Comparison of Immunoglobulin Heavy Chain (IgH) Locus of Zebrafish and Fugu

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Introduction

Immunoglobulins are composed of light (IgL) and heavy chain (IgH) genes. The respective chains are created by somatic re-arrangement of a limited number of germline genes. The organization of these genes and mechanism by which they recombine vary considerably among different taxa. To understand, how this mechanism evolved and diversified, it is necessary to study the immunoglobulin heavy and light chain loci in different vertebrates. The genomic organization of IgH locus has not been fully understood in teleosts. Recently, the genomic data sequence release of pufferfish *Fugu rubripes* [http://fugu.hgmp.mrc.ac.uk] and zebrafish *Danio rerio* [http://www. sanger.ac.uk/] has helped in the acceleration of gene discoveries. In this study, we have analyzed the detailed structure of immunoglobulin heavy chain locus of fish using sequences from these databases.

The heavy chain variable (V_H) region

From zebrafish genomic contig of 1104655 bp in length, V_H segments were identified. We found 49 V_H segments in a 100 kb locus, and could be classified into thirteen families. The identified V_H segments were in the same transcriptional

orientation in relation to the μC constant genes. Among the 49 V_H genes, at least 13 genes could be linked to the expressed genes registered in DDBJ/EMBL/GenBank databases. On the other hand, 10 genes, which had truncation's, encountered termination and no recombinantion signal sequences (RSS) in the V_H regions, were characterized as pseudo-genes. In fugu, we could not determine the total number of variable regions due to the incomplete genomic contig derived from the database.

Diversity (D_H) regions

In zebrafish, nine D_H segments were recognized by the analysis of the genomic region and the transcripts of IgZ and IgM. Four D_H segments were placed downstream of V_H region, and another five were present downstream of Tm2 region of the Cζ gene. In fugu, upstream of the Cτ and Cμ regions one ($D_{H_τ-1}$) and four ($D_{H_μ-1}$ to $D_{H_μ-4}$) D_H segments were identified, respectively. These D_H segments were composed of 10 to 42 bp of coding nucleotides and 12±1 bp gap between RSS elements with conserved heptamers and nonamers.

Joining (J_H) region

The J_H segments have a characteristic WGXG motif.. In this investigation, we found a total of 7 J_H segments in front of IgZ and IgM domains in zebrafish. Two JH segments were placed in front of IgZ region, other five were upstream of Cμ1. Each J_H segment has an upstream recombination signal sequence, which includes a T-rich nonamer, a 22 to 23 spacer, and a heptamer. In fugu, One J segment was located at in front of IgT region, and other five J segments were IgM region.

Constant regions

τ/ζ region

A novel immunoglobulin isotype was discovered from the heavy chain loci in zebrafish (IgZ) and fugu (IgT). These domains are placed between V_H and J_H regions upstream of Cμ. The zebrafish IgT possesses, four constant domains and a transmembrane region, present as four (ζC1 to ζC4) and two exons (ζTM1 and ζTM2) encoding respective regions. The comparative amino acid sequence identities of IgT were: 16.7% to zebrafish IgM, 15.1% to fugu IgM, 14.7% to Atlantic salmon (*Salmo salar*) IgM, 7.8% to zebrafish IgD, 8.2% to fugu IgD, 4.7% to Atlantic salmon IgD, and 9.4% to shark IgW. When compared with human immunoglobulin isotypes, the identities to zebrafish IgZ were: 16.0% to IgM, 12.1% to IgA, 12.0% to IgE, 13.3% to IgG and 16.0% for IgD. Thus, these results are all suggestive that the zebrafish IgT is new isotype of immunoglobulin heavy chain. However, in fugu the novel IgH was made up of two constant domains (C1-C2) and two transmembrane regions (TM1 and TM2). The percentage amino acid identity to zebrafish IgZ was very low (14.1%).

μ region

Fish μ gene is composed of four constant region exons (Cu1 to Cu4), and two TM exons (μTM1 and μTM2). The μ gene in fish conforms to the organizational pattern seen in other vertebrates. Zebrafish and fugu μ genes are also composed of four constant region and two trans-membrane encoding exons.

δ region

Fish IgD is composed of seven constant region exons (Cδ1 to Cδ7) and two TM exons (δTM1 and δTM), except in Atlantic cod where the IgD is composed of three

δ exons (Cδ1, Cδ2 and Cδ7). The zebrafish IgD gene is located downstream of the IgM genes and consists of 16 constant domains and two TM regions. This gene is organized as follows in the genome of zebrafish: δ1-[δ2-δ3-δ4]₂-δ5-δ6-δ7-δtm1-δtm2. On the other hand, fugu IgD consists tandem duplications of δ genes (Cδ1-Cδ2-Cδ3-Cδ4-Cδ5-Cδ6)₂ and transmembrane region (δtm1-δtm2). The gene structure of IgD has already been reported from catfish, *Ictalurus punctatus* (Wilson et al., 1997) Atlantic salmon (Hordvik, 2002), Atlantic cod *Gadus morhua* (Stenvik and Jorgensen, 2000), Atlantic halibut *Hippoglossus hippoglossus* (Hordvik, 2002) and Japanese flounder *Paralicthys olivaceus* (Srisapoome et al., 2004).   Tandem duplications of Cδ1-Cδ2 in Atlantic cod and Cδ2-Cδ3-Cδ4 in Atlantic halibut, Atlantic salmon and catfish, have already been reported.   On the other hand, no duplications of δ domains have been seen in Japanese flounder.   Interestingly, the first Cδ2 domain in zebrafish has an internal stop codon.   We have been unsuccessful in identifying transcripts of δ gene in zebrafish from cDNA database and cDNA libraries.   Thus, it is still not known whether zebrafish IgD transcripts are expressed.

Conclusions

We have characterized the IGH loci in zebrafish and fugu.   This study has lead to the identification of three immunoglobulin class (IgM, IgD and IgT/Z)).   The transcripts of this gene has already reported by Steiner et al.(2005) and Savan et al.(2005).   Further investigation should be needed to determine the functions of these isotypes in fish.

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