Growth hormone-stimulated insulin-like growth factor-1 expression in rainbow trout (Oncorhynchus mykiss) hepatocytes is mediated by ERK, PI3K-AKT, and JAK-STAT

Katie M. Reindl, Jeffrey D. Kittilson, Heather E. Bergan, and Mark A. Sheridan
Department of Biological Sciences, North Dakota State University, Fargo, North Dakota

Submitted 24 June 2010; accepted in final form 7 April 2011

Although growth hormone (GH) regulates numerous processes in vertebrates, including feeding, metabolism, reproduction, osmoregulation, immune function, and behavior, perhaps the most thoroughly studied actions of GH are those related to promotion of organismal growth (3, 7, 16, 19, 20). Many of the growth-promoting actions of GH are mediated through insulin-like growth factor-1 (IGF-1). The GH-IGF-1 system has been well studied in mammals and fish, and the main elements appear to be highly conserved (4, 26). Circulating GH stimulates the synthesis and secretion of IGF-1 from the liver and other sites. In this study, we used hepatocytes isolated from rainbow trout as a model system in which to determine the molecular signaling events of GH in fish. GH directly stimulates the phosphorylation of ERK, protein kinase B (Akt), a downstream target of phosphatidylinositol 3-kinase (PI3K), JAK2, and STAT5 in hepatocytes incubated in vitro. Activation of ERK, Akt, JAK2, and STAT5 was rapid, occurring within 5–10 min, and was concentration dependent. GH-induced ERK activation was completely blocked by the ERK pathway inhibitor, U0126, and the JAK2 inhibitor, 1,2,3,4,5,6-hexabromocyclohexane (Hex), and was partially blocked by the PI3K inhibitor LY294002 (Hex). GH-stimulated Akt activation was completely blocked by LY294002 and Hex, but was not affected by U0126; whereas, STAT5 activation by GH was blocked only by Hex, and was not affected by either U0126 or LY294002. GH stimulated hepatic expression of IGF-1 mRNA as well as the secretion of IGF-1, effects that were partially or completely blocked by U0126, LY294002, and Hex. These results indicate that GHR linkage to the ERK, PI3K/Akt, or STAT pathways in trout liver cells requires activation of JAK2, and that GH-stimulated IGF-1 synthesis and secretion is mediated through the ERK, PI3K/Akt, and JAK-STAT pathways.

Materials and Methods

Materials. All chemicals and reagents used were purchased from Sigma (St. Louis, MO) unless stated otherwise. Antibodies for the phosphospecific and total (recognizing both phosphorylated and non-phosphorylated protein) forms of Akt, ERK1/2, JAK2, and STAT5, horseradish peroxidase (HRP)-linked anti-rabbit IgG antibody, biotinylated molecular weight marker, anti-biotin-HRP antibody, mitogen-activated protein kinase kinase 1/2 (MEK1/2) inhibitor U0126 (MEK1 and -2 are directly responsible for the activation of ERK), PI3K inhibitor LY294022 (PI3K produces phosphatidylinositol phosphates that are critical for activation of Akt by phosphoinositide-dependent kinase 1),
and cell lysis buffer were all obtained from Cell Signaling Technology (Beverly, MA). The JAK2 inhibitor 1,2,3,4,5,6-hexabromocyclohexa-2-ene (Hex), as well as the STAT5 inhibitor N’-[4-oxo-4H-chromen-3-yl]methylene]nicotinohydrazide (Nico), and the Akt inhibitor IL6-hydroxyoxymethyl-chiro-inositol-2-(R)-2’-O-methyl-3-(R)-octadecyl-snglycerocarbonate (Carb), were obtained from EMD Chemicals (Gibbstown, NJ). Molecular weight markers were purchased from Bio-Rad Laboratories (Hercules, CA). Salmonid GH was generously provided by Prof. Akiyoshi Takahashi and Dr. Shunsuke Moriyama (Kita-sato University, Japan).

Experimental animals and conditions. Juvenile rainbow trout of both sexes were obtained from Dakota Trout Ranch (Carrington, ND) and transported to North Dakota State University where they were maintained in 800-liter circular tanks supplied with recirculated (100% replacement volume per day) dechlorinated municipal water at 14°C under a 12:12-h light-dark photoperiod. Fish were fed to satiation twice daily with AquaMax Grower (PMI Nutrition International, Brentwood, MO), except 24 to 36 h before experimental manipulations. Fish were acclimated to laboratory conditions for at least 4 wk prior to experimentation. All procedures performed were in accordance with the Guide for Care and Use of Laboratory Animals (National Research Council, Washington, DC) and were approved by the North Dakota State University Institutional Animal Care and Use Committee.

