^{172}, T^{175}, F^{176}, and R^{178}) of the GH molecule act to maintain binding to the GH-R.[4]

X-ray crystallographic analysis, mutational, and biophysical binding studies have demonstrated that a single molecule of GH binds to two molecules of GH-R or GH-BP, yielding a 1:2 stoichiometric ratio. The 'sequential dimerization model' proposes that GH binding to the GH-R occurs in sequential fashion, with SITE 1 initially binding to one GH-R, followed by SITE 2 interacting with a second GH-R, resulting in dimerization, a prerequisite for GH-R activation.[5] This model predicts that antagonists of GH can be generated by enhancing binding at

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SITE 1, at the expense of preventing binding at SITE 2. Evidence for this model is observed at high concentrations of GH *in vitro*, where excess GH binding at SITE 1 blocks dimerization with a second GH-R at SITE 2, as all GH-Rs are bound to GH at SITE 1.[6] A GH antagonist generated by mutating G^{120} to R results in a molecule ($G^{120}R$ GH) with high SITE 1 binding affinity and decreased SITE 2 binding affinity.[5] In support of the model, transgenic mice expressing $G^{120}R$ GH are phenotypically small;[7] although $G^{120}R$ GH may exhibit partial lactogenic agonist activity *in vitro*[8] and *in vivo*,[9] possibly through interaction with the prolactin receptor. $G^{120}R$ GH can induce GH-R dimerization and internalization, but is incapable of Stat transactivation.[10] GH binding to GH-R may induce an intracellular conformational change that cannot be mimicked by $G^{120}R$ GH.[11] Deletion of eight carboxy-terminal residues produces a GH analogue with increased SITE 1 affinity and biologic potency.[12]

GH-R STRUCTURE

Since its cloning a decade ago, GH-R has subsequently been found to be a member of the cytokine receptor superfamily, a group of receptors characterized by a single membrane-spanning domain, defined sequence homologies in the extracellular domain and juxtamembrane intracellular region and the absence of innate tyrosine kinase activity.[13] Class I cytokine receptors, which include GH-R, possess three pairs of disulfide bonds and a signature WSXWS motif in the proximal extracellular region (minimally conserved in GH-R as YGEFS).[1] Many of the cytokine receptors share common signal transduction mechanisms; prominent among these are interactions with members of the Jak-Stat pathway.[14]

The open reading frame for human GH-R is 638 amino acids long, including an 18 amino acid signal peptide, a 246 residue extracellular domain, a 24 amino acid transmembrane domain, and a 350 residue intracellular domain. GH-R is glycosylated, with an apparent molecular mass of 120 kDa, and is widely expressed in many tissues including liver, heart, kidney, intestine, skeletal muscle, pancreas, brain and testis.[15] Expression of GH-R generally increases around the perinatal/postnatal period, and continues to rise until reaching a plateau by adulthood.

Alanine scanning mutations of the extracellular domain of the GH-R have identified nine out of 33 residues located at the interface with the GH SITE 1 binding site that contribute to the binding affinity of the GH-R.[2, 4] The function of the WSXWS-like motif (YGEFS) in GH-R has also been analysed by alanine substitution: mutations of Y^{222} and S^{226} diminished ligand binding and abolished signal transduction, whereas substitution of G^{223}, E^{224}, and F^{225} had no effect.[16] A schematic summary of the GH-R is depicted in Fig. 1.

ACTIVATION OF JAK2 KINASE

A common property shared by many members of the cytokine receptor superfamily is the utilization of the Jak kinases (Jak 1, 2, 3, and Tyk2), a group of non-receptor tyrosine kinases ranging in molecular weight from 125 to 135 kDa.[14] Jak kinases are composed of an amino-terminal domain that interacts with the cytoplasmic portion of various cytokine receptors, a carboxy-terminal domain that possesses tyrosine kinase activity, and pseudokinase domain of unknown function. GH predominantly activates Jak2 *in vitro*[17] and *in vivo*,[18] although it can also stimulate Jak1 to a lesser extent *in vitro*.[19]

