REVIEW

Growth hormone receptor: structure and signal transduction

Marie-Catherine Postel-Vinay and Joëlle Finidori

INSERM Unité 344, Endocrinologie Moléculaire, Faculté de Médecine Necker, Paris, France


The growth hormone receptor (GHR) belongs to the superfamily of transmembrane proteins that includes the prolactin receptor and a number of cytokine receptors. Two forms exist for the GHR: the full-length membrane-bound human receptor is a protein of 620 amino acids with a single transmembrane region; and the GH binding protein (GHBP) is a short soluble form corresponding to the extracellular domain of the full-length receptor. In rodents, GHBP is encoded by a specific mRNA of 1.2–1.5 kb, whereas in man and other species GHBP is believed to result from proteolytic cleavage of the membrane receptor. Growth hormone binding protein prolongs the half-life of GH but other functions for GHBP remain to be demonstrated. Recombinant GHBP complexed to human GH shows a 2:1 stoichiometric crystal structure. Growth hormone-induced dimerization of the cell surface GHR appears to be a prerequisite for biological activity of the hormone. JAK2 has been identified as a tyrosine kinase associated with GHR and other receptors of the superfamily. Binding of GH to its receptor results in dimerization of the GHR, phosphorylation of JAK2 and of the GHR. Other substrates for JAK2 have to be identified. Transcription factors belonging to the STAT (signal transducers and activators of transcription) family are involved in the transcriptional effects of GH. The activity of mutants of the GHR has been measured in functional tests to identify sequences of the cytoplasmic domain of the receptor that are important for signal transduction. A proline-rich sequence, called Box I, conserved among members of the receptor family has been shown to be crucial for GH effects on gene transcription. MAP kinase activity and cell proliferation. The C-terminal region of the GHR is required for tyrosine phosphorylation of the receptor and for a hormonal effect on gene transcription, whereas only 46 membrane proximal amino acids of the cytoplasmic domain are necessary for activation of JAK2 and transduction of the GH proliferative signal. Much work remains to be done to identify other protein kinases and signalling molecules involved in the mechanism of action of GH.

M-C Postel-Vinay, INSERM Unité 344, Faculté de Médecine Necker, 156 rue de Vaugirard, 75730 Paris Cedex 15, France

Growth hormone (GH), along with prolactin and placental lactogen, belongs to a family of polypeptide hormones. Growth hormone has a wide spectrum of actions but is best known for its effects on growth of skeletal and soft tissues and for its metabolic effects. Growth hormone is produced in the pituitary gland and is carried in the circulation to target organs, where the first step in hormone action is binding to receptors localized in cell plasma membranes. Our knowledge of the GH receptor (GHR) has made considerable progress after cloning of the receptor complementary (c) DNA. New perspectives were opened to the study of the structure/function relationship of the receptor.

In 1987, W. Wood and co-workers at Genentech purified and sequenced GHR and GH binding protein (GHBP) in the rabbit and then cloned the cDNAs encoding the GHR in rabbit and human livers (1). Soon after, the prolactin receptor was cloned and the two receptors were shown to belong to the same family of transmembrane proteins (2). Amino acid sequence identity of the GHBP with the extracellular domain of the GH membrane receptor was demonstrated (3). The receptor family has expanded and includes receptors of a number of cytokines to form the GH/PRL/cytokine receptor family (4).

Until recently, very little was known about the mechanism of action of GH. Two crucial steps in signal transduction have now been identified: dimerization of the receptors and association with the tyrosine kinase JAK2.

Different forms of the GHR

As deduced from the nucleotide sequence, the human GHR is a protein of 620 amino acids comprising an extracellular hormone binding domain of 246 amino acids, a single 24-amino acid transmembrane region and a long cytoplasmic domain. The extracellular domain contains seven cysteine residues and five potential N-linked glycosylation sites (1). A 84% identity has been found in the amino acid sequences of the rabbit and the human GHRs: the cDNAs of the GH receptors of several species subsequently have been cloned (5, 6), and the homology in the primary
sequences of the receptors is high, of the order of 70%.

Between the members of the GH/PRL/cytokine receptor family, in addition to a low overall amino acid identity in the extracellular domain of the receptors, common features are found: two pairs of cysteines in the N-terminal end and a conserved WSXWS motif (tryptophan, serine, any amino acid, tryptophan, serine); this motif is found near the C-terminus of the extracellular region, in all receptors of the superfamily except the GHR where it is replaced by YGEFS (tyrosine, glycine, glutamate, phenylalanine, serine) (4, 7).

Although there is little conservation of primary sequence in the cytoplasmic domains, some regions known as Box 1 and Box 2 are present in several receptors of the superfamily and have been shown to be important for signal transduction (8). Box 1 (amino acids 280–287 in the GHR) is a juxtanembranous proline-rich sequence, well conserved in the superfamily of receptors. Box 2 is made up of hydrophobic residues and charged residues. The cytoplasmic domain does not contain any known tyrosine kinase or nucleotide binding motifs (1).

