Division of Seasonal Biology (Adjunct)

Adjunct Professor
YOSHIMURA, Takashi

Specially Appointed Assistant Professor: *SHINOMIYA, Ai*
SHIMMURA, Tsuyoshi
Visiting Graduate Student: *ADACHI, Daisuke*
SHIMO, Takayuki
NAKATSUKASA, Mana
SENGA, Takumi
Technical Assistant: *BABA, Nayumi*
AKAMA, Akiko
Secretary: *OKUBO, Masayo*

Animals living outside the tropics adapt various physiology and behavior to seasonal changes in the environment. For example, animals restrict breeding to specific seasons to maximize survival of their offspring in temperate zones. As animals use changes in day length and temperature as seasonal cues, these phenomena are referred to as photoperiodism and thermoperiodism, respectively. We use comparative approaches to understand these mechanisms. Japanese quail and medaka provide excellent models 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. Mechanism of seasonal testicular regression

Birds have evolved highly sophisticated mechanisms of seasonal regulation, and their testicular mass can change a hundred-fold within a few weeks. Recent studies revealed that seasonal gonadal development is regulated by central thyroid hormone (TH) activation within the hypothalamus depending on the photoperiodic changes. By contrast, the mechanisms underlying seasonal testicular regression remain unclear. We examined the effects of short day and low temperature on testicular regression in quail. Low temperature stimulus accelerated short day (SD)-induced testicular regression by shutting-down the hypothalamus-pituitary-gonadal (HPG) axis and inducing meiotic arrest and germ cell apoptosis. Induction of triiodothyronine (T_3) coincided with the climax of testicular regression. Temporal gene-expression analysis over the course of apoptosis revealed suppression of LH response genes and activation of T_3 response genes involved in amphibian metamorphosis within the testis. Daily i.p. administration of T_3 mimicked the effects of low temperature stimulus on germ-cell apoptosis and testicular mass. We concluded that birds utilize low temperature-induced circulating T_3 not only for adaptive thermoregulation but also to trigger apoptosis in order to accelerate seasonal testicular regression.

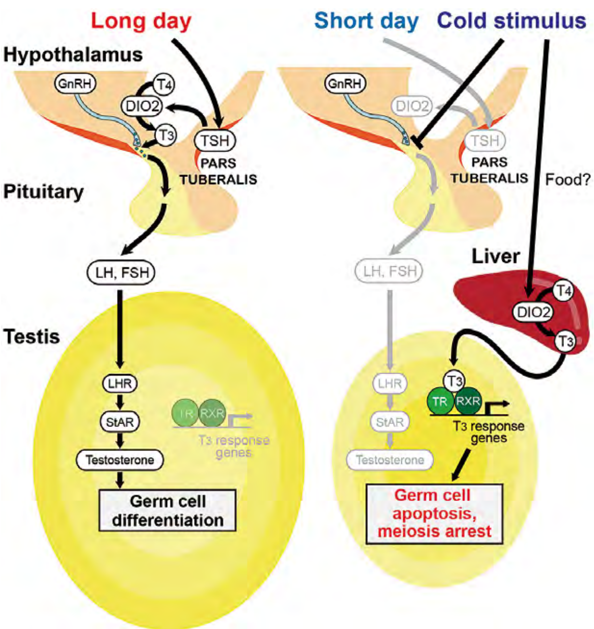

Figure 1. Mechanisms regulating seasonal testicular development and regression in birds.

II. Ontogeny of the saccus vasculosus, a seasonal sensor in fish

Thyroid-stimulating hormone (thyrotropin: TSH) secreted from the pars distalis (PD) of the pituitary gland stimulates the thyroid gland. In contrast, TSH secreted from the pars tuberalis (PT) of the pituitary gland regulates seasonal reproduction in birds and mammals. The ontogeny of thyrotrophs and the regulatory mechanisms of TSH are markedly different between the PD and PT. Interestingly, fish do not have an anatomically distinct PT, and the saccus vasculosus (SV) of fish is suggested to act as a seasonal sensor. Thus, it is possible that the SV is analogous to the PT. We examined the ontogeny of the pituitary gland and SV using rainbow trout. A histological analysis demonstrated the development of the pituitary anlage followed by that of the SV. Transcription factors *Lhx3* and *Pit-1*, which are required for the development of PD thyrotrophs, clearly labelled the pituitary anlage. The common glycoprotein α subunit (*CGA*) and TSH β subunit (*TSHB*) genes were also detected in the pituitary anlage. In contrast, none of these genes were detected in the SV anlage. We then performed a microarray analysis and identified *Parvalbumin* (*Pvalb*) as a marker for SV development. Because *Pvalb* expression was not detected in the pituitary anlage, no relationship was observed between the development of the SV and pituitary gland. In contrast to embryos, *Lhx3*, *Pit-1*, *CGA*, and *TSHB* were all expressed in the adult SV. These results suggest that the morphological differentiation of SV occurs during the embryonic stage, but that the functional differentiation into a seasonal sensor occurs in a later developmental stage.

