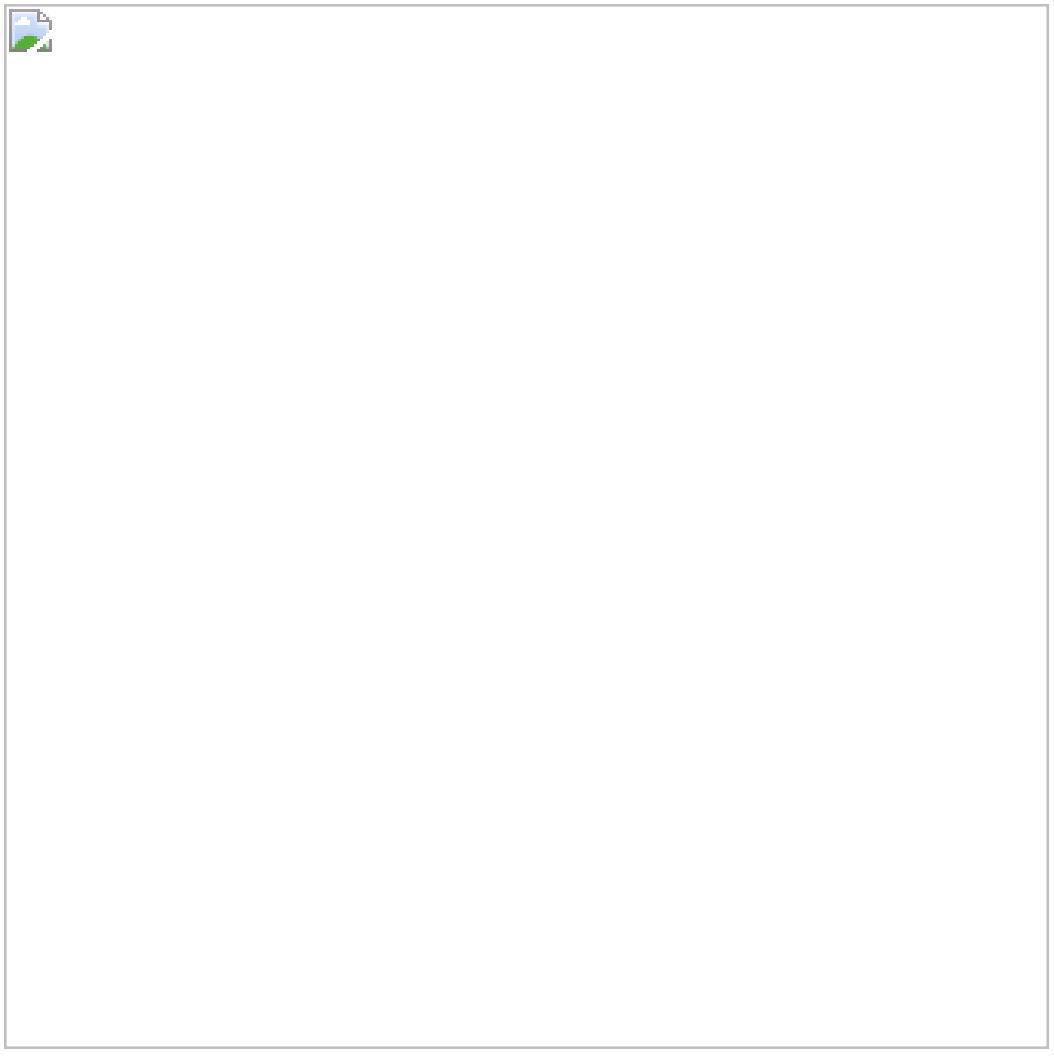

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[RAPID COMMUNICATION]

Insulin-like Growth Factor I Gene Expression during Parr-Smolt Transformation of Coho Salmon

