Somatropin and Growth Hormone Deficiency

Tian Xiao, Fan Jue

Abstract

Somatropin is a type of drug used to complement natural somatotropin secreted by human pituitary gland in treating growth hormone deficiency. Its working mechanism is identical to somatotropin - binding to somatotropin receptors, activation of receptors and therefore activation of two signaling pathways, namely JAK-STAT pathway and MAPK pathway. Somatropin is conventionally produced through recombinant DNA technology, which is time-consuming and requires daily administration. More advanced techniques have been developed to improve such drawbacks.

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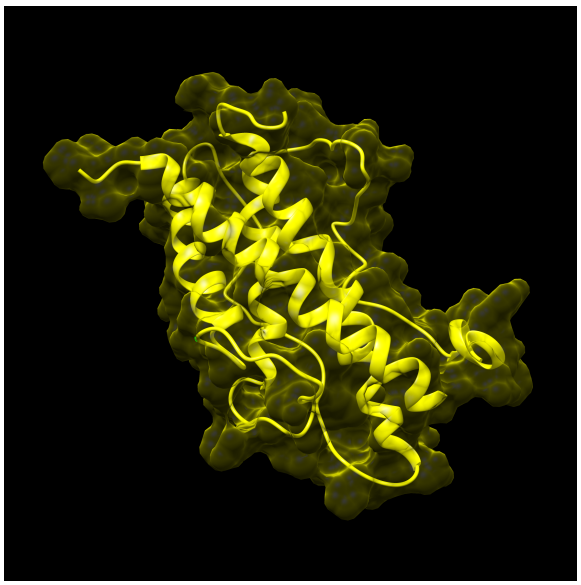
1. Introduction

Growth hormone deficiency (GHD) is a condition with symptoms such as short height, reduced muscle mass, reduced bone density, lipid abnormalities and their consequent psychological problems. GHD is caused by lack of somatotropin in the human body. Somatotropin is a protein synthesised, stored and secreted by somatotropic cells within the lateral wings of anterior pituitary gland, which stimulates the growth and metabolism of muscle, bone and cartilage cells (Nicoll et al., 1986). To tackle GHD, recombinant human growth hormone (rhGH) called somatropin, which has equivalent bioavailability and pharmacokinetics to pituitary somatotropin (Moore et al., 1988), is used as a complement to ensure normal cell growth in the human body. In this report, we discuss the mechanism of how the biochemical structures of somatotropin and somatropin allow them to bind to their receptors and activate downstream pathway in order to stimulate cell growth, as well as how the structures characterise the production and usage process of somatropin.

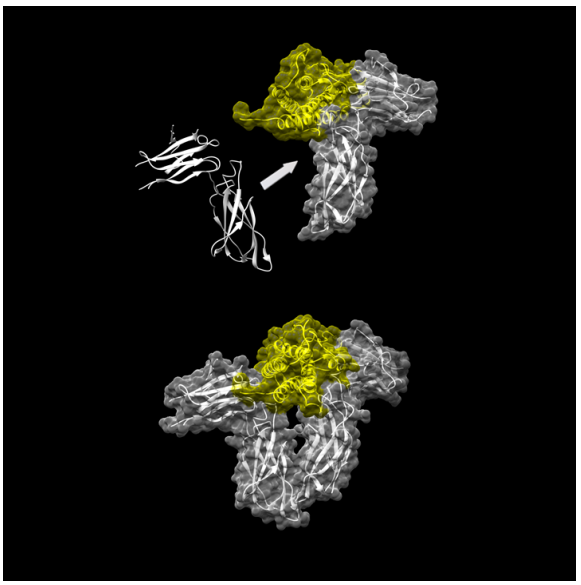
2. Binding of Somatotropin and Its Receptor

Somatotropin[1] is a protein of 191 amino acids with four helices effective on its interaction with growth hormone receptors (GHR). The first two helices are parallel to each other, and antiparallel to the remaining two, as shown in Figure 2.1a. On the other hand, GHR is a transmembrane protein of 638 amino acids which consists of three domains: extracellular domain, transmembrane domain and intracellular domain. Mechanism of somatotropin always starts from the binding between somatotropin and GHR. The structure of somatotropin consists of two allosterically coupled binding sites (Site 1 and Site 2) of different binding affinity (Walsh et al., 2004). As a result, one somatotropin naturally binds to two GHR to form a 1:2 stoichiometric crystal structure, as shown in Figure 2.1b (Postel-Vinay, 1995).

[1] In this report, somatotropin refers to the major isoform of human pituitary growth hormone unless specifically stated.



(a) Structure of somatotropin[2]. The helix at the front is Helix 1, followed by Helix 2, Helix 3 and Helix 4.

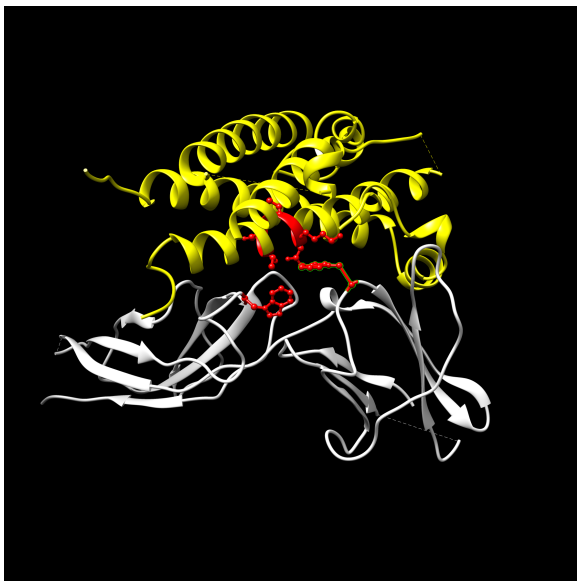


(b) GHR (white) binds to a somatotropin (yellow)-receptor intermediate complex to form a 1:2 crystal structure[3].

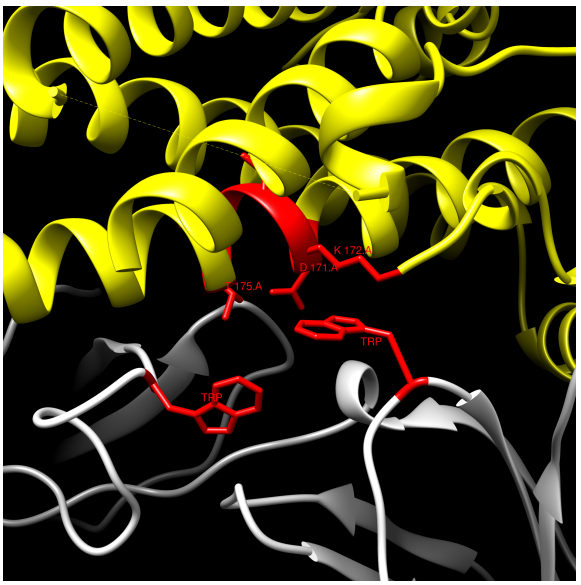
Figure 2.1: Brief view of structure of somatotropin and natural somatotropin-receptor complex.

In order to analyse the binding between somatotropin and GHR clearly, a variant of somatotropin G120R is made by introducing a mutation in the reception stem region S201C such that binding occurs only at Site 1 and a 1:1 complex is formed as shown in Figure 2.2a (Cunningham, 1993). The binding starts with a random and rapid collision process, followed by the functioning of side chains which keeps somatotropin and GHR bound at the binding site. The most critical interaction at the binding site is hydrophobic contact between a small set of buried side chains on both the hormone and its receptor, all of which are located near the centre of the contact interface. For example, as shown in Figure 2.2b, the aliphatic portions of side chains on somatotropin pack against the tryptophans on the receptor to form a hydrophobic contact (Wells, 1996). Polar and charged interactions are also found in the contact interface, but they are considered much less important in maintaining the binding affinity.

