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Gene regulation by growth hormone

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Abstract

Since the somatomedin hypothesis of growth hormone (GH) action was first formulated more than 50 years ago, the key roles of both GH and insulin-like growth factor-I (IGF-I) in human growth have been extended to include important effects on tissue maintenance and repair. More recent observations have revealed that this pathway has a negative side, as it has been implicated as a potential contributor to the development of several human cancers and has been linked to diminished lifespan in experimental animals. This brief review focuses on fundamental aspects of gene regulation by GH, as long-term hormonal effects all require changes in gene expression. Topics to be discussed include GH-stimulated signal transduction pathways, mechanisms of gene activation and gene repression by GH, and an analysis of control of IGF-I gene transcription by the GH-stimulated transcription factor, signal transducer and activator of transcription (Stat)5b.

Keywords

Growth hormone; GH; Growth regulation; Insulin-like growth factor-I; IGF-I; Stat; Stat5b; Gene transcription

Overview

Since the somatomedin hypothesis of growth hormone (GH) action on growth was first formulated in 1957 [1], much has been learned about the physiology of the GH – insulin-like growth factor-I (IGF-I) – growth axis [2–4]. The central roles of this pathway in normal pre- and postnatal growth in mammals have been extended to include actions on tissue maintenance, regeneration, and repair in the adult [3, 4]. Along with a clearer definition of positive actions of GH and IGF-I, an appreciation of their potentially harmful effects has been established. The negative roles of excessive GH on glucose metabolism and on cardiovascular function in acromegaly have been long known [5], as have the pathogenic effects of both GH and IGF-I in proliferative diabetic retinopathy [6]. GH and IGF-I actions

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also have been linked in experimental animals to accelerated aging [7], and IGF-I has been found in epidemiological studies in humans and in experimental models in animals to contribute to several types of cancer [8–10]. Taken together, these studies emphasize the importance of understanding the basic biochemistry and molecular biology of the GH – IGF-I pathway in order to modulate its effects on human health and disease.

The focus of this brief review is on fundamentals of gene regulation by GH from both molecular biological and physiological perspectives, with an emphasis on IGF-I as a central target of GH actions. Topics discussed include GH-activated signal transduction pathways, mechanisms of gene activation and gene repression by GH, and an analysis of control of IGF-I gene transcription by the GH-stimulated transcription factor, signal transducer and activator of transcription (Stat)5b.

Introduction: GH and IGF-I in growth and tissue maintenance

It has long been known that both GH and IGF-I are critical for normal human growth [2]. Natural mutations of the GH gene or impaired development of pituitary somatotrophs cause growth deficiency and short stature in children [11], as do mutations in the GH receptor gene [12, 13]. Mutations that disrupt the IGF-I or IGF-I-receptor genes also lead to profound growth failure that is not responsive to GH [14, 15]. Unlike subjects with GH or GH-receptor deficiency, where growth defects are manifested postnatally, these latter individuals have intrauterine growth failure and exhibit other abnormalities that are consistent with a broader role for IGF-I in both growth regulation and tissue development than as just a mediator of GH actions. Analyses in mice with a targeted deletion of IGF-I or the IGF-I receptor have further established the critical importance of IGF action for normal development in the embryo and fetus [16, 17], whereas knockout of the GH receptor gene has confirmed its key roles in postnatal growth and controlling IGF-I production [18].

GH and IGF-I also play major roles in tissue and organ growth during childhood and in tissue maintenance and repair in the adult. For example, mice lacking the GH receptor have diminished cortical bone formation and trabecular bone turnover that is nearly completely corrected within weeks by systemic infusion of IGF-I [19], demonstrating the significance of the endocrine actions of GH and IGF-I in bone, as initially postulated by the original somatomedin hypothesis [1]. Similarly, engineered loss of IGF-I gene expression in the liver combined with genetic deficiency of the serum carrier molecule acid labile subunit (ALS) [liver-specific IGF-I-deficient (LID) plus ALS knockout (KO) mice] [20] also leads to defects in wholebody and long bone length that are ameliorated by exogenous IGF-I treatment [20], further supporting an endocrine role for IGF-I in bone. Moreover, mice with a systemic IGF-I gene knockout also exhibit impaired bone formation [21] and show a minimal anabolic response to intermittent treatment with parathyroid hormone (PTH) [21], which normally stimulates increases in bone mineral density [22, 23]. As PTH induces IGF-I messenger RNA (mRNA) and protein expression in osteoblasts [24], these latter results highlight a role for locally produced IGF-I in mediating some of the actions of PTH in bone [21] and are consistent with the modified somatomedin hypothesis, which emphasizes autocrine and paracrine IGF actions [2–4]. Other experimental models have provided additional support for direct effects of IGFs in bone growth. As an example, targeted

