Differences in MHC class I genes between strains of rainbow trout (Oncorhynchus mykiss)

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In rainbow trout there is only one dominant classical MHC class I locus, Onmy-UBA, for which four very different allelic lineages have been described. The purpose of the present study was to determine if Onmy-UBA polymorphism could be used for strain characterisation. This was performed by lineage-specific PCR investigation of 30 fish, each of the Nikko and Donaldson strains, and by sequence analysis of 25 of the amplified DNA fragments. Two new MHC class I lineages were detected in addition to the four previously described lineages, thus six distinct lineages were observed within the fish examined (Sal-MHCia*A–F). The distribution of lineages appeared to be strain-specific. For example, the lineage Sal-MHCia*A was very common in the Nikko strain but could not be detected in the Donaldson strain. Analysis of MHC class I variation may help to elucidate relationships between strains and the roles of MHC alleles in disease resistance. © 2002 Elsevier Science Ltd.

Key words: MHC, polymorphism, lineages, strain, rainbow trout.

I. Introduction

Classical MHC class I molecules are involved in cell surface presentation of peptides, derived from intracellular proteins, for recognition by T cell receptors (TCR) of CD8 positive T lymphocytes [1, 2]. When presented non-self peptides T cells can be stimulated, this results in killing the presenting cell. This screening mechanism is the primary immune defence against many viral diseases [3, 4].

One characteristic of classical MHC class I is extensive polymorphism. The polymorphism results in the presentation of different sets of antigenic peptides by each allomorph (protein expressed by an allele), and may result in
differences in disease resistance. Well-characterised correlations between MHC class I alleles and disease resistance are described for Marek’s disease in chickens [5, 6], and malaria [7] and HIV [8] in humans.

In aquaculture there is extensive interest in the characterisation of unique strains within different fish species [9, 10]. Characterisation of strains with respect to their histories and relationships is expected to guide strain improvement, and knowledge regarding polymorphic genes could aid this characterisation.

Classical MHC class I is known to be one of the most polymorphic genes [11], and its polymorphism has been successfully used to characterise populations of several species including humans [12], horses [13], and cattle [14]. Natural populations of chinook and coho salmon have been distinguished by their MHC class I polymorphism [15–18]. One advantage of MHC class I as a genetic trait marker is that allelic differences may relate to differences in disease resistance.

Previously, it has been shown that there is only one dominant expressed classical MHC class I locus in rainbow trout, designated *Onmy-UBA* [19]. Among 14 fish, of which five were distinct homozygous clones, 10 different *Onmy-UBA* alleles were detected [19]. The alleles of the locus were shown to be classical by conservation of key amino acids, high variability in the a1 and a2 domains and ubiquitous expression. A remarkable variability in the a3 domain was observed, whereas for MHC class I loci in higher vertebrates and sharks [20] this region is quite well conserved. This may be due to the ancient character of the *Onmy-UBA* locus or to different stringencies with respect to interactions with T lymphocytes. The 10 alleles in rainbow trout were placed into four lineages (Sal-MHCIA*–D) based on similarity of a1, a2 and a3 domains [19]. MHC class I sequences of other salmonid species could also be placed into these lineages [19]. In the present study significant differences are shown in the *Onmy-UBA* alleles of the Nikko and Donaldson rainbow trout strains present in Japan.

### II. Materials and Methods

**FISH**

The fish used in this study were randomly selected 30–100 g Nikko or Donaldson strain rainbow trout from the National Research Institute of Aquaculture (Nikko, Japan). Fish were held in 9°C flowing water. Both strains were maintained by moderate inbreeding using 5–10 males and 20–30 females for annual reproduction, which is a common practice in Japan. The Nikko strain was imported from the U.S.A. before 1951, but its exact origin is unclear [21]. The Nikko strain fish have classic rainbow trout characteristics. The Donaldson strain [22] was imported from the U.S.A. in 1966 [21]. Large size, rapid growth and high fecundity characterise these fish.

**PRIMERS USED**

The specificities of the primer combinations used for detection of the Sal-MHCIA*–F lineages in Nikko and Donaldson strains are detailed below,
followed by their specificities for other lineages detected by us (in other strains) or by other research groups.