[2] Protein Data Bank (PDB) ID 1HGU (Chantalat et al., 1995).
[3] PDB ID 3HHR (de Vos et al., 1992).



(a) Full view of the complex[4]. Somatotropin is coloured yellow; GHR is coloured white; Residues involved in hydrophobic contact are coloured red.

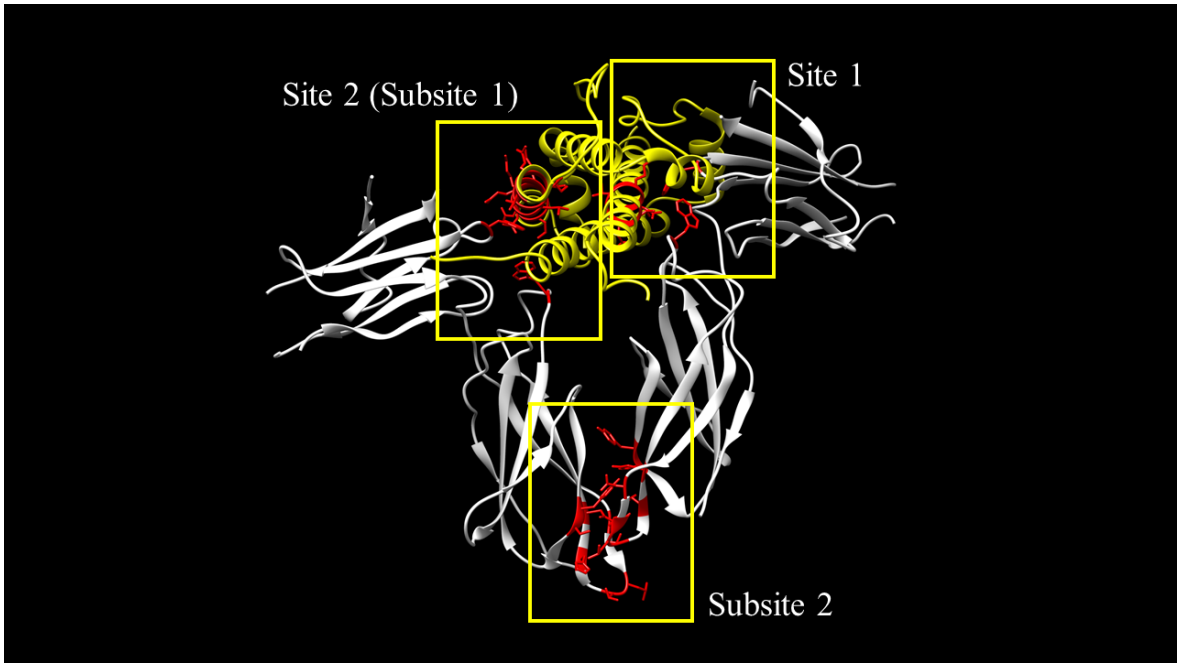


(b) Close view of the contact interface. Aliphatic portions of residues D171, K172 and T175 pack against the tryptophans on GHR. No buried water is found.

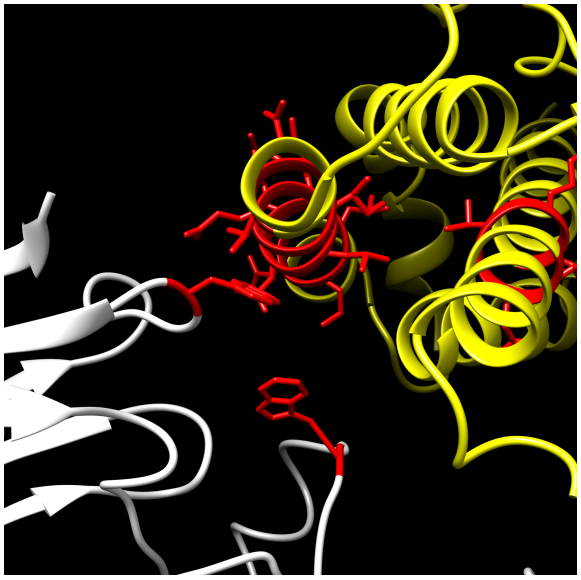
Figure 2.2: Hydrophobic contact interface in a 1:1 somatotropin-receptor complex. For demonstration purposes, residues from T60 to S71 on somatotropin have been hidden.

Similarly, the natural 1:2 crystal somatotropin-receptor complex is formed via a sequential binding process. The surface area of the contact interface at Site 1 is higher than that at Site 2, leading to higher binding affinity at Site 1. Therefore, as shown in Figure 2.3a, a GHR binds to an unbound somatotropin firstly at Site 1 to form a stable 1:1 complex. This structure subsequently provides two spatially distinct subsites Subsite 1 and Subsite 2, which are respectively weak but jointly form a strong association. Subsite 1 (as shown in Figure 2.3b) is located opposite to Site 1 on the surface of somatotropin, which forms a weaker hydrophobic contact, whereas Subsite 2 (as shown in Figure 2.3c) is located at the C-terminus of the two GHRs, which forms a region of pseudo-2-fold symmetrical interaction (stem-stem interaction) (Walsh et al., 2004).

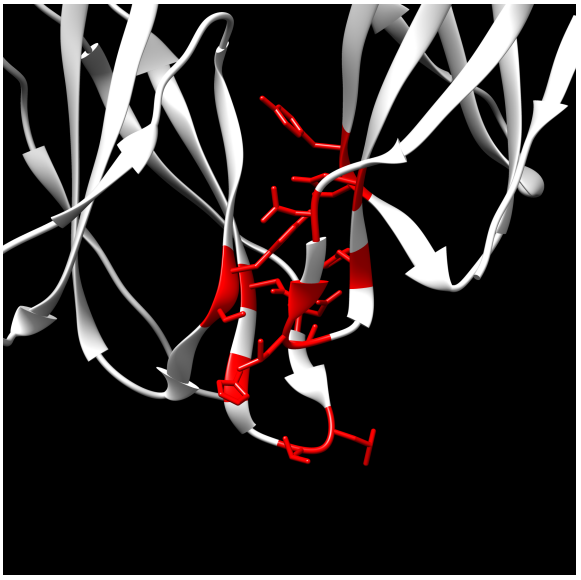
[4] PDB ID 1A22 (Clackson et al., 1998).



(a) Full view of the complex. Somatotropin is coloured yellow; GHRs are coloured white, where on the right is GHR 1 and on the left is GHR 2; Residues involved in binding are coloured red. Site 1 is the hydrophobic contact area between somatotropin and GHR 1, which has previously been mentioned. Site 2 of somatotropin (Subsite 1) and C-terminus FNIII domains (stem region) of GHRs (Subsite 2) together maintain the binding affinity between 1:1 somatotropin-receptor complex and GHR2.



(b) Close view of Subsite 1. This interface contains hydrophobic interaction similar to Figure 2.2b, but much weaker.



(c) Close view of Subsite 2. The internal core of stem-stem interface is made up of six hydrogen bonds (Bernat et al., 2003).

Figure 2.3: Interactions at binding sites in a 1:2 somatotropin-receptor complex.

