연제 5

Characterization of chicken ovalbumin gene 5′-flanking region

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Introduction

The chicken ovalbumin gene is an excellent model system to study the molecular mechanisms that underlie the hormonal, developmental, and tissue-specific regulation of gene expression(Sanders et al.,1995). This study was conducted to characterize 5′-flanking region of ovalbumin gene and to specially locate tissue-specific regulatory elements resided within the 5′-flanking region of the chicken ovalbumin gene by gene transfer into the chicken embryonic fibroblasts (CEFs) and oviduct tubular gland cells.

Materials and Methods

The chicken embryonic fibroblasts(CEFs) and HeLa cell line were transfected with human estrogen receptor and plasmid vectors containing the promoter and various length of 5′-flanking region of the chicken ovalbumin gene. Chicken oviduct cells were transfected with pOVCAT-3.5 and pOVLacZ-O.7 investigated responsiveness to estrogen ($5X10^{-9}$, $5X10^{-7}$, $5X10^{-5}$M), corticosterone and insulin. To determine the chicken ovalbumin gene expression, it was conducted that CAT ELISA, CAT Staining, X-gal staining, immunohistochemistry assay for ovalbumin.

Results and Disscussion

The constructs containing -1200 bp and -1350 bp region of 5′-flanking region(Fig.1) were induced by estrogen, corticosterone, insulin in CEFs and increased at higher concentrations of Estrogen. There was a remarkable reduction in the CAT activity when the ovalbumin 5′ -flanking region was extended from -1200 to -1350 bp. It was also not expressed when ovalbumin 5′ -flanking region was extended from -3500 bp. The construct containing -3500 bp region

was expressed in primary chicken oviduct cells, but repressed in CEFs and HeLa cell line. So, We confirmed the existence of *cis*-acting elements that are necessary for hormonal induction in 5 flanking region. It was observed remarkable reduction in the CAT activity when the ovalbumin 5′ -flanking region was extended from -1200 to -1350 bp, suggesting existence of another negative regulatory element(NRE). The construct containing -2800 bp region of 5′-flanking region was expressed both CEFs and HeLa cell line. however pOVCAT-3.5 was not expressed in both cells. As reported (Muramatsu, 1998), therefore, the tissue-specific silencer element may reside between -2800 bp and -3500 bp that represses the chicken ovalbumin gene expression in CEFs and HeLa cell line, but not in chicken oviduct cells

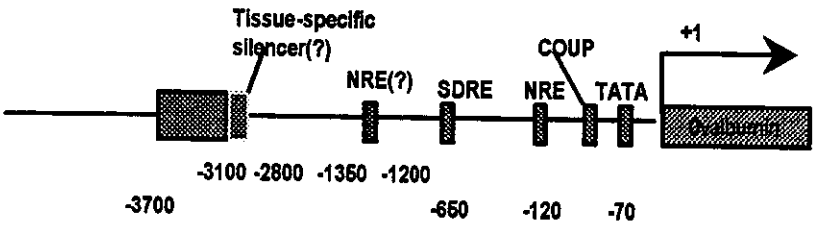

Fig.1 Putative tissue-specific silencer element and negative response element in the 5-flanking region of the chicken ovalbumin gene.
Abbreviations: 1/2EREDR - half estrogen response element direct repeats, SDRE-steroid dependent response element, NRE-negative response element, COUP-chicken ovalbumin upstream promoter, TATA-TATA box

(Key Words; CEF, HeLa cell line, chicken oviduct cells, hormonal induction, tissue-specific silencer)

References

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