overexpression of IGF-I in osteoblasts in transgenic mice caused a doubling of the rate of bone formation in the distal femur and increased the volume of trabecular bone [25]. Taken together, these observations demonstrate key roles for systemic GH and IGF-I in bone formation and osteoblast function and show that agents such as PTH depend in part on local production of IGF-I to mediate bone growth.

GH and IGF actions also are important for normal skeletal muscle growth and repair. Mice lacking IGF-I or the IGF-I receptor have severely diminished muscle mass [17, 26], and mice deficient in the GH receptor also have muscle defects [27]. Similarly, transgenic mice expressing a dominant-negative IGF-I receptor in muscle exhibited a decrease in muscle mass and strength that was compensated in part by increased numbers of myonuclei per muscle fiber [28]. In contrast, overexpression of IGF-I in muscle of transgenic mice caused muscle fiber hypertrophy [29], prevented muscle loss during aging [30–32], and compensated for the decline in muscle mass seen in experimental muscular dystrophy [33]. Other evidence suggests that locally derived IGFs facilitate muscle repair both after injury and in response to increased muscle force and work. IGF-I mRNA and protein have been detected in replicating myoblasts after ischemic or toxic injury [34, 35], and expression of IGF-I and IGF-II were increased as early events in work-induced muscle hypertrophy [36, 37]. Thus, sustained local GH and IGF actions are important for muscle growth, maintenance, repair, and regeneration.

GH signaling and actions

GH-activated signal transduction pathways

A single GH molecule binds sequentially with high affinity to the extracellular domains of two GH receptors [38], leading to rapid activation of receptor-associated intracellular tyrosine protein kinase Jak2 [39, 40]. Jak2 then initiates a series of protein phosphorylation steps that culminate in induction of several intracellular signaling networks, including the Ras – Raf – Mek – Erk and PI3-kinase – Akt pathways [39, 40]. Among downstream signaling molecules acutely activated by the GH receptor and Jak2 are several transcription factors [41], including members of the Stat family [42], and these proteins in turn are responsible for many of the effects of GH on gene expression [41, 42]. Stats were identified originally as components of interferon (IFN)-α- and IFN-γ-signaling pathways [43] and are now known to be activated in response to many cytokines and hormones, including GH [39, 40]. GH induces Stats 1, 3, 5a, and 5b in a variety of cell types [44–50]. Once activated, these factors form homodimers (and heterodimers for Stats 1 and 3), enter the nucleus, and bind to response elements in the chromosomal DNA of a range of target genes [43].

Stats and GH actions

The putative roles of Stats in mediating GH-regulated somatic growth were investigated initially in gene knockout experiments in mice. Inactivation of Stat1 did not alter growth, and Stat3 deficiency caused early embryonic death (reviewed in [43]). Joint knockout of Stat5a and Stat5b impaired growth of male and female mice to 60–70% of wild type and led to a 50% decline in serum IGF-I levels [51]. Stat5b deficiency alone was associated with an ~20% decrease in postnatal growth and to diminished IGF-I levels in male mice but had no

effect on growth rates or IGF-I in females [51, 52]. Subsequent studies investigating potentially novel causes of growth defects in children identified inactivating mutations of Stat5b as the responsible molecular lesions [53–55]. In addition to clinical features consistent with severe phenotypic GH-deficiency, these individuals also appear to have immunological dysfunction that is manifested in part by recurrent and chronic infections [55]. Other more biochemical experiments directly connected GH-activated Stat5b to induction of IGF-I and other gene transcription (see below).