The sequential binding principle limits the association activity between somatotropin and GHR. One somatotropin is able to diffuse in three-dimension to bind to the first GHR, but subsequently it is only able to diffuse in two-dimension along the cell membrane because the complex is tethered by the transmembrane domain of GHR.

3. Activation of Growth Hormone Receptor

Phosphorylation of proteins by tyrosine kinases is an important and well-known mechanism to communicate signals within a cell and hence regulate cell activities. Many receptors intrinsically contain tyrosine kinases for signalling, except for cytokine receptors. Instead, cytokine receptors usually contain binding sites which allow them to bind with other non-receptor tyrosine kinases for the same purpose.

GHR, member of class I cytokine superfamily, is a long homodimeric receptor with one extracellular homology domain, one single-pass transmembrane domain and one cytoplasmic intracellular domain (Dehkhoda et al., 2018). The intracellular domain of GHR contains a highly conserved proline-rich motif termed Box 1, and another less conserved motif termed Box 2. Box 1 contains binding sites with tyrosine kinases.

Although binding with somatotropin stimulates dimerisation of GHR, GHR dimer is also able to form on the cell surface before binding with somatotropin (Ross et al., 2001), and dimerisation alone is not sufficient for activation (Rowlinson et al., 1998).

Figure 3.1 demonstrates how a GHR dimer is activated by somatotropin. Before binding with somatotropin, GHR homodimers exist but are inactive. This is because the tyrosine kinase domain in Box 1 is inhibited by pseudokinases from the opposite receptor. Binding with somatotropin reorients the pre-existing homodimer, hence induces a conformational change in the structure, involving a rotation and a vertical movement. This reorientation is then transmitted through the transmembrane domain and causes a separation of the intracellular domains (Brown et al., 2005; Poger & Mark, 2010). This consequently leads to a repositioning of tyrosine kinase domain at Box 1, which dissociates its previous association with pseudokinases, brings together the two tyrosine kinase domains and consequently activates GHR.

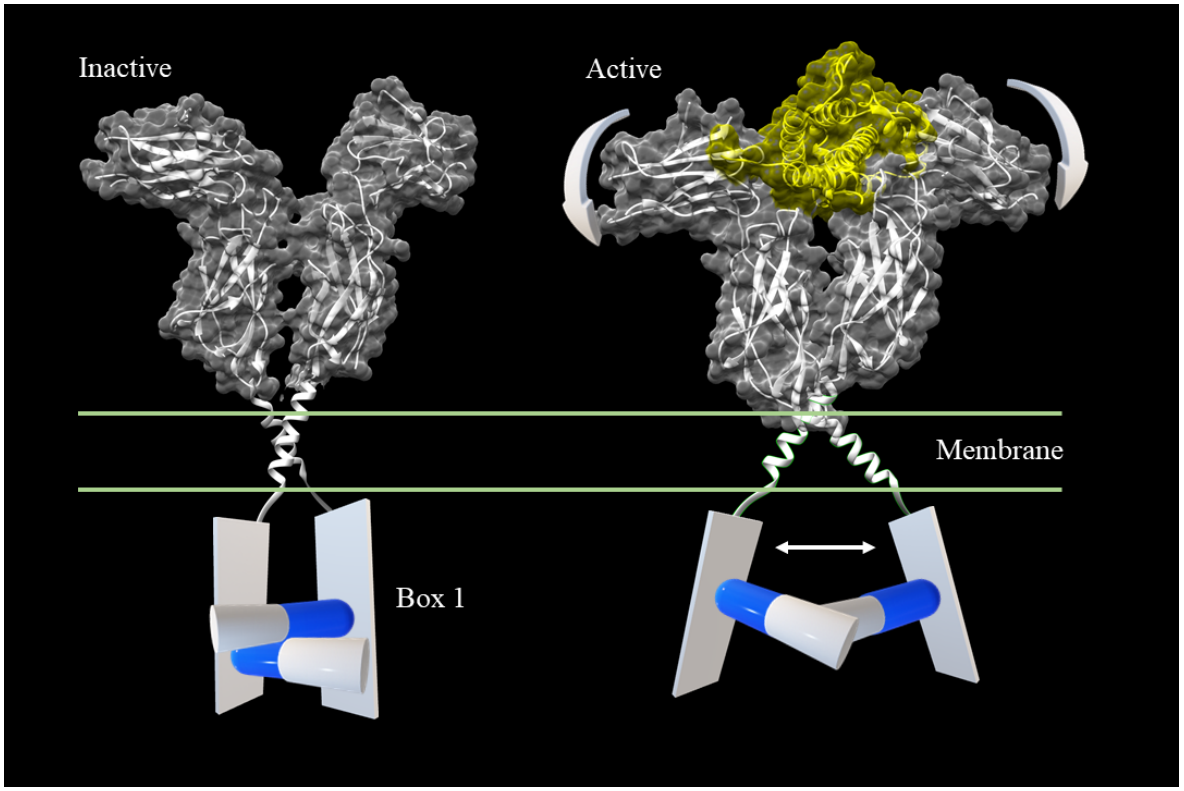
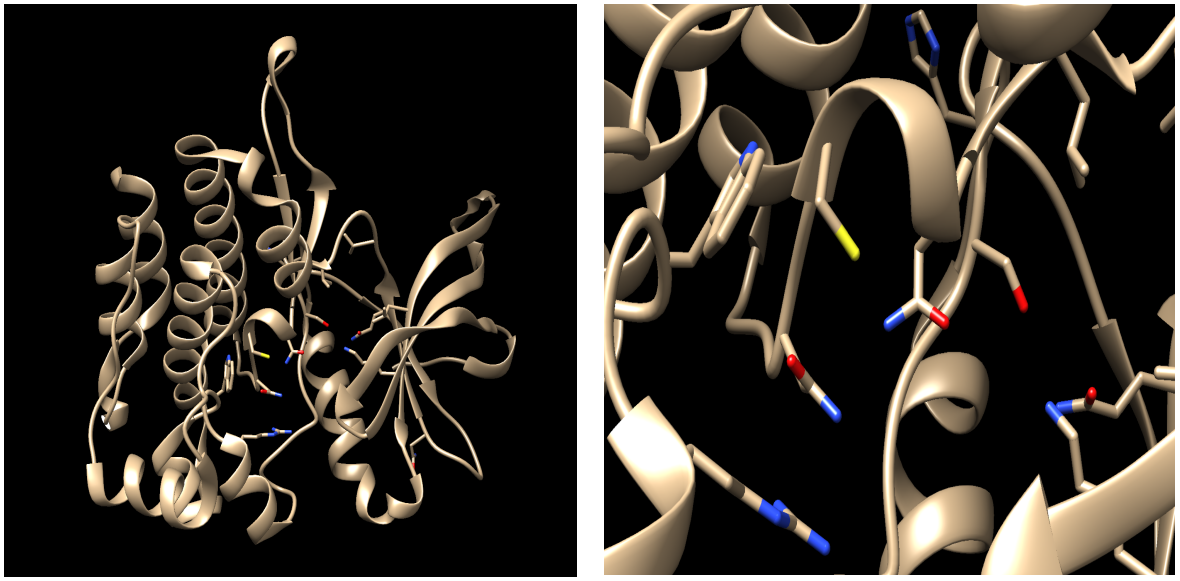


Figure 3.1: Structure of GHR dimer before and after activation. In each Box 1, blue cylinder represents pseudokinases and white cylinder represents tyrosine kinases. Before activation, each tyrosine kinases contact with pseudokinases of the opposite receptor. After activation, the two tyrosine kinases contact with each other.

