General and Comparative Endocrinology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Regulation of skeletal muscle growth in fish by the growth hormone – Insulin-like growth factor system

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Skeletal muscle
Growth
Growth hormone
Insulin-like-growth factors
Receptors
Binding proteins
Signaling pathways
Fish
Teleosts

ABSTRACT

The growth hormone (GH)-insulin-like growth factor (IGF) system is the key promoter of growth in vertebrates; however, how this system modulates muscle mass in fish is just recently becoming elucidated. In fish, the GH induces muscle growth by modulating the expression several genes belonging to the myostatin (MSTN), atrophy, GH, and IGF systems as well as myogenic regulatory factors (MRFs). The GH controls the expression of *igf1* via Janus kinase 2 (JAK2)/signal transducers and activators of the transcription 5 (STAT5) signaling pathway, but it seems that it is not the major regulator. These mild effects of the GH on *igf1* expression in fish muscle seem to be related with the presence of higher contents of truncated GH receptor1 (tGHR1) than full length GHR (flGHR1). IGFs in fish stimulate myogenic cell proliferation, differentiation, and protein synthesis through the MAPK/ERK and PI3K/AKT/TOR signaling pathways, concomitant with abolishing protein degradation and atrophy via the PI3K/AKT/FOXO signaling pathway. Besides this, these signaling pathways control the expression of several genes belonging to the atrophy and IGF systems. Particularly, IGFs and amino acid control the expression of *igf1*, thus, suggesting other of alternative signaling pathways regulating the transcription of this growth factor. The possible role of IGF binding proteins (IGFBPs) and the contribution of muscle-derived versus hepatic-produced IGF1 is also addressed. Thus, a comprehensive overview on the GH-IGF system regulating fish skeletal muscle growth is presented, as well as proposals for future research in this field.

1. Introduction

The main regulator of muscle growth in vertebrates is the growth hormone (GH)-insulin-like growth factor (IGF) system (Glass, 2003, 2005; Velloso, 2008; Wood et al., 2005). In mammals, the role of this system and its signaling pathways in muscle mass accretion has been established in considerable detail (Glass, 2003, 2005; Velloso, 2008), whereas in teleost fish, knowledge of the molecular regulatory mechanisms is limited. This is due to the relative lack of species-specific molecular tools and functional studies. Also, an extra level of complexity is observed due to whole genome duplication (WGD) and a rediploidization event which teleost fish have undergone during evolution (Volff, 2004). This has resulted in both ortholog and paralogue genes being retained in extant species (Amores et al., 1998; Jaillon et al., 2004). In salmonids, a second whole genome duplication was experienced, thus there can be up to four functional gene copies (Volff, 2004; Macqueen et al., 2013). The function of the large majority of these paralogue genes is unknown.

The GH-IGF system has been previously reviewed in fish (Björnsson et al., 2002; Reindl and Sheridan 2012; Reinecke 2010; Wood et al., 2005) and in mammals (Glass, 2003, 2005; Velloso, 2008), as well as fish muscle grows (Johnston 1999, 2006, Johnston et al., 2011; Mommsen 2001; Rescan 2001, 2005; Watabe 1999). Therefore, in the first part of the review only a general overview is given on these subjects to provide a context for the subsequent sections that review the functional research on the GH-IGF system of fish in relation to the regulation of muscle growth, with emphasis on the current understanding of signaling pathways that control skeletal muscle mass. Future research perspectives are also provided.