Mutational analysis of the GH-R has defined a conserved membrane-proximal domain termed "BOX 1", a hydrophobic, proline-rich motif required for GH-R binding and activation of Jak2.[20] Partial activation of GH-R is maintained by individual point mutations of P residues within BOX 1, but substitution of all four P residues abolishes GH-stimulated Jak2 activation. GH-R tyrosyl phosphorylation does not modulate Jak2 activation, since mutant GH-Rs devoid of intracellular Y residues were capable of Jak2 activation.[21,22] Mutant GH-Rs that lack the ability to activate Jak2 are unable to support mitogenic proliferation, induce DNA-protein interactions or stimulate gene expression[23,24] To date, GH-induced oscillations of intracellular calcium are the only biologic effect independent of BOX 1.[25]

The function of an adjacent motif termed "BOX 2" has not been well-defined for the GH-R. In some cytokine receptors (e.g. erythropoietin, granulocyte-colony stimulating factor, and members that interact through gp 130), this domain is required for ligand-stimulated mitogenesis.[26] GH-R truncation mutants lacking BOX 2 are capable of GH-stimulated mitogenesis, although this effect was augmented by the inclusion of BOX 2. A point mutation of within BOX 2, F^{346} to A, disrupts GH internalization, but does not block GH-R signal transduction or GH activation of gene expression by GH[27] (see below).

Mutational analysis of Jak2 has shown that deletion of the amino-terminal domain (residues 2–239) impairs GH-R signal transduction, perhaps by removal of a site required for Jak2 association with GH-R.[28,29] Deletions of the carboxy-terminal (kinase) domain of Jak2 also impair GH-R signaling. A GH-R/Jak2 chimera containing the GH-R transmembrane region (lacking BOX 1) and the kinase domain of Jak2 was able to stimulate a two- to three-fold increase in c-*fos* promoter/reporter gene expression in response to GH.[28]

Attenuation of Jak2 activity may be mediated in part by SHP-1 (also known as PTP-K, SHPTP1, and HCP).[30] Following GH-R activation, SHP-1 associates with tyrosyl phosphorylated Jak2. *In vivo*, hepatic nuclear extracts from SHP-1 deficient ("moth-eaten") mice display prolonged Stat activation after GH administration. *In vitro*, GH-R mutants lacking residues 520–540 display pro-

