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Somatotropic axis genes are expressed before pituitary onset during zebrafish and sea bass development

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Abstract :

The somatotropic axis, or growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis, of fish is involved in numerous physiological process including regulation of ionic and osmotic balance, lipid, carbohydrate and protein metabolism, growth, reproduction, immune function and behavior. It is thought that GH plays a role in fish development but conflicting results have been obtained concerning the ontogeny of the somatotropic axis. Here we investigated the developmental expression of GH, GH-receptor (GHR) and IGF-1 genes and of a GH-like protein from fertilization until early stages of larval development in two Teleosts species, Danio rerio and Dicentrarchus labrax, by PCR, in situ hybridization and Western blotting. GH, GHR and IGF-1 mRNA were present in unfertilized eggs and at all stages of embryonic development, all three displaying a similar distribution in the two species. First located in the whole embryo (until 12 hpf in zebrafish and 76 hpf in sea bass), the mRNAs appeared then distributed in the head and tail, from where they disappeared progressively to concentrate in the forming pituitary gland. Proteins immunoreactive with a specific sea bass anti-GH antibody were also detected at all stages in this species. Differences in intensity and number of bands suggest that protein processing varies from early to later stages of development. The data show that all actors of the somatotropic axis are present from fertilization in these two species, suggesting they plays a role in early development, perhaps in an autocrine/paracrine mode as all three elements displayed a similar distribution at each stage investigated.

Highlights

► Targets the simultaneous presence of the 3 components of the somatotropic axis in developing embryos of two Teleost fish. ► GH, GH receptors and IGF-1 are expressed in unfertilized eggs from zebrafish and sea bass. ► After fertilization expression of all three components is detected before pituitary differentiation. ► GH gene expression is accompanied by GH protein detection. ► Clarifies previous contradictory results on this matter.

Keywords : Growth hormone GH, Insulin-like growth factor-1 IGF-1, Pituitary, Development, Zebrafish, Sea bass

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1. Introduction

The pituitary constitutes an anatomical and physiological link between the nervous and endocrine systems. In fish, it controls diverse physiological functions including somatic growth, metabolism, osmoregulation, reproduction, and behavior (Herzog et al., 2003). Among the hormones produced by the pituitary, growth hormone (GH) belongs to a hormonal superfamily which also includes somatolactin (SL) and prolactin (PRL). In fish GH has pleiotropic actions, including ionic and osmotic balance, reproduction and immune function, as well as different aspects of behavior (Perez-Sanchez, 2000; Reinecke et al., 2005).

The general organization of the somatotropic axis or growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis is similar between mammals and fish, and includes GH, GH receptor (GHR), insulin-like growth factor (IGF), IGF receptor (IGF-R) and IGF binding protein (Gabillard et al., 2003; Moriyama et al., 2000; Reinecke, 2010). GH is expressed and synthesized in somatotropic cells from the adenohypophysis in adults, but expression has also been detected in several extra hypophyseal tissues (Miura et al., 2011). Because of its involvement in growth, GH has also been studied during development. Studies in birds and mammals indicated GH gene expression may appear before pituitary differentiation (Murphy and Harvey, 2001; Pantaleon et al., 1997). In fish, there is evidence that it is transcribed and translated early during development either before or after pituitary differentiation (Ayson et al., 1994; Herzog et al., 2003; Ozaki et al., 2006; Yang et al., 1999). However, the role GH plays during fish embryonic and

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to be corrected, names and surnames have been inverted

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larval development remains unclear (Fuentes et al., 2008; Gabillard et al., 2003; Zhu et al., 2007). A clear-cut picture cannot yet be provided because the studies relate to a very limited number of species, focusing either on mRNA or on protein detection, seldom on both, and barely consider the somatotropic system as a whole (*i.e.*, the GH/GHR/IGF system). This probably accounts for the differences observed from a study to another. In addition, conflicting results have been reported concerning a same species (*e.g.*, in trout Jones et al., 2001; Yang et al., 1999). More information is thus clearly needed to further elucidate the role GH/IGF play in fish larval growth and differentiation. In the present study we investigated the expression of the somatotropic axis genes encoding GH, GHR and IGF-1 during early stages of development in the zebrafish *Danio rerio* and sea bass *Dicentrarchus labrax*. These species were chosen because they have high value for the scientific community (zebrafish) as well as for the farming industry (sea bass), and tools are available for both species. A better knowledge of the GH/GHR/IGF system may help improving the conditions for embryo and larval development in these species. Here we investigated the expression of GH, GHR and IGF-1 transcripts at different stages of early development, including unfertilized eggs. Localization of the sites of expression was achieved using whole-mount *in situ* hybridization. Finally, we developed an antibody directed against GH protein from sea bass in order to see whether the presence of transcripts was associated with GH protein production.

2. Material and methods

2.1. Animals

Adult zebrafish, *D. rerio* were reared at 28 °C on a 12 h light/12 h dark (12L/12D) cycle. Fertilized eggs were incubated under similar LD and temperature conditions. Mature adult sea bass, *D. labrax*, were reared at the "Station IFREMER" of Palavas (France). After fertilization, embryos and larvae were reared under a 19L/5D cycle and natural temperature. Embryos to be used in whole-mount *in situ* hybridization (ISH) studies were placed at 24 hours post-fertilization (hpf) in 0.2 mM phenylthiourea to prevent pigmentation.