GH actions and gene regulation

Gene activation by GH

Several studies have begun to catalog genes activated by GH under a variety of different experimental situations [56–61], and efforts have been initiated to identify transcription factors responsible for induction of each GH-regulated gene, although in several of these studies, it has not been established whether GH is acting at transcriptional or posttranscriptional steps (e.g. to alter mRNA stability). In publications in which investigators employed mRNA profiling to define GH-regulated gene expression, variably sized cohorts of genes appear to be activated by GH, and with different kinetics. For example, in the 3T3 adipocyte cell culture model, Huo et al. found that GH induced the expression of 13 transcripts at 30 min but only two mRNAs at 4 h [59]. In contrast, 37 transcripts were up-regulated after 48 h of hormone exposure [59], suggesting that secondary signaling pathways are activated during chronic GH treatment. Similarly, Thompson et al. found that only eight genes were stimulated by GH in the liver within 3 h of a single hormone injection into GH-deficient rats [56], whereas Vidal et al. measured 52 hepatic mRNAs that increased in abundance by 2 h after GH treatment [60]. Of this latter group of acutely GH-activated genes, ~20% appeared to be dependent on Stat5b [60], and one of these genes was IGF-I (see below).

No comparable studies have been performed in humans. However, when given as a single bolus or short-term infusion to healthy individuals, GH is able to acutely stimulate gene expression, including inducing mRNAs for suppressor of cytokine signaling (SOCS)-1, -2, and -3 in both muscle and fat, and for IGF-I in muscle [62, 63].

In several mammalian species, most notably rodents, the pattern of GH secretion from the pituitary exerts a significant impact on gene expression in the liver and other tissues [64]. In the rat, adult males secrete GH in a highly pulsatile way, leading to peaks and troughs of plasma GH levels, whereas in females, in which GH secretion is more continuous, plasma hormone levels are relatively constant [64]. These gender-specific differences in exposure of cells to GH lead to sexually dimorphic programs of gene expression. Using gene expression profiling, Wauthier and Waxman showed that GH deficiency eliminated most of these differences [65], whereas Ahluwalia et al. found that 44/49 mRNAs whose levels of expression were higher in the liver of male versus female rats were reduced to female levels after 7 days of continuous (female pattern) GH treatment [66], implying that the chronic pattern of GH signaling has a significant impact on gene regulation.

Gene repression by GH

As GH plays a central role in controlling somatic growth, tissue repair and regeneration, intermediary metabolism, and other biological actions, it is not surprising the GH-activated signaling leads to both induction and inhibition of gene expression. Only recently have experiments addressed potential mechanisms by which GH impairs gene expression. Using GH-receptor-deficient mice as the model, Rowland et al. found that the chronic absence of GH signaling led to alterations in the abundance of 330 hepatic genes compared with receptor-intact control mice, with 269 transcripts being increased and 61 being reduced [58]. These results clearly illustrate that GH-mediated signaling exerts a significant inhibitory effect on gene expression, but they do not illumine the relevant biochemical or molecular mechanisms. Zhou and Waxman were the first to show that Stat5b could function as a GH-induced repressor of gene expression. They found that genes induced by several other transcription factors, including peroxisome proliferator-activated receptor (PPAR)-α, -γ, and -δ and the thyroid hormone receptor were inhibited by Stat5b [67, 68]. Using a global gene expression profiling approach, we subsequently demonstrated that Stat5b appeared to be responsible for the vast majority of genes acutely repressed by GH in the liver of rats (89/97 genes inhibited by GH within 2 h of hormone treatment were Stat5b-dependent [69]). Among these GH-inhibited hepatic genes was IGFBP-1, which encodes IGF binding protein-1, a modulator of the bioavailability of IGF-I in the blood [3, 70]. We found that GH-activated Stat5b repressed IGFBP-1 gene transcription by interfering with the actions of forkhead box O1 (FoxO1), which is the major transcriptional inducer of *IGFBP-1* [71]. The biochemical mechanisms by which GH inhibits IGFBP-1 gene expression thus differ from those of insulin, which represses IGFBP-1 gene transcription through activation of Akt, which phosphorylates FoxO1, thus causing its exit from the nucleus [71, 72]. Bioinformatic analysis of the 89 genes inhibited by GH via Stat5b revealed that only ~20% contained putative FoxO1 binding sites in their gene promoters [69], indicating that interference with FoxO1 is only one of several potential mechanisms of gene inhibition by Stat5b. Studies by Clodfelter et al. also concluded that Stat5b could repress gene expression, as they found that the abundance of several hundred mRNAs was increased in the liver of mice lacking this transcription factor compared with wild-type controls [61].

