The Structure and Regulation of Expression of the Mouse Growth Hormone Receptor and Binding Protein (43754)

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Abstract. **The mouse growth hormone receptor (mGHR) and the mouse growth hormone-binding protein (mGHBP) are products of a single gene which are generated by alternative splicing. The factors that regulate the expression of mGHR and mGHBP mRNA and protein during pregnancy in the mouse are incompletely understood. During pregnancy in the mouse, there are parallel increases in circulating mouse growth hormone (mGH), liver mGHR, and serum mGHBP. The increase in both hepatic mGHR and serum mGHBP begins on Day 9 of gestation and by late gestation the hepatic mGHR content has increased 8-fold and serum mGHBP has increased 30-fold compared with values in nonpregnant controls. A parallel increase occurs in the steady state levels of liver GHR and GHBP encoding mRNAs. The increase in both messages begins on Day 9 of gestation; however, the GHR mRNA reaches maximum levels by Day 13, while the GHBP mRNA continues to increase until the end of pregnancy. The magnitude of the increase in the GHR-encoding message is 15- to 20-fold between nonpregnant and late pregnant mice, and the magnitude of the increase in the GHBP-encoding message is 30- to 50-fold. Both pituitary mGH and the number of conceptuses influence the receptors and binding protein for mGH during pregnancy.**

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Within the last decade, a considerable expansion of knowledge relative to the biology of growth hormone (GH) has occurred. This includes the cloning of cDNAs for GH receptors (GHRs) from a variety of species (see 1 for a review), determination of the three-dimensional structure of the complex of human GH (hGH) and the hormone-binding domain of the hGHR (2), and the fact that unlike the human, the GHR and GH-binding protein (GHBP) in rats and mice are generated by alternative splicing mechanisms (1). In this minireview, discussion will be restricted to our knowledge of the structure and regulation of expression of the mouse GHR (mGHR) and the mouse GHBP (mGHBP).

Mouse GH (mGH) is a single chain polypeptide hormone which contains 190 amino acids with two disulfide loops (3). mGH is a member of a group of evolutionarily related protein hormones that includes pituitary prolactin and placental lactogen I and II (4). In order to elicit its biological response, mGH interacts with the mGHBP and the mGHR. The presence of mGHBP was first suggested by Peeters and Friesen (5). Subsequently, mGHR was purified from mouse liver (6), and antibodies raised against the mGHR were shown to recognize the serum mGHBP (7). Hepatic GH receptors in mice (8) and rats (9) were first demonstrated utilizing heterologous ligands. The homologous specificity of mGH with its receptor was demonstrated by the use of covalent cross-linking studies utilizing radiolabeled mGH to a microsomal membrane prepared from late pregnant mouse hepatic tissue (10). These studies demonstrated that the GHR was present in 17-day pregnant mouse liver microsomal membranes in three glycosylated forms, displaying cross-linked molecular weight values of 125,000, 62,000, and 56,000, and corresponding to receptor sizes of approximately molecular weight 103,000, 40,000, and 34,000, respectively.

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In late-pregnant mouse liver, multiple RNA species were observed by Northern analysis of total RNA (11). One RNA species corresponded to 3.9 kb and the other corresponded to 1.2 kb. *In vitro* translation of size-fractionated RNA followed by immunoprecipitation using an anti-mGHR antiserum yielded two proteins of molecular weight 95,900 and 31,800. The larger protein was detected when *in vitro* translation products of RNA in the size range of 3.9 kb were immunoprecipitated. The smaller protein was detected when *in vitro* translation products in the size range of 1.2 kb were immunoprecipitated. cDNA clones were isolated from a late-pregnant mouse liver cDNA library using a probe derived from the rabbit GHR (12). Two clones were identified that were identical in the 5′ region but diverged in the 3′ regions. The larger of the two clones encoded a protein of predicted molecular weight 72,782 with a single transmembrane region. The N-terminal extracellular domain contained five potential N-linked glycosylation sites and eight cysteines. The predicted protein encoded by the larger clone was found to have an overall identity of 70%, 76%, 71%, and 85% with the human, rabbit, sheep, and rat GHR, respectively (1). The smaller clone was found to be identical to the larger clone in nucleic acid sequence in the 5′ region but diverged in the 3′ region. The divergence of the smaller clone occurred just prior to the region encoding the predicted transmembrane domain of the larger clone and encoded a "hydrophilic tail" of 26 amino acids. The predicted protein would have a molecular weight of 34,104 and would contain the GH-binding domain but no transmembrane domain. It was predicted that this smaller clone was responsible for the generation of the mGHBP. Northern blot analysis of liver RNA from pregnant mice with either a probe common to both classes of cDNA clones, a probe specific to the large class, or a probe specific to the small class of cDNA clones demonstrated that the large class of clones is encoded by the 3.9-kb message and the small class is encoded by the 1.2-kb message (12). Utilizing COS-7 cells, both the large and small cDNA clones were transiently expressed under the control of the SV-40 promoter (Cramer and Talamantes, unpublished). Expression of the small clone resulted in specific binding of [^{125}I]bGH in the medium while expression of the large clone resulted in specific binding in the membrane fraction. These results demonstrated that the mGHBP was generated by an mRNA species separate from the mRNA encoding the mGHR.