2.2. Eggs, embryos and larvae sampling

Unfertilized eggs as well as embryos and larvae were collected at different times of their development. They were either frozen in liquid nitrogen (Western blotting or PCR purposes) or immersed in RNA Later solution (Ambion Biosystems; Austin, TX) (PCR purposes only); they were then stored at −80 °C. Other samples were fixed overnight in freshly prepared 4% paraformaldehyde (PFA) in phosphate buffer saline (PBS) at +4 °C, for ISH or immunocytochemical (ICC) studies. ISH samples were stored in methanol at −20 °C. ICC samples were cryo-protected by successive baths of glycerine/sucrose in PBS at +4 °C as follows: 2 × 30 min in 4% sucrose, 30 min in 5% glycerine/10% sucrose, 1 h in 10% glycerine/15% sucrose, and overnight in 10% glycerine/30% sucrose; finally they were frozen in −50 °C isopentane.

2.3. Amplification from different developmental stages

The sequences of the genes investigated in this study were available from the data bases (Supplementary Table 1; partial sequence in the case of *dlIGF-1*). We therefore designed specific primer sets for further amplification from the respective cDNAs (Supplementary Table 1). Total RNA of embryos and larvae from each developmental stage was extracted using the Trizol® method (Invitrogen; Cergy Pontoise, France). One µg of total RNA was incubated with 1 unit of DNAse I (Roche; Meylan, France) for 30 min at 37 °C. After DNAse inactivation (10 min at 65 °C), RNA was reverse transcribed using Powerscript Reverse transcriptase (Invitrogen; Cergy Pontoise, France) in a total volume of 20 µl. The polymerase chain reaction (PCR) was performed on the obtained cDNA in a total volume of 25 µl, using the primers and conditions described in Supplementary Tables 1 and 2, respectively. In the controls, the template cDNA was replaced by water. The PCR products were loaded in a 2% agarose gel, in the presence of DNA size markers (DNA ladder 1 kb, Promega; Charbonnières, France). Fragments of the expected size were extracted, subcloned in a pGEM-T Easy vector (Promega; Charbonnières, France) and sequenced by Cogenics (Meylan, France) for verification.

2.4. Western blots

Total protein content from pooled sea bass larvae were sonicated at 4 °C in PBS solution containing protease inhibitors (Complete Solution; Roche, Meylan, France). A pool of 3 adult sea bass pituitaries was used as a positive control. Protein content in the homogenates was quantified using the Bradford assay and bovine serum albumin (BSA) as a standard Bradford, 1976.

Proteins were resolved on a 12.5% polyacrylamine gel in a Tris/glycine electrophoresis system (Biorad; Marnes-La-Coquette, France). Gels were run at 100 V for 1 h. Markers (Precision-Plus Protein Standard's; Biorad) were used to determine the molecular weight of the proteins. The proteins were transferred on a polyvinylidene Immobilon-P membrane (Millipore; Molsheim, France) in a Tris–glycine/20% methanol buffer with a semi-dry Fisherbrand blotting system (Thermofisher; Illkirsch, France) following the manufacturer's protocol. After transfer, the membranes were placed for 2 h PBS (pH 7.4) containing 10% nonfat dry milk and 0.2% Tween (PBST). They were then incubated overnight (18 h at 4 °C) in the primary antibody dilution (1/5000) in PBS containing 1 mg BSA fraction V. After washing in PBST and then in PBS, the membranes were submitted to horseradish peroxydase conjugated goat antirabbit IgG (0.0083 µg/ml in PBST containing 0.1% normal goat serum). The membranes were then washed in PBST (3 × 10 min) and in PBS (3 × 5 min) and immunodetection was performed using the ECL$^+$ detection kit (Life Technologies, Saint Aubin, France), and finally observed under a Vilbert Lourmat apparatus (Marne-la-Vallée, France).

The antibody directed against sea bass GH (dlGH-antibody) was obtained and validated by Eurogentec (Liège, Belgium) by rabbit immunization against two peptide sequences from sea bass GH (GANQDGAEMFPDSSTL and WEFPSRSLSVPGAARN).

2.5. In situ hybridization (ISH)

For all species, embryos and larvae were fixed overnight by immersion in 4% paraformaldehyde in PBS at 4 °C, washed in buffer, transferred to 100% methanol and stored at −20 °C. Whole-mount ISH was performed using digoxigenin-labeled riboprobes made using a commercially available kit (Roche, Meylan, France) according to the manufacturer's instructions. The antisense and sense probes (Supplementary Table 2) were generated using a cDNA fragment of the genes of interest. Sea bass IGF-1 antisense and sense probes were produced from a cDNA fragment (291 bp) obtained by a 5′,3′-rapid amplification of cDNA ends (RACE)–PCR (SMART RACE cDNA Amplification kit, Clontech; Mountain View, CA) with the specific primer dlIGF-1-F GGTCGACACGCTGCAGTTTGTG under the following conditions: 95 °C (1 min), then 10 cycles of 94 °C (20 s), 67 °C (1 min), 68 °C (1 min), followed by 20 cycles of 94 °C (10 s), 65 °C (1 min), 68 °C (90 s), then 68 °C (7 min) and finally 4 °C. The amplified fragment was purified, subcloned in PGEM-T Easy Vector (Promega, Charbonnières, France) and sequenced.

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All probes were used at a concentration of 1 μg/ml, and digoxigenin was immunodetected as indicated in the kit protocol (Roche; Meylan, France).

