Atlantic salmon myostatin: Characterization of the promoter and the activin IIB receptor.

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Myostatin is a negative regulator of muscle growth

Myostatin is a negative regulator of skeletal muscle growth and belongs to the TGF-β superfamily of growth and differentiation factors. Myostatin functions by inhibiting myoblast proliferation and differentiation (Thomas, 2000; Rios, 2001; Taylor, 2001; Langley, 2000). Mutations in the myostatin gene are found in cattle such as Belgian Blue and Piedmontese (McPerron et al, 1997;Kambadur, 1997; McPerron and Lee, 1997) with the "double muscled" phenotype, and recently in a human child (Schuelke, 2004).
Myostatin encoding sequences have been analysed in a large number of species and is remarkably well conserved throughout evolution. The myostatin sequence is cloned and studied in Atlantic salmon (Salmo salar), and is found to exist in two copies (Østbye et al, 2001). In most vertebrates only a single myostatin gene is reported, and this is almost exclusive expressed in skeletal muscle. The two myostatin genes of Atlantic salmon are expressed in a wide range of tissues. Myostatin show many characteristics in common with other members of the TGF-β superfamily. The predicted myostatin protein sequence has a putative N-terminal signal sequence for secretion, a RXXR proteolytic processing site, and a C-termial domain following the processing site, which contains nine cysteine residues with the characteristic spacing (McPerron and Lee, 1997). Proteolysis of myostatin gives rise to a N-terminal propeptide and a C-terminal mature myostatin. Dimers of the mature myostatin and the propeptide remain non-covalently associated after cleavage.

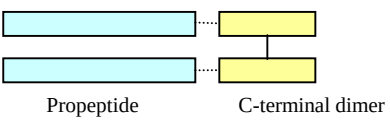

Figure 1
The propeptide and the C-terminal myostatin dimer remain non-covalently bound in a latent complex. Cleavage gives rise to a mature myostatin dimer.

This latent complex functions to avoid binding of myostatin to its receptor ActRIIB (Lee and McPerron, 2001;Thies, 2001). Upon activation the mature

myostatin dimer is released from its propeptide and binds to the ActRIIB (Chang, 2002). Analogous to other TGF-β superfamily members, myostatin bound ActRIIB then recruits type I receptor. Once the active ligand–receptor type I / II complex is formed, ActRIIB activate type I receptor by phosphorylation. The activated type I receptor propagates the signal through the phosphorylation of the SMAD proteins (Zhang, 1999). The activated SMAD complexes are translocated into the nucleus where they regulates transcription in the context of additional factors including transcription factors, co-activators, co-repressors and other molecules that modulate the activity of the transcriptional complex (Rebbapragada, 2003).

To get more knowledge about how the two myostatin genes are regulated, we have cloned and characterized a 2,4 kb fragment of the myostatin I and a 0,9 kb fragment of the myostatin II promoter. Alignment of the myostatin I and II promoters show a large internal region in myostatin II that is missing in myostatin I. Analysis of the promoter sequences show differential distribution of several putative E-boxes that might be important for transcription. E-boxes are binding sites for the myogenic regulatory factors, MRF (Lassar et al, 1991; Murre et al, 1989). To determine if the myostatin I and II promoters can control expression and to find out which E-boxes that is important for expression, a luciferase assay with a luciferase reporter gene is in progress.

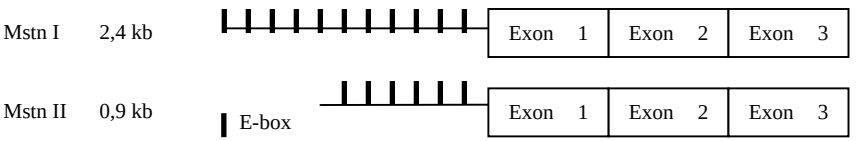

Figure 2
Mstn I and II promoters
The promoter sequences show differential distribution of E-boxes. The two myostatin genes in A. salmon is 93 % identical, both containing three exons.

The activin receptor IIB in Atlantic salmon is cloned and the tissue expression studied. The gene is expressed in all the tissues examined, red and white muscle, heart, intestine, brain, liver, gills, kidney, spleen, tongue, eye, and gonads.

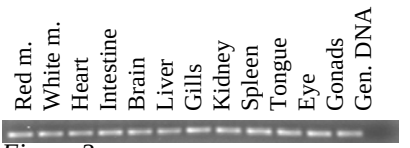

Figure 3
Expression of actRIIB in Atlantic salmon.

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