2. The growth hormone-insulin-like growth factor system: a brief overview

2.1. The growth hormone system

The GH is the main regulator that controls somatic growth in vertebrates (Björnsson et al., 2002; LeRoith et al., 2001). The GH

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0016-6480/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ygcen.2013.06.009

mediates its biological functions by binding, with high affinity, to the full-length GH receptor (flGHR). The main target tissue for the GH is the liver, however, the GH can also significantly affect other peripheral tissues such as muscle (Butler and Le Roith, 2001; Herrington and Carter-Su, 2001; LeRoith et al., 2001). In mammals, the primary transcript that generates flGHR mRNA may undergo processing that includes post-transcriptional modifications, which generate mRNAs of various lengths. These can encode for short forms of the GHR, such as truncated GHRs (tGHRs) and GH binding proteins (GHBPs) (Edens and Talamantes, 1998). tGHRs are membrane-anchored proteins with an extracellular GH binding site, but they lack the majority of intracellular domain necessary for signal transduction. Therefore, they may abolish the GH signal and act as dominant negatives of GH function. GHBPs are soluble proteins containing only the extracellular domain of the flGHR and are able to transport and regulate the bioavailability and half-life of the GH in circulation (Edens and Talamantes, 1998). In mammals, when the GH binds to the flGHR monomer, a homodimerization of the receptor is induced, triggering post-receptor signaling events that include recruitment and activation by the phosphorylation of Janus kinase 2 (JAK2). This, in turn, activates other downstream signaling molecules, including transducers and activators of the transcription family (STAT), the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT), and the extracellular signal-regulated kinase (ERK) (Piwien-Pilipuk et al., 2002). These signaling pathways modulate the transcription of several target genes, including *igf1* (Davey et al., 2001; Piwien-Pilipuk et al., 2002). The GH can also activate other signaling molecules such as JAK1, STAT2, and STAT3, among others (Piwien-Pilipuk et al., 2002), thus making the GH signaling network particularly complex.

2.2. The insulin-like growth factor system

IGFs are peptides structurally related to insulin that include IGF1 and IGF2 (Duan et al., 2010; Wood et al., 2005). In mammals, the main target tissue of these growth factors is skeletal muscle, although IGFs can also affect other tissues (LeRoith et al., 2001). In muscle, IGFs directly stimulate muscle cell proliferation, differentiation, and hypertrophy and inhibit muscle atrophy (Duan et al., 2010; Glass, 2003, 2005). In circulation, IGF1 and IGF2 are bound to specific binding proteins (IGFBPs) which transport them and increase their half-life, concomitant with regulating the actions of IGFs (Clemmons et al., 1998). In mammals, there are six IGFBPs which can differentially inhibit and/or potentiate IGF actions depending on the species, tissue, cell type, and physiological conditions (Duan et al., 2010).

The mitogenic and anabolic effects of IGF1 on muscle cells in mammals are mediated through specific binding with the IGF1 receptor (IGF1R) (Duan et al., 2010; Glass, 2003, 2005; LeRoith et al., 2001). This ligand receptor interaction promotes the activation of two major intracellular signaling pathways, MAPK/ERK (RAS/RAF/MEK/ERK) and PI3K/AKT (Glass, 2003). The activation of the MAPK/ERK signaling pathway in mammals promotes muscle cell proliferation (Coolican et al., 1997) and terminal differentiation (Li and Johnson, 2006). The activation of PI3K/AKT signal transduction stimulates protein synthesis (Rommel et al., 2001), myoblast differentiation (Coolican et al., 1997), and muscle hypertrophy (Bodine et al., 2001b). The PI3K/AKT pathway regulates these processes by activating the target of rapamycin (TOR) which is also regulated by nutrients (e.g. amino acids) and energy status (i.e. [AMP:ATP] via AMPK) (Hall and Loewith, 2011). Once the TOR is activated, it regulates the 70-kDa ribosomal protein S6 kinase (P70S6K) and eIF4E-binding protein1 (4EBP1), which control translation initiation and elongation (Glass, 2003, 2005). Concomitant with its crucial anabolic role in stimulating protein synthesis and muscle mass growth, the PI3K/AKT pathway has also a significant role in inhibiting protein degradation for mammals. PI3K/AKT abolishes protein breakdown by phosphorylating FOXO transcription factors, thus promoting their translocation from the nucleus to cytoplasm (Bodine et al., 2001a; Sandri et al., 2004). This triggers that the E3-ubiquitin ligases, also called "atrogenes" *murf1* and *atrogin1*, are not expressed, which then prevents the polyubiquitination of proteins (Bodine et al., 2001a; Glass, 2005; Sandri et al., 2004). IGF1 also activates other signaling pathways in muscle, such as the MAPK/P38, phospholipase C gamma (PLC γ)/inositol 1,4,5-triphosphate (IP3), calcineurin/nuclear factor of activated T cells (NFAT) (Valdés et al., 2012) and MAPK/P38 (Ren et al., 2010). PI3 K/AKT/glycogen synthase kinase 3beta (GSK3β)/initiation factor of translation (eIF2B) (Ren et al., 2010; Rommel et al., 2001; Valdés et al., 2012).