2.6. Immunocytochemistry (ICC)

Pituitary glands from adult sea bass were fixed as indicated above. They were then rinsed, dehydrated and embedded in paraffin (Martoja and Martoja, 1967). ICC detection of GH was performed on 10 μm thick paraffin sections. Paraffin was removed and the sections were rehydrated and processed as previously described for the detection of GH on trout pituitaries (Falcón et al., 2003) using a dlGH-antibody dilution of 1/2500.

3. Results

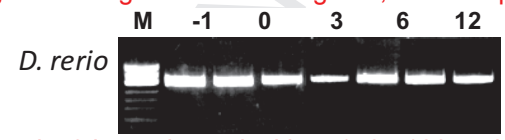
3.1. Growth hormone (GH) during development

The presence of GH mRNA was assessed by RT-PCR in extracts from zebrafish and sea bass samples (Fig. 1). Transcripts were detected in unfertilized eggs and at all developmental stages studied.

In situ hybridization showed a ubiquitous distribution from 0 to 6 hpf in zebrafish (Fig. 2). At 12 hpf (6-somite) to 24 hpf (26-somite) Kimmel et al., 1995 the labeling was restricted to both ends of the embryo; it remained ubiquitous in the head while the notochord seemed labeled at the level of the tail (Fig. 2). Only the anterior part still expressed some labeling at 30 and 36 hpf. After pituitary formation, a strong labeling was seen only at the level of this organ (51 hpf; Fig. 2). A similar situation was described in the developing sea bass embryo and larvae (the sea bass developmental stages at 13/15 °C (Barnabé et al., 1976) were compared to the zebrafish developmental stages at 28.5 °C (Kimmel et al., 1995). Fig. 3 shows the specific *vs.* non-specific labeling observed in sea bass at 4 hpf (morula), 44 hpf (shield), 68 hpf (bud/3-somite), 116/124 hpf (long-pec) and 188 hpf (pec-fin).

In order to make sure the GH RNA expression evidenced by RT-PCR and ISH traduced into a GH protein, we produced a polyclonal sea bass anti-GH antibody in order to detect the protein during early stages of sea bass development (Fig. 4). The specificity of the antibody was tested by Western blots as indicated in materials and methods, as well as by ICC. Fig. 5 shows that the antibody labeled the cells located in the presumptive GH area of the adenopituitary gland. In Western blots from adult pituitary gland extracts two main bands were detected: a major one at 19 kDa and a minor one at 20 kDa. The 20 kDa band was also detected in extracts from unfertilized eggs (−1 hpf) as well as from 0 to 288 hpf (Fig. 4). Although no quantification was performed, it is noteworthy that the intensity of the band decreased from 0 to 24 hpf, to reach almost undetectable levels at 48–72 hpf. Thereafter it increased again (from 96 to 288 hpf). Other immune-reactive bands were also detected by the antibody at molecular weights that varied depending on the developmental stage. Thus a band at 23 kDa was detected from −1 to 24 hpf; other minor bands were also seen and 40 and 50 kDa from 96 hpf that progressively decreased in intensity in later stages.

errors in the legends. 3 legends are lacking: 24 , 48 and hpf for D rerio



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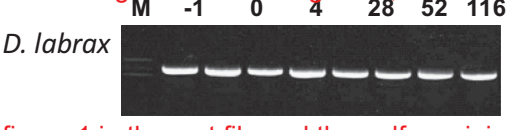

See the original figure 1 in the .ppt file and the .pdf one joined.

Fig. 1. Expression of GH mRNA in extracts from *Danio rerio* and *Dicentrarchus labrax* unfertilized (−1) and fertilized (0) eggs and embryos at different hours post-fertilization (hpf). RT-PCR. M: markers.

3.2. Growth hormone receptor (GHR) and Insulin-like growth factor-1 (IGF-1) during development

RT-PCR as well as ISH allowed detection of GHR expression in unfertilized eggs as well as embryo and larvae at all stages investigated, in both species (Figs. 6–8). As seen for GH, the GHR signal was first seen all over the embryo at early stages (0 and 3 hpf in zebrafish (Fig. 7) and 4 hpf in sea bass (Fig. 8)). Later it concentrated first at both ends (12 hpf in zebrafish and 76 hpf in sea bass) and then at the level of the head (72 hpf in zebrafish and 196 hpf in sea bass) (arrows in Figs. 7 and 8). In the zebrafish, transcripts were seen along the spinal cord (arrow heads in Fig. 7). In both fish, the labeling of the head was particularly concentrated at the interface with the trunk (Figs. 7 and 8). Interestingly, the low PCR amplification observed in RT-PCR from sea bass extracts at 28 and 52 hpf (Fig. 6) corresponded to a low detection signal with ISH at the same stages (Fig. 8).

Rather similar results were obtained with an IGF-1 probe although the labeling appeared more spread out than with the GH and GHR probes (Figs. 9 and 10).

