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## **XI. SPAWNING MIGRATION AND EXPRESSION OF NEUROPEPTIDE GENES IN SALMONIDS**

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### **Abstract**

Migration of salmonid fish, particularly homing migration for spawning, is one of the most attractive innate behaviors whose intrinsic central mechanisms invite clarification. Recent progress in molecular studies of salmonid hypothalamic neurohormones has enabled us to investigate at the molecular level neuroendocrine mechanisms of various biological events underlying the migratory behavior. In this paper, we briefly review our recent molecular studies on basic aspects of salmonid peptidergic neurohormones.

### **Introduction**

It is well established that many hypothalamic neurohormones are involved in central control of stereotyped innate behavior. A neuroanatomical basis of this fact is the presence of immunoreactive extrahypothalamic projections of neurosecretory fibers in many brain areas. These immunoreactive fibers may arise from neurosecretory neuron somata which are localized mainly in the ventral telencephalon and the hypothalamus.

The wide distribution of fine varicose fibers immunoreactive to an antiserum against gonadotropin-releasing hormone (GnRH) is well known in brains from teleosts to mammals (see review, Urano, 1988) including salmonids (Amano et al., 1991). Extrahypothalamic projections of immunoreactive vasotocin fibers occur in the brains of the Japanese toad (Jokura and Urano, 1987) and eel (Fujiwara et al., unpublished).

Physiological roles of vertebrate neurohormones as neurotransmitters or neuromodulators were examined by use of electrophysiological methods not only in the mammalian brain, but also in the toad and fish brains. GnRH applied by microiontophoresis increased discharge rates of toad preoptic neurons (see Urano, 1988). In addition, vasotocin and isotocin enhanced discharge of individual preoptic neurons (Sugita and Urano, 1986). Since involvement of these peptidergic neurohormones in reproductive behavior is well known in teleosts, anuran amphibians and mammals (see Urano, 1988; Urano et al., 1994), it is highly probable that they have some regulatory roles in salmonid spawning migration, which can be regarded as an innate reproductive behavior.

The primary structure of peptidergic neurohormones is encoded in genetic information, so that they have to be synthesized through the typical intracellular pathway of protein synthesis, i.e., gene transcription and translation. Thus the first events in the initiation of

particular phases of salmonid migratory behavior should be increases or decreases in expression of corresponding neurohormone genes. To detect such changes in gene expression, we developed an *in situ* hybridization method in which synthetic deoxyoligonucleotides are used as hybridization probes (Hyodo et al., 1988), cloned and analyzed the sequences of salmonid cDNAs for vasotocin and isotocin (Hyodo et al., 1991; Suzuki et al., 1992; Hiraoka et al., 1993), and salmon GnRH (Suzuki et al., 1992; Ashihara et al., 1995), and analyzed changes in the amounts of mRNAs for these peptides.

### **1. Expression of the vasotocin and isotocin gene family**

Changes in the levels of vasotocin (VT) and isotocin (IT) mRNAs were determined in chum salmon caught during spawning migration at Atsuta (sea water, SW) and Chitose (freshwater, FW), Hokkaido, in 1993 and 1994. The levels of VT and IT mRNAs were determined by quantitative Northern blot analysis. Patterns of changes in the levels of VT and IT mRNAs were remarkably different between males and females. In males, no significant differences were seen in the levels of both VT-I and VT-II mRNAs between SW and FW fish. In contrast, in females, the levels of VT-I and VT-II mRNAs in FW fish were significantly lower than those in SW fish. These sexually dimorphic changes in the levels of VT mRNAs, a significant decrease in females but not in males, were coincidentally observed in both 1993 and 1994. Hence we believe that the sexual difference in VT-I and VT-II gene expression is a naturally repeated phenomenon in homing chum salmon. One possible biological meaning of the sexual dimorphism is an involvement of VT in the control of spawning behavior rather than of osmoregulation.

Changes in the levels of VT and IT mRNAs were also different between pre-spawning males and females caught in the Sanriku coast of the Pacific ocean, the northern part of Honshu Island. In males, changes in the levels of VT and IT mRNAs were essentially the same during spawning migration. Even after the hypo-osmotic stimulation, VT and IT mRNAs were maintained at similar levels or else increased. In contrast, in females, levels of VT and IT mRNAs tended to decrease during the last stages of spawning migration from off-shore Sanriku to the Otsuchi river, and were decreased by both hyper- and hypo-osmotic stimulation. Such sexually dimorphic gene expression may be a naturally-occurring phenomenon in

pre-spawning chum salmon. Furthermore, these sexual differences were reproducible by experimental treatments, suggesting that the sexual dimorphism of VT and IT gene expression may be a physiologically important phenomenon. The control of VT and IT gene expression may be different between males and females in pre-spawning migration. Further, such sexual difference may be caused by the innate genetic program in pre-spawning chum salmon.

The innate program in part may include regulation of gene expression by steroid hormones, because several steroid-responsive elements are present in the 5'-upstream

regions of the VT-I and IT-I genes (Satomi et al., 1994; Kuno et al., 1995), and also plasma levels of steroid hormones in spawning chum salmon showed sexually different profiles.

## 2. Expression of the salmon (s) GnRH genes

Because of tetraploidy, many salmonid species may have two types of genes for precursors of sGnRH (Ashihara et al., 1995). When their nucleotide sequences were compared, the structure of 5' upstream regions differ considerably, despite homologous coding regions between the two genes. A series of in situ hybridization studies by Amano and colleagues have shown that regulation of sGnRH gene expression is a consequence of various physiological stimuli. However, it is not yet clear whether expression of the two sGnRH genes differs. Such information is a requisite for studies aimed at revealing regulatory mechanisms of sGnRH gene expression, and also at an understanding of the molecular bases of spawning migration in salmon.

### Acknowledgments

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## XII. EXPANSION AND CONTRACTIN OF MIGRATION RANGE OF THE JAPANESE SARDINE IN THE NORHTWESTERN PACIFIC

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### Abstract

The population of the Japanese sardine *Sardinops melanostictus* resurged from a minimum low in the mid-1960s to a maximum peak in the mid-1980s, and then started a steep decline at the end of the 1980s. With the drastic resurgence and the precipitous decline of the population, the ranges of feeding and spawning migration of the sardine expanded and then contracted. The eastern limit of the feeding migration in the Oyashio waters was located around southern Hokkaido, when the population was small but it expanded to as far as the date line when the population was maximum. The spawning ground of the sardine was located in the coastal waters on the continental shelf along the western and eastern Japan in the 1970s, which shifted westerly and then expanded offshore toward the oceanic Kuroshio currnet in the 1980s. High productivities in the Oyashio and