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**The GH-IGF Axis in *Sparus aurata*: Possible
Applications to Genetic Selection**

B. Funkenstein, C. Duan

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**The GH-IGF axis in *Sparus aurata*:
possible applications to genetic selection**

U.S. - Israel BARD, IS-2769CR

Final scientific report

The GH-IGF axis in *Sparus aurata*: possible applications to genetic selection

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Cooperation, briefly explain whether synergistic, complementary or supportive.

Cooperation was both complementary and synergistic.

Abstract

Many factors affect growth rate in fish: environmental, nutritional, genetics and endogenous (physiological) factors. Endogenous control of growth is very complex and many hormone systems are involved. Nevertheless, it is well accepted that growth hormone (GH) plays a major role in stimulating somatic growth. Although it is now clear that most, if not all, components of the GH-IGF axis exist in fish, we are still far from understanding how do fish grow. In our project we used as the experimental system a marine fish, the gilthead sea bream (*Sparus aurata*), which inhabits lagoons along the Mediterranean and Atlantic coasts of Europe, and represents one of the most important fish species used in the mariculture industry in the Mediterranean region, including Israel. Production of *Sparus* is rapidly growing; however, in order for this production to stay competitive, the farming of this fish species has to intensify and become more efficient. One drawback, still, in *Sparus* extensive culture is that it grows relatively slow. In addition, it is now clear that growth and reproduction are physiological interrelated processes that affect each other. In particular sexual maturation (puberty) is known to be closely related to growth rate in fish as it is in mammals, indicating interactions between the somatotrophic and gonadotropic axes.

The goal of our project was to try to identify the rate-limiting components(s) in *Sparus aurata* GH-IGF system which might explain its slow growth by studying the ontogeny of growth-related genes: GH, GH receptor, IGF-I, IGF-II, IGF receptor, IGF-binding proteins (IGFBPs) and Pit-1 during early stages of development of *Sparus aurata* larvae from slow and fast growing lines. Our project was a continuation of a previous BARD project and could be divided into five major parts: i) obtaining additional tools to those obtained in the previous project that are necessary to carry out the developmental study; ii) the developmental expression of growth-related genes and their cellular localization; iii) tissue-specific expression and effect of GH on expression of growth-related genes; iv) possible relationship between GH gene structure, growth rate and genetic selection; v) the possible role of the IGF system in gonadal development.

The major findings of our research can be summarized as follows: 1) The cDNAs (complete or partial) coding for *Sparus* IGFBP-2, GH receptor and Pit-1 were cloned. Sequence comparison reveals that the primary structure of IGFBP-2 protein is 43-49% identical to that of zebrafish and other vertebrates. Intensive efforts resulted in cloning a fragment of 138 nucleotides, coding for 46 amino acids in the proximal end of the intracellular domain of GH receptor. This is the first fish GH receptor cDNA that had been cloned to date. The cloned fragment will enable us to complete the GH receptor cloning. 2) IGF-I, IGF-II, IGFBP-2, and IGF receptor transcripts were detected by RT-PCR method throughout development in unfertilized eggs, embryos, and larvae suggesting that these mRNAs are products of both the maternal and the embryonic genomes. Preliminary RT-PCR analysis suggest that GH receptor transcript is present in post-hatching larvae already on day 1. 3) IGF-1R transcripts were detected in all tissues tested by RT-PCR with highest levels in gill cartilage, skin, kidney, heart, pyloric caeca, and brain. Northern blot analysis detected IGF receptor only in gonads, brain and gill cartilage but not in muscle; GH increased slightly brain and gill cartilage IGF-1R mRNA levels. 4) IGFBP-2 transcript were detected only in liver and gonads, when analyzed by Northern blots; RT-PCR analysis revealed expression in all tissues studied, with the highest levels found in liver, skin, gonad and pyloric caeca. 5) Expression of IGF-I, IGF-II, IGF-1R and IGFBP-2 was analyzed during gonadal development. High levels of IGF-I and IGFBP-2 expression were found in bisexual young gonads, which decreased during gonadal development. Regardless of maturational stage, IGF-II levels were higher than those of IGF-I. 6) The GH gene was cloned and its structure was characterized. It contains minisatellites of tandem repeats in the first and third introns that result in high level of genetic polymorphism. 7) Analysis of the presence of IGF-I and two types of IGF receptor by immunohistochemistry revealed tissue- and stage-specific expression during larval development. Immunohistochemistry also showed that IGF-I and its receptors are present in both testicular and ovarian cells.

Although at this stage we are not able to pinpoint which is the rate-limiting step causing the slow growth of *Sparus aurata*, our project (together with the previous BARD) yielded a great number of experimental tools both DNA probes and antibodies that will enable further studies on the factors regulating growth in *Sparus aurata*. Our expression studies and cellular localization shed new light on the tissue and developmental expression of growth-related genes in fish.