The biological effects of IGF2 in mammals are mediated by the specific binding with either IGF1R or IGF2/mannose 6 phosphate (M6P) receptors (Wu et al., 2011). IGF2 activates the MAPK/ERK, PI3K/AKT, and MAPK/P38 signaling pathways by binding the IGF1R in mammalian myogenic cells (Erbay et al., 2003; Ren et al., 2010; Wilson and Rotwein, 2006), whereas the biological effects of IGF2 binding to the IGF2R/M6P in mammalian skeletal muscle are not well understood. However, in cardiomyoblasts IGF2 can activate the PLC calcium/calmodulin-dependent protein kinase II (CaMKII) signaling pathway in a G-protein dependent manner through binding the IGF2R/M6P, leading to hypertrophy (Chu et al., 2008).

3. Fish muscle growth: a general overview

Myogenesis, or skeletal muscle formation, in fish as in other vertebrates, involves the specific control of several myogenic regulatory factors (MRFs) which control processes such as specification, activation, and differentiation of myogenic cells. Specifically, quiescent myogenic cells express markers such as the paired-box protein 7 (*pax7*), whereas committed and activated cells express helix-loop-helix transcription factors such as myogenic factor 5 (*myf5*) and the myoblast determination factor (*myod*) (Watabe, 1999). Once myogenic cells are activated they proliferate and subsequently differentiate. The expression of myogenin (*myog*), myogenic regulatory factor 4 (*mrf4*), and myocyte enhancer factor-2 (*mef2*) marks the starting of differentiation (Rescan, 2001). Finally, the expression of several genes that encode for structural muscle proteins such as myosin light chain (*mlc*), *actin*, and myosin heavy chain (*mhc*) is upregulated, marking a sarcomeric assembly in the later stages of differentiation (Johnston, 2006).

The maintenance of already formed muscle fibers is a complex, dynamic, and controlled process regulated by a balance between protein synthesis and protein degradation. In vertebrates, atrophy occurs when the protein degradation rate exceeds that of protein synthesis, leading to a loss in muscle mass. Conversely, hypertrophy occurs when the protein synthesis rate is greater than the protein degradation rate, resulting in an increased muscle fiber size (Glass, 2005). In fish, skeletal muscle growth can also be achieved by hyperplasia (i.e. the recruitment of new muscle fibers), and hypertrophic and hyperplastic muscle growth can take place concomitantly in fish throughout the lifecycle (i.e. indeterminate growth) (Stickland, 1983; Weatherley et al., 1988). This sets fish apart from other vertebrate classes, where postnatal growth is determinate and just involves the hypertrophy of the fibers formed prior to birth (Rowe and Goldspink, 1969). For this reason, fish represent an important and unique model for studying fundamental growth regulatory mechanisms in vertebrates. Moreover, understanding muscle growth in fish is of great economic importance as muscle constitutes 50–70% of the body weight of most commercially important fish species (Weatherley and Gill, 1985). In finfish

Please cite this article in press as: Fuentes, E.N., et al. Regulation of skeletal muscle growth in fish by the growth hormone – Insulin-like growth factor system. Gen. Comp. Endocrinol. (2013), http://dx.doi.org/10.1016/j.ygcen.2013.06.009