Regulation of IGF-I gene expression by GH

IGF-I protein and gene structure

IGF-I is a single-chain 70 amino acid peptide with a highly conserved amino acid sequence and a complicated and conserved gene structure (reviewed in [73]). In mammals, the single-copy IGF-I gene, located on chromosome 12 in humans, consists of six exons and five introns and spans more than 80 kb of chromosomal DNA [73]. IGF-I gene expression is governed by several biochemical mechanisms that lead to the production of multiple IGF-I mRNAs through the combination of distinct promoter use, variable transcription start sites, alternative RNA splicing, and differential RNA polyadenylation (reviewed in [73]). In mammals, individual promoters reside 5′ to IGF-I exons 1 and 2 (P1 and P2, respectively), whereas in nonmammalian vertebrates, a single promoter is adjacent to exon 1. P1, the major promoter in mammals, is active in all tissues in which IGF-I is expressed [74, 75]. P1 regulates IGF-I transcripts containing exon 1, and nucleotide sequences of rat and human

exon 1 also are very similar to each other (95% identity), as is exon 1 of other species [74, 76–78]. Exon 1 contains the 5′ nontranslated region of IGF-I mRNA and the first 21 codons of the IGF-I signal peptide. P2 governs transcripts containing exon 2 and is active primarily in the liver, where it is responsible for ~25% of IGF-I mRNAs under normal physiological conditions [75, 79]. Exon 2 also encodes a 5′ nontranslated region and the initial six codons for an alternative signal peptide [73]. Both P1 and P2 regulate transcription over dispersed start sites [73], and GH activates both promoters to an equivalent extent in the liver [80, 81]. In mammals IGF-I transcripts containing either exons 1 or 2 are spliced onto exon 3 [73]. Exon 3 encodes the distal common 27 amino acids of the signal peptide and the first 25 amino acids of mature IGF-I. The remainder of 70 residue IGF-I is located in exon 4, which also contains the common part of two distinct carboxylic acid (COOH)-terminal peptide extensions that are found in IGF-I precursor proteins (E domains [73]). Interested readers are referred to a more comprehensive review of IGF-I gene and protein structure [73].

Stat5b and control of IGF-I gene transcription by GH

It has been known for many years that GH rapidly and potently induces IGF-I gene transcription in vivo [82, 83]. Despite this knowledge and despite much effort by several groups, the use of conventional assays of promoter function and DNA protein-binding studies has yielded little about biochemical mechanisms of IGF-I gene activation by GH beyond identification of promoter elements needed for basal transcriptional control [84–87]. The only molecular clue to regulation by GH was identification of a GHstimulated alteration in chromatin structure in the second IGF-I intron by our group [82, 83]. This single GHregulated DNase-I hypersensitive site (HS7) appeared just prior to induction of IGF-I gene transcription by GH and disappeared coincident with the fall in IGF-I transcription by ~6 h after single hormone pulse to GH-deficient rats [82, 83]. Subsequent studies with recombinant adenoviruses expressing modified versions of rat Stat5b showed that a dominant-negative Stat5b completely blocked GH-induced IGF-I gene transcription in the liver of rats and prevented accumulation of IGF-I mRNA, whereas a constitutively active Stat5b stimulated IGF-I gene expression even in the absence of GH [80, 81]. Based on these observations, we soon identified two adjacent putative Stat5 binding sites in the HS7 region of the rat IGF-I gene that were conserved in IGF-I genes from other mammalian species, and we showed that GH rapidly induced binding of Stat5b to this DNA segment in vivo, with onset just prior to initiation of IGF-I gene transcription from both promoters in the liver [80, 81]. Subsequent experiments by our group and others have mapped several potential Stat5b binding sites to chromatin within both the rat and human IGF-I loci [88–90]. Taken together, these observations provide a framework for elucidating the biochemical mechanisms by which GH induces IGF-I gene transcription through Stat5b under normal physiological conditions. Figures 1 and 2 present an outline of mechanisms of regulation of gene expression by GH.

Summary and conclusions

GH and IGF-I play multiple roles in human and animal physiology and disease. They are essential for normal preand postnatal growth and are key factors in normal tissue repair and regeneration throughout the lifespan. Conversely, actions of GH and IGF-I have been linked

to accelerated aging and to cancer development and metastasis. A more comprehensive understanding of the basic biochemistry and molecular biology of GH and IGF-I actions is needed to devise ways to separate therapeutically useful from deleterious effects of this potent hormonal signaling system.

