◀Review▶

Molecular Bases for Seasonal Reproduction in Birds

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Appropriate timing of various seasonal processes is crucial to the survival and reproductive success of animals inhabiting temperate regions. When seasonally breeding animals are subjected to annual changes in daylength, dramatic changes in neuroendocrine-gonadal activity ensue. However, the molecular mechanism of photoperiodic (or seasonal) time measurement (PTM) is not well understood for any organism living. Japanese quail is an excellent model for studying PTM because of the rapid and dramatic response to photoperiod. Here I describe recent progress in understanding the molecular mechanism of PTM in Japanese quail.

Key words : circadian clock, Japanese quail, photoperiodism, thyroid hormone

Introduction

Reproduction of many temperate-zone birds is under photoperiodic control. Although melatonin has an important role for the regulation of photoperiodic time measurement (PTM) in mammals, no effect of melatonin manipulations is observed in birds (Gwinner *et al.*, 1997). Japanese quail is an appropriate model system for the investigation of the PTM because of the rapid and dramatic response to photoperiod (Follett and Sharp, 1969 ; Wada, 1979). Therefore, a considerable number of studies have been made on Japanese quail over the past few decades. Numerous reports have suggested that the mediobasal hypothalamus (MBH), including the infundibular nucleus (IN), the inferior hypothalamic nucleus (IH), the median eminence (ME) and nucleus hypothalamicus posterior medialis (NHMP) is an important center controlling the PTM (Fig. 1). For example, lesions of the IN, the ME, or NHMP within the MBH

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Fig. 1. The mediobasal hypothalamus (MBH) (dashed line) of Japanese quail. Cb : cerebellum ; IH : inferior hypothalamic nucleus ; IN : infundibular nucleus ; ME : median eminence ; NHPM : nucleus hypothalamicus posterior medialis ; OC : optic chiasm ; POA : preoptic area.

can block the rise of luteinizing hormone (LH) and testicular growth under long photoperiods (Sharp and Follett, 1969 ; Davies and Follett, 1975 ; Ohta and Homma, 1987). In the lesion study, block of the photoperiodic response is effective even though the GnRH system has been left intact (Juss, 1993). In addition, electrical stimulation of IN and IH increases LH secretion (Konishi *et al.*, 1987) and induces testicular growth (Ohta *et al.*, 1984). Furthermore, it has been shown that the expression of Fos-like immunoreactivity in IN, IH and ME by photostimulation of one long day photoperiod (Meddle and Follett, 1995 ; 1997). In addition to these reports, deep brain photoreceptors are thought to be localized in the IN (Silver *et al.*, 1988).

Where is the photoperiodic clock ?

It is an established fact that the circadian clock is involved in the PTM (Pittendrigh, 1972). Circadian (~ 24 h) rhythms are a fundamental property of living systems and impose a 24 h temporal organization regulating the physiology and biochemistry of most organisms. The intracellular clock mechanism is composed by interacting positive and negative transcriptional feedback loops that drive ~ 24 hour rhythms in the RNA and protein levels of key clock components called "clock genes" (Dunlap, 1999 ; Reppert and Weaver, 2002). Recently, homologues of mammalian circadian clock genes have been cloned in birds, which have provided a way to examine the molecular link between the circadian clock and photoperiodism in bird (Yoshimura *et al.*, 2000 ; Okano *et al.*, 2001 ; Yamamoto *et al.*, 2001 ; Fu *et al.*, 2002). Avian circadian rhythms are regulated by a multiple oscillatory system consisting of the eye, the pineal gland, and the suprachiasmatic nucleus (SCN) (Ebihara *et al.*, 1987 ;

Yoshimura *et al.*, 2001). However, enucleation or pinealectomy or lesion around the SCN has no effect on the photoperiodic response of gonads in birds (Davies and Follett, 1975 ; Siopes and Wilson, 1974).