For experiments, fish were anesthetized by immersion in 0.05% (vol/vol) 2-phenoxethanol and euthanized by transaction of the spinal cord. Hepatocytes were isolated by the in situ perfusion method of Mommsen et al. (17). The isolated cells were allowed to recover in incubation medium [in mM: 136.9 NaCl, 5.4 KCl, 0.81 MgSO4, 0.44 KH2PO4, 0.33 Na2HPO4, 10 HEPES, 5 NaHCO3, and 1.5 CaCl2, pH 7.6, with 2% (wt/vol) defatted BSA, 3 mM glucose, 2 ml Gibco MEM amino acid mix (50×/100 ml), and 1 ml Gibco nonessential amino acid mix (100×/100 ml) for 3 h at 14°C with gryatory shaking (100 rpm) under 100% O2. The viability of the cells was assessed by trypan blue dye exclusion and ranged between 93–97% for all experiments. After the recovery period, hepatocytes were collected by centrifugation (500 x g for 3 min at 14°C) and resuspended in incubation medium to a final concentration of 2 x 106 cells/ml, and aliquotted into 24-well plates (2 x 106 cells/well). Cells were incubated in medium alone (control) or in medium containing the designated hormone treatment under the same conditions as those used for recovery (14°C with gryatory shaking at 100 rpm under 100% O2) for various times as specified in the figure legends. In combination experiments involving U0126, LY294002, Hex, Nico, or Carb, the specific inhibitor was added 2 h prior to hormone treatment at concentrations recommended to be maximally effective by the manufacturer and/or which were used previously (30). Incubations were stopped by centrifugation (500 g for 3 min at 14°C); cell pellets were either prepared immediately for Western blot analysis or resuspended in 0.5 ml Tri Reagent (Molecular Research Center, Cincinnati, OH), frozen on dry ice, and stored at −80°C for later extraction of RNA. The hepatocytes used in replicates for a given experiment were obtained from different fish.

Western blot analysis. Following incubation, cell pellets were washed with 0.5 ml of phosphate-buffered saline, recollected by centrifugation (500 g for 3 min at 14°C), and lysed with 100 µl 1× cell lysis buffer (Cell Signaling Technology). The lysate was incubated on ice for 5 min then centrifuged at 16,000 g for 15 min at 4°C. The protein concentration of the resulting supernatant was determined by the Bio-Rad dye-binding assay for microplates. Samples containing ~50 µg of total protein were separated on a 7.5% SDS-PAGE gel and transferred to a 0.45-µm nitrocellulose membrane (Bio-Rad) for Western blot analysis under the conditions described previously (9). For most experiments, membranes were visualized with enhanced chemiluminescence according to the manufacturer’s instructions (GE Healthcare, Buckinghamshire, UK) followed by exposure of X-ray film (cat. no. F-BX-57; Phenix Research Products, Hayward, CA); the film was scanned and the bands were quantified with an Alphalager (Alpha Innotech, San Leandro, CA). In the antibody validation experiment (Fig. 1), biotinylated molecular weight marker was run and transferred to membranes along with cell lysates. In these instances, membranes were cohybridized with anti biotin-HRP antibody and anti IgG-HRP secondary antibody, washed, and visualized with enhanced chemiluminescence; chemiluminescence was detected directly and the bands quantified with a FluorChem FC2 imager (Alpha Innotech). The abundance of phosphorylated Akt, ERK1/2, JAK2, or STAT5 was normalized to total Akt, ERK1/2, JAK2, or STAT5, respectively. It should be noted that doublet bands of ERK were sometimes observed in Western blots and that individual bands within a doublet were combined in the quantitative analyses.

The amino acid identities between the synthetic antigens (based on the human sequences) used to generate the ERK, Akt, JAK2, and STAT5 antisera and the counterpart regions in salmonid sequences were 100%, 100%, 95%, and 100%, respectively. The ability of antibodies to human ERK, Akt, JAK2, and STAT5 to detect their counterparts in hepatocytes isolated from rainbow trout compared with Chinese hamster ovary-K1 cells (American Type Culture Collection, Rockville, MD), cultured as described previously (25), was assessed by Western blot analysis (Fig. 1). For all antibodies, bands corresponding only to the predicted size were observed for each signal element (~40–44 kDa for ERK 1/2, 60 kDa for Akt, 90 kDa for STAT5, and 125 kDa for JAK2), thereby validating the use of the antibodies in the rainbow trout system.

