Receptor Domains Involved in Signal Transduction of Prolactin and Growth Hormone (43759)

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Abstract. **Prolactin (PRL) and growth hormone (GH) receptors are members of a superfamily that include receptors for a number of cytokines. GH and its receptor form an unusual homodimer consisting of one molecule of GH and two molecules of receptor. A similar homodimer of the PRL receptor is probably required for biological effects to be seen. Using specific assays to measure the functional activity of PRL and GH receptors, a 25 amino acid juxtamembrane region has been identified as essential but not sufficient for normal action. More detailed studies have limited the region to eight amino acids, rich in prolines, that is highly conserved in many members of the receptor superfamily. Finally, GH and PRL have been shown to induce the rapid tyrosine phosphorylation of an associated kinase, Janus kinase 2, and of the receptor itself.** [P.S.E.B.M. 1994, Vol 206]

Prolactin (PRL), growth hormone (GH), and placental lactogen (PL) form a family of hormones which have been shown to be derived by duplication of an ancestral gene (1). A wide spectrum of functions have been reported for PRL (more than 85 in various vertebrate species), whereas GH is best known for its effects on the growth of skeletal and soft tissues, and for its metabolic actions.

The initial step in the action of PRL and GH involves binding to a cell surface receptor. Very little is known about their mechanism of action, i.e., the steps following receptor binding, with the exception of some recent work discussed at the end of this manuscript. The following sections describe the expanded family of PRL/GH/cytokine receptors of the receptors, and the role of receptor dimerization and tyrosine phosphorylation in signal transduction.

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The PRL/GH/Cytokine Receptor Family

Short and long forms of PRL and GH receptors have been identified in a number of tissues (2–4). For PRL receptors, the short form is membrane bound, with a cytoplasmic region of ~50 amino acids (aa). For GH receptors, the short form represents the extracellular region of the receptor and is found in serum as a GH-binding protein. This protein can be produced by proteolysis of the membrane-bound receptor or by alternative splicing of RNA transcripts in mice and rats (5, 6). Determination of GH-binding protein in human serum represents a direct approach to measure human GH (hGH) receptor levels in man. The family that originally included PRL and GH receptors has expanded to include receptors for a number of cytokines (for review, see 7). Although the overall amino acid identity is low between members of this receptor family, there is a significant (14%–25%) identity over ~200 aa of the extracellular region of these receptors. In addition, two characteristic features are present: the first is the presence of two pairs of cysteines, usually in the N-terminal region of the molecule, which have been shown to be linked sequentially. In addition, near the C-terminal extremity of this homologous region, is a highly conserved WSxWS motif (tryptophan,

