The mechanism of transcriptional regulation of Müllerian inhibiting substance in Japanese flounder (*Paralichthys olivaceus*)

by

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**ABSTRACT.** - Müllerian inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), is a glycoprotein belonging to the transforming growth factor β superfamily. In Japanese flounder (*Paralichthys olivaceus*), a teleost fish that has XX(female)/XY(male) sex determination system, *mis* mRNA is expressed in the somatic cells of the gonads during gonadal sex differentiation. However, the mechanism of transcriptional regulation of the *mis* gene in the flounder has yet to be clarified. To elucidate the mechanism of transcriptional regulation of the *mis* gene in Japanese flounder, we first isolated the 5'-flanking region of the *mis* gene. Next, we investigated whether the *mis* gene expression was regulated by the steroidogenic factor 1 (SF1) and the liver receptor homologue 1 (LRH1), orphan receptors that are involved in the expression of MIS gene in mammals. As a result, electrophoretic mobility shift assay showed that both SF1 and LRH1 bound to a potential Ad4 site of the *mis* promoter region. Therefore, we suggest that SF1 and LRH1 are directly involved in transcriptional regulation of the *mis* gene in Japanese flounder.

**Key words.** - Japanese flounder - Müllerian inhibiting substance - Anti-Müllerian hormone - Steroidogenic factor 1 - Liver receptor homologue 1.

**Introduction**

Müllerian inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), is a glycoprotein belonging to the transforming growth factor β superfamily. In mammals, MIS is responsible for regression of Mullerian ducts, anlagen of the female reproductive ducts, in the male fetus. In Japanese flounder (*Paralichthys olivaceus*), a teleost fish which does not possess the Müllerian ducts, *mis* mRNA is expressed in the somatic cells of the gonads during gonadal sex differentiation (Yoshinaga *et al.*, 2004). However, the mechanism of transcriptional regulation of the *mis* gene in the flounder has yet to be clarified. As a first step to analyze the mechanism of transcriptional regulation of the *mis* gene in Japanese flounder, we investigated whether the *mis* gene expression was regulated by the steroidogenic factor 1 (SF1) and the liver receptor homologue 1 (LRH1), orphan receptors that are involved in the expression of MIS gene in mammals.

**Methods**

We first isolated the 5'-flanking region of the *mis* gene in Japanese flounder as described previously (Kitano *et al.*, 2006), and investigated using electrophoretic mobility shift assay (EMSA) whether SF1 and LRH1 bind to the Ad4 site in the putative promoter of the *mis* gene in the flounder. EMSA was performed by incubating Japanese flounder recombinant SF1 or LRH1 protein with the labeled *mis* promoter probe for 60 min. Competition experiments were conducted by adding 25-fold molar excess of unlabeled probes before the addition of the labeled probe.

**Results and discussion**

The 5'-flanking region of the *mis* gene isolated from the Japanese flounder was 701-bp in length (DDBJ accession no. AB304922). Comparing the 5'-flanking region of the flounder *mis* gene with the 5'-end of the flounder *mis* cDNA (Yoshinaga *et al.*, 2004), the *mis* promoter region was 656-bp in length from the putative transcription initiation site, and contained a potential Ad4 site (5'-CCAAGGACA-3'). EMSA showed that both SF1 and LRH1 bound to the Ad4 site of the *mis* promoter region (Fig. 1). These results indicate that SF1 and LRH1 have the capacity for binding to the site in the *mis* promoter in the flounder.

**Figure 1.** - Confirmation of bindings of the recombinant SF1 and LRH1 to an Ad4 site in the flounder *mis* promoter by EMSA. Arrows show the position of the protein-DNA complex.
Conclusions

EMSA showed that SF1 and LRH1 bound to a potential Ad4 site of the mis promoter region in Japanese flounder. Therefore, we suggest that SF1 and LRH1 are directly involved in transcriptional regulation of mis gene in Japanese flounder.

References