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ABSTRACT—The status of insulin-like growth factor I (IGF-I) mRNA in the liver and gill of coho salmon (*Oncorhynchus kisutch*) during the parr-smolt transformation (smoltification) was examined in relation to changes in plasma levels of thyroxine, cortisol, growth hormone and somatolactin. Smoltification coincided with silvering and an increase in gill Na^+/K^+-ATPase activity occurred during mid-April and May [22]. The levels of IGF-I mRNA in the liver rose steadily from the first sampling date in February to maximum levels in April. Increases in IGF-I mRNA in the gill occurred later in smoltication. Plasma cortisol, thyroxine and growth hormone increased during the latter half of April and May. Plasma somatolactin declined in April. These results demonstrate that IGF-I gene expression in both liver and gill increased during smoltification and suggest that IGF-I expression in both organs may be involved in salmonid smoltification.

INTRODUCTION

Insulin-like growth factors (IGFs) stimulate growth and differentiation of a variety of cell types [16]. Growth hormone (GH) regulates the level of insulin-like growth factor-I (IGF-I) peptide and/or mRNA both in mammals and teleosts [5, 7, 10, 13, 23, 26, 29]. Whereas considerable evidence indicates that IGF-I is a major mediator of GH action, other hormonal factors such as insulin, thyroid hormones and the recently-discovered somatolactin (structurally similar to GH and prolactin), tissue-specific and developmental factors, and nutritional status, may all modulate IGF-I gene regulation [7, 12–14]. Several studies with mammals and birds have demonstrated that the ontogeny of IGF-I expression precedes that of GH, suggesting that signals other than GH may regulate IGF-I gene expression in early development [7, 20]. However, there is little information on developmental regulation of IGF expression in lower vertebrates.

Anadromous salmonids undergo morphological and physiological changes referred to as the parr-smolt transformation (smoltication), which prepare the fish for entry into seawater and for growth and migration to feeding areas in the ocean [4, 18]. A number of accompanying endocrine changes have now been identied. The most clearly and consistently defined are increased plasma levels of thyroid hormones and cortisol. Increased GH levels have been reported in both coho and Atlantic salmon [4, 8, 34]. IGF-I produced in the liver is an important mediator of the growth-promoting actions of GH in teleosts as in other vertebrates [1]. GH stimulates hypoosmoregulatory ability in salmonids at least in part by inducing local expression of IGF-I in osmoregulatory organs such as gills and kidney [26, 27].

In view of the potential importance of IGF-I in bringing about physiological changes and also of the possible multihormonal regulation of IGF-I biosynthesis during teleost development, the present study was undertaken to document the changes in IGF-I gene expression in liver and gill during smoltication of coho salmon in relation to the circulating levels of several hormones: thyroxine, cortisol, GH and somatolactin. The results suggest specific roles for IGF-I expression in liver and gill during salmonid smoltification, and the possible involvement of signals other than GH in regulation of IGF-I gene expression.

MATERIALS AND METHODS

Yearling coho salmon, *Oncorhynchus kisutch*, were obtained from Iron Gate Hatchery, California Department of Fish and Game, in November 1991. They were maintained at the Bodega Marine Laboratory at ambient temperature in a concrete raceway supplied with filtered pond water; fish were fed with Oregon Moist Pellets (Moore-Clarke, LaConner, WA, U.S.A.) *ad libitum*. Samples were taken from February to July. After being stunned by a blow to the head, the fish (in groups of 10) were weighed, and blood was collected from the caudal blood vessels into microhematocrit tubes. Plasma was obtained after centrifugation, frozen and stored at −70°C. Tissues were removed immediately, frozen in liquid nitrogen, and kept at −70°C. The increase in gill Na^+,K^+-ATPase activity

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and mean body weight of these coho salmon at this time has been reported in Madsen and Bern [22].