Achievements

Achievements: The goal of the original research proposal was to clone additional growth-related genes to those cloned during the first BARD from the marine fish *Sparus aurata* and to study the developmental expression of these genes in relation to growth selection and try to understand the reasons for the slow growth of *Sparus aurata*.

During the three years of this project, which was a continuation to a previous BARD project, we fulfilled much of this general objective and were able also to investigate aspects that were beyond the scope of the original project.

1. Two cDNAs coding for IGFBP-2 and GH receptor were cloned and sequenced. To date, no fish GH receptor was cloned.
2. The GH gene was structurally characterized. The analysis revealed an extensive polymorphism of the first intron, never found before in a vertebrate GH gene. This phenomenon was used for analysis of cultured and natural populations of *Sparus aurata* for genetic variation. Studies were also initiated to dissect the possible role of GH first intron in regulating of the GH gene (promoter) activity.
3. The developmental expression of IGF-I, IGF-II, IGF-1R, IGFBP-2, Pit-1 and GH receptor genes were studied. In addition, tissue specific expression of IGF-1R and IGFBP-2 mRNA and the tissue-specific effect of GH on the expression of these two genes was determined.
4. A detailed immunohistochemistry study was conducted for the cellular localization of IGF-I and two types of IGF receptor during early stages of *Sparus aurata* larval development. This study sheds new light on the important role of IGFs in the development of specific organs in fish.
5. The expression of IGF-I, IGF-II, IGFBP-2 and IGF-1R was studied during gonadal development of *Sparus aurata*. In addition, immunohistochemistry was used for cellular localization of IGF-I and two types of IGF receptor during gonadal development. The presence of IGFs, their binding protein and their receptor, suggests an autocrine/paracrine role for IGFs in gonadal physiology.
6. Experiments were initiated to express *Sparus aurata* IGF-II in *E. coli*, which will be used for raising antibodies in order to develop an RIA or ELISA for quantification of this protein in the circulation and for cellular localization studies. The protein will also be used for further analysis of the two types of IGF receptor identified in *Sparus aurata*.

The research activities during this project provided us with additional molecular tools with which we were able to gain new information regarding the possible important roles of components in the GH-IGF axis during fish early development and enabled us also to shed some light on the mechanism of GH action in fish. In addition, our sequence information provided us with the possibility to assess the degree of conservation of these mRNAs along the evolution of vertebrates in general, and fish in

particular. The characterization of the GH gene and the identification of unique features in the structure of its introns, in particular the extensive polymorphism and the presence of half-site steroid response elements will be useful in future studies of analysis of their importance in relation to the activation/inhibition of the GH gene. The development of specific antibodies achieved during the first BARD project provided us in the present project with a valuable tool with which to identify sites where growth factors may play important role during fish development.

Cooperation: This research project was based on a close collaboration between the research groups at IOLR and at the University of Michigan. Since the experimental fish used for this project was the gilthead sea bream (*Sparus aurata*), which is grown in Israel but not in the USA, a close relationship had evolved between the two laboratories. Cloning of the different cDNAs carried out in this project was accomplished by mutual efforts. The tissue distribution studies and the experiment of the effects of GH were performed by the two groups, using fish tissues or RNA samples collected and prepared by the group at IOLR or by the American partner. The American partner cloned IGFBP cDNA using RNA extracted from tissues brought by Dr. Funkenstein and a liver cDNA library which was constructed by the former BARD partner and was made available for this project. The studies on the hormonal regulation of IGF-1R and IGFBP-2 by GH were commenced at IOLR by injecting fish with GH, extraction of RNA samples from different tissues and shipping to Ann Arbor for IGFBP-2 expression analysis by Northern blots.

The two groups communicated with each other on a regular basis by e-mail, fax and mail. The Israeli PI visited once Ann Arbor and in addition the two PIs met on three occasions during International Symposia and discussed the progress of the project and made plans for future experiments. Materials and information were exchanged often, when needed. The final report submitted hereby was prepared by the two groups from IOLR and from the University of Michigan.

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8. Funkenstein, B., Tsai, W., Maures, T. and Duan, C. Insulin-like growth factor binding protein-2 (IGFBP-2) in *Sparus aurata*: ontogeny, tissue distribution and hormonal regulation. In preparation.

Papers presented in conferences and meetings (national and international):

1. Funkenstein, B. and Almuly, R. Growth hormone and insulin-like growth factor I mRNA detection in *Sparus aurata* larvae by *in situ* hybridization. The 18th Conference of the European Society for Comparative Endocrinology, Rouen, France, 1996.
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10. Funkenstein, B., Tsai, W.S. and Duan, C. Expression of insulin-like growth factor binding protein 2 (IGFBP-2) in a marine fish, *Sparus aurata*. The 20th Conference of the European Society for Comparative Endocrinology, Faro, Portugal, 2000.
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