Red jungle fowl (Gallus gallus) as a model for studying the molecular mechanism of seasonal reproduction

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ABSTRACT

Photoperiodism is an adaptation mechanism that enables animals to predict seasonal changes in the environment. Japanese quail is the best model organism for studying photoperiodism. Although the recent availability of chicken genome sequences has permitted the expansion from single gene to genome-wide transcriptional analysis in this organism, the photoperiodic response of the domestic chicken is less robust than that of the quail. Therefore, in the present study, we examined the photoperiodic response of the red jungle fowl (Gallus gallus), a predecessor of the domestic chicken, to test whether this animal could be developed as an ideal model for studying the molecular mechanisms of seasonal reproduction. When red jungle fowls were transferred from short-day- to long-day conditions, gonadal development and an increase in plasma LH concentration were observed. Furthermore, rapid induction of thyrotropin beta subunit, a master regulator of photoperiodism, was observed at 16 h after dawn on the first long day. In addition, the long-day condition induced the expression of type 2 deiodinase, the key output gene of photoperiodism. These results were consistent with the results obtained in quail and suggest that the red jungle fowl could be an ideal model animal for the genome-wide transcriptional analysis of photoperiodism.

Key words: luteinizing hormone (LH), photoperiodism, red jungle fowl, thyrotropin (TSH), type 2 iodothyronine deiodinase (DIO2).

INTRODUCTION

Organisms measure changes in day length (photoperiod) to adapt to seasonal changes in the environment and limit the breeding time to an appropriate season. This ensures that offspring are born only in spring and summer when food is abundant. Thus, this phenomenon is called photoperiodism. Among the various vertebrate species, Japanese quail (Coturnix japonica) have proven to be the best model animal to study photoperiodism because of their rapid and dramatic response to photoperiod (Follett & Sharp 1969; Nicholls et al. 1983; Follett et al. 1998). When quail is maintained under short-day conditions, testicular length is maintained at around 2–5 mm. However, once they are transferred to long-day conditions, their testes grow very rapidly, and testicular length reaches about 15–20 mm within 2 weeks. Thus, a detailed understanding regarding photoperiodic time measurement is obtained from studies on Japanese quail. The center controlling the photoperiodic response is considered to be localized within the mediobasal hypothalamus (MBH). Lesions around the MBH block the photoperiodic response of the gonads, although the gonadotropin-releasing hormone (GnRH) neurons are

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left intact (Sharp & Follett 1969; Juss 1993). In addition, it is thought that the MBH contains a deep brain photoreceptor (Silver et al. 1988) and a clock that measures day length (Yasuо et al. 2003). Furthermore, recent molecular analysis of the quail MBH revealed molecular gene cascades regulating the photoperiodic response of gonads. When quail is transferred from short-day- to long-day conditions, induction of the thyrotropin beta subunit (TSHβ) gene is observed within the pars tuberalis of the pituitary gland at about 14 h after the dawn on the first long day (Nakao et al. 2008). In the pars tuberalis, common glycoprotein alpha subunit (CGA or α-GSU) is rhythmically expressed and forms thyrotropin (thyroid stimulating hormone, TSH) with TSHβ. In the pars tuberalis, long-day induced TSH acts as a master regulator of photoperiodism and induces the expression of the type 2 iodothyronine deiodinase (DIO2) gene about 4 h later in the ependymal cells lining the ventral wall of the third ventricle and the infundibular nucleus (which is anatomically homologous to the mammalian arcuate nucleus) of the MBH. This TSH-induced expression of DIO2 is mediated by the TSH receptor (TSHR)-cAMP signaling pathway (Nakao et al. 2008). DIO2 is a thyroid hormone-activating enzyme, which converts prohormone thyroxine (T₄) to its bioactive form triiodothyronine (T₃) (Yoshimura et al. 2003). Increased T₃ concentration within the MBH has been demonstrated to induce morphological changes in GnRH nerve terminals and glial processes, which result in secretion of the luteinizing hormone (LH) and testicular growth (Yoshimura et al. 2003; Yamamura et al. 2004, 2006).

The recent availability of chicken genome sequences and microarrays has permitted the expansion from single gene to genome-wide transcriptional analysis in chickens. However, since the photoperiodic response of the domestic chicken is less robust than that of the quail (Wilson & Sharp 1975; Dunn et al. 1990; Sharp 1992; Sreekumar & Sharp 1998), functional genomic analysis of photoperiodism was performed in Japanese quail by using a high-density chicken oligonucleotide microarray (Nakao et al. 2008). Although this analysis was successful and signals for over 83% of the probes were statistically indistinguishable between the chicken and quail samples, eventually, it would be ideal if chickens could be used as a model for understanding the molecular mechanism of photoperiodism.