4. Discussion

The present study brings new information on the GH/GHR/IGF system of fish during development. It complements and clarifies previous studies that have reported different developmental patterns of expression, depending on the gene and species investigated, and on the method used. On debate is the question to know whether maternal GH transcripts are present or not in the unfertilized egg and whether GH transcription starts before or after pituitary organogenesis. It has been though for long that production of GH was associated with the formation of the pituitary gland (Cambre et al., 1990; Volckaert et al., 1999). In zebrafish, studies report detection of GH transcripts at ~48 hpf, *i.e.*, after internalization of the forming pituitary and 24 h after the expression of SL, PRL and proopiomelanocortin (POMC) had started Chen and Ge, 2012; Herzog et al., 2003; Li et al., 2009; Pogoda and Hammerschmidt, 2009; in the Japanese eel *Anguilla japonica* (Ozaki et al., 2006) and sea bream *Sparus aurata* (Funkenstein and Cohen, 1996; Herrero-Turrión et al., 2003), GH mRNA were detected neither in unfertilized/fertilized eggs, nor in newly hatched larvae. Conversely, in other species, including *Oncorhynchus mykiss* (Yang et al., 1999), *Atractosteus spatula* (Revol et al., 2005) and *Epinephelus coioides* (Li et al., 2005), GH mRNA from maternal origin has been evidenced in unfertilized fish eggs as well as in embryo and larvae. However, the situation is not so simple that some fish would express GH from fertilization and others later during development. In this regard trout *O. mykiss* provides a case study: early investigations indicating the presence of GH transcripts in unfertilized eggs and embryos prior to pituitary organogenesis (Li et al., 2006; Yang et al., 1999) have been challenged by others (Jones et al., 2001) who found no expression before stage 29 (organogenesis). Today our results on zebrafish also challenge previous studies that found expression of GH gene only from 48 hpf (Chen and Ge, 2012; Herzog et al., 2003; Pogoda and Hammerschmidt, 2009). We show here early expression in unfertilized and fertilized eggs and embryos, far before pituitary formation in zebrafish as well as in sea bass.

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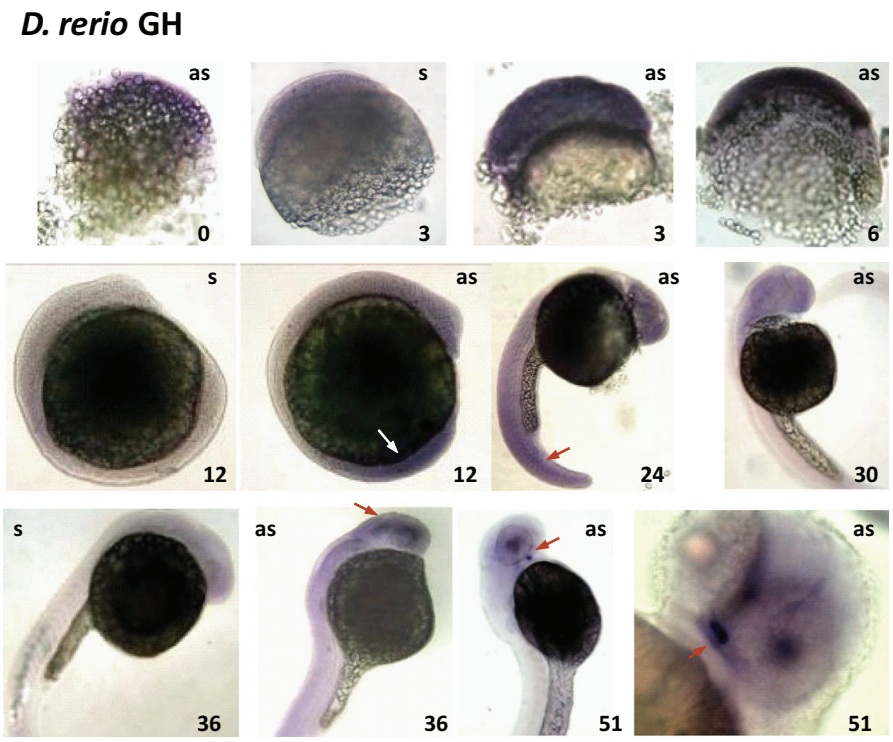

Fig. 2. Whole-mount ISH for GH mRNA during zebrafish development. s: sense probe; as: antisense probe. Hours post-fertilization are indicated at the bottom of the picture.