4. Activation of JAK-STAT Signaling Pathway

One of the two major signaling pathways activated by somatotropin is the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway which stimulates the production of Insulin-like Growth Hormone-1 (IGF-1) to promote cell proliferation. JAK proteins are a family of tyrosine kinases that associate with the receptors and are brought into close proximity for activation when Somatotropin binds to the receptor (Aaronson et al., 2002). JAK proteins also contain a kinase domain which is essential for their phosphorylation capability and an Src homology 2 (SH2) domain for binding with cytokine receptors (as shown in Figure 4) (Dehkhoda et al., 2018). Two main domains in the structure of STAT proteins that are vital to the discussion of Somatotropin are SH2 domains and DNA-binding domains which both serve the purpose of binding to different regions (Figure 4.2) (Aaronson et al., 2002).



(a) Overview of the crystal structure of JAK protein[5].

(b) Close view of the SH2 domain of the JAK protein.

Figure 4.1: Crystal structure of JAK protein with SH2 domain highlighted.

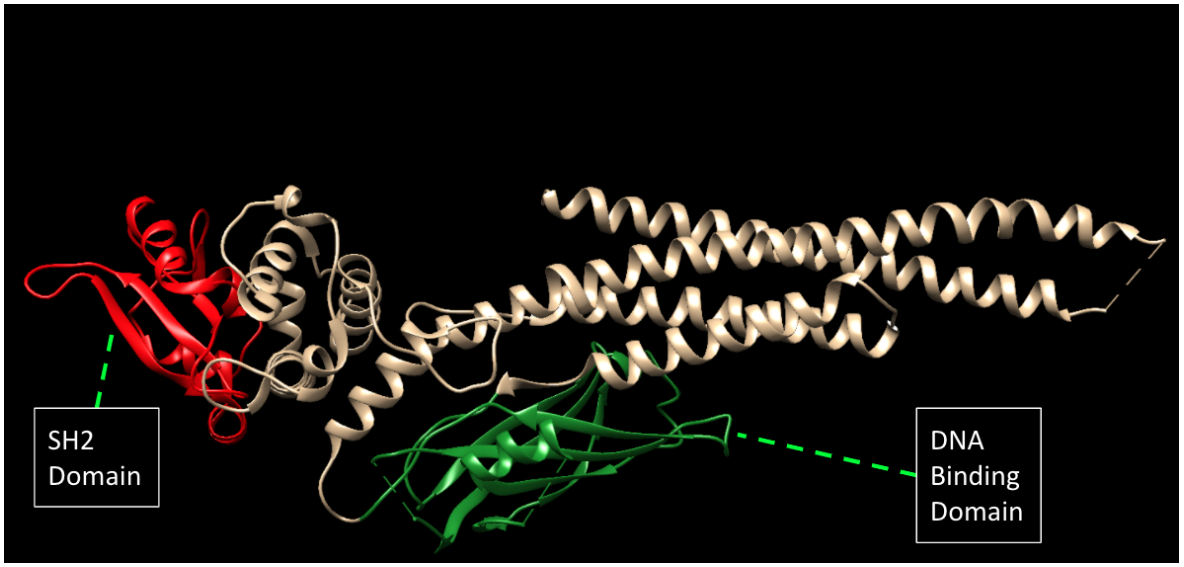


Figure 4.2: Crystal structure of STAT protein with SH2 domain (red) and DNA binding domain (green) highlighted[6].

[5] PDB ID 5UT1 (Publeo & Schlesinger, 2017).
[6] PDB ID 6MBZ (de Araujo et al., 2019).

The brief summary of the JAK-STAT signaling pathway is demonstrated in Figure 4.3. The pathway contains three main stages, the first of which is the phosphorylation of JAK proteins and subsequently STAT proteins (Dehkhoda et al., 2018). Afterwards, phosphorylated STAT proteins dissociate from the receptor and dimmerize, after which STAT dimers start translocation to the nucleus for DNA binding (Dehkhoda et al., 2018).

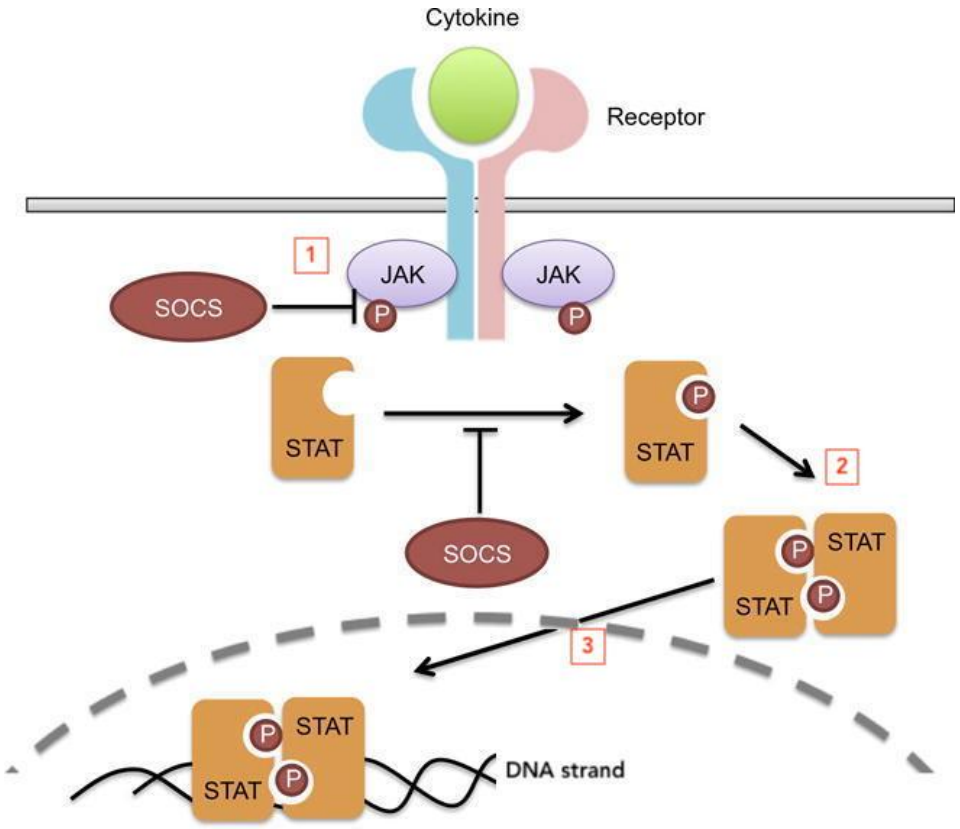


Figure 4.3: Brief overview of the activation mechanism through JAK-STAT signaling pathway (Yan et al., 2018). ① Phosphorylation of JAK proteins, tyrosine residues such as SOCS, and STAT proteins. ② Dimerization of phosphorylated STAT proteins. ③ Nuclear Translocation of STAT dimmers for DNA Binding.

After being activated, JAKs initiate transphosphorylation with universal phosphoryl donor adenosine triphosphate (ATP) to increase the activity of their kinase domains, which allow them to phosphorylate tyrosine residues on the receptor (Seif et al., 2017). Phosphorylated tyrosine residues will attract the SH2 domain of STAT proteins, which are present as inactive monomers in the cytoplasm before activation (Morri et al., 2018). Unphosphorylated STAT proteins bind to the phosphorylated tyrosine residues through the SH2 domains (Seif et al.,

2017). Studies have substantiated that residues at position 487, 534, 566, and 627 are sufficient for the phosphorylation of STAT proteins (Babon et al., 2014). The bound STAT proteins then undergo phosphorylation, hence becoming activated.

