Identification and gene expression profiling of chicken Pumilio family, Pum1 and Pum2

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Abstract

Members of the Pumilio are the RNA binding proteins acting as translational repressors and required for germ cell development and asymmetric division. We identified chicken Pum1 and Pum2 that are similar to mouse and human in highly conserved C-terminal RNA-binding domain and eight tandem repeats. The comparative sequence analysis of Pum1 and Pum2 from fly, chicken, mouse and human shows high degree of evolutionary conservation in the homology of the peptide sequence and the structure of PUM-HD (Pumilio homology domain) with similar spacing between adjacent Pum repeats. Also, structures of chicken Pum1 and Pum2 genes are almost identical to those of mouse and human. We revealed that the expression levels of Pum1 and Pum2 were the highest in hatched female gonad among various embryonic tissues, and Pum2 expressed highly in 12-day and hatched gonad by real-time RT-PCR. These results suggest that Pum1 and Pum2 might have an effect on the development of chicken gonad.

▸ **Key words** : chicken, Pumilio, RNA binding protein

Introduction

The Pumilio (Pum) genes were first identified in Drosophila (Lehmann and Nusslen-Volhard, 1987). Members of the Pumilio are required for germ cell development and asymmetric division. Asymmetric cell division is thought to play a critical role in self-renewal of somatic and germ-line stem cells (Jan and Jan, 1998; Morrison et al., 1997). Fem-Binding Factor (FBF)-1 and -2 protein, Pumilio homologs in C. elegans, play a critical role for germ-line stem cell maintenance and the sperm - oocyte switch (Crittenden et al., 2002). Also, it was suggested that a conserved mechanism of Pum function extends to the newly described mammalian members of the Pumilio family (human Pum1 and Pum2, and mouse Pum1 and Pum2), that could have an important role in cell development, fate specification and differentiation (Spassov and Jurecic, 2003). In the present study, we performed the sequence analysis and initial characterization of Pum1 and Pum2 in the chicken and we described the expression pattern of Pum genes in various tissues during chicken embryonic development.

Materials & Methods

Each mRNAs was obtained from adult tissues in 24-30 weeks, embryo tissues in stage X, 6-day, 12-day and hatched chick tissues of White Leghorn. To identify chicken Pum genes, we predicted the chicken Pum mRNA from Chicken BLAT Genome database. We performed the cloning of chicken Pumilio genes by RT-PCR amplification of pooled mRNA from chicken adult tissues. The genomic and protein sequences of chicken Pum1 and Pum2 were compared with Pum of Drosophila, mouse, and human

using clustal X 1.83. A neighbor-joining tree of the Pum protein sequences was constructed to the rooted tree by using PAUP*4.0. RT-PCR and SYBR Green quantitative real-time RT-PCR were performed to analyze the expression pattern of chicken Pum1 and Pum2. The relative quantification of gene expression is calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Result

We identified the transcripts of chicken Pum1 (3867bp) and Pum2 (3329bp). Interestingly, the comparison of Pum proteins shows high degree of evolutionary conservation in the peptide sequence and the structure of PUM-HD. The structure of chicken Pum1 and Pum2 is almost identical with the structure of mouse and human, having highly conserved exon size and exon/intron boundaries. Also, the evolutionary distance among Pum1 proteins is closer than that of Pum2 proteins. In real-time RT-PCR analysis, the expression amount of normalized Pum1 and Pum2 was the most (4.13 and 8.81-fold) in hatched female gonad among various embryonic tissues. In particular, the expression amount of Pum2 was the largest in hatched female gonad followed by hatched male gonad (5.25-fold), 12-day female gonad (3.38-fold) and 12-day male gonad (2.82-fold) in descending order. Among the adult tissues, the expression amount of normalized Pum1 and Pum2 was large in muscle (3.04 and 3.09-fold) and testis (2.48 and 4.33-fold).

적 요

Pumilio 유전자는 생식 세포의 발달과 분화에 중요한 역할을 한다고 알려져 있다. 우리는 이러한 Pumilio family인 Pum1, Pum2 유전자를 닭에서 클로닝하여 그 Pumilio homology domain의 구조와 단백질 염기서열이 초파리, 생쥐, 인간과 유사하다는 것을 밝혔고 이를 통해 이 유전자가 진화적으로 보존되어 있다는 것을 증명하였다. 또한 닭의 Pum1과 Pum2 genome 구조 역시 생쥐와 인간 Pum 유전자들의 구조와 일치하는 것을 보여주었다. Real-time RT-PCR 결과 닭의 배아의 여러 조직들 중 Pum1과 Pum2 유전자 모두 부화한 암컷 생식선에서의 발현 수준이 유의적으로 높았고, 특히 Pum2 유전자의 경우 부화한 병아리의 생식선뿐만이 아니라 12일령의 생식선에서도 발현 수준이 높았다. 결과적으로, 다른 동물에서 알려진 바와 같이 닭에서도 Pumilio 유전자들이 생식선 발달에 관여할 가능성이 크다는 것을 알 수 있다.

Reference

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