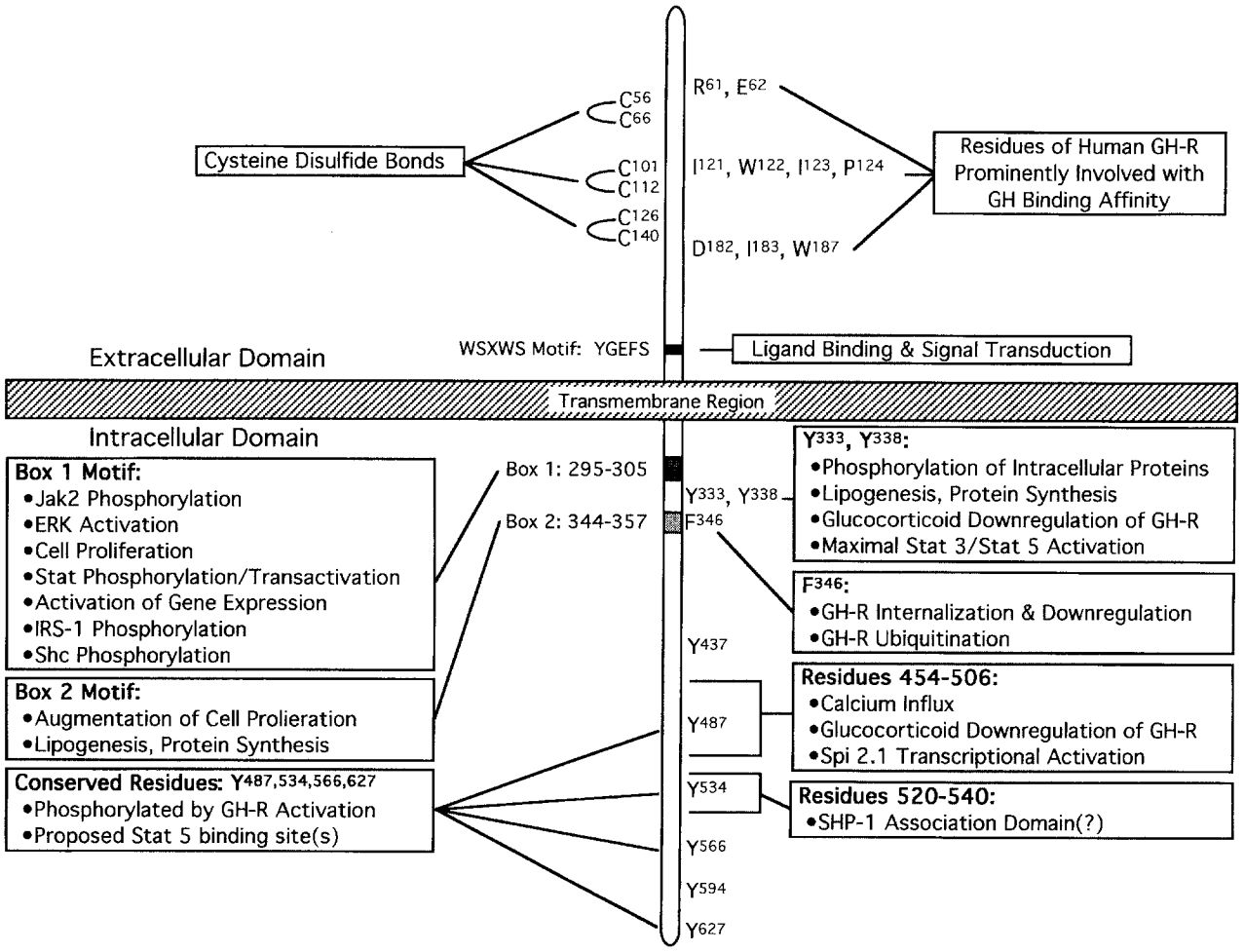

Fig. 1 Summary of GH-R domains in GH binding and signal transduction. Numbering system of amino acid residues corresponds to rat GH-R (ref. 15), except where noted.

longed Jak2 tyrosyl phosphorylation; however, it is unclear if SHP-1 is constitutively associated with GH-R, or whether other phosphatases participate in attenuating intracellular phophorylations induced by GH.[30] A schematic summary of GH-R signal transduction pathways is depicted in Fig. 2.

ACTIVATION OF INTRACELLULAR SIGNALING CASCADES

Following the activation of Jak2, several intracellular proteins, including the cytoplasmic domain of the GH-R, undergo tyrosine phosphorylation. Specific point mutations of various tyrosine residues in the intracellular region of the GH-R disable signal transduction. Mutation of Y^{332} to F in a truncated GH-R had no effect on GH-stimulated proliferation[31] but a double mutation of Y^{332} and Y^{338} to F residues blunted GH-induced tyrosine phosphorylation of certain intracellular proteins, lipogenesis, and protein synthesis,[32] but had little or no effect on ligand binding, Jak2 phosphorylation, GH-R internalization, MAP kinase activation, or induction of reporter gene expression.[33] Y^{332} and Y^{338} are required for maximal tyrosyl phosphorylation of Stats, and this domain may also serve an accessory role for binding Stats.[19]

In vivo studies have shown that GH treatment stimulates tyrosine phosphorylation and nuclear translocation of at least eight different nuclear proteins, including ERKs 1 and 2, and Stats 1, 3, and 5.[34–36] *In vitro* studies have identified at least 13 proteins in whole cell lysates that are tyrosyl phosphorylated by GH.[37] Stably transfected CHO cells containing truncated or mutated GH-Rs have demonstrated that BOX 1 is required for tyrosine phosphorylation of at least four proteins: p121 (Jak2), p42 and p39 (ERKs 1 and 2), and p97 (Stat 5?).[20] Of these, only p97 failed to display GH-inducible tyrosyl phosphorylation when a truncated GH-R mutant (residues 1–454) was studied.

