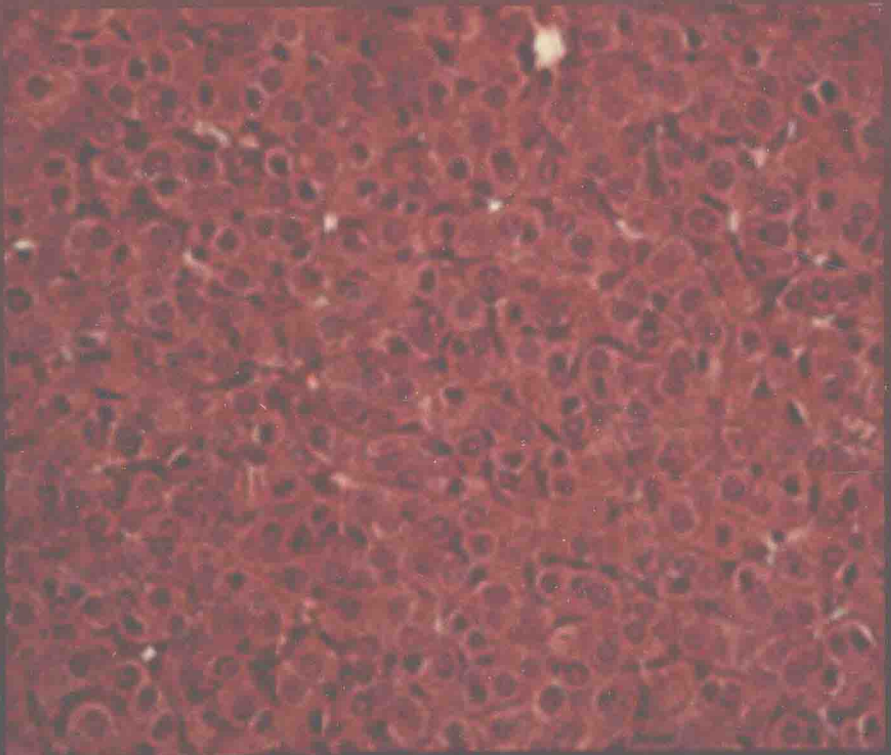




Current Topics in
**Developmental
Biology**



Volume 68

Edited by

Gerald P. Schatten

Current Topics in Developmental Biology

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Contents

Contributors xi

1 ---

Prolactin and Growth Hormone Signaling

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I. Introduction	2
II. Growth Hormone	2
III. Prolactin	5
IV. The Jak/Stat Pathway	7
V. Putative Jak2/RUSH Pathway	12
VI. Alternatives to Jak/Stat Signaling	15
VII. Negative Regulators of Jak/Stat	15
VIII. Conclusions	17
Acknowledgments	18
References	18

2 ---

Alterations in cAMP-Mediated Signaling and Their Role in the Pathophysiology of Dilated Cardiomyopathy

Matthew A. Movsesian and Michael R. Bristow

I. Introduction	26
II. cAMP-Mediated Signaling in Dilated Cardiomyopathy	27
III. What Inferences Can be Drawn from Clinical Studies?	30
IV. Probing More Deeply in Transgenic Animals	31
V. Evidence that Attenuation of cAMP-Mediated Signaling is Pathogenetic	31
VI. Evidence that the β -Adrenergic Receptor-Mediated Signaling Changes Are Compensatory	32

VII. Inferences from Studies in Transgenic Animals	33
VIII. The Compartmentation of cAMP-Mediated Signaling in Cardiac Myocytes	34
IX. Evidence for the Potential Benefits of Increasing the Phosphorylation of Individual PK-A Substrates	36
X. The Compartmentation of cAMP-Mediated Signaling in Dilated Cardiomyopathy in Humans	37
XI. Implications	38
Acknowledgments	41
References	41

3

Corpus Luteum Development: Lessons from Genetic Models in Mice

Anne Bachelot and Nadine Binart

I. Introduction	50
II. Development of the Corpus Luteum	50
III. Luteolysis	61
IV. Factors Affecting the Maintenance or the Development of the Corpus Luteum: Mouse Models	66
V. Conclusion	78
References	79