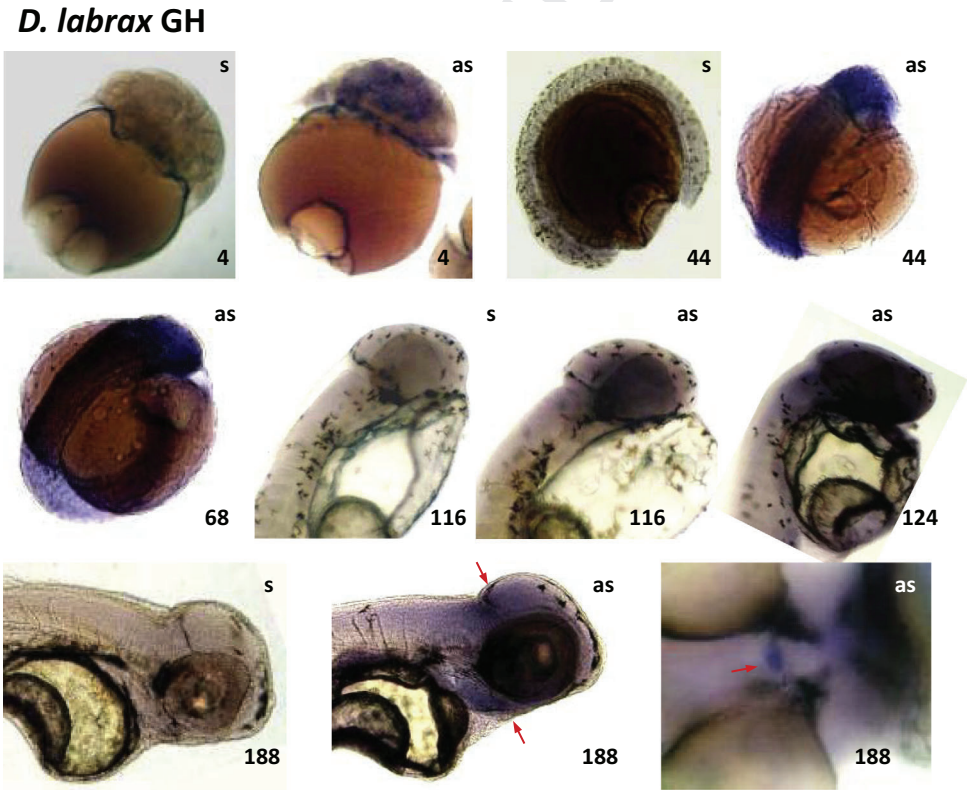

Fig. 3. Whole-mount ISH for GH mRNA during sea bass development. s: sense probe; as: antisense probe. Hours post-fertilization are indicated at the bottom of the picture.

Studies in the trout (Li et al., 2006) and sea bass (Cambre et al., 1990) had shown that the early presence of GH transcripts was not necessarily accompanied by detection of the corresponding protein. Here we demonstrate that the dlGH-antibody used recognized a major band at 19 kDa and another minor band at 20 kDa in pituitary extracts, while in unfertilized eggs and embryos only the 19 kDa band was detected with an additional minor band at 23 kDa. Twenty three kDa is the expected size of sea bass GH

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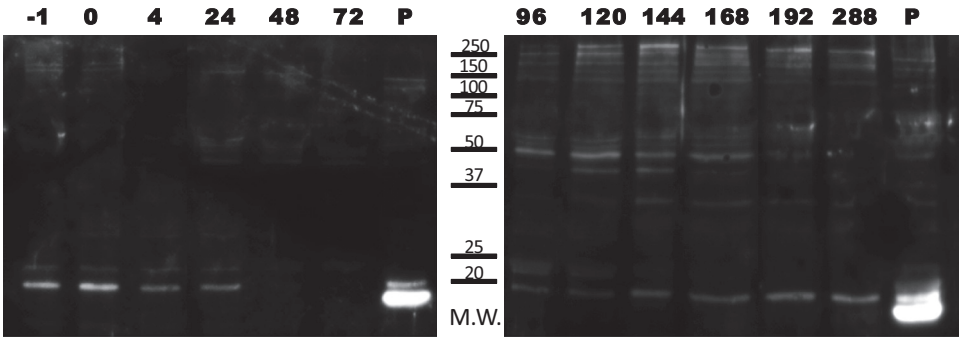
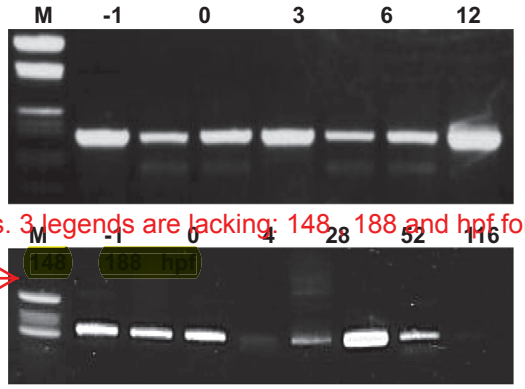


Fig. 4. Western blot analysis of GH protein from developing sea bass embryos and larvae. Hours post-fertilization are ~~indication~~ at the top (−1: unfertilized eggs, 0: fertilization); P: pituitary extract; M.W.: molecular weight markers.

Fig. 5. Immunocytochemical detection of a GH like protein in a section from a sea bass pituitary (right). Control section (left) (Bar: 150 μm).

errors in the legends. 3 legends are lacking: 24 , 48 and hpf for D rerio



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lost legends are here !!

Fig. 6. Expression of GH receptor mRNA in extracts from *Danio rerio* and *Dicentrarchus labrax* unfertilized (−1) and fertilized (0) eggs and embryos at different hours post-fertilization (hpf). RT-PCR. M: markers.