Insulin-like growth factor-1 mRNA expression. Total RNA was extracted using TRI Reagent as specified by the manufacturer (Molecular Research Center, Cincinnati, OH). Isolated RNA was dissolved in 75 µl RNase-free deionized water. Total RNA was quantified by ultraviolet (A260) spectrophotometry and diluted to 100 ng/µl in RNase-free deionized water. RNA samples were then stored at −80°C until further analysis. From 200 ng total RNA, endogenous poly(A)+ RNA was reverse transcribed in a 10 µl reaction using a iScript cDNA Synthesis kit (Bio-Rad, Knightdale, NC) containing a RNase H− reverse transcriptase and a blend of oligo(dt) and random hexamer primers according to the manufacturer’s instructions. Reactions without reverse transcriptase were included as negative controls; no amplification was detected in negative controls.

Steady-state levels of IGF-1 mRNA were determined by real-time RT-PCR using Stratagene Brilliant II and a STRATAGENE MX3000P Detection System (Stratagene, La Jolla, CA) with primers and probes described previously (23). Briefly, real-time reactions were carried out as described above.

![Fig. 1. Western immunoblot analysis of lysates from Chinese hamster ovarian-K1 (CHO) cells and rainbow trout hepatocytes (RT) using antibodies to total ERK (tERK), total protein kinase B (tAkt), total STAT5 (tSTAT5), or total JAK2 (tJAK2). Cell lysates were separated by SDS-PAGE, transferred to nitrocellulose, and hybridized individually with the specific primary antibody. All blots were subsequently hybridized with a mixture of anti-rabbit IgG-horse-radish peroxidase (HRP) secondary antibody for the detection of signal elements and anti-biotin-HRP antibody for the detection of the biotin-labeled molecular weight marker (M) by enhanced chemiluminescence. Blots were visualized with a FluorChem imager.](http://ajpregu.physiology.org/Downloadedfrom)
for samples, standards, and no-template controls in a 10 μl volume, containing 1 μl cDNA from the reverse transcription reactions, 5 μl Brilliant II QPCR Master Mix, and 1 μl of each forward primer (900 nM), reverse primer (900 nM), and probe (150 nM). Cycling parameters were set as follows: 95°C for 10 min and 45 cycles of 95°C for 15 s and 60°C for 1 min. A sample copy number was calculated from the threshold cycle number (CT), and the CT was related to a gene-specific standard curve followed by normalization to -actin. No difference (P > 0.05) was observed in -actin expression among the various treatment groups.

**Results**

GH activates the ERK, PI3K, and JAK-STAT signaling pathways. The activation of cell signaling pathways was studied on lysates from hepatocytes probed with phosphospecific antibodies.

**Radioimmunoassay of IGF-1.** Specific assays validated for rainbow trout were used to determine concentrations of IGF-1 in acid-ethanol extracts of culture medium (29). Assay components for the IGF-1 RIA were obtained from GroPep (Adelaide, Australia).

**Statistics.** After natural logarithmic transformation to obtain normally distributed data with homogeneous variance, statistical differences were estimated by one-way or two-way ANOVA, as appropriate. In all cases, main effects were significant, and no significant interactions were observed between main effects in two-way ANOVAs. Pairwise comparison of simple effects was assessed by Duncan’s multiple range test; statistical notations on the figures reflect such comparisons. A probability level of 0.05 was used to indicate significance. All statistics were performed using SigmaStat version 1.0 (SPSS, Chicago, IL), and graphs and curve fitting models were constructed with SigmaPlot version 8.0 (SPSS). Quantitative data are shown relative to control for ease of comparison and are expressed as means ± SE.
and control antibodies. Treatment of hepatocytes in vitro with GH activated the ERK pathway in a time- and concentration-dependent manner (Fig. 2). GH induced phosphorylation of ERK1/2 rapidly, with maximum phosphorylation occurring within 10 min; thereafter, phosphorylation of ERK1/2 subsided. Significant activation of ERK1/2 by GH was observed at 1 ng/ml; the ED$_{50}$ for ERK1/2 activation was ~13 ng/ml. GH also activated the PI3K/Akt pathway in hepatocytes in a time- and concentration-related manner (Fig. 3). GH induced a rapid phosphorylation of Akt, a downstream target of PI3K; maximum phosphorylation occurred within 5 min, but after 60 min no appreciable phosphorylation was observed. As was the case with ERK activation, significant phosphorylation of Akt was induced by GH at a concentration as low as 1 ng/ml; the ED$_{50}$ for Akt activation was ~18 ng/ml.