Phosphorylation causes STAT to dissociate from the receptor and also promotes the formation of STAT dimers from STAT monomers through reciprocal intermolecular phosphotyrosine-SH2 domain interactions (Figure 4.4) (Liu & Nash, 2012). Dimerization plays an important role in increasing the DNA binding affinity of STAT proteins (Ihle, 1996). Specifically, there is a hydrophobic interface on the SH2 domain that helps to prevent the tyrosine-phosphorylated tail segment of the STAT protein bound to it from rapid inactivation due to phosphatases (Fahrenkamp et al., 2016). Therefore, the dimer structure is favorable to sustain the phosphorylation of STAT proteins.

Phosphorylation and dimerization make STAT dimers capable of quickly translocating to the nucleus (Braunstein et al., 2003). This is because STAT proteins are large molecules that are not capable of spontaneously diffusing through the nuclear pore complexes, so they require facilitated transport mediated by nuclear localization signals and disrupt the permeability barrier (Wente & Rout, 2010). It has been substantiated that dimerization and phosphorylation are necessary conditions for importin-alpha5 to bind to the DNA binding domain of STAT protein to serve as nuclear localization signals (Bousoik & Montazeri Aliabadi, 2018), allowing STAT dimers to pass through the nuclear pore complexes (Braunstein et al., 2003; McBride et al., 2002). The GHR is also significant in the nuclear translocation of STAT dimers. Before the dissociation of STAT protein, the GHR recognizes a nuclear export signal which helps to shift the STAT protein to specific DNA sequence in the nucleus (Wingelhofer et al., 2018). In this way, STAT dimers translocate to the cell nucleus.

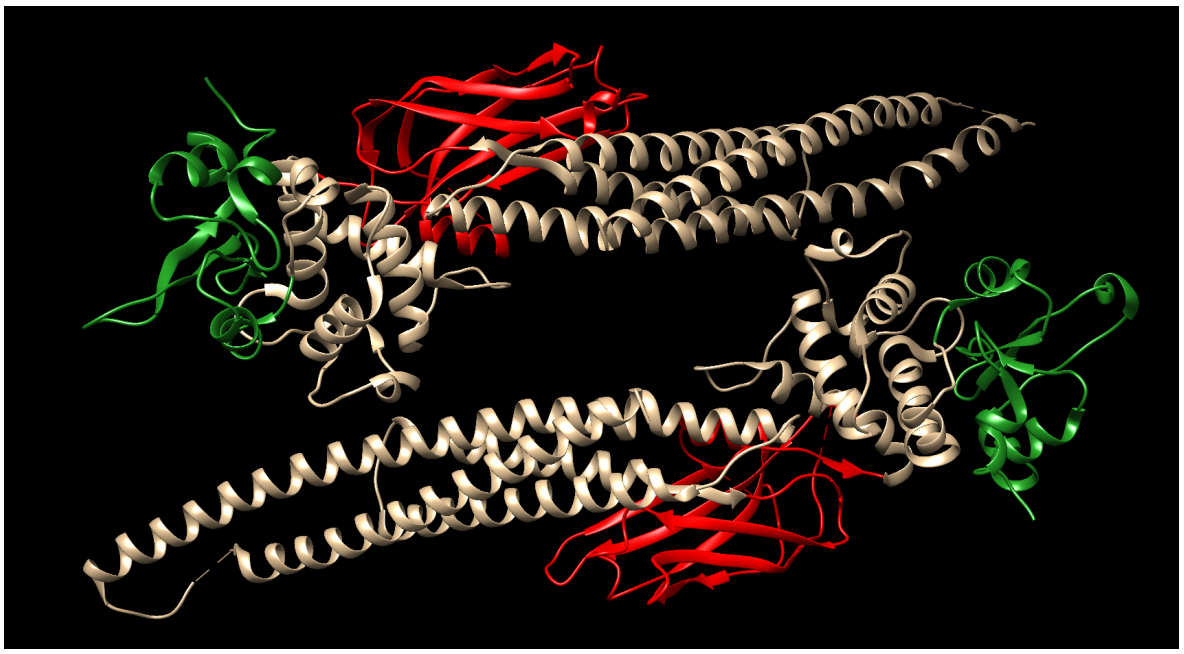


Figure 4.4: STAT dimers formed through the interaction between the SH2 domain (green) of one protein with the tyrosine-phosphorylated tail segment of another STAT protein.

After entering the nucleus, DNA dimers bind to gamma-activated sites (GAS), a consensus DNA-recognition motif in the promoter region of cytokine-inducible genes, through DNA-binding domain which contains a S-type immunoglobulin fold that facilitates sequence-specific binding (Figure 4.5) (Mitchell & John, 2005).

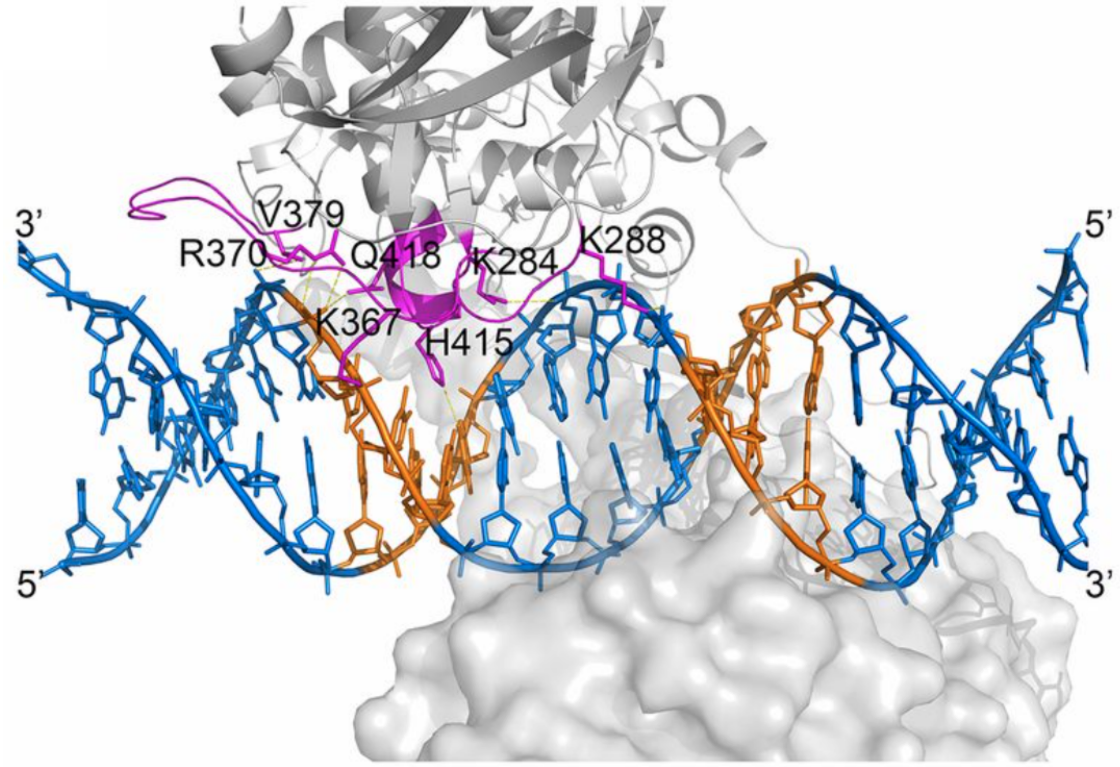


Figure 4.5: Binding of STAT protein to the consensus DNA-recognition motif by its DNA binding domain (pink) (Li et al., 2016).