An isoform of the human GHR (hGHRd3) has been found in the placenta (9); its expression was first thought to be tissue specific but now appears to be widespread (10, 11). Human GHRd3 mRNA differs from the liver GHR mRNA by the deletion of 66 base pairs encoding exon 3. Human GHRd3 is probably a fully functional receptor, able to bind the ligand and transduce the GH signal. The physiological significance of the two human GHR forms remains to be clarified.

In addition to the membrane full-length receptor, a soluble GHR is present in blood (12) and other biological fluids, such as milk (13); it has been called GHBP. Growth hormone binding protein, identified in the serum of man and many other species (12, 14), corresponds to the extracellular domain of the membrane receptor and is thus a soluble short form of the GHR (3). A schema of the two forms of the GHR is presented in Fig. 1.

Growth hormone binding protein can be produced through two mechanisms. In rat and mouse, alternative splicing of a single primary transcript results in two distinct mRNAs: the 4.5-kb transcript encodes the full-length receptor and the 1.2-kb species encodes the binding protein in which the transmembrane region has been replaced by a hydrophilic tail (15, 16). The second mechanism, which has been proposed for the GHBP generation, is proteolytic cleavage of the membrane receptor (1). In man and many species, a single mRNA transcript of about 4.5 kb has been detected by Northern blot analyses (17); moreover, the C-terminal sequence of the binding protein appears to be identical to that of the extracellular domain of the receptor. In CHO cells transfected with a cDNA encoding the full-length rabbit GHR, the membrane form of the receptor was expressed and the soluble form of the receptor was detected in the culture medium (18).

![Fig. 1. Schema of the full-length membrane-bound growth hormone receptor (GHR) and growth hormone binding protein (GHBP).](image-url)

Only the membrane receptor was present when the cDNA of the rat GHR was transfected in the cells. These results suggest that in rabbit, man and probably most non-rodent species, GHBP results mainly from proteolysis of the membrane receptor. The protease involved in this cleavage remains to be identified. The mechanism of regulation of the cleavage is important to determine in order to interpret fully the changes observed in plasma GHBP levels in pathophysiological situations.

### Binding determinants in the extracellular domain of the GHR

The three-dimensional structure of the complex GHBP–hGH (19) has shown that the extracellular domain of the receptor consists of two regions containing seven antiparallel β-strands each, grouped in a β-sheet sandwich, with a topology similar to that of immunoglobulin domains, as predicted by Bazan for the extracellular regions of the receptors of the superfamily (7). The N-terminal subdomain contains six cysteine residues, which are linked to form three disulfide bridges; this first subdomain contributes to the binding interface with the hormone.

The strategy of homolog and alanine scanning mutagenesis was used to provide a functional map of the side chains in hGHBP important for binding of the ligand (20). Four important binding determinants are located in the cysteine-rich region; they form a patch in the four loops connecting the β-strands. Tryptophan at position 104 has more contact with the hormone than any other receptor residue, consistent with the result that mutation of this residue to alanine virtually abolished binding (20).
The characteristic WS motif is located near the C-terminus of the extracellular domain, away from all binding interfaces; however, it is required for the normal binding affinity of several receptors of the superfamily, including the prolactin receptor (21). In the GHR, the analogous motif YGEFS, at position 222–226, is also important for normal binding activity. In sex-linked dwarf chicken, a point mutation resulting in substitution by isoleucine of the last invariant serine of the motif was identified and the mutant receptor was shown to have a decreased binding affinity (22). In a recent work (23), each residue of the YGEFS sequence of the GHR was mutated to alanine; the two mutants Y222A and S226A displayed lower ligand binding affinity and decreased signal transduction.

**Dimerization of the GHR**

The three-dimensional crystal structure of GHBP in complex with hGH has confirmed that one hormone molecule binds two receptor molecules (19). Human GH is made up of four helix bundles, with the first two helices parallel to each other and antiparallel to the last two helices. The hGH molecule has two receptor sites, identified as site 1 and site 2. Site 1 consists of about 24 residues on helix 1 and a large part of helix 4. The second site, composed of 13 residues, is formed by the N-terminus of helix 1 and residues on helix 3. The two receptors contribute the same regions to the interfaces with the hormone. The interface between the two receptor molecules involves the C-terminal end of their extracellular domains (24).