The rat GHR cDNA was cloned by two different laboratories (13, 14). Both groups reported cDNA with a sequence encoding a protein similar to the mouse, rabbit, and human GHRs. One group (13) reported finding an additional cDNA that predicted the coding of a protein similar to the protein encoded by the smaller cDNA found in mice. Further studies by this group suggested the same presence of an alternatively spliced message encoding the GHBP in the rat as was suggested for the mouse (11).

Like the human, the mGHR gene was determined to be a single-copy gene (15). Southern blot analysis of somatic hybrids between mouse cells and Chinese hamster or rat cells demonstrated that the mGHR gene was located on Chromosome 15. The human GHR gene was cloned and demonstrated to consist of nine exons spanning 87 kb of genomic DNA (16). Alignment of the mouse and rat cDNA clones encoding the secreted GHBP with the hGHR gene reveals that the point of divergence, in mice and rats, of the GHBP cDNA with the GHR cDNA corresponds to the splice junction of Exon 7 and 8 in the hGHR gene. In the mouse it has been demonstrated that the exon encoding for the hydrophilic tail does indeed exist between Exon 7 and 8 (Edens and Talamantes, unpublished). Structural analysis of the GHR receptor has revealed that this receptor is a member of the hematopoietin receptor superfamily (17).

The factors that regulate the expression of mGHR and mGHBP mRNA and protein during pregnancy in the mouse are not completely understood. During pregnancy in the mouse, there are parallel increases in circulating mGH (18), liver GHR (6), and serum mGHBP (7). The increase in both hepatic mGHR and serum mGHBP begins on Day 9 of gestation; by late gestation the hepatic mGHR content has increased 8-fold, and serum GHBP has increased 30-fold compared with values in nonpregnant controls (18). A parallel increase occurs in the steady state levels of liver GHR and GHBP encoding mRNAs (18). The increase in both messages begins on Day 9 of pregnancy; however, the GHR mRNA reaches maximum levels by Day 13, while the GHBP mRNA continues to increase until the end of pregnancy. The magnitude of the increase in GHR-encoding message is 15- to 20-fold between nonpregnant and late-pregnant mice, and the magnitude of the increase in the GHBP-encoding message is 30- to 50-fold. In addition, there is a 10- to 16-fold increase in GH-binding capacity in liver microsomes and a 30- to 50-fold increase in serum mGHBP between nonpregnant and late pregnant mice (19). The increase in hepatic GH-binding capacity began on Day 9 of pregnancy and reached a plateau on Day 11, which was maintained until the end of pregnancy. In contrast, the increase in serum mGHBP which is observed to begin occurring on Day 9 of pregnancy continues to increase until Day 17 of pregnancy. No significant changes in mGHR and mGHBP affinity were noted between nonpregnant and pregnant mice. The mGHR was observed to have a 20-fold greater affinity for mGH than did the mGHBP. The amount of free circulating mGH in the serum appears to be very

tightly regulated below 0.5 n*M*, despite increases in total GH concentration in late pregnancy of greater than 4.0 n*M*. At the same time, the amount of GHBP with bound mGH is less than 10% of the total GHBP that is available. These data demonstrate that there is a large excess of free GHBP relative to the amount of total GH in the late pregnant mouse and is suggestive of the idea that GHBP acts as a reservoir for GH increases.

The nature of the regulatory factors that modulate the increases in mRNA and protein for the mGHR and mGHBP during pregnancy in the mouse are only beginning to be elucidated. mGH has been shown to regulate the expression of the mGHR and mGHBP (20). Pregnant mice hypophysectomized on Day 11 of pregnancy and then administered GH by osmotic minipumps have demonstrated that GH regulates the mGHR and mGHBP at the protein and mRNA level. In addition, further studies have shown that the number of conceptuses influences the receptors and binding protein for GH at the protein and message level (21). Recently, the mouse placenta has been shown to express the message for the mGHR and mGHBP (22). This study demonstrates similar increases in steady state mRNA for the mGHR and mGHBP during pregnancy. In addition, *in vitro* studies of mouse placental cells in culture demonstrate that while the mGHBP mRNA is expressed, the actual protein is not secreted by the cells as is the case for the liver.

