

## **Molecular cloning of an immune responsive gene (LMP7) in flounder**

Jung Jong Cho, Jae Hyung Lee, and Young Tae Kim  
Department of Microbiology, Pukyong National University

### **Introduction**

There are many evidences in similarities between the immune system of teleosts and higher vertebrates. For example, antigen-presenting cells and T and B like lymphocytes have been found in teleosts (Clem *et al.*, 1991) and specific proliferation of mitogen activated leukocytes can occur by the immunization experiments. The molecular mechanisms of immune response are being elucidated by the isolation of several fish cytokines. Teleosts have MHC (major histocompatibility complex) I and II gene clusters, however, not linked like in mammals (Trowsdale, 1993).

### **Materials and Methods**

**CELL ISOLATION AND PRIMARY CULTURE :** Head kidney, spleen tissue and blood were immediately removed and placed in culture media HBSS prior to isolation of leukocytes. Leukocytes were plated on tissue culture bottle and incubated at 15°C for 24 hours as primary cultures.

**RNA ISOLATION AND LIBRARY CONSTRUCTION :** Media were removed from the cell culture and total RNA was isolated using TRIzol reagent (Gibco). The construction of cDNA library was performed using ZAP-cDNA synthesis Kit (Stratagene).

**Screening of LMP7 and sequence analysis :** Leukocyte cDNA clone was screened using mass *in vivo* excision method and excised according to the manufacturer's instructions (Stratagene). The isolated cDNA clone was reacted with ABI PRISM™ Dye reagent (Perkin Elmer) and sequenced with ABI 310

Genetic Analyzer (Perkin Elmer).

**PHYLOGENETIC ANALYSIS:** The phylogenetic tree was constructed by using the program of the GeneDoc and TreeView.

**REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION :** Total RNA was isolated from the tissues of brain, liver, spleen, heart, kidney, and leukocyte. One  $\mu\text{g}$  of total RNA was used as a template. Access RT-PCR System (Promega) was used for RT-PCR.

## Results and Discussion

Fish are the largest and most diverse vertebrates. Their evolutionary position relative to other vertebrates and their ability to adapt to a wide variety of environments make them ideal for studying both organismic and molecular evolution. The low molecular weight protein (LMP7) is a subunit of the proteasomal complex and is specifically associated with the generation of peptides from intracellular proteins before they are presented on the MHC class I. Several LMP genes have been studied in fish, and some have analyzed at the molecular level.

In this study, the results provide us the phylogenic evidence on the LMP7, which may be essential to understand the molecular evolution of this gene in vertebrates. It would be further necessary to conduct a comparative analysis of the structure, expression and function of the gene in order to elucidate the mechanism that is responsible for the immune system by the new LMP7. In conclusion, the flounder LMP7 cDNA sequence is well conserved with those of other teleost fish and LMP7 mRNA is expressed in lymphoid tissues including spleen, leukocyte, kidney, liver and heart.

## References

- Clem, L W., Miller, N. W. and Bly, J. E. (1991). Evolution of lymphocyte populations, their interactions and temperature sensitivity. In *Phylogensis of the immune system*. (G.W. Warr & n. Cohen, eds), pp.191-213, CRC Press, Boca Raton, FL, U.S.A.
- Trowsdale, J. (1993). Genomic structure and function in the MHC. *Trends in Genetics* 9, 117-122.