This same truncated GH-R mutant (residues 1–454)

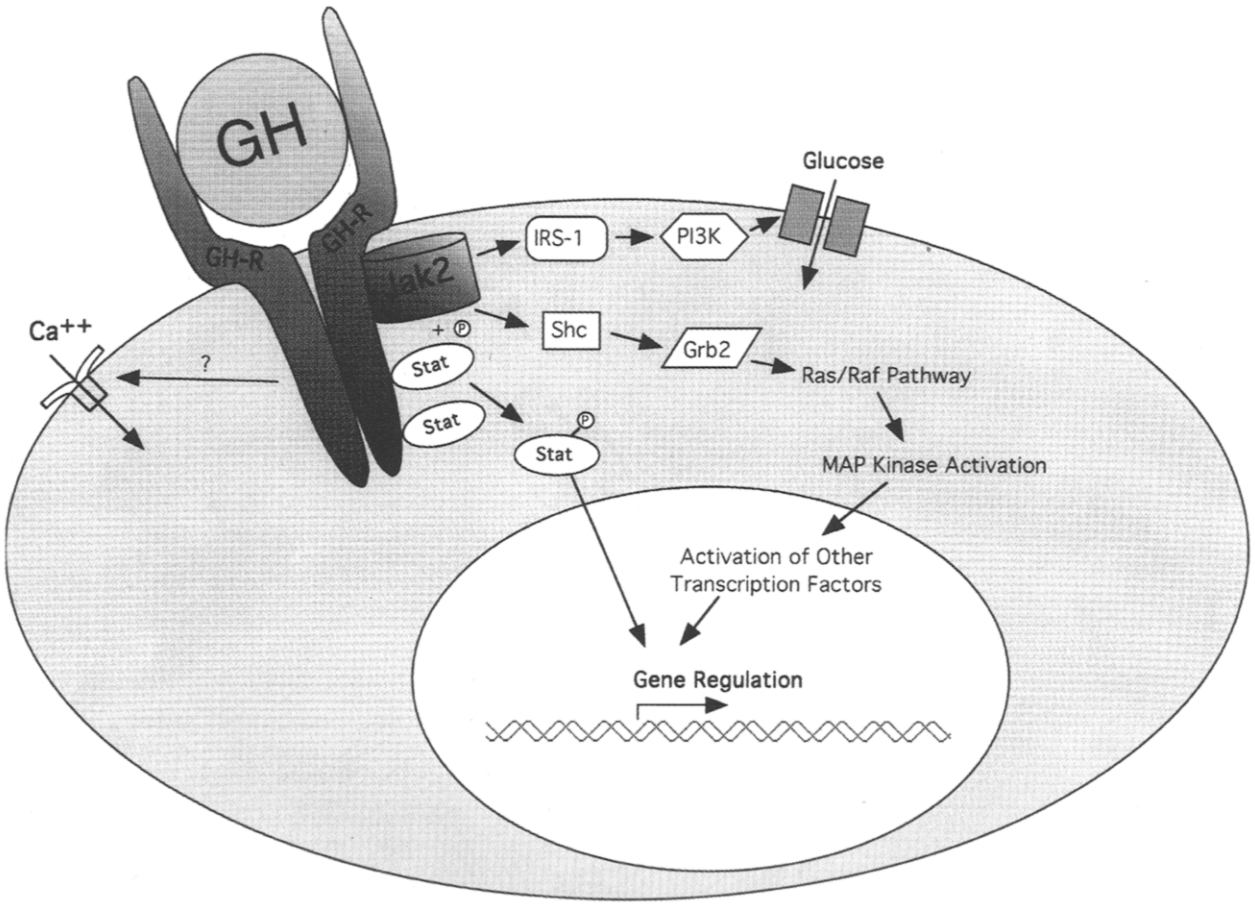

Fig. 2 Summary of intracellular signaling pathways activated by GH/GH-R. Details of the mechanisms and abbreviations are described in the text.

maintains its ability to activate ERKs, but truncation to residue 380, or mutations that destroy BOX 1 result in loss of ERK activation, suggesting that BOX 1 or adjacent regions are important for stimulation of the ERK pathway by GH-R.[20] However, other studies have observed ERK activation with a truncated rabbit GH-R (residues 1–317, containing BOX 1).[38] Jak2 activation is required for GH stimulation of MAP kinase activity, and appears to utilize the Ras-Raf pathway.[39] Mutant GH-Rs devoid of all intracellular Y residues maintained the capability of ERK activation.[21]

ACTIVATION OF STATS

Virtually all members of the cytokine receptor superfamily activate a family of transcription factors known as Stats (signal transducers and activators of transcription).[40] There are currently seven Stats (1, 2, 3, 4, 5a, 5b, and 6), which share 28–40% sequence identity within their initial ~700 amino acids, but differ widely at the carboxy-terminus.[14] Stats can form homodimers and heterodimers with other Stats, and at least one non-Stat protein, p48.[41] In the unstimulated state, Stats reside in the cytoplasm but are rapidly transactivated by tyrosine phosphorylation by Jak kinases (and serine phosphorylated by non-Jak kinases) and translocated to the nucleus.[2–44] Stat transactivation enables binding to cognate sequences of DNA termed GAS (γ-interferon activated sequences) elements, *cis*-acting elements that minimally consist of a core consensus sequence of $TT(N)_5AA$. GAS elements reside in the promoter/regulatory regions of genes whose expression is modulated by various cytokines.