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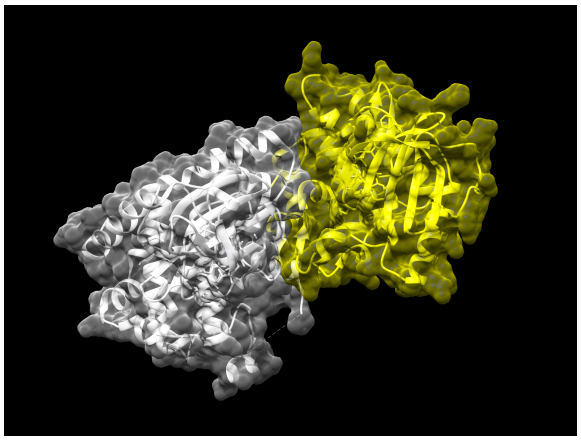
After binding to an interferon GAS-like core sequence in the DNA of target cells (TTCT/CNA/GGAA) (Wingelhofer et al., 2018), STAT regulates transcription of a range of target genes. The gene that is the most important in our discussion is the Igf1 gene which stimulates the production of IGF-1. IGF-1 is responsible for stimulating systematic body growth and it can promote the proliferation of almost every cell in the human body (Wrigley, Arafa, & Tropea, 2017). IGF-1 is also a peptide that binds to a tyrosine kinase receptor (IGF1R) and promotes the phosphorylation of the insulin-receptor substrate-1, hence activating mitogen-activated protein kinase (MAPK) pathways which will be further elaborated on in the next section (Ferreira Mendes et al., 2020).

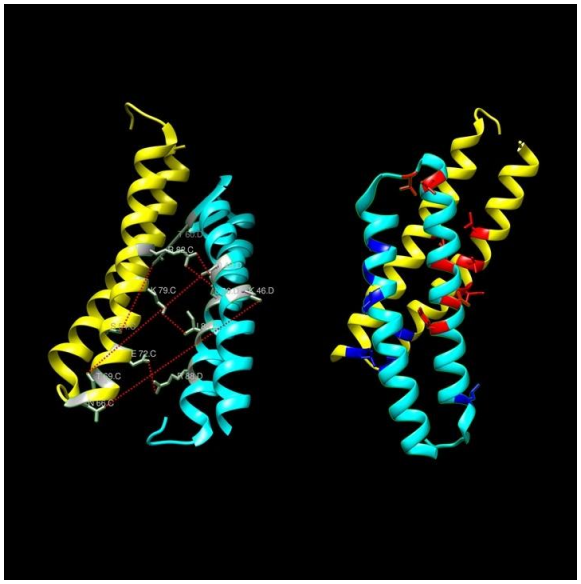
5. MAPK Signaling Pathway

Another important signaling pathway activated by somatotropin is the mitogen-activated protein kinases (MAPK) pathway, through which somatotropin directly stimulates cell growth at chondrocytes of cartilage. MAPK pathway is a multi-tiered cascade, involving MAP kinase kinase kinase (MAP3K), MAP kinase kinase (MAP2K) and MAPK. There are

scaffold proteins such as MAP2K partner 1 (MP1) and kinase suppressor of Ras (KSR) binding to two or more of MAP3Ks, MAP2Ks and MAPKs and hence bringing them to close proximity such that phosphorylation can occur within themselves along the three tiers (Chuderland & Seger, 2005). Figure 5.1 demonstrates how KSR can bind to MAP3K and MAP2K respectively.



(a) Binding of MAP2K (white) and KSR1 (yellow)[7]. This complex can allosterically activate RAFs when they bind to each other.



(b) Binding of BRAF (cyan) and KSR1 (yellow)[8]. BRS domain of BRAF and CC-SAM domain of KSR1 is shown. Binding affinity is maintained by both polar and hydrophobic contact. On the left the red lines show the polar contact between residues (grey) and on the right two hydrophobic patches (red and blue) are shown.

Figure 5.1: KSR, as a scaffold protein, can bind to different tiers of MAPKs such as MAP2K, as well as B-rapidly accelerated fibrosarcoma (BRAF) which is a type of MAP3K, hence bring them into close proximity. This as a result stimulates the phosphorylation of MAP2K by MAP3K.

When GHR is activated by somatotropin, tyrosine phosphorylation happens at Box 1 and this involves a variety of protein kinases including JAK2. The phosphorylated protein kinases then continue to phosphorylate their neighbour kinases, which forms a chain of

[7] PDB ID 7JUW (Khan, 2020).
[8] PDB ID 5VYK (Lavoie et al., 2018).

phosphorylation. Such chains are able to reach MAPK1 (previously called ERK2) and MAPK3 (previously called ERK1) (Bazan, 1989; Anderson, 1992), which are both MAPKs. One example chain leading from GHR towards MAPK involves Src homology and collagen (SHC) proteins, growth factor receptor-bound protein 2 (Grb2), son-of-sevenless (Sos), Ras proteins and reaches the scaffold of MAPKs. Figure 5.2 demonstrates how the MAPK pathway communicates signals from cell membrane to cell nucleus in steps.

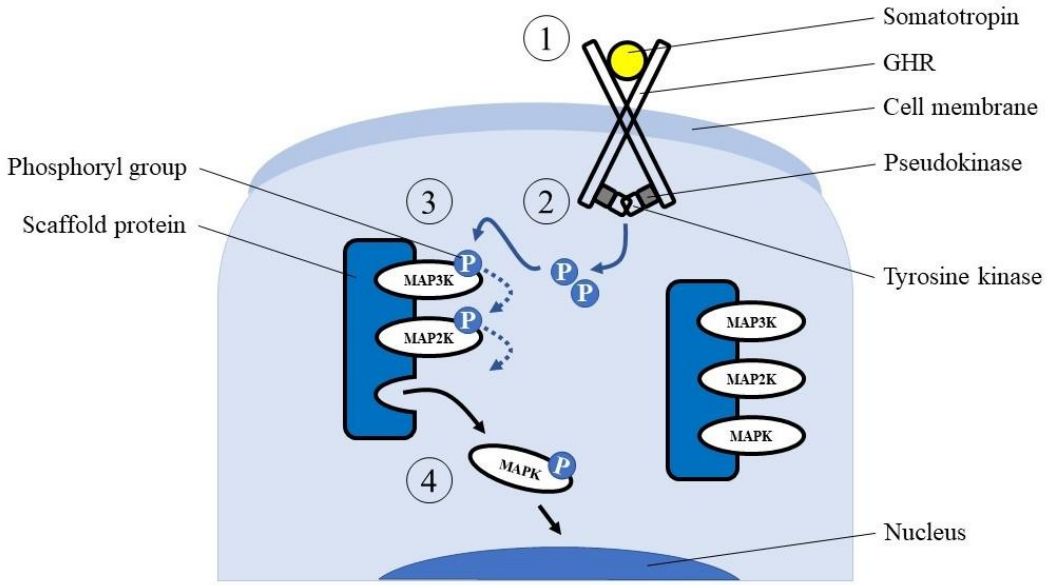


Figure 5.2: Steps of MAPK signaling pathway. ① Somatotropin binds to GHRs and causes a conformational change in the homodimer. ② Tyrosine kinase domains are brought together and activate each other through phosphorylation. A chain of phosphorylation starts from the activated kinases and reaches the scaffold of MAP3K, MAP2K and MAPK. ③ The scaffold proteins facilitates further phosphorylation within the scaffold, until MAPK is phosphorylated. ④ Phosphorylated MAPK forms a dimer, translocates to cell nucleus and regulates activities of transcription factors through phosphorylation.