protein, deduced from the peptide sequence. There are reasons to believe the 19 and 20 kDa bands also correspond to GH. First the antibody identified the bands specifically in pituitary extracts. Second, this antibody labeled only the GH producing cells in ICC from pituitary sections (identified by their location in the tissue (Cambre et al., 1986). Third, in enzyme-linked immune-absorbent assays (ELISA) the immunogen peptides or media dilutions from cultured pituitary glands displaced linearly antibody binding (not shown). Finally the co-existence of several forms of GH has been shown in tetrapods as a result either of gene duplication or expression of splice variants or post-translational modifications of the native protein (Aramburo et al., 2000; Komatsu et al., 2007). Although gene duplication is common in Teleost fish, there is to date no evidence for the presence of two GH in sea bass as is the case in zebrafish, and in contrast with trout (Gabillard et al., 2003). We therefore believe that the bands detected in this study at 19 and at 20 kDa result from proteolytic cleavage of a native 23 kDa protein, as observed in the chicken and human GH (Aramburo et al., 2000; Komatsu et al., 2007). Interestingly, the Western blots from sea bass extracts suggest that the cleavage operates at different levels during development: a major (20 kDa) and a minor (23 kDa) band were detected in eggs and embryos up to 24 hpf, only the 20 kDa band remained from 144 to 288 hpf while adults extracts display again two bands but at 20 and 19 kDa. Ontogenetic changes in the proportions of the different variants have already been reported to occur in chicken (Aramburo et al., 2000). The transient appearance of bands at higher molecular weights (40–50 kDa) between 96 and 168 hpf might reflect the presence of GH dimers of different cleavage products, as reported for tetrapods (Aramburo et al., 2000; Grigorian et al., 2005; Luna et al., 2004). The decrease in bands intensity observed from 0 to 48 hpf and disappearance at 48 and 72 hpf might correspond to the extinction of GH material from maternal origin followed at 86 hpf and onwards by endogenous production. Altogether our results suggest that GH transcripts translate into GH protein in the developing embryo. The low levels detected by Western blot could explain that ICC methods did not allow detection of GH cells before pituitary formation in sea bass (Cambre et al., 1990) and other species (Li et al., 2005; Ozaki et al., 2006).

A GH protein being present at the stages investigated, it was of interest to investigate the expression of the corresponding receptors. GHR have been identified in several fish species, and two forms are reported to exist (Di Prinzio et al., 2010; Ozaki et al., 2006; Saera-Vila et al., 2005, 2009). In zebrafish, expression of receptors (GHRa and GHRb) was detected at all stages investigated except at 96 hpf (Di Prinzio et al., 2010). Our results agree with these data and extend to sea bass the conclusion that GHR are

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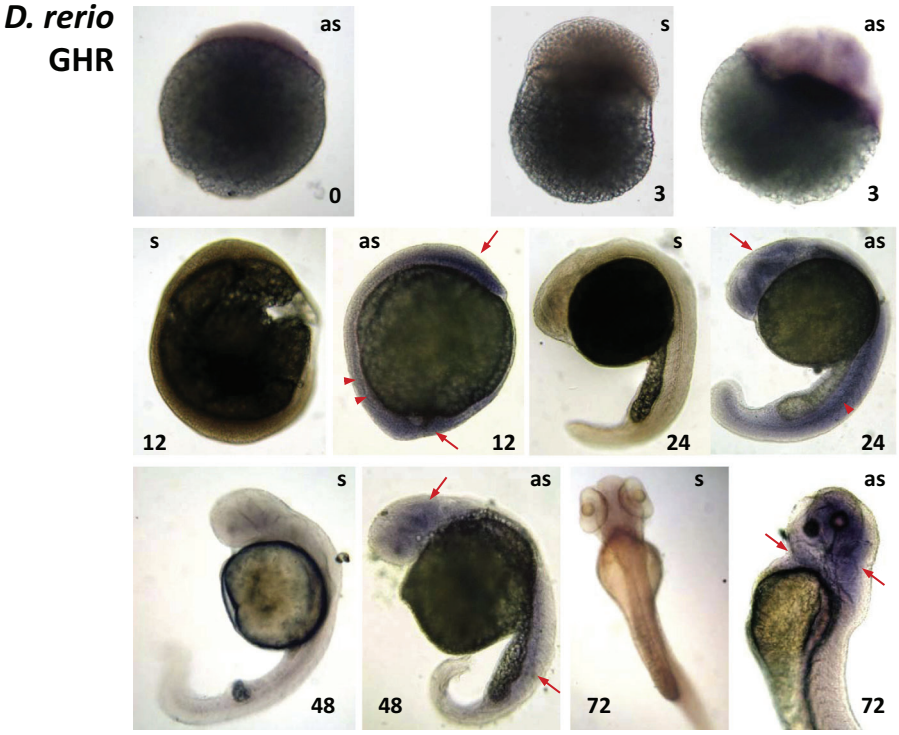

Fig. 7. Whole-mount ISH for GH receptor mRNA during zebrafish development. s: sense probe; as: antisense probe. Hours post-fertilization are indicated at the bottom of the picture.