In addition, GH also activated the JAK-STAT pathway in isolated hepatocytes as demonstrated by phosphorylation of both JAK2 and STAT5. Activation of JAK2 by GH occurred in a time- and concentration-dependent manner (Fig. 4, A and B). GH induced a rapid but transient phosphorylation of JAK2; maximum phosphorylation was observed within 5 min, and although the extent of phosphorylation declined thereafter, it remained higher than control levels up to 60 min after GH treatment. Significant phosphorylation of JAK2 by GH was observed at 1 ng/ml; the ED$_{50}$ for JAK2 activation was ~7 ng/ml. GH activated STAT5 in a time- and concentration-related manner similar to that observed for JAK2 (Fig. 4, C and D). Maximum phosphorylation of STAT5 was observed within 5 min and then declined but remained elevated compared with control levels up to 60 min after GH treatment. Significant phosphorylation of STAT5 was induced by GH at a concentration as low as 1 ng/ml; the ED$_{50}$ for STAT5 activation was ~10 ng/ml.

**Linkage of the ERK, PI3K, and JAK-STAT signaling pathways to GH-stimulated IGF-1 production.** The roles of the ERK, PI3K, and JAK-STAT signaling pathways on GH-stimulated IGF-1 production in isolated hepatocytes was investigated using inhibitors specific to these pathways. Initial experiments examined the effects of specific inhibitors alone and in combination on GH-stimulated phosphorylation of ERK, Akt, and STAT5. Data are presented as representative immunoblots (insets) and as means ± SE. Six blots quantified with a FluorChem imager. Groups with different letters are significantly different from each other ($P < 0.05$).
The ERK pathway inhibitor, U0126 (which specifically inhibits MEK), significantly abolished GH-induced ERK phosphorylation (Fig. 5A). Interestingly, GH-induced phosphorylation of ERK also was partially blocked by the PI3K inhibitor, LY294002, and completely blocked by the JAK2 inhibitor, Hex. The addition of U0126 or Hex in combination with LY294002 resulted in the complete abolishment of GH-induced ERK activation. GH-induced activation of Akt was not affected by the MEK inhibitor U0126 but was completely blocked by either the PI3K inhibitor LY294002 or by the JAK2 inhibitor Hex (Fig. 5B). By contrast, GH-induced phosphorylation of STAT5 was only blocked by the JAK2 inhibitor Hex; neither the MEK inhibitor U0126 nor the PI3K inhibitor LY294002 had any effect of GH-induced STAT5 phosphorylation except when administered in combination with Hex (Fig. 5C).

As previously observed, GH increased steady-state levels of IGF-1 mRNA in isolated hepatocytes incubated in vitro. Pretreatment of hepatocytes with the MEK inhibitor U0126 partially blocked GH-stimulated IGF-1 mRNA expression (Fig. 6). The PI3K inhibitor LY294002 and the specific Akt inhibitor Carb partially blocked GH-stimulated IGF-1 expression to a similar extent. Notably, the inhibitory effects of LY294002 and Carb on GH-stimulated IGF-1 mRNA expression were greater than those of U0126 (Fig. 6). Pretreatment of hepatocytes with the JAK2 inhibitor Hex completely abolished GH-stimulated IGF-1 mRNA expression. However, the specific STAT5 inhibitor Nico only partially blocked GH-stimulated IGF-1 mRNA expression (Fig. 6).

Also, as previously observed, GH stimulated the release of IGF-1 into culture medium from hepatocytes incubated in vitro. Both the MEK inhibitor U0126 and the PI3K inhibitor LY294002 retarded GH-stimulated IGF-1 release (Fig. 7). The JAK2 inhibitor Hex completely blocked GH-stimulated IGF-1 release to levels similar to those released from control-treated cells.

**DISCUSSION**

GH was previously shown to stimulate hepatic IGF-1 expression and to increase levels of IGF-1 in the plasma of several species of fish, including carp, coho salmon, rainbow trout, and tilapia (33, 36). The present results confirm the effects of GH on IGF-1 production in isolated trout hepatocytes incubated in vitro and support our starting hypothesis that the ERK, PI3K/Akt, and JAK2-STAT5 signaling pathways mediate GH-stimulated IGF-1 mRNA expression and GH-stimulated IGF-1 secretion. These findings establish the mechanisms through which GH exerts its growth-promoting actions and provide insight into the signaling pathways that may underlie the many other actions of GH in fish.
Significant differences \((P < 0.05)\) across time for a given treatment are denoted by different letters. Significant differences \((P < 0.05)\) between treatments at a given time are denoted by different typographical symbols (no symbol, *, +).