4

Comparative Developmental Biology of the Mammalian Uterus

Thomas E. Spencer, Kanako Hayashi, Jianbo Hu, and Karen D. Carpenter

I. Introduction	86
II. Development of the Müllerian Duct and Uterus	86
III. Comparative Development of the Uterus	91
IV. Mechanisms Regulating Prenatal Development of the Müllerian Duct	96
V. Mechanisms Regulating Postnatal Development of the Uterus	101
VI. Developmental Disruption	111
VII. Summary	113
References	114

5

Sarcopenia of Aging and Its Metabolic Impact

Helen Karakelides and K. Sreekumaran Nair

I. Introduction	124
II. What Is Aging?	125
III. Age-Related Muscle Changes in Humans	125
IV. Aging Muscle and Hormones	138
V. Summary	142
References	143

6

Chemokine Receptor CXCR3: An Unexpected Enigma

Liping Liu, Melissa K. Callahan, DeRen Huang, and Richard M. Ransohoff

I. Introduction to Chemokines and Chemokine Receptors	150
II. Cloning, Characterization, and Regulation of CXCR3 and Its Ligands	151
III. CXCR3: Roles in T-Cell Chemotaxis and Effector Functions	157
IV. Introduction to CNS Inflammatory/Autoimmune Disorders and Corresponding Animal Models	159
V. Complex Roles of CXCR3 and Its Ligands in Diverse Disease Models	161
VI. The Role of CXCR3 and Its Ligands in CNS Inflammatory/Autoimmune Disorders	170
VII. Concluding Remarks	173
Acknowledgments	173
References	174

7

Assembly and Signaling of Adhesion Complexes

Jorge L. Sepulveda, Vasiliki Gkretsi, and Chuanyue Wu

I. Introduction	184
II. Integrin-Mediated Cell-ECM Adhesion	184
III. The PINCH-ILK-Parvin Complexes	186
IV. Adhesion Complexes in Cardiac Myocytes	195
Acknowledgments	210
References	210

8

Signaling Mechanisms of Higher Plant Photoreceptors: A Structure-Function Perspective

Haiyang Wang

I. Introduction	228
II. Phytochromes	229
III. Cryptochromes	236
IV. Phototropins	243
V. Other Plant Photoreceptors	249
VI. Concluding Remarks	250
Acknowledgments	251
References	251

9

Initial Failure in Myoblast Transplantation Therapy Has Led the Way Toward the Isolation of Muscle Stem Cells: Potential for Tissue Regeneration

Kenneth Urish, Yasunari Kanda, and Johnny Huard

I. Introduction	264
II. Myoblast Transplantation	264
III. Initial Limitations of Transplantation	265
IV. Improved Skeletal Muscle Regeneration	267
V. Mechanism of Improved Muscle Regeneration	269
VI. MDSCs and the Development of Muscle	272
VII. Tissue Engineering and MDSCs	274
References	275

10

Role of 14-3-3 Proteins in Eukaryotic Signaling and Development

Dawn L. Darling, Jessica Yingling, and Anthony Wynshaw-Boris

I. 14-3-3 Introduction	282
II. 14-3-3 in Yeast Development	285
III. 14-3-3 in Plant Development	289
IV. Role of 14-3-3 in <i>Drosophila</i> Development	292
V. Role of 14-3-3 in <i>Xenopus</i> Development	299

VI. Role of 14-3-3 in Mammalian Development	301
VII. 14-3-3 in Human Development	305
VIII. Summary	306
References	306

11

The Evolution and Function of Blue and Red Light Photoreceptors

Angela Falciatore and Chris Bowler

I. Introduction	318
II. Blue Light Perception and Signaling	319
III. Red Light Perception and Signaling	334
IV. Concluding Remarks	344
Acknowledgments	345
References	345
Index	351
Contents of Previous Volumes	367

Prolactin and Growth Hormone Signaling

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- I. Introduction
- II. Growth Hormone
 - A. Hormone Synthesis and Secretion
 - B. Receptor Isoforms and Function
- III. Prolactin
 - A. Hormone Synthesis and Secretion
 - B. Receptor Isoforms and Function
- IV. The Jak/Stat Pathway
 - A. Jak2/Stat5
 - B. Jak-Receptor Interactions
 - C. Stat-Target Gene Interactions
- V. Putative Jak2/RUSH Pathway
 - A. Comparison of Jak2/RUSH with Jak2/Stat5a
 - B. RUSH-Target Gene Interactions
- VI. Alternatives to Jak/Stat Signaling
- VII. Negative Regulators of Jak/Stat
- VIII. Conclusions
- Acknowledgments
- References