Once MAPKs translocate to the nucleus, they can phosphorylate different transcription factors, such as ternary complex factor (TCF) Elk-1, serum response factor accessory protein Sap-1a, Ets1, C-Myc, Tal, etc (Zhang, 2002), which subsequently bind to DNA and regulate cell activity. Figure 5.3 demonstrates how Elk-1 binds to a DNA sequence.

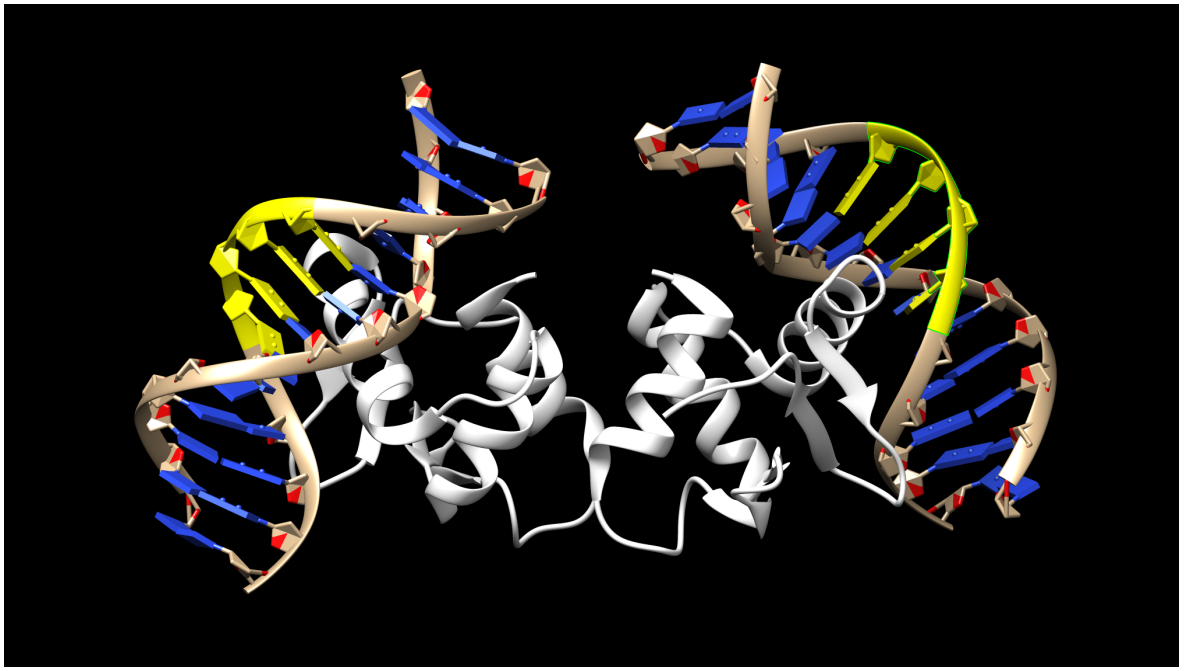


Figure 5.3: Erythroblast transformation specific (ETS) domain of Elk-1 (white) binds to DNA sequence GGAA (yellow), where guanine residues are the most critical contact points[9].

Although both JAK-STAT signaling pathway and MAPK signaling pathway are able to occur independently, they can also integrate with each other and hence enhance the activation of each other (Rowlings et al., 2004). As demonstrated in Figure 5.4, activated JAK can phosphorylate growth factor receptor-bound protein 2 (GRB2), which is a protein involved in the chain of phosphorylation discussed earlier. Continuation of the remaining chain from GRB2 leads to MAPK pathway. On the other hand, activated MAPKs can also phosphorylate a serine near the C-terminus of most STATS, which enhances their transcriptional activities.

[9] PDB ID 1DUX (Mo et al., 2000).

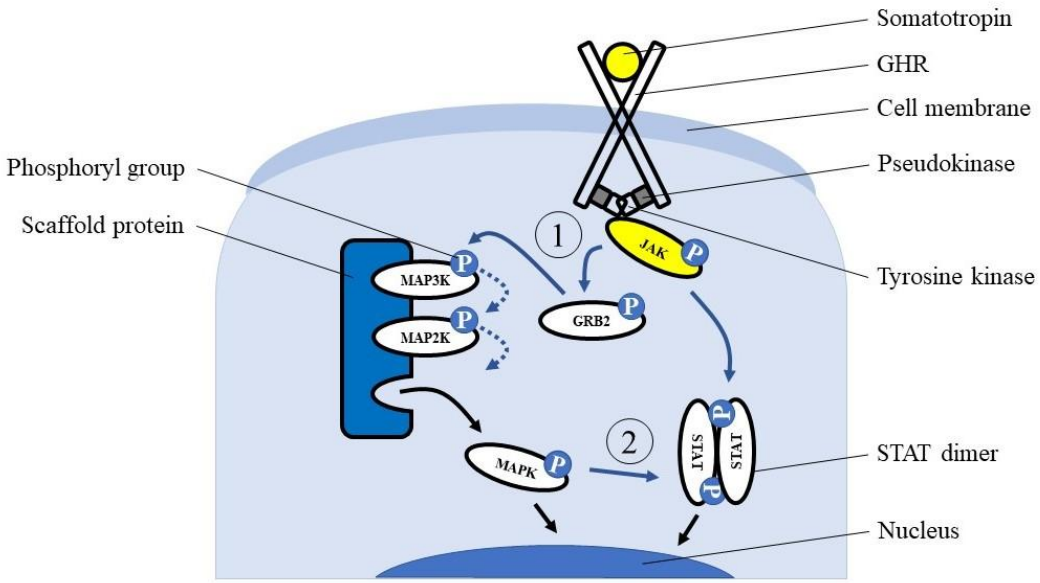


Figure 5.4: Integration between JAK-STAT signaling pathway and MAPK signaling pathway. ① Activated JAK phosphorylates GRB2, which subsequently leads to the MAPK pathway. ② Phosphorylated MAPK enhance the transcriptional activity of STAT.

6. Somatropin and Its Developments

Somatotropin was firstly isolated from human pituitary gland in 1956, and its biochemical structure was elucidated by 1972, which became the catalyst for the development of recombinant human growth hormone, somatropin (Blizzard, 2012). In 1981, Genentech developed the first somatropin by a biosynthetic process. 1985 is the year when somatropin replaces pituitary somatotropin as the approved drug by US Food and Drug Administration (FDA) to conduct growth hormone therapy, because the latter is found to connect with the fatal Creuzfeldt Jacob Disease (CJD) due to contamination (Appleby, 2013). Up till now, over 13 types of somatropin have been introduced to the market for growth hormone therapy.

Somatropin is conventionally produced via recombinant DNA technology. Just like many other cloning techniques, required DNA from somatotropin is cleaved by restriction enzymes and combined with vector plasmid isolated from a strain of *E. coli*. This forms a circular structure and the recombinant plasmid is inserted back to *E. coli*, which is then transformed to synthesise somatropin (Flodh, 1986). The process is demonstrated in Figure 6.1. After further fermentation, purification, filtration, capping and labelling, the product is ready to use.