Further studies have demonstrated that binding of the receptors to hGH occurs sequentially: a first receptor binds to site 1 and then a second receptor can bind to site 2 of the hormone, and interaction between the two receptor molecules forms the homodimer complex (19). Assays of hGH mutants have provided additional support for this dimerization mechanism. The myeloid leukemia cell line FDC-P1 is able to proliferate in response to interleukin 3. A chimeric receptor consisting of the extracellular domain of the GHR and the transmembrane and intracellular domains of the G-CSF receptor was prepared. The cDNA of the chimeric receptor was transfected into FDC-P1 cells and GH was then able to stimulate proliferation of the transfected cells (25). Human GH mutants with a non-functional site 1 are not able to stimulate the proliferation of cells expressing the GH/G-CSF hybrid receptor. A hGH mutant in site 2 (G120R), which binds well to the receptor, failed to stimulate cell proliferation: it is a potent hGH antagonist. Moreover, several antibodies to GHBP were potent agonists, whereas their Fab fragments were not (25). These results strongly suggest that the hormone-induced dimerization of the receptor could be a necessary step for the hormonal effect on proliferation. Moreover, receptor dimerization could also be critical for other GH effects, such as stimulation of lipogenesis in rat adipocytes and receptor down-regulation in IM-9 lymphocytes (26).

More evidence that receptor dimerization is crucial for GH signal transduction has been obtained with the identification of a mutation of the GHR gene in two families with Laron syndrome (27). Most of the gene abnormalities identified so far in patients with Laron syndrome lie in the extracellular domain of the GHR and result in a receptor that has lost its binding activity (28). Recently, several patients presenting with the clinical and biological features of Laron syndrome, but with normal GH binding activity in their plasma, have been reported (29). In two such patients, a point mutation with the substitution Asp→His at position 152 has been detected. This mutation does not affect the binding activity of the GHR but prevents hormone-induced dimerization of the receptor and results in a state of extreme GH resistance (27).

**Signal transduction for the GHR**

**Molecules involved in signalling**

Several years ago, it was shown that GH stimulates tyrosine phosphorylation of a protein with a molecular weight of 120000 (pp120), which was thought to be the GHR itself (30). However, the primary sequence of the receptor contains no known kinase domain or consensus sequence for ATP binding. Further studies have shown that tyrosine-phosphorylated pp120 actually consists of the GHR itself and a closely associated protein (31). C Carter-Su and co-workers...
identified the GHR-associated kinase as JAK2 (32). JAK2 belongs to the Janus kinase family, whose first members were initially identified by the polymerase chain reaction (33) or low stringency hybridization (34), and was shown subsequently to be involved in signal transduction of cytokine receptors (35). These 120–135 kD cytosolic kinases, devoid of SH2 and SH3 regulatory domains, have two kinase-like domains in tandem in their carboxyterminal region, the most distal being the only one functionally active. Four members of the Janus kinase family have been identified: JAK1, JAK2, JAK3 and Tyk2. JAK2 is activated in response to GH binding to its receptor; it is also involved in erythropoietin, prolactin, IL-3, IL-5, GM-CSF and γ-interferon signalling. Activation of JAK2 after GH binding is a very rapid and transient event. It is not clear whether the association of JAK2 with the GHR is constitutive, the activation being induced by trans-conformation of the receptor after ligand binding, or if the ligand induces association/activation of the kinase. One major substrate for the JAK2 kinase is the receptor itself. We have shown that carboxy-terminal tyrosines of the GHR are phosphorylated, presumably by JAK2 (36). JAK2 serves in signal transduction by phosphorylating other proteins that could associate with the receptor.

The mitogen-activated protein (MAP) kinases have also been reported to be activated by GHR (37, 38). However, the pathways allowing GH activation of MAP kinases have not been defined. It is possible that both the Ras pathway and the protein kinase C (PKC) route are involved.

Growth hormone has also been shown to stimulate tyrosine phosphorylation of Shc (39); this SH2 domain-containing protein, upon tyrosine phosphorylation, can serve as a signalling molecule in pathways such as that of the MAP kinases. Another mechanism of signalling for GH could be related to an increase in calcium influx; GH is able to increase the cytosolic free calcium ion concentration in IM-9 cells, independently of protein tyrosine phosphorylation or PKC activation (40).

Signal transducers and activators of transcription (STATs) are a family of transcription factors that couple ligand binding to the activation of gene expression (41). The first members of this family were identified several years ago, from experiments exploring the transcriptional induction of interferon receptors. Seven members of this family have been identified so far. Most of them were originally reported to be activated in response to stimulation by a specific cytokine: STAT1α, STAT1β and STAT2 by interferons; STAT3 by IL-6, STAT5 by prolactin (42) and STAT6 by IL-4. It appears now that various cytokines can induce the activation of the same STAT molecules (41). The specificity of the cytokine response could be linked to specific combinations between these molecules. Following cytokine stimulation and JAK activation, STAT proteins associate with the receptor complexes via their SH2 domains, and are then phosphorylated. Conformational changes occur leading to the dissociation of the STAT from the receptor complex and to STAT protein dimer formation. Each molecule interacts via its SH2 domain with the tyrosine phosphorylated residue of an identical molecule to form a homodimer or of a different member of the family to form a heterodimer. The dimeric form then translocates to the nucleus to bind specific sequences of cytokine regulated genes (43). STAT proteins are also involved in transcriptional effects of GH and very recently, it was shown that GH, like prolactin, is able to activate the DNA binding of STAT5 (Mammary gland factor) in transfected COS-7 cells (44).