Prolactin (PRL) and growth hormone (GH) act by way of their receptors as either hormones (systemically) or cytokines (locally). The Jak2/Stat5 pathway is the principal route by which PRL/GH activate target genes. The availability of knockout mice for each member of this signaling cascade has provided opportunities to understand their unique interactions. Jak2 is important in alternative signal transduction schema such as the MAP kinase and PI3K/Akt pathways. The putative Jak2/RUSH pathway is based on the fact that RUSH mediates the ability of PRL to augment progesterone-dependent gene transcription. New evidence shows that suppressors, regulators, and degraders control Jak2/Stat5. This review focuses on the most recent advances in the field of PRL/GH signal transduction. © 2005, Elsevier Inc.

I. Introduction

Prolactin (PRL), growth hormone (GH), and placental lactogen (PL) form the PRL/GH/PL peptide hormone family. Family members are derived from a common ancestral gene by duplication and sequence divergence (Cooke *et al.*, 1981; Niall *et al.*, 1971), and they bind to highly conserved cell surface receptors. Structural similarities between these receptors and those of a larger class of colony-stimulating factors, interleukins, and cytokines resulted in the recent assignment of PRL/GH/PL to the extended family of hematopoietic cytokines (Horseman and Yu-Lee, 1994). Despite the diversity in this superfamily, the three-dimensional architecture of its members is subject to evolutionary selection by receptor-binding constraints based on common motifs in the extracellular and intracellular domain structure of the receptors (Forsyth and Wallis, 2002). Inside target cells, PRL and GH share the Jak2/Stat5a/b signal transduction pathway (Aaronson and Horvath, 2002; Grimley *et al.*, 1999; Herrington *et al.*, 2000; Ihle, 2001; Kisseleva *et al.*, 2002; Leung *et al.*, 2004). The putative Jak2/RUSH pathway is unique to PRL signaling (Hewetson *et al.*, 2002). As a progesterone-regulated, SWI/SNF-related transcription factor and putative helicase, RUSH-1 α is remarkably different from the Stat5a proteins known to mediate PRL action. In contrast to the cross talk between Jak/Stat and other cytokine signaling pathways, RUSH coordinates two independent signaling pathways; one is direct transcriptional regulation by the progesterone receptor, and the other is the extended protein phosphorylation cascade of the PRLR. SOCS family members suppress Jak2/Stat5 signaling by PRL and GH (Alexander and Hilton, 2004; Johnston, 2004). Other negative regulators include PTP and PIAS (Greenhalgh and Hilton, 2001; Wormald and Hilton, 2004). Negative regulation by RUSH may include competitive inhibition by the alternative splice variant, RUSH-1 β .

For this review, some aspects of hormone structure and regulation were addressed to set the stage for an evaluation of proximal signaling networks for PRL/GH. In addition to the reviews already referenced in this section, the interested reader might also enjoy the following on GH/GHR (Anderson *et al.*, 2004), PRL/PRLR (Goffin *et al.*, 2002; Schuler *et al.*, 2001), and alternative signaling (Herrington and Carter-Su, 2001).

II. Growth Hormone

A. Hormone Synthesis and Secretion

Release of GH, a 22-kDa polypeptide hormone, from the somatotropes of the anterior pituitary into the circulation is stimulated by GH-releasing hormone and ghrelin (acting by way of the GH secretagogue receptor) and

inhibited by somatostatin (Frohman *et al.*, 2000; Kojima *et al.*, 2001). Evidence from a variety of animal models supports the idea that GH secretion is also influenced by gonadotropin-releasing hormone, insulin-like growth factor 1, leptin, pituitary adenylate cyclase-activating polypeptide, and thyrotropin-releasing hormone. GH circulates as a monomer, and it is generally accepted that it functions in monomeric form even though it can aggregate. Excess GH results in acromegaly (Colao and Lombardi, 1998; Daughaday and Harvey, 1995; Melmed *et al.*, 1995), and GH deficiency results in diminished postnatal growth (Reiter and Rosenfeld, 2003).