GH stimulates tyrosine phosphorylation of Stats 1, 3, and 5.[34–36, 45–50] Stably transfected GH-R BOX 1 deletion mutants have impaired GH-inducible Stat binding to GAS sequences. Truncated GH-R mutants retain this ability, although some studies have noted a requirement for BOX 2 for GH-inducible binding to a GAS element.[51] The ability of GH-R to transactivate Stat 5 appears dependent on the presence of at least one Y residue in the carboxy-terminal domain of the GH-R.[21,22] Stat 5 may associate

with GH-R through DXY motifs in this region.[19] Stat 3 seems to associate with the membrane-proximal domain of the GH-R, and can be transactivated in the absence of the carboxy-terminal domain.[19,52] There is also some evidence that Stat 3 (but not Stat 5) can associate with Jak2 directly.[53] The identity of GH-R intracellular domains regulating Stat 1 transactivation has not been elucidated.[19]

Temporal patterns of GH secretion are sexually dimorphic in rodents and present another level of complexity in GH regulation of Stat activation. Adult male rats display intermittent, high amplitude, low frequency GH patterns of release, whereas adult female rats possess low amplitude, high frequency (continuous) patterns of GH release; these patterns are sex steroid dependent. Stat 5b activation is downregulated by continuous GH administration, whereas a single GH pulse desensitizes Stat 1 and 3 (but not Stat 5) tyrosyl phosphorylation.[54,55] These differences might contribute to sexually dimorphic patterns of GH gene expression (see below).

ACTIVATION OF TARGET GENE EXPRESSION

Long-term consequences of GH action are modulated by the induction or repression of expression of target genes, which can occur at the transcriptional or post-transcriptional level. Several genes known to be regulated by GH are summarized in Table 1.

GH rapidly and transiently induces expression of the immediate early gene c-*fos*,[35,56] which requires at least two *cis*-acting elements in the c-*fos* promoter region: the serum response element (SRE, which binds serum response factor, SRF, and ternary complex factor, TCF)[57] and the c-*sis* inducible element (SIE), which binds Stats 1 and 3. GH does not alter the ability of the SRF/TCF to bind to the SRE,[35] however, GH induces binding of Stats 1 and 3 with the SIE.[35,48] The GH-R BOX 1 motif and Jak2 amino-terminal domain are required to activate c-*fos* gene expression. GH can also activate c-*jun* gene expression but to a lesser extent than c-*fos*.[58]

Many other GH target genes utilize the Stat signaling pathway to regulate transcription. Rat hepatic Spi 2.1 and 2.2 genes are regulated by at least two independent GH-responsive *cis*-acting elements in the proximal promoter. A GH-inducible DNase I hypersensitive site is located in the 5′ flanking region of the Spi 2.1 promoter,[59] a domain that contains two adjacent GAS-like elements (GLE, also GHRE) that bind Stat 5.[60] A more proximal *cis*-acting element also contributes a modest induction by GH, and may be due to the presence of a CCAAT enhancer binding protein (C/EBP) site[61] or GAGA box[62] in this region. Cotransfection studies with a Stat 5 expression vector stimulated Spi 2.1 promoter activity, but mutation of Stat 5 Y^{694} blocked induction by GH.[63] A GH-R BOX 1 motif is required for activation to occur, however, additional cytoplasmic domains between residues 454 and 506 also appear to be needed. At least four other promoters of GH-regulated genes appear to have GLE motifs, β casein,[49] p450 3A10/lithocholic acid 6 β hydroxylase,[64] insulin,[65] and the acid labile subunit of the insulin-like growth factor (IGF)-binding protein 3 complex.[66]