In summary, in the mouse the GHR and GHBP are generated by alternative splicing from one gene. Pregnancy results in a dramatic increase at the protein and at the message level for GHR and GHBP. The expression of the mGHR and mGHBP during pregnancy is regulated by factors such as GH and the number of fetal-placental units.

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1. Cramer S, Talamantes F. The growth hormone receptor and growth hormone binding protein: Structure, functions and regulation. In: Shreibman MP, Scanes CG, Pang PKT, Eds. The Endocrinology of Growth, Development, and Metabolism in Vertebrates. New York: Academic Press, pp117–149, 1993.
2. Devos AM, Ultsch M, Kossiakoff AA. Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. Science **255**:306–312, 1992.
3. Linzer DIH, Talamantes F. Nucleotide sequence of mouse prolactin and growth hormone mRNAs, and expression of these mRNAs during pregnancy. J Biol Chem **260**:9574–9579, 1985.
4. Southard JN, Talamantes F. Placental prolactin-like proteins in rodents: Variations on a structural theme. Mol Cell Endocrinol **79**:C133–C140, 1991.
5. Peeters S, Friesen H. A growth hormone binding factor in the serum of pregnant mice. Endocrinology **101**:1164–1183, 1977.
6. Smith WC, Colosi P, Talamantes F. Isolation of two molecular weight variants of the mouse growth hormone receptor. Mol Endocrinol **2**:108–116, 1988.
7. Smith WC, Talamantes F. Gestational profile and affinity cross-linking of mouse serum growth hormone binding protein. Endocrinology **123**:1489–1494, 1988.
8. Posner B. Characterization and modulation of growth hormone and prolactin binding in mice liver. Endocrinology **98**:645–654, 1976.
9. Kelly PA, Posner BI, Tsushima T, Friesen H. Studies of insulin, growth hormone and prolactin binding: Ontogenesis, effects of sex and pregnancy. Endocrinology **95**:532–539, 1974.
10. Smith WC, Talamantes F. Identification and characterization of a heterogeneous population of growth hormone receptors in mouse hepatic membranes. J Biol Chem **262**:2213–2219, 1987.
11. Smith WC, Linzer DIH, Talamantes F. Detection of two growth hormone receptor. mRNAs and primary translation products in the mouse. Proc Natl Acad Sci **85**:9576–9579, 1988.
12. Smith WC, Kuniyoshi J, Talamantes F. Mouse serum growth hormone binding protein has growth hormone receptor extracellular and substituted transmembrane domains. Mol Endocrinol **3**:984–990, 1989.
13. Baumbach WR, Homer DL, Logan JS. The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. Genes Dev **3**:1199–1205, 1989.
14. Mathews LS, Enberg B, Norstedt G. Regulation of growth hormone receptor gene expression. J Biol Chem **264**:9905–9910, 1989.
15. Barton DE, Foellmer BE, Wood WI, Franke U. Chromosome mapping of the growth hormone receptor gene in man and mouse. Cytogenet Cell Genet **50**:137–141, 1989.
16. Godowski PJ, Leung DW, Meacham LR, Galgani JP, Hellmiss R, Keret R, Rotwein PS, Parks JS, Laron Z, Wood WI. Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with laron-type dwarfism. Proc Natl Acad Sci **86**:8083–8087, 1989.
17. Bazan FJ. A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors and the p75 IL-2 receptor β-chain. Biochem Biophys Res Commun **164**:788–795, 1989.
18. Cramer SD, Barnard R, Engbers C, Ogren L, Talamantes F. Expression of the growth hormone receptor and growth hormone-binding protein during pregnancy in the mouse. Endocrinology **131**:876–882, 1992.
19. Smith WC, Talamantes F. Gestational profile and affinity cross-linking of mouse serum growth hormone binding protein. Endocrinology **123**:1489–1494, 1988.
20. Sanchez-Jimenez F, Fielder PJ, Martinez R, Smith WC, Talamantes F. Hypophysectomy eliminates and growth hormone maintains the mid-pregnancy elevation in growth hormone receptor and serum binding protein in the mouse. Endocrinology **126**:1270–1275, 1989.
21. Cramer SD, Wong L, Kensinger RS, Ogren L, Talamantes F. Regulation of the hepatic growth hormone receptor and serum growth hormone-binding protein during pregnancy in the mouse: effects of litter size. Endocrinology **131**:2914–2920, 1992.
22. Barnard R, Thordarson G, Lopez MF, Yamaguchi M, Edens A, Cramer SD, Ogren L, Talamantes F. Expression of growth hormone (GH)-binding protein with hydrophilic carboxy-terminus by the mouse placenta: Studies *in vivo* and *in vitro*. J Endocrinol (in press).