B. Receptor Isoforms and Function

GH receptor (GHR) and GH-binding protein (GHBP) mediate the somatogenic and metabolic effects of GH. GHR, a membrane-embedded protein that binds GH, is required for cells to respond to GH. Alternative processing of GHR transcripts yields circulating GHBP and truncated GHRs. Cloned in 1987 by Leung *et al.*, GHR is a 100–130-kDa member of the cytokine/hematopoietin superfamily characterized by a single hydrophobic transmembrane pass that connects the extracellular hormone-binding domain to the intracellular signaling domain. Synthesized as a preprotein, with a short N-terminal signal peptide, GHR is directed to the cell surface, where it binds GH with high affinity and specificity. GHR transcripts are encoded by 10 exons, average 4.7-kb in size, and predominate in hepatocytes (Godowski *et al.*, 1989; Moffat *et al.*, 1999). Although there is tremendous variability in the 5'-UTR of the *GHR* gene, translation results in the synthesis of the same receptor protein (Edens and Talamantes, 1998). Laron syndrome (Laron, 2004), an inherited disease with GH resistance, ultimately became a unique clinical model for the study of GHR defects. In an effort to create an experimental model for Laron syndrome, Zhou *et al.* (1997) generated a GHR/GHBP-deficient mouse and demonstrated that GHR is the physiologically relevant receptor.

Cloning the GHR provided few clues to the conformation of hormone-receptor binding. Although several biochemical methods showed a complete 1:2 binding interaction between the hormone and its receptor (Cunningham *et al.*, 1991), characterization of the co-crystal structure of human GH and the extracellular domain of the receptor ultimately showed the complex is composed of one molecule of hormone and two molecules of receptor (Ultsch *et al.*, 1991). Within the complex, both receptors contribute identical residues to interact with two structurally dissimilar binding sites on the hormone (de Vos *et al.*, 1992). The structure of the unliganded extracellular domain of the GHR is poorly defined. Thus, it remains to be determined whether the native receptor exists as a preformed homodimer awaiting GH

binding, undergoes dimerization in response to GH binding, or participates in other GH-induced conformational changes. The extensive contact surface between the carboxyl-terminal domains of the receptors could permit sequential dimerization. The crystal structure of the closely related erythropoietin receptor (EpoR) shows unliganded receptors exist as preformed dimers in an open scissor-like configuration, which keeps the cytoplasmic domains apart (Frank, 2002). Presumably then, ligand occupancy brings both the extracellular and intracellular domains into close proximity to facilitate signaling. Collectively, GHR, EpoR, PRL, and thrombopoietin receptors constitute a unique subset of the class I cytokine receptor group, because they bind alone to their cognate ligands rather than as part of a heterooligomeric complex.

Alternative splicing of the human GHR yields two short receptor membrane-embedded isoforms (GHR1-277 and GHR1-279) with extracellular/hormone-binding domains and truncated cytoplasmic domains that are incapable of signal transduction (Dastot *et al.*, 1996; Ross *et al.*, 1997). The GHR1-277 product results from skipping exon 9 such that the C-terminal residues are three frame-shifted codons from exon 10 plus an in-frame stop codon. The GHR1-279 product results from the use of an alternative 3'-acceptor site that is 26-bp downstream in exon 9. As a result, the predicted C-terminal residues are six frame-shifted exon 9 codons plus an in-frame stop codon. When the truncated isoforms heterodimerize with full-length receptor, they invoke dominate negative inhibition of GHR signaling (Ayling *et al.*, 1997; Iida *et al.*, 1998; Ross *et al.*, 1997). The short isoforms also contribute to GHBP production (Allevato *et al.*, 1995; Amit *et al.*, 1999).

Evolutionary divergence in GHBP generation (Dastot *et al.*, 1998) is suggested by the fact that GHBP is produced either by proteolytic cleavage (humans, rabbits) of the GHR extracellular/hormone-binding domain (Baumann, 2001; Wang *et al.*, 2002), or alternative splicing (rodents) of the *GHR* gene (Baumbach *et al.*, 1989; Edens *et al.*, 1994; Smith *et al.*, 1989). GHBP is found in the circulation, in intracellular locations, and associated with cell membranes. In the circulation, GHBP prolongs the half-life of GH. GHBP levels are higher in females than males, where an estimated 40–50% of circulating GH complexes with GHBP in humans (Baumann and Shaw, 1988, 1990) compared with 20–50% in mice (Turyn *et al.*, 1997). The percentage of GH bound is greater than 90% during pregnancy (Cramer *et al.*, 1992). In addition, there is a diurnal fluctuation in the percentage bound because of the pulsatile release of GH from the anterior pituitary (Veldhuis *et al.*, 1993). Intracellular GHBP is purported to mediate transport of GH (Baumann *et al.*, 1987), whereas cell membrane-associated GHBP competes with tissue receptors for GH binding (Mannor *et al.*, 1991).

