

Dissertation Abstract

Hildahl, Jon (2007). Endocrine regulation of flatfish metamorphosis – growth hormone, insulin-like growth factor-I and their receptors in Atlantic halibut.

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Background and aims: The endocrine system is largely responsible for regulating development and growth in vertebrates, with growth hormone (GH) and insulin-like growth factor-I (IGF-I) being of major importance. Atlantic halibut (*Hippoglossus hippoglossus*) is a commercially important flatfish species which undergoes dramatic metamorphosis, transforming the symmetrical and pelagic larva into an asymmetric benthic juvenile. Successful metamorphosis requires precise endocrine regulation of major cranial remodeling as well as most physiological processes. As little prior data exist, the aim of this thesis has been to elucidate the role of the GH-IGF-I system in larval development and metamorphosis of the Atlantic halibut.

Methods: The main approach has been to characterize genes coding for GH and IGF-I receptors (GHR and IGF-IR, respectively) and to establish molecular techniques to quantify and localize receptor gene expression. PCR was used to amplify genes of interest for sequencing and identification of multiple GHR and IGF-IR genes. These were used to design primers and probes for use in quantitative rt-PCR (qrt-PCR) and to synthesize riboprobes for *in situ* hybridization. Sequence information was also used to design a peptide for immunization and production of a polyclonal GHR antibody used to detect GHR by immunohistochemistry. A radioimmunoassay was established to quantify IGF-I tissue content. A complementary approach to elucidate the interaction between thyroid hormones, prolactin (PRL) and the GH-IGF-I system was to treat larvae *in vivo* using *Artemia* enriched with thyroxine (T_4), a T_4 -blocker, methimazole, and a PRL-blocker, bromocriptine.

Results and Conclusions: Atlantic halibut possess genes for type I GHR (*hhGHR*), a truncated type I GHR (*hhGHRtr*), a putative *hhGHBP*, type II *hhGHR* and two isoforms of IGF-IR (*hhIGF-IR1* and *hhIGF-IR2*). Multiple variants of GHRs and IGF-IRs have been identified in many teleost species, but Atlantic halibut are the first species found to have all the GHR variants. The GH-IGF-I system is established by the start of endogenous feeding in Atlantic halibut larvae when GH and IGF-I protein content as well as type I *hhGHR*, type I *hhGHRtr*, *hhIGF-IR1* and *hhIGF-IR2* mRNA are detected. Moreover, all above parameters except GH content is down-regulated in abnormally developing larvae, suggesting that the GH-IGF-I system is required for successful metamorphosis. These proteins/genes are differentially regulated during metamorphosis with larval IGF-I body content and type I *hhGHR*, type I *hhGHRtr*, and *hhIGF-IR2* mRNA decreasing, while *hhIGF-IR1* mRNA increases. Type I *hhGHR*, type II *hhGHR*, and *hhIGF-IR* transcripts as well as GHR protein are found throughout metamorphosis in tissues involved in eye migration. T_4 -treatment decreases GH and IGF-I content and advances settling behavior and pigmentation.

It is concluded that the GH-IGF-I system is a regulator of Atlantic halibut larval development and metamorphosis and hypothesized that T_4 interacts with the GH-IGF-I system during metamorphosis in order to divert energy expenditure from growth to tissue remodeling.

Keywords: *Hippoglossus hippoglossus*, growth, development, growth hormone receptor, insulin-like growth factor-I receptor, quantitative reverse transcription PCR, *in situ* hybridization

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