The red jungle fowl is considered to be the predecessor of the domestic chicken, which originated in Southeast Asian countries, and is expected to show wild original characteristics (West & Zhou 1989). Therefore, in the present study, we examined the photoperiodic response of the gonads, gonadotropin (LH) secretion, and TSHβ and DIO2 expression in the red jungle fowl to test whether this animal is a good model for studying the molecular mechanism of seasonal reproduction.

MATERIALS AND METHODS

Animals and sampling procedures

Male and female red jungle fowls were reared in floor pens under natural day length (lat 35°31’N) until November 2006 when they became 11 weeks old. Thereafter, the birds were transferred to individual cages and maintained under short-day conditions [6-h light, 18-h dark (6L : 18D)] for 2 weeks in a light-tight room maintained at 16 °C. Light was supplied by fluorescent lamps at an intensity of 200 lux. At the age of 13 weeks, all birds were transferred to long-day conditions [20-h light, 4-h dark cycle (20L : 4D)], because photoperiodic control of LH secretion is reported in juvenile bantams well before somatic maturity (Sreekumar & Sharp 1998). Plasma samples, brains, and gonads were collected from 3 male and 3 female birds at 6-, 16-, and 20 h after dawn (i.e. zeitgeber time; ZT 6, ZT 16, and ZT 20) on the first long day and 6 h after dawn (ZT 6) on the 14th long day. Since birds sacrificed at ZT 6 on the first long day never experienced long-day stimulus, they were referred to as short-day animals, while birds sacrificed at ZT 6 on the 14th long day were referred to as long-day animals in the present study. The MBHs (4 mm in diameter) were punched out from 4-mm-thick chicken brain slices. Food and water were available ad libitum throughout the experiment. The present study was approved by the Committee on Animal Experiments of the Graduate School of Bioagricultural Sciences, Nagoya University.

LH radioimmunoassay

Plasma LH concentrations were determined by radioimmunoassay (RIA) using the chicken LH RIA kit as previously described (Takagi et al. 2007).

Quantitative real-time RT-PCR (Q-PCR)

Total RNA was prepared from each MBH using TRIzol reagent (Invitrogen, Tokyo, Japan). Reverse transcription was performed on total RNA (0.5 μg) using ReverTra Ace (Toyobo, Osaka, Japan) and oligo-dT primers. Samples contained 1X SYBR Premix Ex Taq (Takara, Osaka, Japan), 0.3 mmol/L gene-specific primers, and 1/20 synthesized cDNA in a volume of 25 μL. Q-PCR was performed in duplicates by using ABI Prism 7000 (Applied Biosystems, Tokyo, Japan) as follows: 1 cycle at 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, 60°C for 31 s. We used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The following gene-specific primers were used: TSHB (NM_205063), sense 5’-ctcgctggtttgcagacgac-3’, antisense 5’-gtgacacgttttgagacag-3’; DIO2 (NM_204114), sense
RESULTS

Statistical analysis

Data are presented as mean ± SE. Gonadal weight, plasma LH level and Dio2 expression were statistically analyzed by Mann-Whitney U-test. Tshb expression was analyzed by one-way analysis of variance (ANOVA).

Photoperiodic response of gonads

To assess the effect of the long-day photostimulation on gonadal development, gonadal weight was measured under short-day- and long-day conditions. A significant increase in gonadal weight was observed in both sexes (Mann-Whitney U-test, \( P < 0.05, n = 3 \)) (Fig. 1).

Photoperiodic changes in LH secretion

To examine the effect of long-day photostimulation on gonadotropin secretion, we compared the plasma LH concentration between short-day- and long-day birds. A significant increase in plasma LH concentration was observed in both sexes (Mann-Whitney U-test, \( P < 0.05, n = 3 \)) (Fig. 2).

Induction of TSHB and Dio2 gene expression by long-day stimulus

We examined the changes in Tshb expression at ZT 6, 16, and 20 on the first long day. Significant induction of Tshb expression was observed at ZT 16 in both sexes (one-way ANOVA and Fisher’s least significant difference (LSD) post hoc test; male: F(2,6) = 3.897029, \( P < 0.05 \) (Fig. 3a); female: F(2,6) = 11.06894, \( P < 0.05 \) (Fig. 3b), \( n = 3 \)).