III. Prolactin

A. Hormone Synthesis and Secretion

PRL, a 23-kDa polypeptide hormone, is synthesized and secreted by lactotrophs of the anterior pituitary and by other cells and tissues in the body. Release of PRL from the lactotrophs into the circulation is under the inhibitory control of dopamine (Samson *et al.*, 2003). Large, reversible PRL aggregates form in the lumen of the trans layer of the Golgi complex (Giannattasio *et al.*, 1975; Rambourg *et al.*, 1992). When released into the circulation, they rapidly dissolve into correctly folded, functionally active, monomeric protein. Attempts to identify authentic PRL-releasing factors (PRF) have failed. None of the potential candidates, including thyrotropin-releasing hormone, vasoactive peptide, oxytocin, endothelin, prolactin releasing peptide, or neuropeptide W, has emerged as the requisite PRF. The tertiary structure of human PRL, as determined by solution heteronuclear nuclear magnetic resonance spectroscopy (Keeler *et al.*, 2003), showed that although the topology of PRL and GH resembles that of other hematopoietic cytokines, they also differ in three structurally important ways. These include the absence of a helix in the first extended loop of prolactin, structural differences in the short loop connecting the second and third helices, and an extension from the N-terminus that has a unique conformation. Excess PRL results in hyperprolactinemia (Colao and Lombardi, 1998), and PRL deficiency results in impaired mammaryogenesis (Ormandy *et al.*, 2003).

B. Receptor Isoforms and Function

Classical, as well as autocrine–paracrine, effects of PRL are mediated by the PRL receptor (PRLR). The major membrane-bound PRLR isoforms are styled short, intermediate, and long because of differences in their cytoplasmic domains. Their extracellular domains are identical. In addition to membrane-bound receptors, soluble prolactin-binding protein (PRLBP) is generated by cellular proteolysis of the extracellular domain of the PRLR. PRLBP binds 30–35% of the available PRL in serum, but it cannot signal.

PRLR isoforms have been described in different species (Clevenger and Kline, 2001; Freeman *et al.*, 2000). In the rat, the three major isoforms are the long (591 amino acids), intermediate (393 amino acids), and short (291 amino acids) forms. In mice, where one long form and three short forms of the receptor exist, the phenotype of PRLR null mice was attributed to the targeted disruption of the long form. In 1989, the human ortholog (598 amino acids) of the long form of the rat receptor was cloned (Boutin *et al.*,