**Growth hormone-responsive genes**

Transcriptions of early response genes c-fos and c-jun are rapidly stimulated by GH (45), as are genes that encode the serine protease inhibitors Spi2.1 and Spi2.3 (46). Transcription of IGF-I gene has also been shown to be induced in vivo within 30 min of GH treatment (47). In male rats, the pattern of P-A50 isozymes is regulated by GH at a transcriptional level (48); the signalling pathways involved are unknown.

The promoter regions of the two GH-responsive genes c-fos and Spi2.1 have been studied more extensively. For the c-fos promoter, two regions, the serum responsive element (SRE) (49) and the sis-inducible element (SIE) (50), apparently bind GH-induced transcriptional complexes. STAT1 and STAT3 are phosphorylated in response to GH and bind to the SIE of the c-fos promoter (51). The molecular mechanism of c-fos transcriptional regulation by GH through the SRE element is not defined. If it is similar to the pathways followed by receptor tyrosine kinases, it could be a downstream event of MAP kinase activation (52).

The Spi2.1 gene encodes a protease inhibitor expressed in rat liver and specifically regulated by GH in vivo (46). The promoter region of the gene has been characterized and two GH-responsive elements have been defined in the 150 bp upstream of the transcription start point (53, 54). In liver and in CHO cells, GH induces a transcriptional complex able to bind the most distal element of the Spi2.1 promoter (GHRE2), which acts as an enhancer. This GH-induced complex contains proteins that share some characteristics with the STAT proteins: the complex is induced rapidly, independently of protein synthesis, and its activation is dependent on tyrosine phosphorylation. However, on the basis of their antigenic properties, the transcriptional factors that bind the GHRE2 element are different from the identified members of the STAT family (55).

**Functional regions in the cytoplasmic domain of the GHR**

Tests to measure receptor activity. Several cellular systems have been developed to measure the functional
activities of mutant forms of the GHR. A first assay measures the transcriptional activity of GH on the gene Spi2.1. We used a construct in which the GH-responsive region of the Spi2.1 promoter was fused to a reporter gene, either chloramphenicol acetyltransferase (CAT) or luciferase. Co-transfections of this construct with the cDNA of the GHR in CHO cells allowed measurement of the activity of wild-type or mutants forms of the GHR to induce Spi2.1 gene transcription (56).

Another test was developed to measure the proliferative effects of GH. The pre-B lymphoma FDC-P1 or BAF-3 cell lines depend on IL-3 for proliferation. However, this ligand dependency can be switched to GH in stable transfectants expressing GHRs (57). These tests have been used to evaluate the function of mutant receptors in parallel to their ability to stimulate the various molecules involved in signal transduction.

Important sequences for signal transduction. For GHR, as for most cytokine receptors, the cytoplasmic domain contains two functional regions. The membrane proximal part, including the proline-rich sequence (Box 1) conserved in the GH/prolactin/cytokine receptor family, appears necessary and sufficient to transduce the proliferating effects of the cytokines. The distal parts of the cytoplasmic domains are required for induction of specific genes.

Using truncated mutants, we have shown that a region of 46 amino acids, adjacent to the membrane and containing Box 1, is sufficient for activation of JAK2 and MAP kinases (36); the membrane proximal region is also sufficient for GH-induced proliferation, as shown by Colosi et al. using FDC-P1 cells (57). Deletion and alanine scanning mutagenesis allowed us to confirm that the integrity of Box 1 is crucial for all hormonal effects. However, an additional part of the cytoplasmic domain of the receptor is necessary for the GH effect on gene transcription. Using various deleted mutants, a correlation between tyrosine phosphorylation of the GHR mutants and their ability to activate transcription was shown (36); these results suggest that phosphorylated residues in the GHR could serve as an anchor for SH2-containing molecules required for the transcriptional effects of GH. Identification of such molecules, whose activation requires the C-terminal part of the receptor, is under current research.

In conclusion, the GHR is a member of the cytokine receptor superfamily. Recent findings have opened new areas of research into signalling pathways for the GHR and for other members of the receptor family. Binding of GH to its receptor induces dimerization of the receptor and the dimerized receptor activates JAK2, a tyrosine kinase that is also associated with several receptors of the superfamily. Molecular mechanisms linking the activated GHR–JAK2 complex to gene transcription are largely unknown. Moreover, the activated GHR may induce distinct nuclear signal transduction pathways to mediate specific regulation of different target genes.

References