Total RNA was extracted from frozen tissues, and poly(A)$^+$ RNA was isolated using oligo(dT)-Latex (Takara Shuzo Co., Kyoto, Japan). Aliquots (1–20 μg) of poly(A)$^+$RNA were denatured and electrophoresed on 1% agarose gels containing 2.2 mol formaldehyde/l. The RNA was transferred to a nylon filter as described previously [26].

A cDNA probe for coho salmon IGF-I was kindly provided by Dr. S. J. Duguay [5]. A chicken β-actin cDNA probe was used as a control. Blots were hybridized and subjected to autoradiography. One major band for IGF-I message was demonstrated at 3.9 kb in agreement with previous observations (Fig. 1) [5, 26]. This band probably represents most of the transcripts coding salmonid IGF-I prohormones. As the size differences among the transcripts have been suggested to be due to insertions or deletions in the E-domain of the prohormone, i.e.,$\leq$0.1 kb, it is highly probable that the transcripts would not be resolved by agarose gel electrophoresis [6, 9, 32]. The intensity of the individual bands was compared with those of known standards on each blot. The analytical procedure was validated by measuring the 3.9-kb IGF-I message and the β-actin message in RNA samples pooled from each tissue. The data obtained by analyzing 1-, 2.5-, 5-, 10-, and 20-μg samples indicated linear dependence on the amount of RNA in the samples. Further characterization of the analysis of IGF-I and β-actin has been published previously [26].

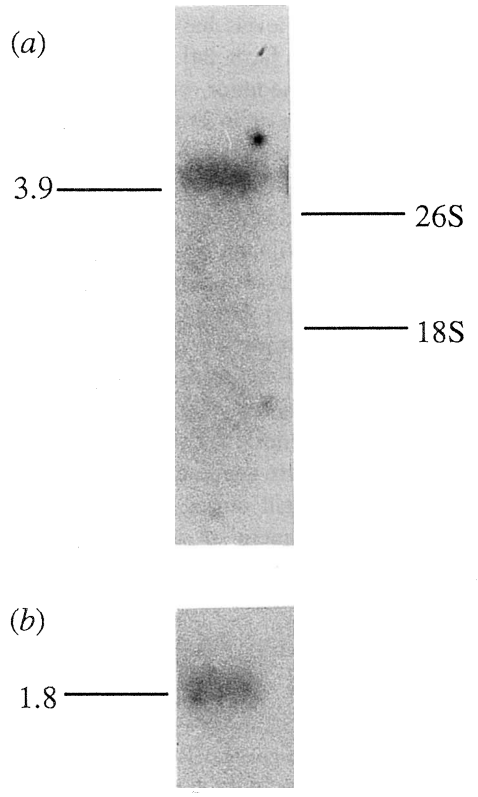

FIG. 1. Detection of IGF-I (*a*) and β-actin (*b*) mRNA in coho salmon liver by Northern blot hybridization using poly(A)$^+$ RNA (2.5 μg) from mid-April fish liver. The probes hybridized to each mRNA (See Materials and Methods, [5, 27]): 3.9 kb for IGF-I (on longer exposure additional bands also could be seen), and 1.8 kb for β-actin. Mobility of molecular size markers (26S and 18S salmonid rRNA) is indicated. Autoradiographic exposure was 1 day for both IGF-I and β-actin.

Plasma GH was measured in a specific homologous assay [3]. Cortisol levels were measured by the method of Takahashi *et al.* [31]. Thyroxine was determined as described [30]. Plasma somatolactin was measured in a specific homologous assay [19].

Differences among groups were evaluated using one-way analysis of variance followed by Duncan's new multiple-range test [15].

RESULTS

Quantified results are presented in Figure 2. On the basis of external morphological characteristics and an increase in gill Na^+,K^+-ATPase activity of the same fish [22], the great majority of these fish were considered to have become smolts during mid-April and May. Plasma cortisol levels remained fairly stable (11–26 μg/l) between February and early April; levels rose significantly ($P<0.01$ compared with values in early April) to about 30 μg/l in mid-April and May. In July, levels declined to 10 μg/l.