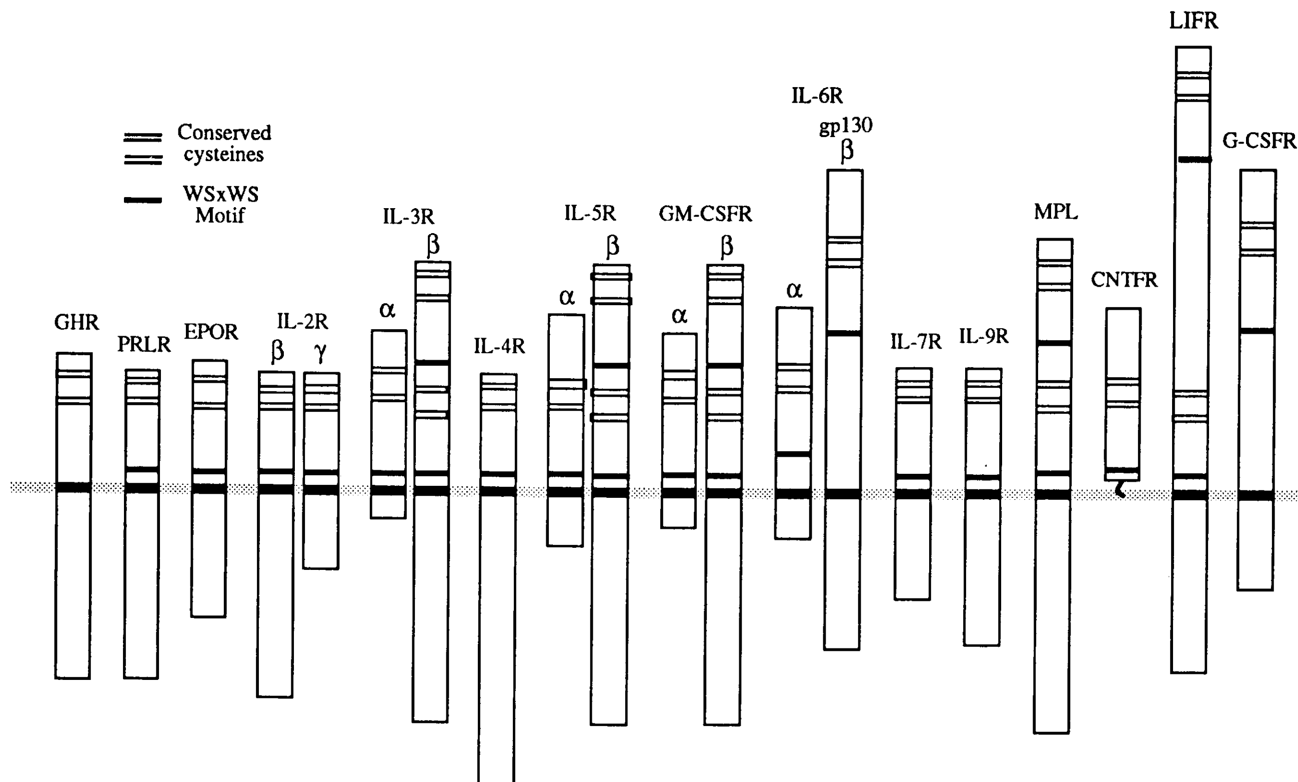

Figure 1. Schematic representation of the GH/PRL/Cytokine receptor family. Abbreviations: GHR, Growth hormone receptor; PRLR, prolactin receptor; EPOR, erythropoietin receptor; IL-2R, interleukin-2 receptor; IL-3R, IL-3 receptor; IL-4R, IL-4 receptor; IL-5R, IL-5 receptor; GM-CSFR, granulocyte-macrophage colony-stimulating factor; IL-6R, IL-6 receptor; gp130, glycoprotein of mol wt 130,000 (or β subunit of IL-6R or oncostatin M receptor); IL-7R, IL-7 receptor; IL-9R, IL-9 receptor; MPL, myeloproliferative leukemia virus or orphan receptor of unknown ligand; CNTFR, ciliary neurotrophic factor receptor; LIFR, leukocyte inhibitory factor receptor; G-CSFR, granulocyte colony stimulatory factor. The plasma membrane is indicated by a stippled rectangle. The transmembrane region is shown in black. The thin black lines indicate the conserved cysteines and the thick black lines the WSxWS motif (tryptophan, serine, any amino acid, tryptophan, serine). Several receptors are formed by subunits, indicated α, β, or γ.

serine, any aa, tryptophan, serine), which is found in all members of the family except the GH receptor, in which some conservative substitutions occur (Fig. 1).

Receptor Dimerization and Action

Recently the three-dimensional crystal structure of the hGH-binding protein and hGH has confirmed that this complex forms an unusual dimer consisting of two molecules of receptor and one molecule of ligand (8). Thus, there are two receptor binding sites on hGH (identified as Sites 1 and 2). Both sites bind to the same region of the hGH receptor. A sequential complex appears to form with the receptor first binding to Site 1, after which a second receptor binds to Site 2 (9). Human GH antagonists have been successfully prepared that are able to specifically inhibit binding to Site 2.

There is strong evidence that activation of PRL receptors also follows a similar mechanism involving dimerization. Monoclonal antibodies to the rat PRL receptor which are able to form receptor dimers have previously been shown to be partial agonists (10). More recently, using adjusted concentrations of second antibody to cross-link receptors, we have been able to show that these same monoclonal antibodies are able to induce a full stimulatory response. In addition, Fab fragments which bind to receptors are devoid of activity, but addition of a second antibody restores their functional activity (Rui *et al.*, submitted). Recently, the binding of hGH to rat or human PRL (hPRL) receptors has been shown to follow the two-site model. Thus, addition of low concentrations of hGH stimulate activity while high concentrations lead to a marked inhibition of activity (11). Although similar studies were attempted with hPRL, the authors failed to see any significant inhibition of activity at high concentrations of ligand. Their interpretation was that not enough PRL was added, due to a limited supply of recombinant material. In fact, we have recently been able to show that higher concentrations of PRL are required. At 10^{-5} to 10^{-4} *M* ovine PRL, a clear inhibition of cell proliferation is observed (Lebrun JJ, *et al.*, submitted[2]). This would appear to support the two-site model of PRL action. Curiously, Gertler *et al.*

[2] Lebrun JJ, Ali S, Sofer L, Ullrich A, Kelly PA. (submitted for publication).

(12) reported that the extracellular domain of the rabbit PRL receptor expressed in insect cells was unable to form a homidimer, as measured by gel exclusion HPLC. On the other hand, Ebner *et al.* (13) recently reported that the extracellular domain of the rat PRL receptor produced in bacteria did in fact dimerize with the same 2:1 receptor-ligand complex seen with hGH and its receptor. Clearly, final confirmation must await three-dimensional crystal structure, but there is strong evidence to suggest that for PRL, and perhaps for other members of the cytokine/GH/PRL receptor family, homodimerization is the first step in the mechanism of action.