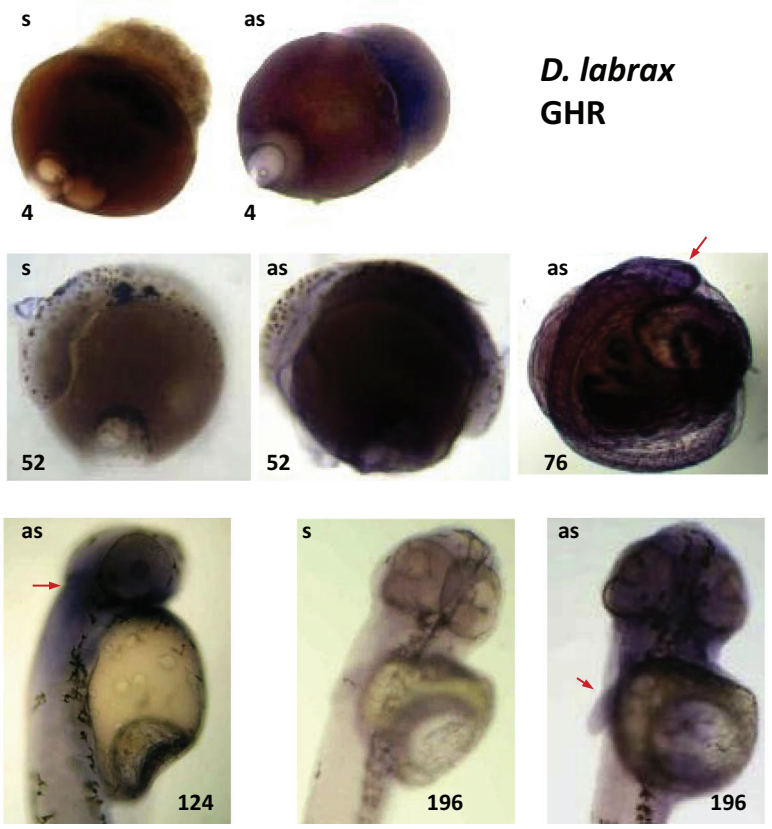

Fig. 8. Whole-mount ISH for GH receptor mRNA during sea bass development. s: sense probe; as: antisense probe. Hours post-fertilization are indicated at the bottom of the picture.

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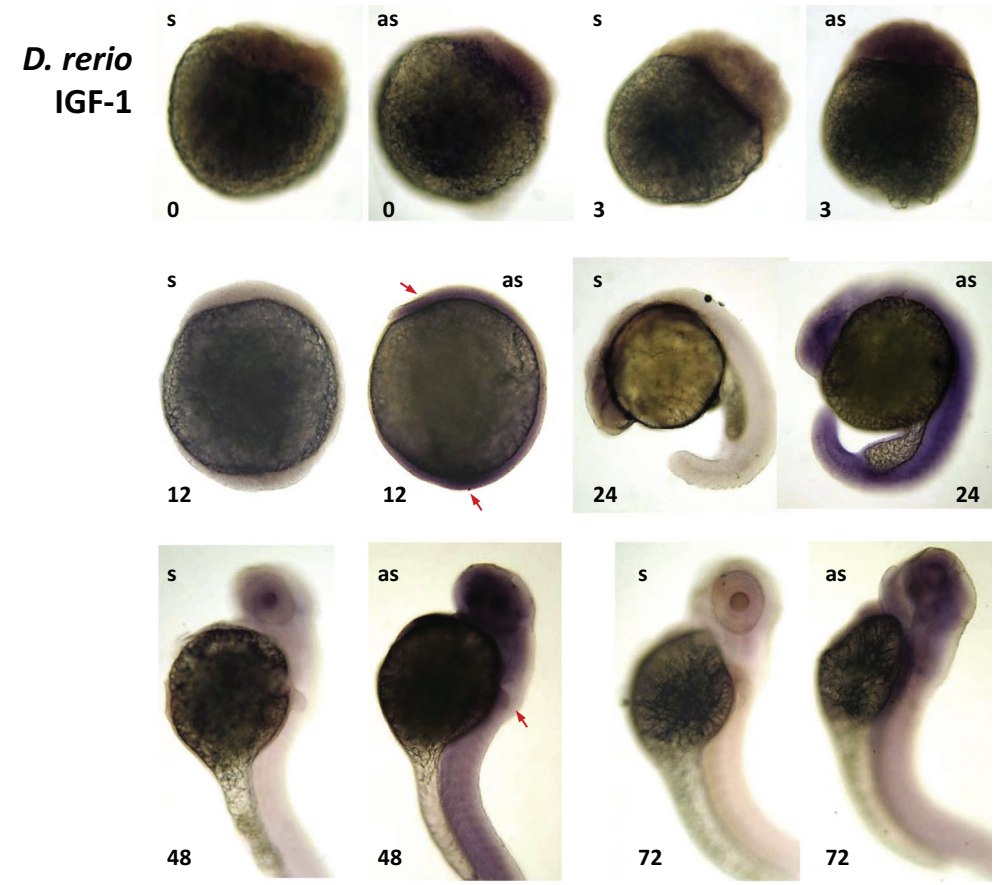

Fig. 9. Whole-mount ISH for IGF-1 mRNA during zebrafish development. s: sense probe; as: antisense probe. Hours post-fertilization are indicated at the bottom of the picture.