Another member of the Spi gene family, Spi 2.3, is postulated to escape regulation by GH due to the presence of a transcriptional repressor located in the 3′ untranslated region.[67] GH also downregulates IGF-binding protein 1 gene transcription,[68,69] through unknown *cis*-acting elements in the 5′ flanking region. Albumin gene expression also displays downregulation by GH.[68]

The CYP2C genes, including 2C7, 2C11, 2C12, and 2C13, are regulated by GH in a sexually dimorphic manner. In adult male rats, pulsatile GH secretion promotes hepatic expression of 2C11 and 2C13, whereas continuous GH secretion in female rats increases 2C12 gene expression. Regulation of 2C12 gene expression may involve cytosolic phospholipase A_2, which generates arachidonic acid in response to activation by ERK.[70] The *cis*-acting elements that mediate regulation by GH are likely to reside in the promoter regions of these genes, but the identity of *trans*-acting factors is not known.[71,72] Recently, several GH-dependent DNA-protein interactions were identified in the 2C12 promoter, termed GH nuclear factor (GHNF), which appear distinct from Stats; but it is not clear what role GHNF plays in transcriptional regulation.[73] Potential HNF-6 sites have also been identified in the promoters of 2C12 and the MUP genes, and

Table 1 Genes regulated by growth hormone

Gene	Proposed *cis*-acting element(s)	Proposed *trans*-acting element(s)
Upregulation		
c-fos	SRE, SIE	SRF/TCF, Stats 1 & 3 C/EBPβ
c-jun	?	?
Spi 2.1, 2.2	GLE, GAGA	Stat 5, Others?
CYP2C7, 11, 12, 13	?	GHNF? HNF-6?
Insulin-like growth factor-I	?	?
β-Casein	GLE	Stat 5
p450 3A10/6 β hydroxylase	GLE	Stat 5
Insulin	GLE	Stat 5
Acid-labile subunit	GLE?	Stat?
BL1A, BS1 (MUP2A, MUP2b)	GLE?	Stat?, HNF-6?
Lipoprotein lipase	AP1	AP1
Downregulation		
IGF-binding protein 1	5′ flanking region?	?
Spi 2.3	3′ untranslated region?	?
Albumin	?	?

Table 2 Transcription factors regulated by growth hormone

Factor	Proposed mechanism
Stat 1	Transactivation
Stat 3	Transactivation
Stat5	Transactivation
C/EBPβ	Translation
C/EBPδ	Transcription
AP1	Transcription
SRF/TCF	Transactivation (?)
HNF-6 (?)	(?)

may be involved in GH-induced transcriptional regulation.[74] Although it is four decades since the discovery of the GH/IGF-I axis, the mechanism(s) by which GH regulates IGF-I gene expression remain unclear. *In vivo*, GH rapidly activates hepatic IGF-I gene transcription through the coordinate use of the major and minor IGF-I promoters.[75–78] Coincident with IGF-I transcriptional activation is the induction of a GH-inducible DNAase I hypersensitive site in the second intron of the IGF-I gene, but the functional significance of this event is not clear. To date, GH-activated *cis*- and *trans*-acting factors that regulate IGF-I gene expression are unknown.

GH regulates the activities of several other transcription factors in addition to Stats. As mentioned previously, GH rapidly stimulates c-*fos* and c-*jun* gene expression, which results in increased AP-1 activity.[58] AP1 in turn may activate expression of other GH-responsive genes, such as lipoprotein lipase.[79] GH stimulates the binding of C/EBPs to an oligonucleotide containing a high affinity C/EBP binding site, and modulates the expression of C/EBP isoforms by several mechanisms, including induction of C/EBPδ expression at the transcriptional level, and translational stimulation of C/EBPβ mRNA (an event dependent on tyrosine kinase, protein kinase A, and protein kinase C pathways).[80] Transcription factors regulated by GH are summarized in Table 2.