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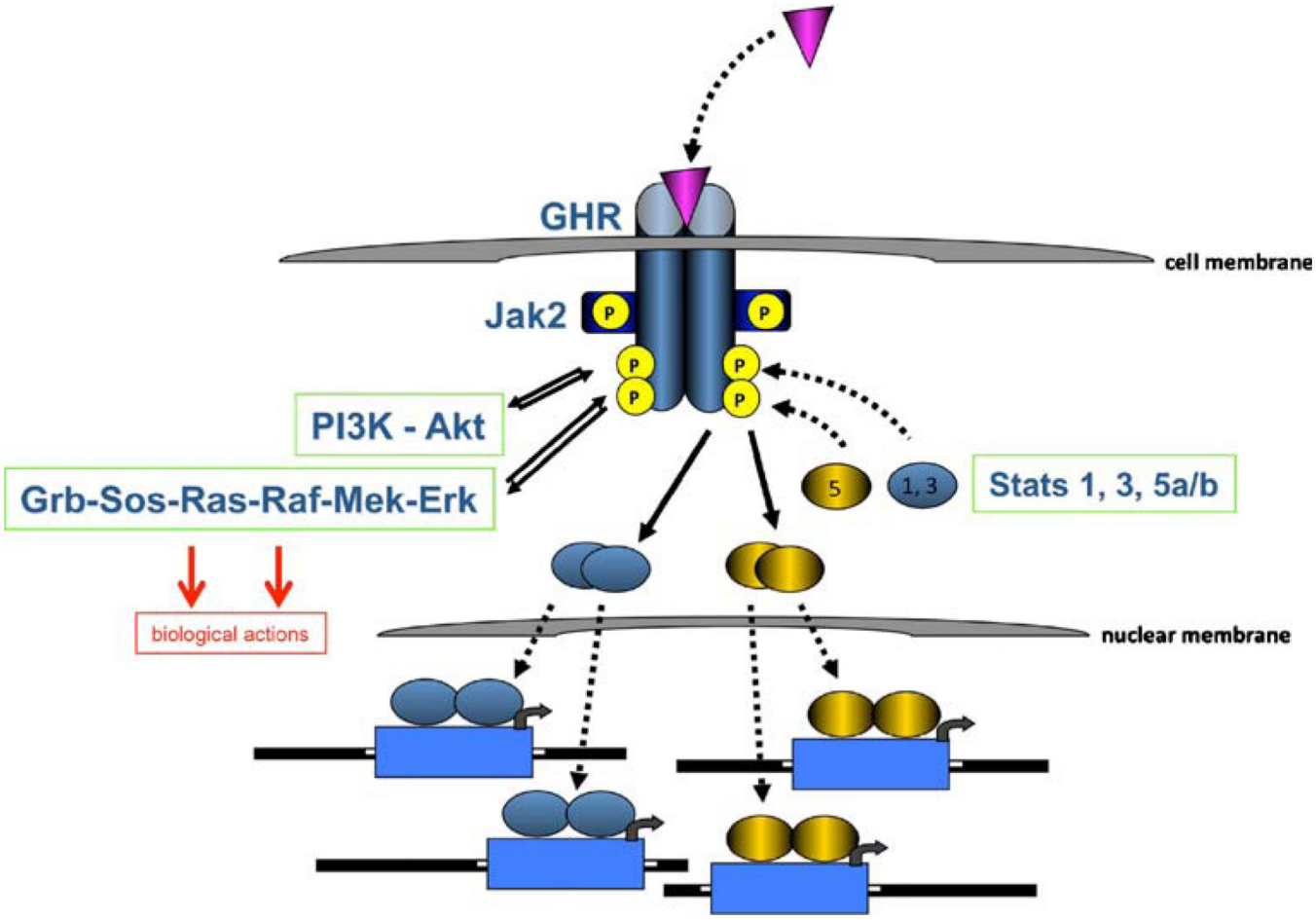

Fig. 1.