III. Forward genetic analysis 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 photoperiod 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 currently performing a forward genetic analysis in medaka populations collected from various latitudes all over Japan.

3-1 Variation in seasonal responses with latitude in medaka fish

To perform a forward genetic analysis, we have collected thousands of medaka fish from all over Japan. We have examined the effects of changing day length to determine the critical day lengths that will cause seasonal responses in the gonad and we found differences in critical day length between medaka from higher latitudes and lower latitudes (Figure 2).

3-2 Quantitative trait loci (QTL) analysis of critical day length

To identify the genes regulating critical day length, quantitative trait loci (QTL) analysis was conducted using more than 700 F2 medaka derived from crosses between Northern and Southern populations. As a result, we identified significant QTLs. We are now performing genome re-sequencing using various medaka strains that show different critical photoperiods.

IV. 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 temperature as a calendar. Medaka provides an excellent model to uncover this mechanism. To elucidate the signal transduction

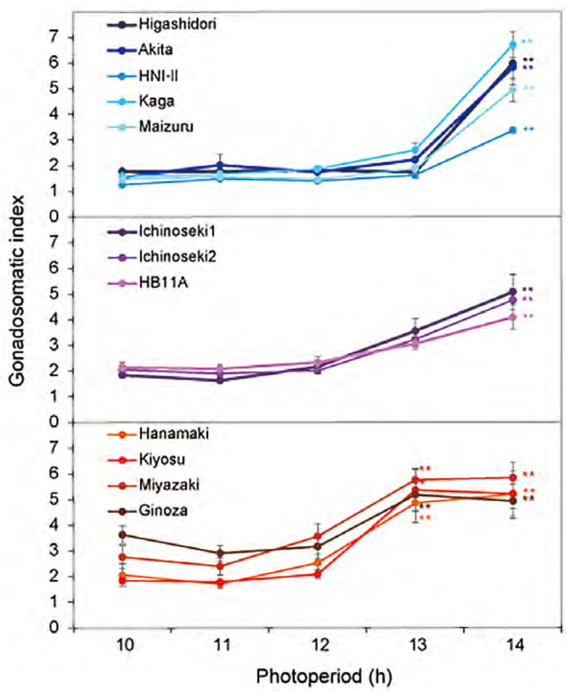

Figure 2. Different critical day length between medaka from higher latitudes and lower latitudes.

pathway regulating seasonal reproduction in medaka fish, we have examined transcriptome analysis using microarrays. We identified hundreds of genes that respond to day length and temperature changes.

Publication List:

〔Original papers〕

- Ikegami, K., Atsumi, Y., Yorinaga, E., Ono, H., Murayama, I., Nakane, Y., Ota, W., Arai, N., Tega, A., Iigo, M., Darras, V.M., Tsutsui, K., Hayashi, Y., Yoshida, S., and Yoshimura, T. (2015). Low temperature-induced circulating triiodothyronine accelerates seasonal testicular regression. Endocrinology *156*, 647-659.
- Maeda, R., Shimo, T., Nakane, Y., Nakao, N., and Yoshimura, T. (2015). Ontogeny of the saccus vasculosus, a seasonal sensor in fish. Endocrinology *156*, 4238-4243.
- Oshima, T., Yamanaka, I., Kumar, A., Yamaguchi, J., Nishiwaki-Ohkawa, T., Muto, K., Kawamura, R., Hirota, T., Yagita, K., Irle, S., Kay, S.A., Yoshimura, T., and Itami, K. (2015). C-H activation generates period shortening molecules targeting cryptochrome in the mammalian circadian clock. Angew. Chem. Int. Ed. *54*, 7193-7197.
- Shimmura, T., Ohashi, S., and Yoshimura, T. (2015). The highest-ranking rooster has priority to announce the break of dawn. Sci. Rep. *5*, 11683.

〔Review article〕

- Stevenson, T.J., Visser, M.E., Arnold, W., Barrett, P., Biello, S., Dawson, A., Denlinger, D.L., Dominoni, D., Ebling, F.J., Elton, S., Evans, N., Ferguson, H.M., Foster, R.G., Hau, M., Haydon, D.T., Hazlerigg, D.G., Heideman, P., Hopcraft, J.G.C., Jonsson, N.N., Kronfeld-Schor, N., Kumar, V., Lincoln, G.A., MacLeod, R., Martin, S.A.M., Martinez-Bakker, M., Nelson, R.J., Reed, T., Robinson, J.E., Rock, D., Schwartz, W.J., Steffan-Dewenter, I., Tauber, E., Thackeray, S.J., Umstatter, C., Yoshimura, T., and Helm, B. (2015). Disrupted seasonal biology impacts health, food security, and ecosystems. Proc. R. Soc. B. *282*, 1817.