Yasuo *et al.* (2003) examined the expression of all known avian circadian clock genes (*Clock*, *Per2*, *Per3*, *Bmal1*, *Cry1*, *Cry2*, and *E4bp4*) in Japanese quail brain and observed all gene expressions in the MBH (Fig. 2). Temporal expression patterns of clock genes in the MBH were stable under various light conditions. Together with the previous lesion studies, their results suggest that the clock in the MBH is the long-sought clock essential for PTM, and appears to enable animals to retain a steady state photoinducible phase (Yasuo *et al.*, 2003 ; Ball and Balthazart, 2003).

Identification of a gene which is responsible for PTM

Now it seems that all the molecular machinery required for PTM is localized in the MBH. Since light pulses at photoinducible phase are known to mimic photoperiodically induced testicular growth (Follett and Sharp, 1969 ; Wada, 1979) (Fig. 3), it is

Fig. 2. Expression of circadian clock gene in the mediobasal hypothalamus (MBH) of Japanese quail. Representative autoradiogram for *Per2* expression of the coronal section.

Fig. 3. Light pulses at photoinducible phase cause testicular growth. Animals were given light pulses for 10 days.

expected that single light pulse at photoinducible phase causes a molecular event in the MBH. To identify the genes that are responsible for the regulation of PTM in birds, Yoshimura *et al.* (2003) performed a differential subtractive hybridization analysis using MBH of birds given a light pulse at photoinducible phase versus birds given no light pulse. Among 150 clones, they found only one gene, *type 2 iodothyronine deiodinase (Dio2)* gene, whose expression was significantly induced by light in the MBH (Yoshimura *et al.*, 2003). It is noteworthy that expression site of *Dio2* in the MBH was entirely consistent with the lesion study (Sharp and Follett, 1969).

Content and target site of thyroid hormone

Dio2 is an enzyme that converts prohormone thyroxine (T_4) to active triiodothyronine (T_3), which is primarily responsible for thyroid hormone action. *Dio2* plays an essential role in the local control of brain T_3 through mechanisms that operate under a variety of situations to maintain T_3 concentrations in a narrow range (Bernal, 2002). To determine the level of T_3 and T_4 in the MBH, Yoshimura *et al.* (2003) measured the contents of the pooled MBH collected from 10 animals in each group under short and long day conditions. Although the plasma concentration of T_3 and T_4 was not significantly different between short and long day conditions, both contents in the MBH were about 10 fold higher for long days than short days. In addition to these results, they examined the expression of thyroid hormone receptors and found expression of $TR\alpha$, $TR\beta$ and the $RXR\alpha$ genes in the IN and the ME. These results indicate that locally generated T_3 acts on the IN and the ME.

Morphological changes in the ME under short and long photoperiods

It was suggested that photoperiodic GnRH release could be controlled at the GnRH terminals by glia (Dawson *et al.*, 2001 ; Meddle and Follett, 1997), and thyroid hormones have a critical involvement in the development, plasticity and function of the central nervous system (Bernal, 2002). Therefore, to validate the effect of T_3 , Yamamura *et al.* (2004) examined ultrastructures of GnRH terminals in the ME under long and short day conditions using transmission electron microscopy (TEM). Immunoelectron microscopy revealed that GnRH nerve terminals are in close proximity to the basal lamina in birds subjected to long day conditions. Conventional TEM demonstrated encasement of nerve terminals by the endfeet of glial processes under short days. This morphological change seems to allow the neurons to secrete GnRH due to the increased access of their terminals to the perivascular area surrounding the fenestrated portal capillaries under long days (Fig. 4). These results support the hypothesis that the photoperiodic response of Japanese quail is regulated primarily at the level of secretion of GnRH rather than by the synthesis of GnRH in the cell bodies (Meddle and Follett, 1997 ; Dawson *et al.*, 2001).

Administration of thyroid hormone induces gonadal growth

To assess whether T_3 mediates the photoperiodic response of the gonads, Yoshimura *et al.* (2003) further studied the effect of the intracerebroventricular (i.c.v.)