Between February and early April, plasma thyroxine levels remained stable at about 2 μg/l; levels rose ($P<0.01$ compared to values from February to early April) to a peak of 8 μg/l in mid-April. Thereafter, levels declined to 3–5 μg/l.

Plasma GH levels varied between 3–7 μg/l between February and early April; levels increased thereafter to 17 μg/l in May ($P<0.01$ compared to values from February to early April) and were maintained until July. On the other hand, somatolactin levels declined significantly ($P<0.01$ compared to March levels) in mid-April.

As predicted from solution hybridization/RNase protection assay data (expressed per μg DNA) from coho salmon smolt [11, 12], IGF-I mRNA in the liver was about 5–10 times more abundant than in the gill.

Levels of IGF-I mRNA in the liver rose steadily, increasing 5-fold from initial levels to a peak in mid-April ($P<0.01$ compared with initial levels) and remained fairly stable thereafter (data not shown). When normalized to β-actin mRNA, the relative IGF-I mRNA in the liver also increased maximally in early April, although the increase was significant ($P<0.05$ compared to initial levels) only in May because of the large variations.

Gill IGF-I mRNA levels gradually increased over the period of sampling, and the increase became significantly higher in July ($P<0.01$ compared with levels in February and early April; data not shown). Owing to high levels of β-actin mRNA in the gill in June and July, the expression of IGF-I relative to that of β-actin mRNA increased to peak levels in May ($P<0.05$ compared with initial levels), and then progressively declined during the last 2 months of sampling. The high level of β-actin mRNA late in smoltication may be associated with branchial chloride cell differentiation in this salmonid species [4, 25].

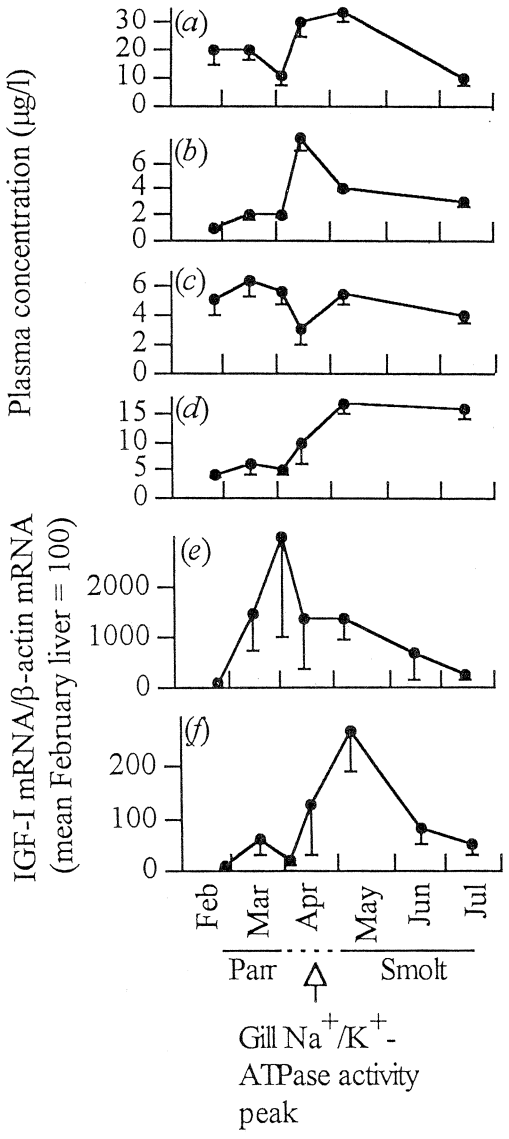

FIG. 2. Changes (February-July) in plasma concentrations of (*a*) cortisol, (*b*) thyroxine, (*c*) somatolactin, and (*d*) GH, and concentrations of IGF-I mRNA normalized to that of β-actin in (*e*) liver and (*f*) gill during the parr-smolt transformation of coho salmon. The stages of development are indicated below x-axis. Poly(A)$^+$RNA was analyzed and the results for 3.9-kb IGF-I mRNA were normalized to levels of β-actin mRNA, as described [27]. Scale is in arbitrary units, with the average value obtained for the liver in February set at 100. Each point represents the mean ± SEM. (n=3–10). Absence of standard error bars indicates that the SEM was smaller than the point.