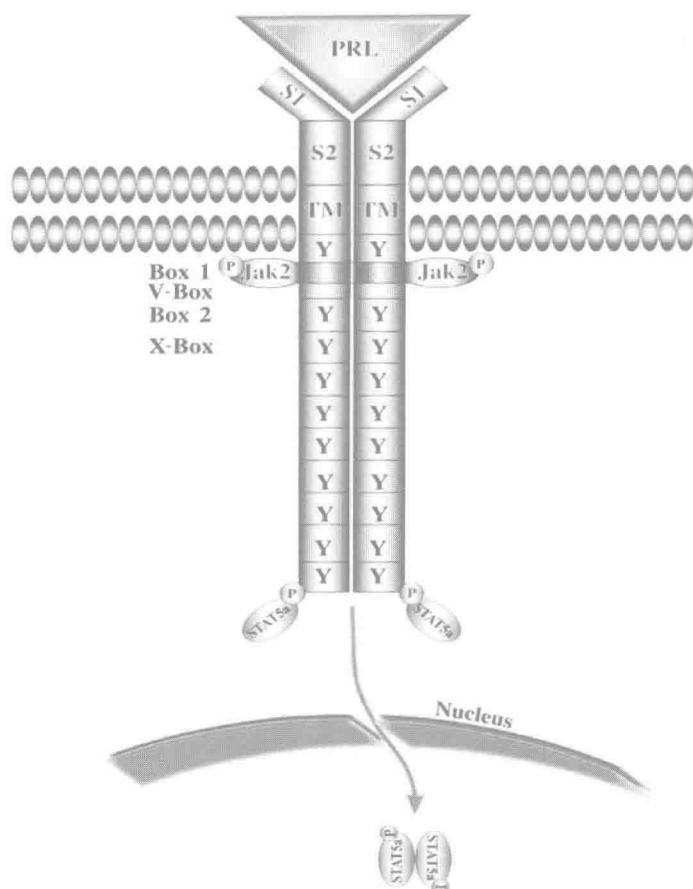


Figure 1 Schematic representation of PRL bound to dimerized (activated) long form of the human prolactin receptor. The extracellular domain of the receptor contains two type 3 fibronectin-like domains (S1 and S2) that have binding determinants for the ligand (PRL). The transmembrane (TM) domain consists of 24 amino acids that are mostly hydrophobic. The Box 1 and Box 2 motifs that characterize the membrane proximal region of the intracytoplasmic domain are highly conserved in the cytokine receptor superfamily. Proline-rich Box 1 is hydrophobic and required for Jak2 binding. Acidic Box 2 is hydrophobic and functionally uncharacterized. Although the intervening variable box (V-Box) and the extended Box 2 (X-Box) are not well conserved or characterized, the V-box together with Box 2 may contribute to Jak2 activation. A total of 10 tyrosine (Y) residues are found in the intracellular domain. The most C-terminal tyrosine residue is purported to interact with the SH2 domain of Stat5a. Stat5a is phosphorylated by receptor-associated Jak2 while docked at the receptor. Individual phosphorylated Stat5a molecules are released from the receptor to homodimerize by way of phosphotyrosine residues with the SH2 domain of another phosphorylated Stat5a or Stat5b molecule. Stat5a/b dimers translocate to the nucleus where they bind GAS (γ -activated sequence) elements in target genes such as β -casein, whey acidic protein, β -lactoglobulin, and α -lactalbumin.

1989). Like the GHR, a single hydrophobic transmembrane pass that connects the extracellular hormone-binding domain to the intracellular signaling domain characterizes the PRLR (Bole-Feysot *et al.*, 1998; Clevenger and Kline, 2001). The extracellular domain (Fig. 1) contains two type 3 fibronectin-like regions termed the S1 and S2 domains. A short transmembrane pass (24 amino acids) connects the extracellular and intracytoplasmic domains (Fig. 1). The latter contains a highly conserved membrane proximal region with Box 1 and 2 motifs, an intervening variable box (V-box), and the extended Box 2 (X-box). Box 1 is hydrophobic and has a proline-rich, SH3-like binding domain required for Jak2 binding. This box is conserved in all PRLR isoforms and required for Jak2 interactions with the receptor. Box 2 is hydrophobic, acidic, and relatively uncharacterized. This box is absent from the short PRLR isoforms. Boxes V and X are poorly understood. The intracellular domain contains 10 tyrosine residues, the most C-terminal (Y587) of which contributes to the engagement of Stat5 proteins. The human intermediate form of the PRLR is identical to the long form from the extracellular domain through the membrane proximal domain including Box 1 and Box 2 motifs. An RNA processing event results in premature truncation of the intermediate form such that it spans only 325 amino acids and is missing 7 tyrosine residues including Y587. Although this deletion had no impact on the activation of Jak2, activation of Fyn was greatly diminished. The Δ S1 isoform is nearly identical to the long form of the receptor except, because of alternative splicing, its extracellular domain is missing the S1 domain. This isoform has reduced PRL affinity but faster signaling capabilities. Nearly all cells express PRLR, and most express more than one receptor isoform. As with GH signaling, one molecule of PRL binds two molecules of PRLR. Homodimerization of the different PRLR isoforms should have different signaling properties, whereas heterodimerization of the different PRLR isoforms blocks signal transduction.

IV. The Jak/Stat Pathway

A. Jak2/Stat5

Binding of class I/II cytokines to their receptors promotes tyrosine phosphorylation through members of the Janus kinase (Jak) family of associated tyrosine kinases because the cytoplasmic tails of the receptors are devoid of intrinsic enzymatic activity (Leung, 2004). Signal transduction continues by means of phosphorylation of the cytosolic Stat (Signal transducer and activator of transcription) family of transcription factors (Fig. 1). Jak2/Stat5 is the best characterized of the GH/PRL signaling cascades. PRL signal transduction begins with hormone-receptor binding, which invokes receptor