DIVISION OF SEASONAL BIOLOGY (ADJUNCT)

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Animals living outside the tropics adapt various aspects of their physiology and behavior to seasonal changes in the environment. For example, animals restrict breeding to specific seasons to maximize the survival of their offspring in temperate zones. The differing approaches that animals use as seasonal cues to recognize changes in day length and temperature are referred to as photoperiodism and thermoperiodism, respectively. We use comparative approaches to understand these mechanisms. Medaka fish provide an excellent model to study these mechanisms because of their rapid and robust seasonal responses. In this division, we are trying to uncover the underlying mechanisms of seasonal adaptation.

I. Underlying mechanism that defines the critical temperature

It is well established that temperature changes are important in seasonal time measurement. However, it remains unknown how animals measure temperature changes regarding seasonal time measurement. It has been reported that medaka populations that are caught at higher latitudes have more sophisticated responses to seasonal changes. To uncover the underlying mechanism of seasonal time measurement, we are currently performing a forward genetic analysis among medaka populations collected from various latitudes across Japan.

1-1 Variation in critical temperatures in line with latitudinal differences in medaka fish

To perform a forward genetic analysis, we have obtained 5 populations including wild populations and closed colonies from all over Japan. We have examined the effects of changing temperatures to determine the critical temperature that will cause seasonal responses in the gonad. In winter, fish were subjected to 14, 16, 18, and 20 degree temperatures over a long day length. Gonadal development was examined to identify the critical temperature.

As a result, we found differences in the critical temperature among medaka populations (Figure 1). It was found that medaka from higher latitudes required higher temperatures while those from lower latitudes required lower temperatures.

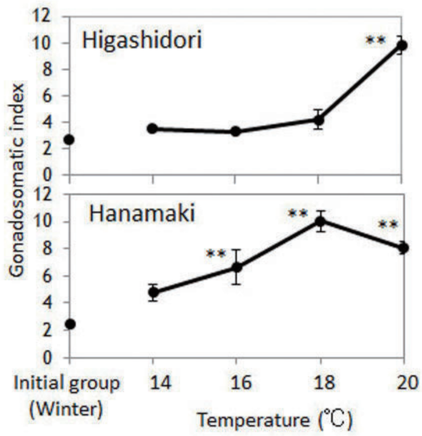

Figure 1. Differences in critical temperature.

1-2 Quantitative trait loci (QTL) analysis of critical temperature

To identify the genes regulating critical temperature, quantitative trait loci (QTL) analysis was conducted using F_2 medaka derived from crosses between Northern and Southern Japanese populations. We identified significant QTL on chromosome 12 using Restriction-site Associated DNA (RAD) markers. We have also performed whole genome re-sequencing using various medaka strains that show different critical temperatures, and identified potential candidate genes that define the critical temperature.

II. Mechanism that determines seasonal breeders and non-seasonal breeders

Animals that reproduce year-round (*e.g.*, human beings and laboratory mice) are so-called non-seasonal breeders. In contrast, most animals living outside of tropical zones reproduce only during a particular period of the year. Therefore, they are called seasonal breeders. However, the underlying mechanism that separates seasonal breeders from non-seasonal breeders remains unknown. To uncover this mechanism, we performed a forward genetic approach.

2-1 Geographic variations in the responses to short day stimulus

When we transferred medaka fish from summer conditions to winter conditions, we noticed that the medaka from lower latitudes' gonads did not regress even under short day conditions. Accordingly, we then examined the responses to short day conditions using 20 populations derived from various latitudes. As a result, we found that populations from higher latitudes showed gonadal regression, while populations from lower latitudes did not regress their gonads.

2-2 QTL analysis of genes determining seasonal breeders and non-seasonal breeders

To identify genes that determine seasonal breeders and non-seasonal breeders, we performed QTL analysis using F_2 generations and identified a significant QTL that determines seasonal breeders and non-seasonal breeders. We are also performing a genome-wide association study to identify responsible genes.

III. Transcriptome analysis of seasonality in medaka fish

In addition to the forward genetic approach, we have performed genome-wide transcriptome analysis of the brain of the medaka fish to understand the underlying mechanism of seasonal adaptation.