Furthermore, we examined Dio2 expression under short-day- and long-day conditions. In both sexes, significant induction was observed following long-day stimulus (Mann-Whitney U-test, \( P < 0.05, n = 3 \)) (Fig. 4).

DISCUSSION

In the present study, we first examined the photoperiodic response of gonads and LH secretion in the red jungle fowl. Marked differences in both the testicular and ovarian weights were observed between short-day- and long-day birds. Although statistically significant differences in gonadal weight have also been reported in 12-week-old bantams (Sreekumar & Sharp 1998), the difference in testicular size was far greater in the red jungle fowl than in the bantams. Moreover, we observed a significant increase in plasma LH levels in both sexes of the red jungle fowl. In bantams, difference in photoinduced LH secretion was observed between sexes; in 12-week-old animals, the induction of LH secretion was more robust in females than in males (Sreekumar & Sharp 1998). However, in the present study on the red jungle fowl, we did not find any apparent differences in the photoperiodic response of the gonads and LH secretion between the sexes.

In Japanese quail, an increase in plasma LH concentration can be observed by the end of the first long day,
and this phenomenon is called the ‘first-day release model’ (Nicholls et al. 1983; Follett et al. 1998). In previous studies, we applied functional genomic analysis to study the first-day release model in quail and identified the molecular gene cascades regulating the photoperiod response of gonads in Japanese quail. We found that long day-induced TSH expression in the pars tuberalis triggers DIO2 expression in the MBH via the TSHR-cAMP signaling pathway, and this results in LH secretion from the pituitary gland. The long day-induced expression of the TSHB in the pars tuberalis is the earliest event in the photoinduction process recorded to date. In the present study, we examined the effects of long-day stimulus on the expression of TSHB and DIO2 in the red jungle fowl. We found rapid induction of TSHB during the photoinduction process on the first long day; this was consistent with the results obtained for Japanese quail. To our knowledge, this is the first demonstration of a first-day response to the photoinduction process in the chicken. In addition to the rapid induction of TSHB, we confirmed the long day-induced expression of the DIO2 gene – the key output gene of photoperiodism. All these results were consistent with that observed in Japanese quail and clearly suggest that the red jungle fowl responds to photoperiod both at the endocrine and molecular levels.

In addition to being a major food source, chickens are one of the predominant model species used for biological research; it bridges the evolutionary gap between mammals and non-mammalian vertebrates. Chicken embryos have been a powerful tool for developmental studies, and chickens have been used in seminal studies in virology, oncogenesis, and immunology (Hutt 1949; Cooper et al. 1966; Stehelin et al. 1976; Brown et al. 2003). Recently, draft sequences and initial analysis of the genome of the red jungle fowl (Gallus gallus) were reported by the International Chicken Genome Sequencing Consortium (2004). Thereafter, commercial chicken microarrays were released. Thus, to date, the red jungle fowl is the best

**Figure 3** Induction of TSHB gene expression during the first long day in (a) male and (b) female red jungle fowls. TSHB gene expression was measured by quantitative real-time RT-PCR (Q-PCR). Different characters indicate statistically significant differences (mean ± SEM, one-way ANOVA and Fisher’s least significant difference (LSD) post hoc test, *P* < 0.05, *n* = 3). Data were normalized with GAPDH expression levels. The bar below the graph shows the light/dark cycle of the first long day; the white bar indicates the 20-h light period, while the black bar indicates the 4-h dark period.

**Figure 4** Induction of DIO2 gene expression by long-day stimulus in (a) male and (b) female red jungle fowls. Expression of the DIO2 gene was measured by quantitative real-time RT-PCR (Q-PCR) (mean ± SEM, *P* < 0.05, by Mann-Whitney U-test, *n* = 3). Short-day birds were maintained under short-day conditions (6L : 18D) and long-day birds were maintained under long-day conditions (20L : 4D) for 2 weeks. The MBH samples were collected 6 h after dawn (ZT 6) both under short-day- and long-day conditions.
model for genome-wide transcriptional analysis among the various avian species. In the present study, we reported a robust photoperiodic response in the red jungle fowl. Thus, the red jungle fowl could be an ideal model organism for studying the molecular mechanism of seasonal reproduction in the future.

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REFERENCES


