

**CLONING OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)
 α -ACTIN AND MYOSIN REGULATORY LIGHT CHAIN 2 GENES
AND α -TROPOMYOSIN 5'-FLANKER.
FUNCTIONAL ASSESSMENT OF PROMOTERS**

Aleksi Krasnov,
Heli Teerijoki and Hannu Mölsä
Institute of Applied Biotechnology, University of Kuopio, P.O.B. 1627, Kuopio
70211 FINLAND. E-mail: krasnov@uku.fi

EXTENDED ABSTRACT ONLY - DO NOT CITE

Cloning and characterization of fish promoters is important for gene transfer research, studies of cell differentiation and regulation of gene expression. Identification of genomic structure of fish genes provides valuable sequence information and adds to understanding of their molecular evolution. We aimed at cloning of rainbow trout regulatory sequences that direct expression of genes in skeletal muscle. To address this task, we chose α -actin (α -OnmyAct), myosin light regulatory chain (OnmyMLC2) and tropomyosin (α -OnmyTM). Cloning of genomic sequences was performed using the Genome Walker system. In brief, high molecular weight genomic DNA of rainbow trout was digested with restriction enzymes leaving blunt ends, which was followed by ligation to adaptors including primer sites. Combination of universal and gene specific primers was used for PCR amplification of these libraries.

Actins are highly conserved structural proteins. Being components of contractile structures and cytoskeleton, actins are distributed ubiquitously. Of three major vertebrate actin groups (α , β and γ), α -actins are specific for striated muscle. Cardiac isoforms are predominant in heart, whereas skeletal α -actins are found in both skeletal and cardiac muscle. We cloned the whole coding part (2.8 kb), 5'-flanker (2.1 kb) and terminator (0.5 kb) of α -OnmyAct. This gene was expressed in both skeletal and cardiac muscle being a predominant isoform in trunk muscle of adult rainbow trout. Its structure of this gene was identical to all known vertebrate skeletal and part of cardiac α -Act genes. The upstream regions

of β -OnmyAct included TATA box and a number of putative regulatory motifs (E-boxes and CArG-boxes). These elements were reported in promoters of α -Act genes of zebrafish (Higashijima et al., 1997), medaka (Kusakabe et al., 1999) and channel catfish (Kim et al., 2000).

Myosin complex is a hexamer of two heavy and four light chains, of which regulatory chain is required for calcium binding. This gene has been cloned from one teleost species, zebrafish (Xu et al., 1999). We cloned two distinct OnmyMLC2 promoters (1.6 and 1.0 kb) and both included transposon-like sequences. The OnmyMLC2 promoters included TATA box and a number of putative regulatory motifs (E-boxes), their number being less than in β -OnmyAct. Number (7) and length of exons in this gene was typical for vertebrate MLC2.

In skeletal muscle, tropomyosin is a dimer of α and β chains mediating interaction between the troponin complex and actin, which is required for regulation of contraction. Unlike actin and myosin, tissue-specific isoforms of mammalian tropomyosin are encoded by a single gene and multiple proteins arise due to usage of different promoters and alternative splicing. We cloned α -OnmyTM promoter (700 bp) which lacked canonical regulatory elements. This was typical for vertebrate α tropomyosins (Wieczorek et al., 1988).

For functional assessment, promoters were cloned with LacZ reporter and these constructs were transferred into rainbow trout eggs. All four promoters were able to control expression of reporter which was detected earliest at the stage of somitogenesis. Reporter expression was found mainly in myotomes and heads and but none of these promoters was strictly muscle-specific. Functionality of four promoters and α -OnmyAct terminator was also confirmed in rainbow primary embryonic cell cultures.

Three vectors containing β -OnmySkAct terminator combined with β -OnmySkAct, OnmyMLC2 or α -OnmyTM promoter were constructed. We cloned rainbow trout glucose transporter type I (OnmyGLUT1) into these vectors and the transgenes were transferred into rainbow trout eggs. Recombinant OnmyGLUT1 transcripts were detected in embryos.

References

Higashijima, S., Okamoto, H., Ueno, N., Hotta, Y. and Eguchi, G. (1997). High-frequency generation of transgenic zebrafish which reliably express

GFP in whole muscles or the whole body by using promoters of zebrafish origin. *Dev. Biol.* 192, 289-299.

Kim, S., Karsi, A., Dunham, R.A. and Liu, Z. (2000). The skeletal muscle alpha-actin gene of channel catfish (*Ictalurus punctatus*) and its association with piscine specific SINE elements. *Gene* 252, 173-181.

Kusakabe, R., Kusakabe, T and Suzuki, N. (1999) In vivo analysis of two striated muscle actin promoters reveals combinations of multiple regulatory modules required for skeletal and cardiac muscle-specific gene expression. *Int. J. Dev. Biol.* 43, 541-554.

Wieczorek, D.F., Smith, C.W. and Nadal-Ginard B. (1988). The rat alpha-tropomyosin gene generates a minimum of six different mRNAs coding for striated, smooth, and nonmuscle isoforms by alternative splicing. *Mol. Cell. Biol.* 8, 679-694.

Xu, Y., He, J., Tian, H.L., Chan, C.H., Liao, J., Yan, T., Lam, T.J. and Gong Z. (1999). Fast skeletal muscle-specific expression of a zebrafish myosin light chain 2 gene and characterization of its promoter by direct injection into skeletal muscle. *DNA Cell. Biol.* 18, 85-95.