GH-R INTERNALIZATION AND TRANSLOCATION TO THE NUCLEUS

Following ligand-receptor binding, many cell surface receptors, including GH-R, undergo internalization.[81, 82] Internalization is dependent upon a cytoplasmic GH-R domain localized to residues 318–380. Mutation of F^{346} to A blocks GH-R internalization, but does not affect Spi 2.1 promoter activation by GH.[27] Other aromatic residues between 318 and 380 can be mutated without effect, and deletion of BOX 1 does not block GH-induced GH-R internalization. After dimerization, GH-R is ubiquitinated and degraded in a GH-dependent manner.[83] Mutation of F^{346} inhibited GH-R ubiquitination, but permitted Jak-Stat activation.[84]

Following internalization, GH-R can be translocated to the nucleus, where it might exert additional unknown effects.[81] The full-length GH-R has been identified in the nuclei of many cell types *in vivo*, an apparent consequence of rapid, ligand-dependent nuclear translocation. Studies using truncated mutant GH-Rs have localized a region between residues 294 and 454 that permits nuclear translocation. Currently, the function of GH-R nuclear translocation is unclear.

GH-R signal transduction is antagonized by glucocorticoids and phorbol myristate acetate (PMA), which both decrease the number of cell surface (but not total cellular) GH-R.[85] A reduction in tyrosyl phosphorylation of GH-R and Jak2, and a decrease in MAP kinase activity and Stat activation accompany GH-R downregulation.[86] Glucocorticoid-induced GH-R downregulation mapped to residues 455–506, and required Y^{333} and/or Y^{338}; but downregulation also occurred with mutant GH-R F^{346}, suggesting a mechanism distinct from GH-R internalization.[85] PMA downregulated all GH-R mutants, particularly those containing residues 507–638.[85]

ADDITIONAL SIGNALING PATHWAYS

The acute metabolic actions of GH mimic insulin; both GH and insulin stimulate glucose uptake, enhance amino acid uptake, and inhibit lipolysis. *In vitro*, GH can stimulate tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), a 185 kDa cytosolic protein that is involved with insulin and IGF-I signal transduction.[87–89] This event occurs in a dose-dependent manner in adipocytes, and coincides with phosphatidylinositol (PI)-3 kinase activation. The GH-R BOX 1 is required for IRS-1 activation, but truncation of the GH-R proximal to residue 380 also impairs IRS-1 activation. GH activation of IRS-1 has not been observed *in vivo*.[18]

Both Shc and Grb2 have been postulated to link GH-R to MAP kinase activation. Shc is a cytosolic protein with at least three isoforms (46, 52, and 66 kDa) and mediates tyrosine kinase receptor signaling.[90] GH treatment inducibly phosphorylates Shc, probably via Jak2; mutation of BOX 1 impairs the ability of the GH-R to phosphorylate Shc.[33] Grb2, a cytosolic adaptor protein that binds Shc and IRS-1 by its SH_2 domains, can stimulate the Ras signaling pathway and MAP kinase activation by interaction with a guanine nucleotide exchange protein, Sos. Grb2 does not appear capable of binding directly to GH-R or Jak2, but displays inducible interaction with Shc and IRS-1, suggesting a link between GH signaling and MAP kinase activation.[33,39]

GH stimulation of voltage dependent L-type calcium channels is another mechanism implicated in GH signal transduction. Truncation mutants in the GH-R proximal to residue 454 cannot stimulate GH-inducible calcium

influx, but deletion or mutation of BOX 1 does not affect this response, suggesting a Jak2-independent GH signaling pathway.[25] Although GH-R residues 454–506 are postulated to contain domains that modulate Stat 5 transactivation and Spi 2.1 transcriptional activation, no direct correlation has been made between these events and calcium influx. A mutant GH-R devoid of all intracellular Y residues also maintained the ability to stimulate calcium influx.[21] Other studies have noted that inhibition of protein kinase A can blunt GH-induced calcium influx, despite no change in intracellular cAMP levels.[91]

CONCLUSIONS

In the past 5 years, GH-R signal transduction pathways have been actively dissected using structural and biochemical studies, molecular biological approaches, and transgenic animals. Key components of GH-R signal transduction have been defined and a series of steps have been identified from the initial binding of GH to the GH-R at the cell surface, to the activation of cytoplasmic signaling cascades, to regulation of gene expression in the cell nucleus. Activation of Jak2 appears to be a key mediator of signal transduction, but other mechanisms, such as calcium influx also appear to play a role. Intracellular signaling pathways influence the long-term metabolic/mitogenic effects of GH, and have led to an initial understanding of the diverse mechanisms utilized by GH to regulate gene expression. Future studies can be expected to build upon this current knowledge, and should lead to a more complete understanding of the molecular physiology of GH action.

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