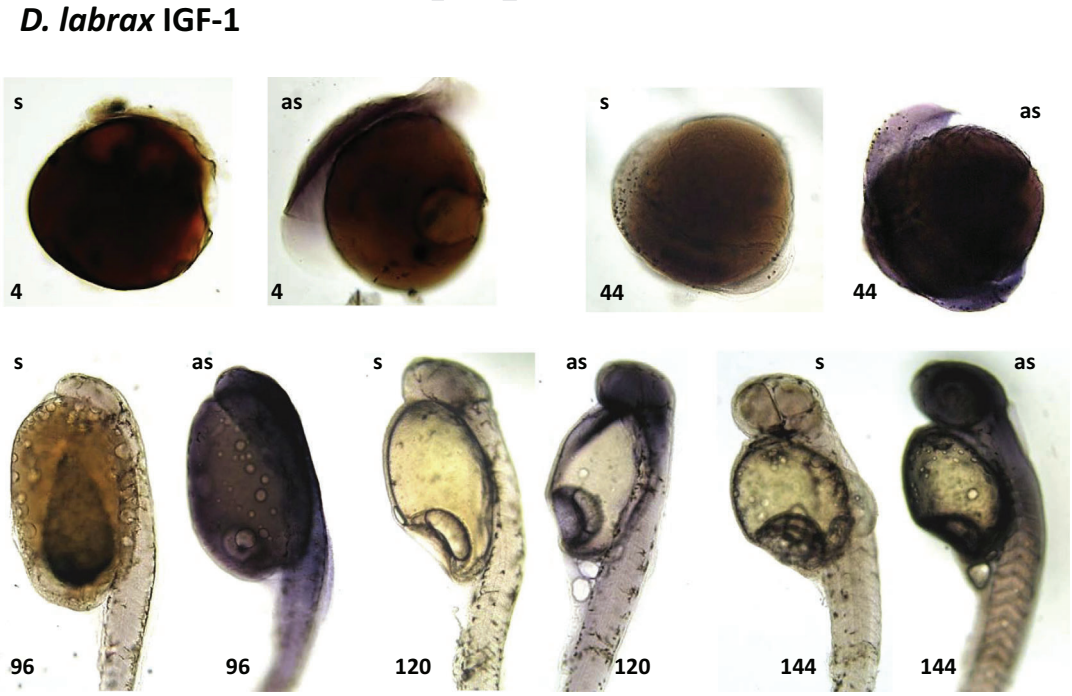

Fig. 10. Whole-mount ISH for IGF-1 mRNA during sea bass development. s: sense probe; as: antisense probe. Hours post-fertilization are indicated at the bottom of the picture.

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expressed from fertilization. GHR transcripts had been detected in immature oocytes of tilapia (*Oreochromis mossambicus*) (Kajimura et al., 2004) and Japanese eel (Ozaki et al., 2006), but their presence has not been described in embryonic stages. An early expression of GHR in zebrafish was previously surprising in view of the fact that no GH expression had been demonstrated before 42 hpf in zebrafish (Herzog et al., 2003). Our current data on zebrafish and sea bass suggest that a functional GH/GHR system is present from unfertilized and fertilized eggs. It is noteworthy that sea bass GHR expression detected both by RT-PCR and ISH seemed to be lower at 28 and 52 hpf, perhaps reflecting extinction of the maternal transcripts.

Many of the growth promoting effects of GH are believed to be mediated by IGF-1 (Funkenstein et al., 2002). Members of the IGF family, including IGF-1, have been cloned in various Teleost fish, but their developmental expression pattern has been investigated only in a very few of them (Berishvili et al., 2006; Escobar et al., 2011; Li et al., 2005; Patruno et al., 2008; Perrot et al., 1999; Reinecke, 2010; Wood et al., 2005; Zou et al., 2009). If GH and GHR are determinant for the expression of IGF-1 as generally admitted (Duval et al., 2002; Sanders and Harvey, 2004) one would expect IGF-1 expression to be concomitant to GH and GHR expression. Whereas this is the case in trout (Li et al., 2006), discrepancies have been reported to exist in other species. Thus, the first GH transcripts and a GH-like protein were seen three days post-hatching in the sea bream, coincident with the end of yolk absorption and first feeding (Funkenstein and Cohen, 1996), while expressions of IGFs and IGFBPs were detected in unfertilized eggs and embryos (Perrot et al., 1999). It was concluded that IGF-1 expression might be GH independent at early stages. A similar lag between the GH (Herzog et al., 2003) and IGF-1 (Chen et al., 2001; Zou et al., 2009) onsets of expression has been reported in zebrafish where two IGF-1 (a and b) have been cloned; IGF-1b is expressed from fertilization and onwards, and IGF-1a shortly after (Zou et al., 2009). The zebrafish 630 mers long IGF-1 probe used in this study covered a sequence corresponding to IGF-1a that differed from IGF-1b mainly by 20 mers at each end (*i.e.*, 40 mers in total). It is therefore possible that it might have labeled both IGF-1a and IGF-1b forms. Whatever it may be, our data indicate that IGF-1 was expressed in eggs, embryo and larvae as was the case for GH and GHR. And we extend this conclusion to the developing sea bass in contradiction with a previous study that reported IGF-1 expression starting in the liver at dph 6 (Patruno et al., 2008).

Previous studies have suggested that either GH, or GHR or IGF act on fish development in an autocrine and/or paracrine manner (Berishvili et al., 2006; Escobar et al., 2011; Patruno et al., 2008). Our results show that all three components from maternal origin are present in the unfertilized egg and during the first hours of development, and are produced as soon as endogenous transcription starts. This strongly supports the view that the GH/GHR/IGF axis is functional at the very early stages of development, playing a role in fish development and differentiation. GH and IGF might have proper actions before transcription starts in the embryo. Put together, our results agree with the hypothesis that the system is important for the development of the anterior and posterior parts of the larvae and particularly of the nervous system, muscle and bone related structures, as suggested for the role of IGF in the Chilean flounder *Paralichtys adsperus* (Escobar et al., 2011) and zebrafish *D. rerio* (Eivers et al., 2004; Zou et al., 2009).

In conclusion, we have shown for the first time that transcripts corresponding to GH, GHR and IGF are inherited and present during early development in zebrafish and sea bass. Our results highlight that the discrepancies regarding onset of GH expression in fish may just result from technical issues. The presence of the GH protein, as shown in sea bass, further supports the idea that GH is important for the early function of the GH/GHR/IGF system. To date GH knocked-down experiments performed by us (not shown) and others (Zhu et al., 2007) did not lead to any discernible change. Thus, further experimentation is needed in order to precisely determine the role GH/GHR/IGF-1 axis plays in early development of fish.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ygcen.2013.08.018.

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