DIVISION OF SEASONAL BIOLOGY (ADJUNCT)

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Animals living outside the tropics adapt various physiology and behavior to seasonal changes in the environment. As animals use changes in day length and temperature as seasonal cues, these phenomena are referred to as photoperiodism and thermoperiodism, respectively. Medaka provides an excellent model to study these mechanisms because of their rapid and robust seasonal responses. In addition, genomic sequences and transgenic approaches are available in this species. In this division, we are trying to uncover the underlying mechanisms of seasonal adaptation using medaka fish.

I. Identification of seasonal sensor in fish

In our previous study, we uncovered the signal transduction pathway regulating photoperiodism in birds and mammals. The pars tuberalis of the pituitary gland and the mediobasal hypothalamus are considered to play a critical role in the regulation of photoperiodism. Long-day induced thyrotropin (thyroid-stimulating hormone: TSH) in the pars tuberalis, a master factor regulating photoperiodism, acts on the TSH receptor in the mediobasal hypothalamus to induce *DIO2* expression. DIO2 (type 2 iodothyronine deiodinase) is a thyroid hormone activating enzyme, which converts prohormone thyroxine (T_4) to bioactive triiodothyronine (T_3).

Most fish living outside the tropics also exhibit a seasonal response and involvement of thyroid hormones in seasonality has been described. However, fish do not possess an anatomically distinct pars tuberalis, which is the regulatory hub of photoperiodism in birds and mammals. Therefore, we tried to identify the photoperiodic center in fish using masu salmon.

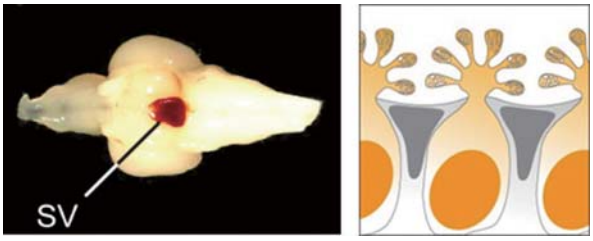

Figure 1. Left, Ventral view of the masu salmon brain. SV: saccus vasculosus. Right, Schematic drawing of the coronet cells.

1-1 Expression analysis of key genes regulating photoperiodism in the masu salmon

Photoperiodic regulation of TSH and DIO2 were observed in the saccus vasculosus of masu salmon (Figure 1). In addition, localization of rhodopsin family genes were observed in the saccus vasculosus. Immunohistochemical studies demonstrated that TSH, DIO2 and photoreceptor proteins are expressed in the coronet cells of the saccus vasculosus, suggesting the existence of a photoperiodic signaling pathway from light input to neuroendocrine output in this organ.

1-2 Functional analysis of saccus vasculosus

We have cultured saccus vasculosus *in vitro* and exposed them to short day or long day conditions. Isolated saccus vasculosus had the capacity to respond to photoperiodic signals. In addition, removal of the saccus vasculosus abolished the photoperiodic response of the gonad *in vivo*. Although the physiological role of the saccus vasculosus has been a mystery for several centuries, these findings indicated that the saccus vasculosus acts as a seasonal sensor in fish.

II. Genome-wide association study of seasonal time measurement

It is well established that the circadian clock is somehow involved in seasonal time measurement. However, it remains unknown how the circadian clock measures day length. Additionally, it is not known how animals adapt to seasonal changes in temperature. It has been reported that medaka populations that were caught at higher latitudes have more sophisticated responses to day length and temperature. For example, medaka fish caught in Hokkaido have a critical day length (i.e., duration of light period required to cause a response) of 13 h, while those caught in Okinawa have an 11.5 h critical day length. To uncover the underlying mechanism of seasonal time measurement, we are planning to perform a genome-wide association study in medaka populations collected from various latitudes all over Japan.

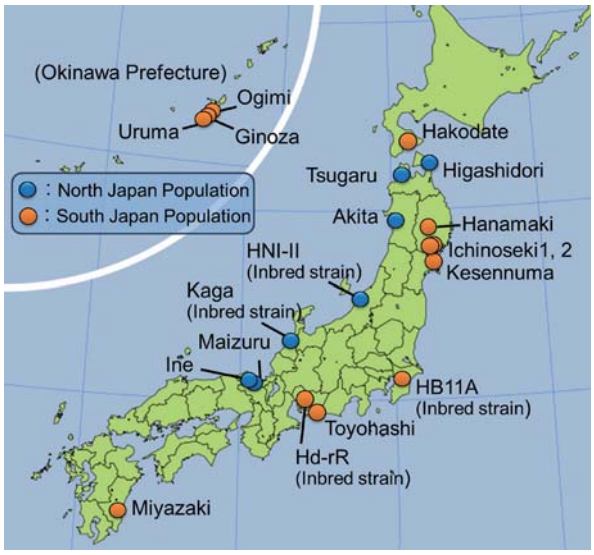

Figure 2. Medaka populations collected and used in our study.

2-1 Variation in seasonal responses with latitude in medaka fish

To perform a genome-wide association study, we have collected thousands of medaka fish from all over Japan (Figure 2). This initial year, we have examined the effects of changing day length and temperature to determine the critical day lengths and critical temperatures that will cause seasonal responses in the gonad.

III. Transcriptome analysis of seasonality in medaka fish

Homeotherms such as birds and mammals do not show clear seasonal responses to changing temperature. In contrast, poikilothermal animals also use changing temeperature as a calendar. Medaka provides an excellent model to uncover this mechanism. To elucidate the signal transduction pathway regulating seasonal reproduction in medaka fish, we have examined transcriptome analysis.

Publication List

〔Original paper〕

- Nakane, Y., Ikegami, K., Iigo, M., Ono, H., Takeda, K., Takahashi, D., Uesaka, M., Kimijima, M., Hashimoto, R., Arai, N., Suga, T., Kosuge, K., Abe, T., Maeda, R., Senga, T., Amiya, N., Azuma, T., Amano, M., Abe, H., Yamamoto, N., and Yoshimura, T. (2013). The saccus vasculosus of fish is a sensor of seasonal changes in day length. Nature Commun. *4*, 2108.

〔Review articles〕

- Ikegami, K., and Yoshimura, T. (2013) Seasonal time measurement during reproduction. J. Reprod. Develop. *59*, 327-333.
- Yoshimura, T. (2013). Thyroid hormone and seasonal regulation of reproduction. Front. Neuroendocrinol. *34*, 157-166.