Fig. 4. Schematics of the morphological changes in the GnRH nerve terminals and endfeet of glial processes in the median eminence under short days and long days.

infusion of physiological doses of T_3 on testicular growth. T_3 infusion induced testicular growth in a dose dependent manner even though the animals were kept under short day conditions, while T_4 infusion had only a minor effect. Interestingly, however, significant testicular growth was not observed in the largest dose of T_3 . Although thyroid hormone is of critical importance in the regulation of photoperiodically induced gonadal growth, this hormone is also known to be somehow involved in the regulation of photorefractoriness (insensitivity to previously stimulatory daylength) (Dawson *et al.*, 2001 ; Dawson and Thapliyal, 2001). The observation that no significant testicular growth is induced at the highest dose of T_3 , suggests the possibility that photorefractoriness occurs as a result of down-regulation of thyroid hormone activity. This hypothesis remains to be clarified in future studies.

It has also been reported that peripheral pharmacological doses of thyroid hormone injection mimics photoperiodically induced gonadal growth. However, in contrast to the central administration, T_4 is more effective than T_3 in peripheral administration (Follett *et al.*, 1988 ; Follett and Nicholls 1988). These reports seem to be in contradictory. However, in blood, about 1/3 and 45% of T_4 is known to be converted to T_3 and reverse T_3 (rT_3), respectively. In addition, T_3 is converted to $3,3'$ - T_2 in the blood (Chopra *et al.*, 1978). Therefore, it seems that most of the peripherally delivered T_4 might be converted to T_3 and act on the central nervous system. The observation that orally administered T_4 mimics photoperiodically induced gonadal growth while orally administered T_3 has no effect further supports this idea (Yasuo *et al.*, 2004a).

Decoding photoperiodic information in the pars tuberalis

Prolactin (PRL) secretion is also regulated by photoperiod. In mammals, the pars tuberalis (PT) in the pituitary is known to be involved in the regulation of photoperiodic regulation of PRL secretion. In birds, however, hypothalamic vasoactive intestinal peptide (VIP) is implicated in PRL secretion, and physiological roles of the avian PT remain unknown. Yasuo *et al.* (2004b) have found expression of *Per2* and

Cry1 genes in the PT of Japanese quail. The peak of *Per2* expression was observed around dawn, while that of *Cry1* was found around dusk under both long and short photoperiods, resulting in a different phase relationship between the two genes. In mice, protein-protein interactions between PER and CRY affect their stability, nuclear entry and subsequent gene transcription (Lowrey *et al.*, 2000, Shearman *et al.*, 2000). Therefore, changes in the phasing of *Per-Cry* expression is a potential mechanism by which the long day and short day light signals can be decoded. The level of coincidence in turn determines the formation of PER-CRY protein complexes that can control the transcription of other clock genes and downstream genes that dictate the secretory activity of the prolactin-releasing factor that drives the lactotrophs to secrete PRL (Lincoln *et al.*, 2003 ; Yasuo *et al.*, 2004b).

Conclusion

Although thyroid hormone was known to be involved in the regulation of PTM in birds, its precise role had been uncertain for several decades (Dawson, 1993 ; 1998 ; Dawson *et al.*, 2001 ; Dawson and Thapliyal, 2001 ; Follett and Nicholls, 1988 ; Follett *et al.*, 1988). Recent findings have finally determined the target site and molecular events that takes place in the brain.

Finally, it is also of interest to note that multiple studies indicate that thyroid hormones are essential for the maintenance of seasonal reproductive changes in a number of mammals (Nicholls *et al.*, 1988). Recently, it is reported that *Dio2* gene is also photoperiodically regulated in the Djungarian hamster, a long day breeder (Watanabe *et al.*, 2004). Thus, recent findings may offer the key to an understanding of the molecular mechanism of PTM in all photoperiodic vertebrates.

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