Schematic diagram of growth hormone (GH) receptor signaling and gene activation. GH (*purple triangle*) binds to its receptor, causing both receptor dimerization and activation of the associated intracellular tyrosine kinase, Jak2, which phosphorylates itself and the GH receptor on tyrosine residues (each indicated as *P* within a *yellow circle*). A series of intracellular signal transduction pathways are activated, which mediate the biological effects of GH. Signal transducer and activator of transcription (Stats) 1, 3, 5a, and 5b are recruited to the GH receptor and become tyrosine phosphorylated, leading to their dimerization and translocation into the nucleus, where they bind to DNA in chromatin of target genes and activate gene transcription (indicated by *bent arrows* at transcription start sites)

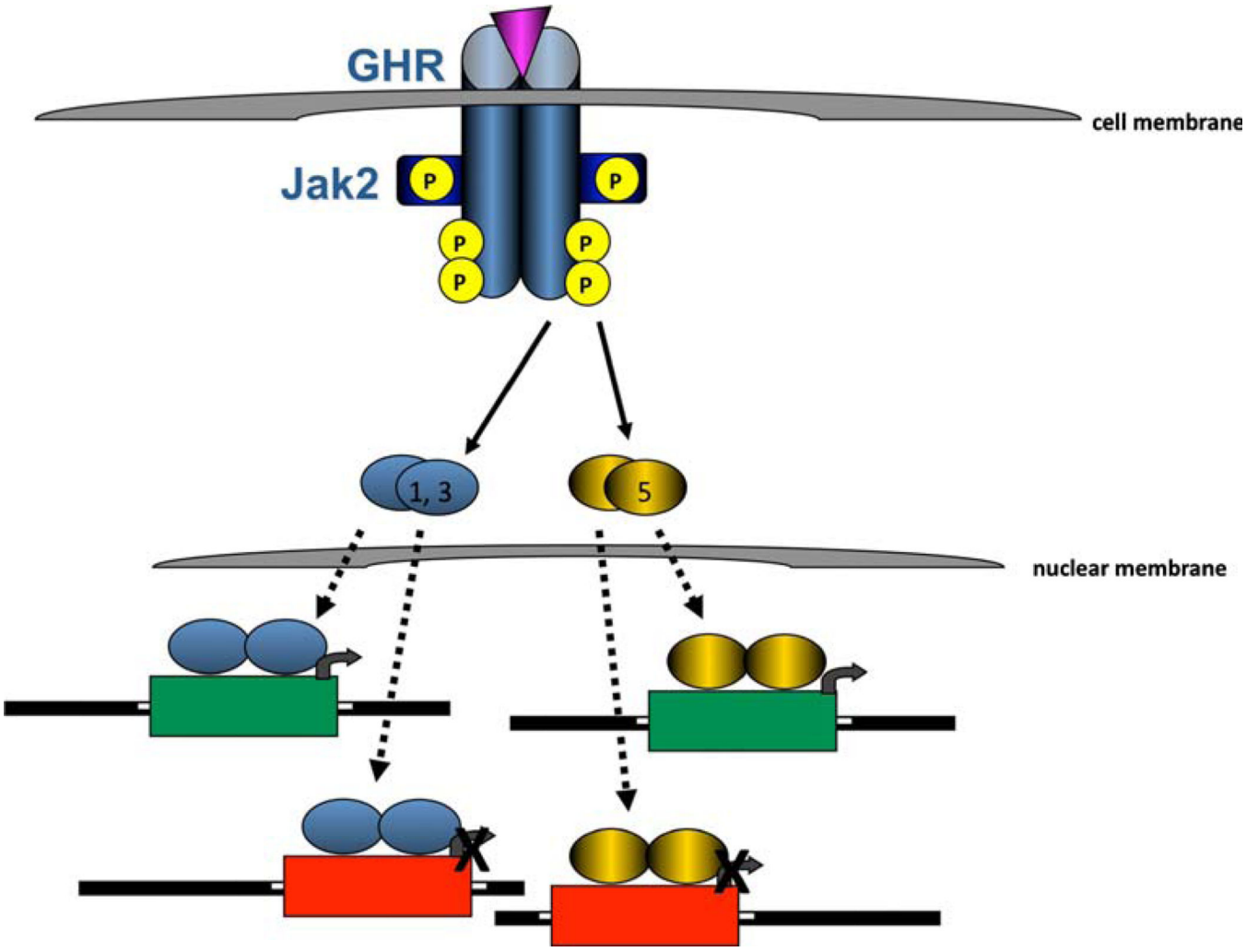

Fig. 2.

Signal transducer and activator of transcription (Stats) mediate growth hormone (GH)-regulated gene activation and gene repression. Activated Stats 1, 3, 5a, and 5b are translocated into the nucleus and bind to DNA in chromatin of target genes, where they can either activate gene transcription (indicated by *bent arrows* at transcription start sites; *green boxes*) or inhibit it (indicated by *Xs overlying bent arrows* at transcription start sites; *red boxes*)