THE EXPRESSION OF PITUITARY GLAND GENES IN LAYING GEESE

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ABSTRACT

The purpose of this study was to detect differential expression of genes in the pituitary gland in laying geese by suppression subtractive hybridization (SSH). Pituitary glands from pre-laying and laying geese were dissected for mRNA extraction. The cDNA from pituitary glands of pre-laying geese was subtracted from the cDNA from the pituitary glands of laying geese (forward subtraction). The reverse subtraction was also performed. We screened 384 clones with possible differentially expressed gene fragments by differential screening. Sixty five clones from the differential screening results were subjected to gene sequence determination and further analysis. We found that at least 19 genes were highly expressed in the pituitary glands of laying geese compared with pre-laying geese. Among these, six genes, including four novel genes were confirmed by virtual Northern analysis. We found that prolactin and visinin-like protein were highly expressed in the pituitary glands of laying geese compared with that from the pre-laying geese ($P < 0.05$). Further investigation is needed to demonstrate specific functions of the novel genes discovered in the current study.

KEY WORDS: Laying geese, Pituitary gland, Prolactin.

INTRODUCTION

The pituitary gland secretes several proteins involved in the function of egg laying in birds. For instance, luteinizing hormone and follicle-stimulating hormone are two of the most important hormones involved in regulating ovulation (Scanes *et al*., 1977). Prolactin is secreted from the anterior pituitary at a high level at the onset of egg laying in chickens and Japanese quail (Sharp *et al*., 1979; Goldsmith and Hall, 1980).

The goose is a short light period reproduction bird. With stimulation from a short lighting program, mature geese start ovulation and oviposition (Wang *et al*., 2005). The lighting program can be used to modulate the egg-laying period in geese (Wang *et al*., 2005). Understanding of gene expression in the pituitary of laying geese is the first step toward improving the low laying performance in geese. Therefore, the purpose of this study was to detect differentially expressed genes in the pituitary gland of laying geese by suppression subtractive hybridization (SSH).

MATERIALS AND METHODS

The animal protocol used in the present experiment was approved by the Animal Care and Use Committee of the National Chung Hsing University. The geese (6 geese per group) were purchased from a commercial goose farm and were raised according to the standard program used at the farm. The pre-laying geese were killed at the age of 5 months (average body wt = 4.2 + 0.6 Kg). The laying geese were killed at the age of 17 months (average body wt = 4.2 + 0.4 Kg). Geese were killed by electrical stunning coupled with exsanguination. Tissue samples were rapidly removed, wrapped in foil, frozen in liquid nitrogen, and then stored at -70^{0}C until analysis. The SSH procedure utilized the PCR Select Kit (Clontech, Palo Alto, CA), as previously detailed (Wang *et al*., 2006). The differential screening procedure followed the PCR-Select Differential Screening Kit User manual (Clontech). Details for the screening procedure were also described by Wang *et al*. (2006). Total RNA was extracted from the goose pituitary gland by the guanidinium-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987) with modifications (Wang *et al*., 2004). The virtual Northern analysis was performed for determining the concentrations of the transcripts of interest. The β-actin probe sequence was from a chicken gene fragment (Accession no. NM_205518). Hybridization results were quantified by phosphor-image analysis as previously described (Ding *et al*., 2004). All data were analyzed by Student's T-test using the procedures of the SAS software (SAS Institute, 2001).

RESULTS AND DISCUSSION

Three hundred and eighty four clones of gene fragments were subjected to differential screening to reduce false positive clones. Sequences of these differentially expressed gene fragments showed that there were at least 19 genes highly expressed in the pituitary glands of laying geese compared with pre-laying geese. Among these genes, six, including four novel genes were confirmed by virtual Northern analysis (Figure 1).

Prolactin mRNA was more than fivefold greater in laying geese compared with the pre-laying geese. The goose is similar to other poultry species in which prolactin is highly expressed in the pituitary gland of the laying birds (Kansaku *et al*., 2005). In avian species, prolactin is involved in reproduction, fat metabolism, and maternal behavior (Meier *et al*., 1965; Scanes *et al*., 1976; Proudman and Opel, 1981).

Visinin-like protein (VILIP) was highly expressed in the pituitary glands of laying geese compared with pre-laying geese (Figure 1; $P < 0.05$). The VILIP belongs to the superfamily of calcium sensor proteins involved in modulation of the activity of the acetylcholine receptor (Lin *et al*., 2002), mitogen-activated protein kinase signaling pathway (Spilker *et al*., 2002), and cAMP functions (Mahloogi *et al*., 2003; Gonzalez-Guerrico *et al*., 2005). The high expression of VILIP in the laying goose pituitary may be involved in regulating functions in aforementioned pathways, or it may be a direct result of photostimulation in the laying goose.

The function of the novel genes (PEUG 1 to 4) is not known, but they were highly expressed in the pituitary gland of the laying goose, suggesting the possible involvement of these genes in goose reproduction. Further investigation is needed to demonstrate specific functions of the novel genes discovered in the current study.

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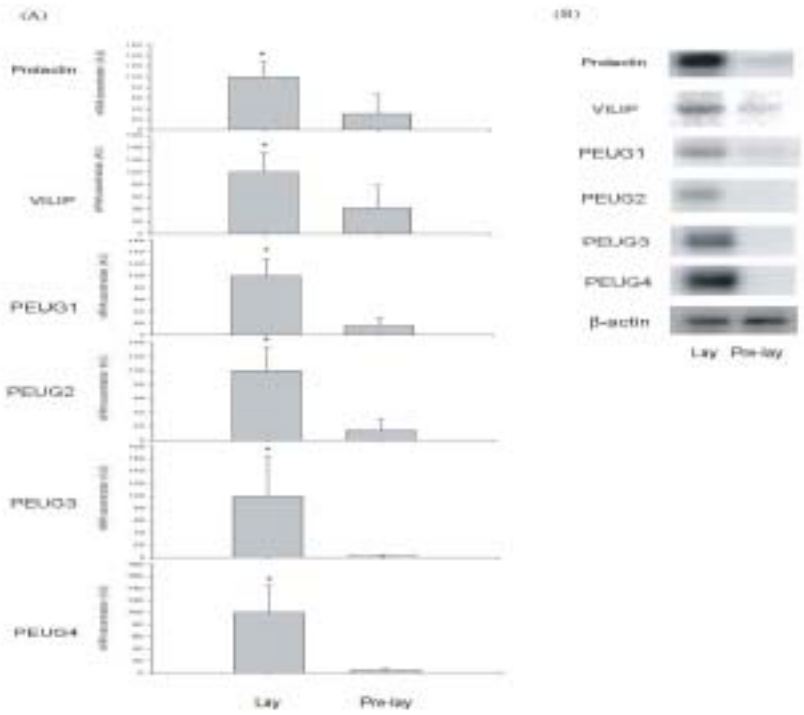

Figure 1. The differential expression of genes in the pituitary gland in laying geese (Lay) compared with pre-laying geese (Pre-lay). Bars in Figure 1(A) are means with SD. *Asterisk* denotes a significant difference between the groups ($P < 0.05$).