Functional Assays of PRL and GH Receptors

We have previously developed assays to measure the functional activity of transfected forms of the PRL receptor. These involve co-transfection of a PRL-responsive gene such as ovine β-lactoglobulin or rat β-casein coupled to a reporter gene, chloramphenicol acetyltransferase (CAT). Chinese hamster ovary (CHO) cells transiently transfected with the PRL receptor cDNA and the milk protein/CAT fusion gene respond to increasing concentrations of PRL in the incubation media (14). Interestingly, the long form, as well as an intermediate form of PRL receptor found exclusively in Nb2 cells, has functional activity, whereas the short form of PRL receptor is devoid of activity (14, 15). We have recently developed similar assays to measure the functional activity of GH receptors, using a fusion gene consisting of either ovine β-lactoglobulin/CAT or the serine protease inhibitor (SPI) 2.1/CAT (16). Cells transfected with both constructs show a marked stimulation in the presence of GH. The advantage of such assays is that they use transient transfection, and are thus well adapted to evaluate the cytoplasmic regions of each receptor required for the response.

Role of Box 1 in PRL/GH Signal Transduction

Truncation and deletion mutants of PRL and GH receptors were prepared and expressed in CHO cells. The presence of ~50% of the cytoplasmic domain is sufficient for full activity of the PRL receptor, while a similar mutant of the GH receptor is inactive. Detection of a 25 aa domain, located just after the transmembrane region of PRL and GH receptors, which is highly conserved between all members of this family, leads to a complete loss of functional activity. A more restrictive region of 8 aa, known as Box 1, has been identified in several members of the cytokine receptor family. Deletion and alanine scanning mutagenesis has confirmed that this region of the cytoplasmic domain is essential for the process of signal transduction for both receptors. Mutation of any two of the four prolines in Box 1 is sufficient to block activity, although a single proline to alanine mutation is without effect for the GH receptor (16). We are currently in the process of examining in detail the amino acids in this region essential for biological action.

It should be mentioned that other tests have been recently developed to measure the functional activity of GH receptors, in general with similar results. Francis *et al.* (17) reported a test involving the transcriptional activation of a lipoprotein lipase promoter in transfected Buffalo rat liver cells. The ability of the GH receptor to stimulate mitogenic activity has been evaluated by transfecting the IL-3-dependent promyeloid cell line FDC-P1 with the full-length or mutated forms of the hGH receptor cDNA (18). Surprisingly, a truncated receptor, with as little as 54 aa in the cytoplasmic domain, was able to stimulate the proliferative signal. Since a similar (but not identical) mutant had no activity in either GH or PRL functional tests involving transcriptional activation, it would appear that more than one signal transduction pathway can be activated in responsive cells.

PRL and GH-Induced Tyrosine Phosphorylation

Growth hormone has been shown to stimulate the phosphorylation of a protein with a molecular weight of ~120,000 in a number of different cell systems. Originally, it was thought that the pp120 represented the GH receptor itself (19). More recently, however, studies have clearly shown that an associated protein, and not the receptor, is the primary and initial tyrosine phosphorylated protein (20). In addition, we have clearly shown using CHO cells stably expressing the rabbit GH receptor, that at least three tyrosine-phosphorylated proteins are induced following stimulation with GH. The receptor itself is also phosphorylated, but the degree of phosphorylation appears to depend on the cell system used. The functional role of the phosphorylated tyrosines in the various functional assays is currently being investigated.

Using Nb2 cells, we and others have demonstrated the rapid stimulation of tyrosine kinase activity by PRL (21, 22). We have identified at least three tyrosine-phosphorylated proteins (pp120, pp97, and pp42) induced by lactogenic hormones. Phosphorylation of pp120 is maximal following incubation of cells with PRL for 1 min. Peak levels of pp97 and pp40 occur at somewhat later periods. The 42- to 44-kDa protein induced by both GH and PRL appears to be MAP kinase, a protein frequently involved in proliferation.

Although neither the GH nor the PRL receptor contain a consensus sequence for ATP/GTP binding, nor a kinase domain, a major advance in the field was made by the identification of Janus kinase 2 (JAK2) as a GH receptor-associated tyrosine kinase. JAK2 is a member of a family that also includes JAK1 and tyk2.

All these proteins share the unusual feature of having two kinase domains. Complementary DNAs encoding these kinases were originally identified a few years ago (23–25), although it was not known how they were activated. Stimulation of various cells expressing the GH receptor was shown to induce tyrosine phosphorylation of a protein with a Mr of 130,000, that could be immunoprecipitated with an antibody specific to JAK2 (26). Erythropoietin is also known to activate rapid tyrosine phosphorylation of a similar sized protein, and this kinase has also been shown to be JAK2 (27). In addition to the phosphorylation of JAK2, the GH and erythropoietin receptors are also phosphorylated. Using GH or erythropoietin receptor mutants, a membrane-proximal region of the cytoplasmic domain was shown to be important for biological activity. In addition to GH and erythropoietin, JAK2 has recently been shown to be the kinase that couples IL-3 (28), and may be implicated for GM-CSF, G-CSF, IFN-γ, and PRL receptors, as the first event in the process of signal transduction.

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