inantly from the scale. In this study, we determined the concentrations of melatonin in the scale of adult female goldfish during reproductive period from December to April. Scales in the dorsal, lateral line, or ventral regions were collected. Melatonin and other indoles in the scale were detected by reversed-phase high-performance liquid chromatography with fluorometric detection. The melatonin levels in the scale exhibited significant variations in all regions ($p<0.01$), with higher levels in early February. Serotonin in the scale was detected in few animals during the period. Indole acetic acid was only detected in December and January. Gonad somatic index gradually increased and peaked in early April. These results suggest that melatonin could be related with vitellogenin synthesis, because early February is thought as yolk formation stage.

MOLECULAR CLONING OF cDNAs ENCODING MARKERS IN OSTEOCLASTS, TRAP AND CATHEPSIN K IN GOLDFISH

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Bone formation in mammalians proceeds through a remodeling process that runs continuously, involving the resorption of old bone by osteoclasts, and the subsequent formation of new bone by osteoblasts. On the other hand, fish scales also have osteoclasts and osteoblasts. Tartrate-resistant acid phosphatase (TRAP) and cathepsin K are characteristic constituents of osteoclasts and, therefore, used as histochemical and biochemical markers for osteoclasts. In the present study, we have successfully isolated cDNAs encoding TRAP and cathepsin K form scales in goldfish by RT-PCR and RACE procedures, and inferred the primary amino acid structures of them. It was found that TRAP and cathepsin K in goldfish consists of 340 and 332 amino acid residues, respectively. The expressions of TRAP and cathepsin K mRNAs with measurement of osteoclastic activity in goldfish scales will also be discussed.

EXPRESSION ANALYSIS OF TIMP-2 DURING OSTEOCLAST ACTIVATION IN THE SCALES OF GOLDFISH

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It is known that the tissue inhibitor of metalloproteinases-2 (Timp-2) has inhibitory activity on bone resorption at higher concentrations in vitro. In contrust, at lower concentrations, Timp-2 directly stimulates the bone-resorbing activity of isolated mature osteoclasts. The two types of cells, osteoclasts and osteoblasts are seen in fish scales. Thus, we consider fish scales as a good model for the study of bone formation. In goldfish scales, 2weeks after intermuscular auto-transplantation, an osteoclast marker; TRAP activity was increased up to 10 times, but an osteoblast marker, ALP activity was hardly increased. To screen genes expressed during the osteoclastic activation, differential display analysis was carried out using auto-transplanted and non-transplanted scales. We obtained the results showing that Timp-2 expression was extremely strong in auto-transplanted scales, but very week in non-transplanted scales. We determined by RT-PCR analysis whether Timp-2 expression changed during the certain time period after intramuscular auto-transplantation. Furthermore, cells expressing Timp-2 mRNA in the scale were identified. The result also been presented.

cDNA CLONING OF SPECIFIC GENES INVOLVED IN THE DIFFERENTIATION OF OSTEOCLASTS IN ALLOTRANSPLANTED SCALES OF THE GOLDFISH

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Multinucleated mature osteoclasts with an ability of absorbing the bone are formed through the fusion of mononuclear precursors. Previously, we have found that both osteoclasts and osteoblasts exist in the goldfish scale. This prompted us to use the scale as a model system for the study of osteoclast differentiation. Scales of male goldfish were allotransplanted to induce osteoclast maturation. On day 1, 2, 3, 5, 7, 10, 13, and 16, the scales were checked for their morphological changes and their activities of tartrate-resistant acid phosphatase (TRAP), a marker enzyme for osteoclast differentiation were determined. On day 7, TRAP activity in the scale was significantly ($p<0.001$) elevated as compared with that in the scale on day 1. Around this time, abundant mononuclear osteoclast-precursors appeared. Multinuclear osteoclasts began to appear on day 10. On day 16, multinucleated mature osteoclasts increased in number. Attempt was made to identify the genes that are specifically expressed during the osteoclast differentiation. Differential display revealed that one of the genes is midkine, a heparin-binding growth/differentiation factor.

EFFECTS OF SALMON GnRH AND SEX STEROID HORMONES ON EXPRESSION OF GENES ENCODING GnRH RECEPTORS IN MASU SALMON PITUITARY CELLS *IN VITRO*

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Effects of salmon GnRH (sGnRH) and sex steroid hormones on expression of genes encoding five subtypes of GnRH receptors (GnRH-Rs) were examined in primary pituitary cell cultures of masu salmon (*Oncorhynchus masou*). Pituitaries were taken from fish in March (early maturation), in May (maturing), in July (pre-spawning), and in September (spawning). The amounts of GnRH-R mRNAs in the pituitary cells were measured by real-time PCR. All five mRNAs showed similar changes in response to sGnRH and sex steroids. sGnRH increased the amounts of GnRH-R mRNAs only in March and May in the females. Estradiol-17 β (E2) increased the amounts in May in the males, while it tended to decrease them in September. In the females, testosterone (T) significantly increased the amounts in May, whereas it decreased them in July and September. These results suggest that sGnRH, E2 and T differentially regulate expression of five subtypes of GnRH-R genes in the pituitary of masu salmon, depending on gender and the stage of reproduction.

HORMONAL ACTIVITY OF SYNTHETIC GONAD-STIMULATING SUBSTANCE (GSS) WHICH IS A GONADOTROPIN OF STARFISH, *ASTERINA PECTINIFERA*

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Similar to most vertebrates, oocyte maturation in starfish is regulated under the hormonal action. Gonad-stimulating substance (GSS), a peptide hormone, which is secreted from radial nerves, is the first trigger to induce oocyte maturation. Previously, we were successful in purification of the GSS from radial nerves of starfish, *Asterina pectinifera*. The purified GSS is a heterodimer composed of two different peptides, A-chain (24 amino acid residues) and B-chain (19 amino acid residues). In this study, we examined whether synthetic GSS exerted gonadotropic activity. Based on amino acid sequences, A and B peptides were chemically synthesized, then the heterodimer was formed by disulfide cross-linkage between both peptides. The synthetic GSS could induce gamete spawning by way of producing 1-methyladenine in follicle cells. Fifty percent induction for spawning was brought about by 2 nM of GSS. Thus, the synthetic GSS is confirmed to mimic the natural GSS action.
*(Mita and Yoshikuni: Equal contribution.)

FIRST IDENTIFICATION OF THE INSULIN-LIKE GENE FAMILY IN *HYDRA*

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The insulin signaling pathway has been characterized in detail in bilaterian animals, where it has been shown to regulate metabolism, growth, and longevity. Although a homologue of the bilaterial insulin receptors is identified in *Hydra*, all the efforts to identify the ligand have not been rewarded. By using a *Hydra* EST database, we carried out bioinformatics search for insulin-like genes and found 3 of them. All of them encode A and B chains and C peptide and have cystein residues in conserved positions, although the identity of amino acid sequences is low. The expression patterns of these genes analyzed by whole mount in situ hybridization are totally different. Gene 1 is expressed only transiently in a small number of ectodermal epithelial cells in the presumptive tentacle regions. Gene 2 is expressed in nerve cells in the ectoderm of the hypostome and lower peduncle, while in the gastric region the endodermal nerve cells express the gene. This is the first marker ever obtained for endodermal nerve cells. Gene 3 is expressed in the ectodermal epithelial cells just under the tentacle zone and in the peduncle.