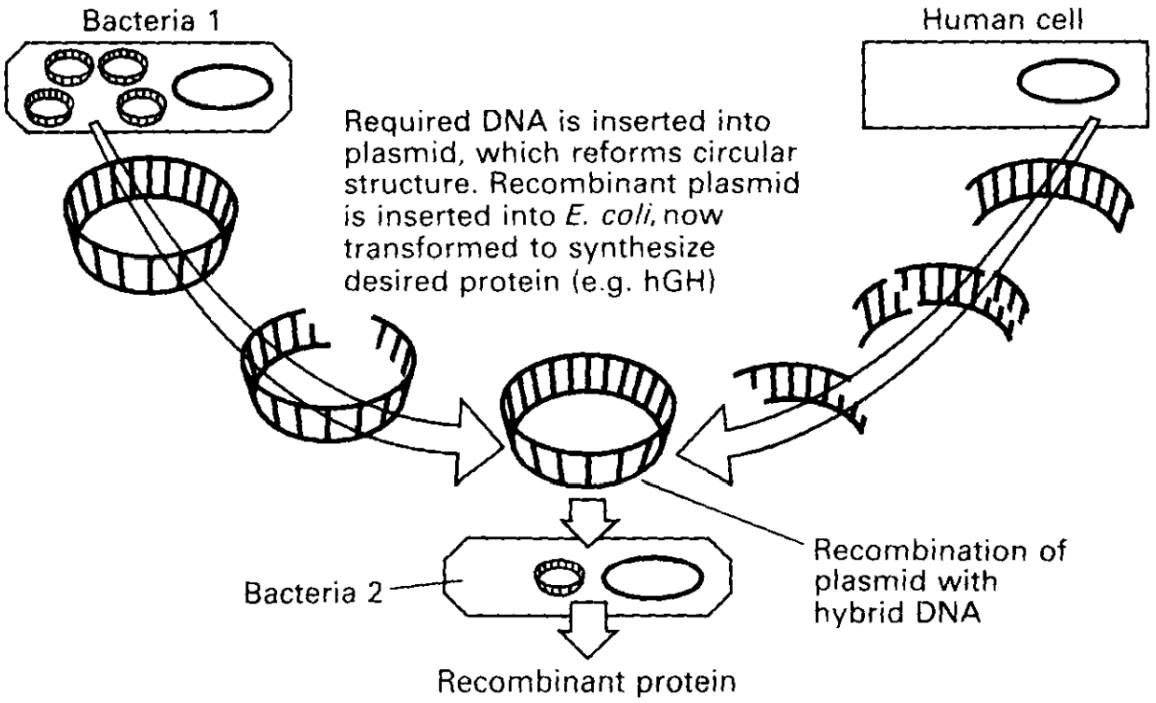
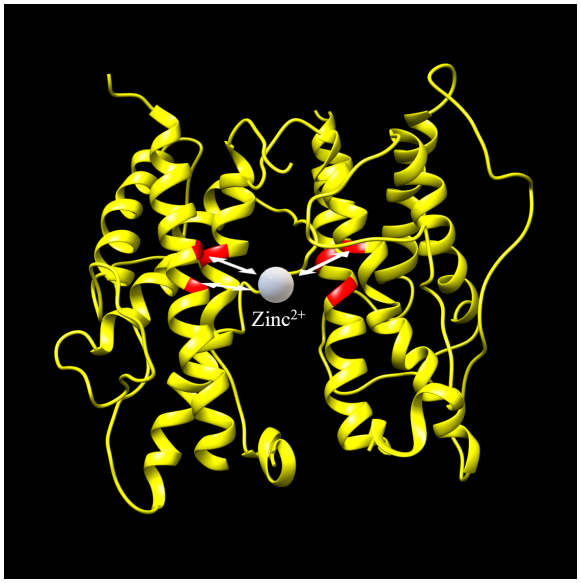


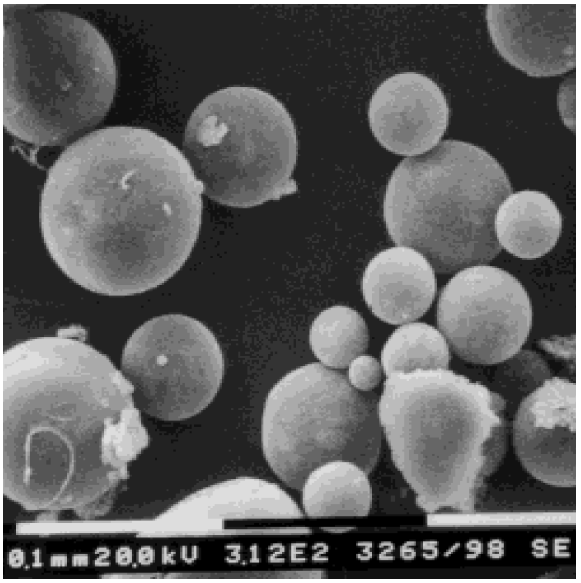
Figure 6.1: Somatropin is produced through conventional DNA recombination process.

Major drawbacks of conventional somatropin include short plasma half-life and time-consuming DNA recombination process. Hence, various analogues have been developed to target such drawbacks respectively.

The plasma half-life of somatropin is around 3.4 hours if administered subcutaneously and 0.36 hour if administered intravenously (Webster et al., 2008). As a result, this requires daily or thrice weekly administration, which is inconvenient and potentially painful for patients (Hermanussen et al., 1985). The major 3 techniques used to overcome this issue are zinc complexes, microspheres formulations and PEGylated analogues (Sueiras-Diaz et al., 2017). Zinc ion could induce the dimerisation of somatotropin as demonstrated in Figure 6.2a (Cunningham et al., 1991). Zn^{2+}-somatropin dimer is much more stable than somatropin monomer and the plasma half-life could be lengthened. Meanwhile, somatropin could also be encapsulated within poly (D,L-lactic-co-glycolic acid) microspheres as demonstrated in Figure 6.2b. Lastly, somatropin could also be pegylated to become long-acting (de Schepper, 2011). These techniques make weekly or twice monthly injection possible.



(a) Two somatropin proteins are bound to zinc ions via disulfide bridge to form a dimer.



(b) Somatropin proteins are encapsulated within poly microspheres.

Figure 6.2: Techniques to lengthen plasmid half-life of somatropin.

Besides, total chemical synthesis of somatropin is also possible through sequential native chemical ligation (Sueiras-Diaz, 2017). This makes the production of somatropin much more time efficient, although the cost may remain high.

7. Side Effect of Somatropin

Somatropin is mostly used in targeting adult human growth hormone deficiency and growth failure because of its efficacy in stimulating cell proliferation. Major side effects are trivial injection-site effects. However, somatropin has also been evidenced to increase the risk of cancer for people under growth hormone therapy. Specifically, the effects of growth hormone (GH) are exerted by its binding to the GH receptors on target cells, which in turn stimulates the production and secretion of IGF-1 from many tissues, mainly the liver. Through triggering a cascade of signal transduction events, IGF-1 promotes also the nuclear translocation of transcription factors that promote cell growth and reduce cell apoptosis like FOXO, GSK3β, MDM2 and mTOR, IGF-1 thus promotes cancer stemness and increases the risk of cancer (Dalla Libera et al., 2004). The JAK-STAT signalling pathway also allows the

transcription of genes such as BLC2 and c-Myc which are involved in cell division for the promotion of cancer cell proliferation (Groner et al, 2017).

8. Conclusion

In summary, we have illustrated the functionality of somatropin through the binding of somatotropin with receptors, the activation of GHRs, and the two signalling pathways for somatotropin to take effect, namely JAK-STAT pathway and MAPK pathway. On top of that, we also discuss the development of somatropin and its side effects in medical usage.

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