3-1. Identification of photoperiodically-regulated transcripts

During the breeding season, animals show stress responses, such as self-protective and escape behaviors, when confronted with potentially dangerous situations such as predation, interspecific competition, and harsh weather. These behaviors are critical for animals to survive changing environments. Photoperiod is the most reliable indicator of the forthcoming season, and seasonal regulation of various physiological and behavioral processes by photoperiod has been known for decades. However, the molecular mechanisms underlying seasonal adaptive strategies are not well understood. Medaka fish serve as an excellent model to study the mechanism of seasonal adaptation, because of their highly sophisticated seasonal responses and the recent availability of genomic information and genome-editing tools. Interestingly, we recently demonstrated that dynamic seasonal changes in color perception alters breeding behavior in medaka. To further understand the molecular basis of seasonal adaptation in medaka, we performed genome-wide transcriptome analysis of their brains during the transition from short day (SD) to long day (LD) conditions and identified several photoperiodically-regulated genes and signaling pathways (Figure 2).

3-2. Photoperiodically-regulated, cycling transcript was a lncRNA

Expression of one transcript named *olvl28m13* was induced and was strongly rhythmic only under long day conditions (Figure 2). Notably, the timing of its induction was much earlier than nearly all up-regulated genes.

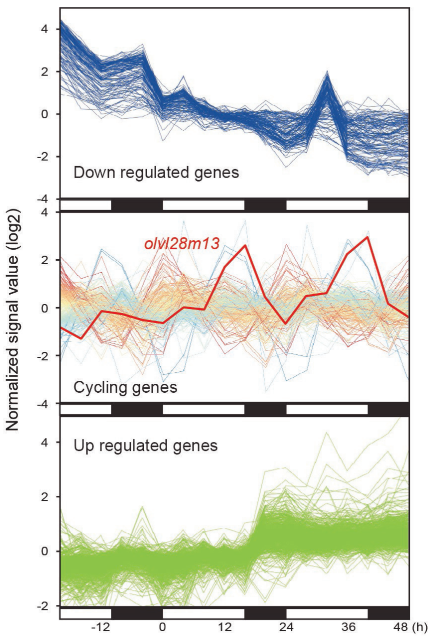

Figure 2. Transcriptional landscape during the transition from short day to long day.

To examine *olvl28m13* further, we next performed strand-specific RNA-seq analysis and confirmed higher expression under LD than SD conditions (Figure 3). This RNA was transcribed from the intronic region of the *LPIN2* gene. We then performed ribosomal profiling analysis (Ribo-seq) to determine the protein-coding potential of this transcript. The result of Ribo-seq indicated that the transcript *olvl28m13* is not associated with ribosomes and most likely not translated into protein. To examine functional significance of this lncRNA, we generated KO medaka using the CRISPR/Cas9 system. Behavioral analysis of the KO fish demonstrated that this lncRNA affects self-protective behaviors, suggesting that lncRNA modulates adaptive behaviors to seasonal environmental changes.

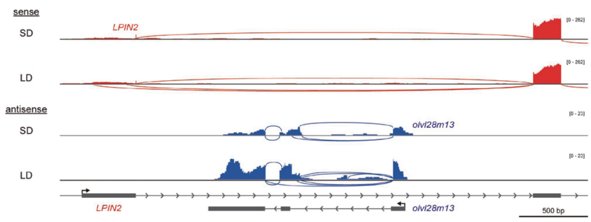

Figure 3. Strand specific RNA-seq identified *olvl28m13* in the intronic region of the *LPIN2* gene.

Publication List:

〔Original papers〕

- Ota, W., Nakane, Y., Hatter, S., and Yoshimura, T. (2018). Impaired circadian photoentrainement in Opn5-null mice. iScience *6*, 299-305.
- Tamai, T.K., Nakane. Y., Ota, W., Kobayashi, A., Ishiguro, M., Kadofusa, N., Ikegami, K., Yagita, K., Shigeyoshi, Y., Sudo, M., Nishiwaki-Ohkawa, T., Sato, A., and Yoshimura, T. (2018). Identification of circadian clock modulators from existing drugs. EMBO Mol. Med. *10*, e8724.

〔Review articles〕

- Nakane, Y., and Yoshimura, T. (2018). Seasonal reproduction: Photoperiodism, Birds. In Encyclopedia of Reproduction 2nd Edition, M.K. Skinner, ed. (Academic Press Inc.) pp. 409-414.
- Nakayama, T., and Yoshimura, T. (2018). Seasonal rhythms: the role of thyrotropin and thyroid hormones. Thyroid *28*, 4-10.
- Shimmura, T., Nakayama, T., Shinomiya, A., and Yoshimura, T. (2018). Seasonal changes in color perception. Gen. Comp. Endocrinol. *260*, 171-174.
- Shinomiya, A., and Yoshimura, T. (2018). Seasonal regulation of reproduction in vertebrates: special focus on avian strategy. In Reproductive and Developmental Strategies, K. Kobayashi, T. Kitano, Y. Iwao, M. Kondo, eds. (Springer Japan) pp. 103-122.