The role of the ERK pathway in GH-stimulated IGF-1 production was supported by several observations. GH directly induced the phosphorylation of ERK1/2 in trout hepatocytes in a time- and concentration-related manner. Blockade of the ERK pathway with U0126, a highly specific inhibitor of MEK1/2, completely abolished GH-induced phosphorylation of ERK1/2. Blockade of the ERK pathway with U0126 also partially inhibited hepatic expression of IGF-1 as well as the release of IGF-1 protein. These findings are consistent with those in mammals in which GH-induced activation of the ERK pathway and the activation of IGF-1 transcription \((1, 11, 22)\). Because U0126 only partially blocked GH-induced IGF-1 production in trout hepatocytes, mechanisms other than the ERK pathway could be operating to influence the growth-promoting actions of GH.

The role of the PI3K/Akt pathway in GH-stimulated IGF-1 production also was supported by several observations. GH directly induced the phosphorylation of Akt in trout hepatocytes in a time- and concentration-related manner. Blockade of the PI3K/Akt pathway with the selective PI3K inhibitor LY294002 completely abolished GH-stimulated phosphorylation of Akt. Blockade of the PI3K/Akt pathway also partially inhibited hepatic expression of IGF-1 as well as the release of IGF-1 protein. That the influence of PI3K on GH-stimulated IGF-1 expression was specifically dependent on Akt was confirmed by the observation that the Akt-specific inhibitor Carb blocked GH-stimulated IGF-1 expression to the same extent as the PI3K inhibitor LY294002. Collectively, these findings are consistent with those in mammals in which GH-induced activation of the PI3K/Akt pathway and the activation of IGF-1 transcription \((1, 11, 22)\). Notably, while the ERK pathway inhibitor, U0126, had no effect on Akt activation, LY294002 blocked, at least in part, the phosphorylation of ERK, suggesting cross-talk between the ERK and PI3K/Akt pathways. This notion was supported by the observation that inhibition of both PI3K (via LY294002) and Akt directly (via Carb) retarded GH-induced IGF-1 production to a greater extent than that of U0126. Cross-talk between the PI3K/Akt pathway and ERK pathway has been shown in mammals, with proto-oncogene serine/threonine protein kinase (c-Raf) serving as a nexus between the two pathways and establishing a PI3K-c-Raf-MEK-ERK cascade \((13)\). As was the case with ERK blockade of GH-induced IGF-1 production in trout hepatocytes, PI3K/Akt blockade was not complete, which suggested that yet other pathways could be operating to influence the growth-promoting actions of GH.

The role of the JAK-STAT pathway in GH-stimulated IGF-1 production also was supported by several observations. GH directly induced the phosphorylation of JAK2 and STAT5 in trout hepatocytes in a time- and concentration-related manner. Blockade of the JAK-STAT pathway with the JAK2 specific inhibitor Hex completely abolished GH-stimulated phosphorylation of STAT5. Blockade of the JAK-STAT pathway completely inhibited hepatic expression of IGF-1 as well as the release of IGF-1 protein. That GH-stimulated IGF-1 production was dependent, at least in part, on STAT5 was confirmed by the observation that the STAT5-specific inhibitor Nico partially blocked GH-stimulated IGF-1 expression. Together, these findings are consistent with those in mammals that demonstrated the role of JAK2 in the activation of STAT5 and the subsequent role of STAT5 in stimulating IGF-1 transcription \((1, 11, 22)\). That JAK2 is most proximal to GHR and is critical for signal propagation through either the ERK or PI3K/Akt pathways in trout hepatocytes was supported by several additional observations. Although the ERK pathway inhibitor U0126 and the PI3K inhibitor LY294002 had no effect on the phosphorylation of STAT5, the JAK2 inhibitor Hex completely inhibited ERK and Akt activation when added alone. Moreover, when Hex was added in combination with either U0126 or LY294002, ERK and Akt activation could be completely blocked. In addition, while U0126 and LY294002 partially inhibited GH-stimulated IGF mRNA expression and IGF-1 release, JAK 2 inhibition via Hex alone completely
blocked GH-induced IGF-1 biosynthesis and secretion to levels observed in control-treated cells. These findings are consistent with the observations that JAK2 activation is the initial step in GH signal transduction in mammalian systems and that activation of the ERK and PI3K pathways are downstream of JAK (22, 35).