DISCUSSION

The increases in plasma thyroxine, cortisol and GH during smoltication are in good agreement with previous work on this and other stocks of coho salmon [8, 33, 34]. Plasma thyroxine rose relatively steeply in mid-April and declined thereafter. A rise in plasma cortisol between mid-April and May coincides with enhanced hypoosmoregulatory ability at the peak of the smoltification period; GH levels also began to increase concomitant with increasing hypoosmoregulatory ability as judged by gill Na^+,K^+-ATPase activity and seawater challenge test, and remained high until mid-June [2, 4, 22]. In parallel with these developmental changes, IGF-I mRNA in both liver and gill rises but was differentially expressed.

Lindahl *et al.* [21] has suggested that plasma IGF-I levels, as measured by a heterologous (human) radioreceptor assay, increased during smoltification of Atlantic salmon. Moriyama *et al.* [23] recently reported higher plasma IGF-I levels in coho salmon smolts than those in parr. Our observation on liver IGF-I mRNA is in accord with these findings, as the liver is considered to be the major source of plasma IGF-I [7, 17, 20, 24, 28].

Liver IGF-I gene expression began to rise relatively early in smoltification, with peak levels occurring before the GH peak, and thus, seems to be regulated not only by GH but also by signals other than GH. The later increases in gill IGF-I mRNA levels coincided to a greater degree with those in plasma GH and cortisol than those of liver IGF-I mRNA. We have already shown in rainbow trout that the liver and osmoregulatory organs respond to GH by increased IGF-I expression [26]. However, tissue-specific factors may also be involved [10]. For example, both somatolactin and thyroid hormones also induced IGF-I mRNA in coho salmon liver [11, 12]. An increase in cortisol during seawater adaptation of the trout may inhibit GH induction of IGF-I mRNA in the liver and stimulate it in the gill [26]. Clearly, the relationship between cortisol and IGF-I in salmonids needs additional experimental analysis. The increased cortisol and decreased somatolactin in mid-April may be related to decreased IGF-I mRNA in the liver from the peak in early April. Also, differential regulation of splicing variants by hormonal and tissue-specific factors and nutritional states suggests multiple regulatory factors, only one of which may be GH: GH increased Ea-1 and Ea-3 transcripts but probably not Ea-4 transcripts in coho salmon [10, 14]. Using a different stock of coho salmon, Duguay *et al.* [10] recently reported observations largely similar to those presented herein: liver and gill IGF-I mRNA increased at about the same time as thyroxine and GH; no increase in IGF-I mRNA was detected in muscle, brain or ovary. Thus, several different mechanisms seem to regulate IGF-I gene expression during salmonid development.

Our present and our earlier observations [22] demonstrate that IGF-I mRNA in the liver and gill is expressed in a tissue-specific manner coincident with increases in thyroid hormones, cortisol and GH, as well as with increased gill Na^+, K^+-ATPase activity during coho salmon smoltication [4]. In addition, IGF-I stimulates gill Na^+,K^+-ATPase *in vitro* depending upon the smoltification stage [22]. Taken together, our studies suggest that IGF-I expressed in the liver and in the gill may serve specific functions in endocrine and in autocrine/paracrine manners, respectively, not only in growth stimulation but also in seawater preadaptation during smoltification.

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Note added in proof

Since submission of this paper, Duan *et al.* have also published on IGF-I mRNA in coho salmon (Duan C, Plisetskaya EM, Dickhoff WW, 1995, Endocrinology 136: 446–453).