MASTS VISITING FELLOWSHIP: January - June 2014.

Final Report

1. Project details

Title: Interaction between growth and stress signalling pathways in skeletal muscle of Atlantic salmon (*Salmo salar*)

Duration: Six months - January to June 2014.

Visiting fellow: Dr Eduardo N. Fuentes

Home Institute at time of fellowship: *Universidad Andrés Bello, Republica 470 piso 1, Región Metropolitana Santiago, Chile.*

Visited:

(a) January – March 2014

Scottish Oceans Institute, University of St. Andrews, East Sands, Fife, KY16 8LB, UK.

*(*b*)* April – June 2014

Institute of Biological and Environmental Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB24 2TZ, UK.

Fellowship hosts:

(1). Professor Ian A. Johnston (*Scottish Oceans Institute, University of St. Andrews, East Sands, Fife, KY16 8LB, UK*)

With support from post-doctoral researcher Dr Daniel Garcia de la Serrana.

(2). Dr Daniel J. Macqueen (*Institute of Biological and Environmental Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB24 2TZ, UK*)

2. Overview of project

The fellowship allowed Dr Fuentes, a post-doctoral researcher from Universidad Andrés Bello (expert in fish muscle biology), to visit two MASTS institutions over a period of six months and perform a range of carefully-designed scientific experiments. The goal was to better understand molecular mechanisms underlying muscle growth in Atlantic salmon muscle (the edible flesh, i.e. main commercial commodity in salmon aquaculture) with a focus on pathways driving catabolic processes such as atrophy and/or remodelling of the tissue for energetic reallocation. Muscle growth depends on a balance between anabolic and catabolic processes, which is regulated by a range of environmental and internal signals. In a commercial context, catabolic outcomes are unfavourable, but expected to arise under stress and disease. Our project aimed to characterize pathways driving catabolic outcomes, using *in vitro* Atlantic salmon primary muscle cell cultures and several catabolic signals as a model system. These pathways remain poorly characterized in teleosts as a group. At the molecular level, our core focus was on better understanding the role of key gene components of the insulin-like growth factor axis as major potential players in the balance between anabolic and catabolic outcomes.

3. Breakdown of the work

The fellowship comprised two major phases, with research stays at the Universities of St Andrews and Aberdeen. During each phase, there was extensive contact between Dr Fuentes and the two hosting researchers, with regular in person and phone meetings taking place (including the whole team) to optimize strategic delivery of project goals.

(a) Phase 1: Scottish Oceans Institute, University of St Andrews. In the first phase of work, Dr Fuentes, under the guidance of Dr Garcia de la Serrana, performed *in vitro* primary cell culture experiments to test the effect of several treatments aiming to induce a catabolic state in Atlantic salmon muscle – separately recapturing the anticipated internal state during disease (interleukin treatment), stress (dexamethasone treatment) or fasting (amino acid deprivation treatment) challenges. He also tested the effect of a treatment designed to induce an anabolic state in the muscle following a period of fasting (amino acids, along with the pro-growth hormone IGF1). The data indicated that the treatments worked as intended (i.e. they either induced atrophy or muscle cell growth, as predicted *a priori*) and a range of samples were taken for molecular gene expression analyses, which were largely performed in Aberdeen (phase 2).

(b) Phase 2: Institute of Biological and Environmental Sciences, University of Aberdeen. In the second phase of work, Dr Fuentes, using samples generated during phase 1, performed an extensive set of quantitative gene expression analyses under the guidance of Dr Macqueen. Genes analysed included those coding key players from the insulin-like growth factor (IGF) system (IGF hormones, IGF binding proteins), ubiquitin ligases (MAFbx and MURF family genes) and muscle structural proteins (e.g. myosin light chain and troponin). We made full efforts to distinguish gene duplicates retained from a genome duplication event in the evolutionary history of salmonid fish. A range of expression responses were observed and statistical analyses performed providing evidence for distinct molecular mechanisms being involved in the different atrophy pathways. This work is currently being prepared for publication (see below, section 4).

During phase 2, an important discovery was made concerning the evolution of genes centrally involved in the breakdown of muscle – namely the MURF family. In our initial characterization of the genes for study in Atlantic salmon, we identified a novel MURF family member conserved across a range of vertebrates. Using comparative genomic and phylogenetic analyses along with cross-taxon expression analyses, we characterized the MURF family and the results were published in a high-ranking molecular biology journal (see below). We are also preparing a follow up manuscript focussed on characterising the MURF gene family of Atlantic salmon - including its regulation under different catabolic signals (see below, section 4).

4. Research outputs

All described outputs acknowledged (or, if unpublished currently, will acknowledge) support received during the MASTS fellowship.

(a) Conference talks:

(1). Macqueen DJ, Johnston IA, Devlin RH, **Fuentes EN**, Alzaid A, Martin SAM. 'Characterizing signalling pathways linking growth & other physiological systems in farmed salmonid fish'. MASTS third Annual Science Meeting. Edinburgh. Aug 2013.

(2) **Fuentes EN**, Valdes J., Molina A, Johnston, IA, Macqueen DJ. '*Evolution of transcriptional responses of the muscle ringer protein family in salmonids'*. XXXVII meeting

of Sociedad de Bioquímica y Biología Molecular de Chile, Puerto Varas, Chile, Sep 30-01, Oct 2014.

(b) Publications:

(1). Macqueen DJ, **Fuentes EN**, Valdés JA, Molina A, Martin SA. 2014. The vertebrate muscle-specific RING finger protein family includes MuRF4--a novel, conserved E3-ubiquitin ligase. FEBS Lett. 588:4390-4397.

Final accepted manuscript freely available at:
http://aura.abdn.ac.uk/bitstream/2164/4125/1/Macqueen_et_al._FEBS_Lett._Manuscript_GREEN_OA.pdf

Further manuscripts in preparation:

(2) **Fuentes EN**, Garcia de la serrana D, Valdés JA, Molina A, Macqueen DJ & Johnston IA. Complex expression responses of the insulin-like growth factor binding protein gene family in Atlantic salmon myotubes exposed to catabolic and anabolic conditions.

(3) Macqueen DJ, **Fuentes EN**, Valdés JA, Molina A, Garcia de la serrana D & Johnston IA. Characterization of the complete expanded MURF gene family from Atlantic salmon.

5. Financial Breakdown

From MASTS fellowship: £16,050. Used to cover the cost of Dr Fuente's travel between Chile and Scotland, along with travel between the Universities of St Andrews and Aberdeen, as well as rented accommodation and living expenses during the 6-month stay.

In kind contributions

(a) Prof. Johnston contributed an estimated £10,500, which was used for primary cell culture experiments, along with sampling of material employed in gene expression analyses. The main cost came from purchasing chemicals and consumables required for cell culture, including staining of cells for phenotyping, as well as RNA extraction.

(b) Dr Macqueen contributed an estimated £5,800 for molecular analyses in order to study the expression of ~50 genes in the different cell culture experiments. The main cost arose from purchasing consumables used for quantitative PCR as well as those required to generate complementary cDNA samples from RNA samples.