A model of the mechanisms activated by GH leading to IGF-1 production in hepatocytes of rainbow trout is depicted in Fig. 8. In this model, the binding of GH to GHR initiates a progression of events. Binding of GH leads to the activation of JAK2, a step that appears essential for propagation of the signal through ERK, PI3K/Akt, and STAT5. The activation of the various signal elements is rapid and transient, and sets into motion the processes necessary for IGF-1 biosynthesis, including transcriptional and translational events, and, ultimately, secretion. The rapid and transient activation of signal elements is consistent with previous studies that examined GH effects on activation of the JAK-STAT, ERK, and PI3K/Akt pathways in mammals (22, 30). The more protracted time course of GH action on steady-state levels of IGF-1 mRNA or IGF-1 protein secretion, taking some 3–6 h to reach maximal levels, is consistent with the time required to activate transcriptional and translational events and is similar to previous reports of GH action on mRNA and functional expression of IGF-1 in fish by us and others (33, 36).

The precise mechanism(s) through which ERK, PI3K/Akt, or STAT5 affects IGF mRNA expression and IGF-1 release are not known. The promoter region of fish IGF-1 genes possess putative c/EBP, HNF1α, HNF-3β, and STAT5 binding sites, which in mammalian systems have been shown to be activated through numerous pathways, including JAK2, ERK, PI3K, and cAMP-protein kinase A (18, 27, 33, 34, 36). ERK, PI3K/Akt, and STAT5 in mammalian systems also have been shown to increase the expression of transcription factors for GH-regulated genes such as c/EBPβ, c-fos, and Spi 2.1 (5). GH has been shown to induce transcription of c/EBPs in trout and of c-fos and Spi 2.1 in seabream (10, 14). Our previous findings that Akt mediated the inhibition of GH-stimulated IGF-1 expression by somatostatins is somewhat enigmatic (9). However, it is conceivable that the influence of Akt on downstream targets is modulated by other elements in the presence of somatostatins. For example, in mammalian systems, c/EBPβ exhibits dual regulation through two different cascades; PI3K/Akt activation leads to dephosphorylation of c/EBPβ at one site, whereas ERK activation promotes phosphorylation at another site of c/EBPβ (22). Other modifications (e.g., acetylation) of c/EBPβ, and interactions of c/EBPβ with p300 and c-fos appear critical for GH-regulated gene transcription (5). The extent to which somatostatin may influence modification of c/EBPβ or the presence/activation of other coactivators involved with GH-regulated gene transcription is not known. The linkage between signaling pathways and the activation of specific transcription factors/coactivators in fish remains to be elucidated.

**Perspectives and Significance**

Despite knowledge that GHRs are widespread and that GH has many actions in fish, including effects on feeding, metabolism, reproduction, osmoregulation, and immune function (3, 19, 31), little is known about the mechanism(s) of action that underlie these various processes. The present findings provide important insight into GH signaling in fish by demonstrating for the first time that GH activates the ERK, PI3K/Akt, and JAK-STAT pathways (Fig. 8). It is conceivable that these pathways mediate actions of GH in fish other than the stimulation of IGF-1 production. The observation that the ERK, PI3K-Akt, and JAK-STAT pathways mediate GH action in teleost fish as in mammals demonstrates that the linkage of the GH receptor to these effector systems arose early during the course of vertebrate evolution. It should be noted, however, that GH signaling in fish, such as rainbow trout, is particularly complex (more complex than in mammals) as a result of the existence of multiple GHRs that have differential ligand binding and agonist-induced regulation features (25, 31). Moreover, the pattern of expression of the distinct GHRs of fish are affected by developmental and nutritional state and environmental salinity (15, 19, 21, 23, 24, 28). Ultimately, therefore, the action of GH in fish may depend on the distribution and abundance of a specific GHR subtype as well as on the signal pathways to which they link under a given developmental/physiological state.

**ACKNOWLEDGMENTS**

We thank Whitney Carlson, Alison Hagemeister, Andrea Hanson, Evan Jones, Lincoln Martin, Lindsey Norbeck, and Chad Walock for their assistance with this study. We are grateful to Prof. Akiyoshi Takahashi and Dr. Shunsuke Moriyama (Kitasato University, Japan) for generously providing salmonid growth hormone.

**GRANTS**

This research was supported by National Science Foundation Grant IOS920116 (to M. A. Sheridan).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


