

**MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I GENES IN
RAINBOW TROUT (*Oncorhynchus mykiss*).**

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Introduction

The Major Histocompatibility Complex (MHC) is a group of genes located together on a single chromosome in mammals which encode highly polymorphic proteins (Klein 1986). These proteins are translocated to the cell surface carrying foreign antigens which they present to T-cells, initiating immune responses. There are two classes of MHC molecules. Class I MHC is a heterodimer expressed on the surface of most cells, consisting of a *Mr* 45,000 alpha chain and a *Mr* 12,000 beta chain, referred to as β 2-microglobulin. Class I MHC antigens present intracellular antigens to cytotoxic T-cells (Klein 1986). Class II MHC molecules are also heterodimers composed of an alpha and beta chain, both of which are approximately *Mr* 30,000. Class II MHC proteins are expressed by specialised antigen presenting cells (APC) and these present extracellular antigens to helper T-cells, which initiate humoral immune responses (Klein 1986). MHC genes have been reported present in all classes of vertebrate, with the exception of the most primitive, the jawless fishes (Kaufman, Salomonsen et al. 1994; Dixon, van Erp et al. 1995; Trowsdale 1995). Our objective is to study all aspects of teleost class I MHC genes and proteins, using the rainbow trout (*Oncorhynchus mykiss*) as a model system.

Teleost MHC genes

The study of MHC genes in teleost fish has, to date, focused on isolating and characterising genomic and cDNA sequences from many species (Dixon, van Erp et al. 1995). MHC sequences have been obtained from nearly 30 species of fish since their first isolation in 1990 (Hashimoto, Nakanishi et al. 1990). Unfortunately, these are usually only fragments of genes, obtained by polymerase chain reaction. Full length clones of MHC genes encoding all

the chains of both class I and class II MHC antigens have been found for only a few species. Rainbow trout MHC genes are quite well studied, with sequences available for MHC class II beta (Glamann 1995), β 2-microglobulin (Shum, Azumi et al. 1996) and four MHC class I alpha sequences, which may correspond to different loci (Shum, Azumi et al. 1996, Shum et al., unpublished data), at least one of which thought to be equivalent to a mammalian "classical" class I gene.

Teleost MHC gene studies have revealed that while the genes and proteins are structurally similar to their mammalian counterparts, they possess many unique features, as well. β 2-microglobulin in mammals is encoded by a single copy gene, located outside the MHC. Due to similarities in sequence and structure, it is thought that β 2-microglobulin shares an evolutionary origin with the other MHC genes, but was translocated out of the MHC at some point in evolution. Initial studies reporting the cloning of β 2-microglobulin from tilapia, carp (Dixon, Stet et al. 1993) and zebrafish (Ono, Figueroa et al. 1993) indicated that it was also a single copy gene. This was not true for rainbow trout, however. Shum et al. (Shum, Azumi et al. 1996) observed multiple bands on Southern blots and cloned 12 distinct cDNAs from a single individual. While the existence of multiple copies of genes may be explained by the tetraploid state of rainbow trout, this cannot explain all of the polymorphism. One possible explanation for this increased polymorphism may be that rainbow trout possess an ancestral MHC organisation in which the β 2-microglobulin gene is still located within the MHC. Our laboratory is attempting to answer this question by screening a genomic library selected for large fragments (>20 kb) and examining the linkage patterns of the resultant clones. We are currently analysing 12 β 2-microglobulin genomic clones and 57 class I genomic clones. We also hope to answer this question using Fluorescence In Situ Hybridisation (FISH).

Teleost Class I MHC genes have evolved in a unique manner. In mammals, class I genes from different species which have diverged up to 70 million years ago are so different that they cannot be grouped into lineages. Teleost MHC class I genes, however, can be grouped into lineages which are well over 150 million years old, and may be up to 350 million years old. Genes from at least three such lineages can be found in the carp, while homologous sequences are present in distantly related species, such as salmon and the coelacanth (van Erp, Egberts et al. 1996). Our laboratory is currently searching for new class I MHC sequences from the rainbow trout in order to fully investigate the novel nature of class I MHC evolution in teleosts.

MHC gene expression in teleosts

Most knowledge of MHC gene expression patterns is derived from Northern blotting or isolation of cDNA sequences, which does not guarantee that MHC antigens are expressed and functional in the cells or tissues examined (Dixon, van Erp et al. 1995). The use of bacterial expression systems to produce recombinant proteins from cDNA clones has allowed the production of polyclonal antisera. These have been used in initial studies of MHC antigen expression in the carp (Rodrigues, Dixon et al. 1996; van Erp, Dixon et al. 1996), but these studies are far from complete.

In general, mRNAs from teleost MHC genes are present in tissues with immunological functions and cells of lymphoid lineage. Both class I and class II mRNAs are present in liver, but are not detectable in other non-lymphoid organs, such as heart, or brain. No MHC class I transcripts are detectable in the muscle or gonad of teleosts, despite the fact that some class I has been detected in human gonads, and mammalian muscles express class I MHC. This may, however, only reflect the nature of the MHC genes examined so far in teleosts, since only a small number of genes have been studied, and the classical or non-classical nature of class I genes can only be inferred from sequence data. The studies using polyclonal antisera raised against recombinant proteins tend to support the Northern blot and cDNA data.

The main differences in MHC expression between teleosts and other vertebrate groups seem to occur in two types of blood cells; thrombocytes, the lower vertebrate equivalent of platelets, and erythrocytes. Thrombocytes do not show any cell surface class I or β 2-microglobulin in carp (Rodrigues, Dixon et al. 1996; van Erp, Dixon et al. 1996), yet

chicken thrombocytes and mammalian platelets both express class I antigen. Mammalian erythrocytes do not express MHC class II on their cell surface, but do express class I. Erythrocytes in lower vertebrates are nucleated and chicken, reptiles, and amphibians all express MHC class I. Axolotl erythrocytes even express MHC class II. Teleost erythrocytes, which are nucleated but transcriptionally silent, do not express any MHC antigens on their cell surface. This may, however, merely reflect the fact that MHC loci or alleles which have not been isolated yet are expressed there.

Mammalian class I MHC and β 2-microglobulin cDNAs have been used to produce recombinant proteins in a variety of systems, including mouse cells (Mage, Lee et al. 1992), *Drosophila* cells (Jackson, Song et al. 1992; Matsumura, Saito et al. 1992; Stura, Matsumura et al. 1992) and *E. coli*. (Parker and Wiley 1989; Garboczi, Hung et al. 1992; Parker, Carreno et al. 1992; Parker, Silver et al. 1992; Pedersen, Stryhn et al. 1995). In the latter reports, investigators were able to combine recombinant class I heavy chain, β 2-microglobulin and peptides in denatured states, then renature them to produce a complete, correctly folded class I complex. This system has been optimised for immunoglobulin family members by adding reduced and oxidised low molecular weight thiol compounds and labilizing agents such as L-arginine to the reconstitution buffer in order to promote the formation of the correct disulphide bonds (Buchner and Rudolph 1991; Garboczi, Hung et al. 1992). This system can be used to renature up to 40% of the added proteins. We are currently producing recombinant class I MHC and β 2-microglobulin, which will be refolded as above and used to produce high quality monoclonal antibodies. We hope to use these antibodies to further investigate MHC expression and function in teleosts.

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