For the generic amplification of sequences belonging to lineages Sal-MHCla*-A, -C, -D, -E, or -F, a primer from the leader peptide encoding region, \(pG-LPf\), \(^5\)-GTATTATCTTGCTGGTGCTGGGAA (forward), was used in conjunction with a primer from the 3’UTR, \(pG-3’UTRr\), \(^5\)-TTATGTTCTTGAGAAGTCCTCTTC (reverse). This primer set was also shown to amplify rainbow trout sequences belonging to lineages Sal-MHCla*-G and -H (data not shown).

For the specific amplification of Sal-MHCla*A sequences, the primer \(pG-LPf\) was used in conjunction with a primer overlapping the end of the \(a3\) region and the start of the transmembrane region \(pI-a3/TMr\), \(^5\)-GGGGACACGTTGGCGCATCCKGG (reverse). Primer \(pI-a3/TMr\) is specific for sequences with a type I \(a3\) region. This primer set also amplified sequences from the lineage Sal-MHCla*H (data not shown).

For the specific amplification of Sal-MHCla*B sequences, the primer \(pI-LPf\), \(^5\)-ATGAAGTCTTTCATCATKTGCTCC (forward), which binds to the leader peptide encoding region of all published sequences with a type IV \(a1\) region, was used in conjunction with primer \(pG-3’UTRr\). This primer set is also expected to amplify sequences from the Sal-MHCla*K lineage.

For the specific amplification of Sal-MHCla*C sequences, the primer \(pI-LPf\), \(^5\)-CTACACCGSATCTTCTGAAGTTCCCA (forward), binding to type I \(a1\) regions, was used in conjunction with primer \(p5-a2r\), \(^5\)-AATGTTTATCCTCCGT CATCAT (reverse). Primer \(p5-a2r\) binds specifically to the \(a2\) region of the Sal-MHCla*C sequences thus far described for rainbow trout.

For the specific amplification of Sal-MHCla*D sequences, the primer \(pI-LPf\) was used in conjunction with a primer overlapping the end of the \(a3\) region and the start of the transmembrane region \(pIII-a3/TMr\), \(^5\)-CATTTGAACCCCTGTTGGTCTTT (reverse). Primer \(pIII-a3/TMr\) is specific for sequences with a type III \(a3\) region.

For the specific amplification of Sal-MHCla*E sequences, the primer \(pII-a1f\), \(^5\)-GCCAAGACTGAGGGGTCTGACTAC (forward), was used in conjunction with primer \(pIII-a3/TMr\).

For the specific amplification of Sal-MHCla*F sequences, the primer \(pVI-a1f\), \(^5\)-TCCAGAAAGCTGAGTGTA TCAGTG (forward), was used in conjunction with a primer overlapping the end of the \(a3\) region and the start of the transmembrane region \(pII-a3/TMr\), \(^5\)-GGGGCTGGGTCATTCCAGTGGTAGTTT (reverse). Primer \(pII-a3/TMr\) is specific for sequences with a type II \(a3\) region.

**CONDITIONS OF RT-PCR**

Total RNA was isolated from whole blood with ‘TRIzol’ reagent (Gibco BRL, Life Technologies, Grand Island, U.S.A.) following the manufacturers recommendations. *Onmy-UBA* expression was analysed by RT-PCR amplification using a ‘RT-PCR high-PLUS’ kit (Toyobo, Osaka, Japan). The 25 \(\mu\)l RT-PCR reaction mixtures were set up as suggested by the manufacturer, with 2·5 mmol Mn(OAc)\(_2\), 1 \(\mu\)mol of each primer and 0·5 \(\mu\)g total RNA. The conditions for
amplification with the different primer sets were: For the primer combinations pG-LPf with pG-3'UTRr, pG-LPf with pI-a3/TMr, and pIV-LPf with pG-3'UTRr: First 60°C for 98 min, then 94°C for 2 min, then 35 cycles of 94°C for 1 min followed by 55°C for 5 min, and finally 55°C for 7 min. For the primer combinations pI-a1f with p5-a2r and pI-a1f with pIII-a3/TMr: First 60°C for 30 min, then 94°C for 2 min, then 25 cycles of 94°C for 1 min followed by 65°C for 1·5 min, and finally 65°C for 7 min. For the primer combinations pIII-a1f with pIII-a3/TMr and pVI-a1f with pII-a3/TMr: First 60°C for 98 min, then 94°C for 2 min, then 35 cycles of 94°C for 1 min, 60°C for 5 min, and finally 60°C for 7 min.

SEQUENCE ANALYSIS

Amplified RT-PCR fragments were cloned into the vector pGEM-T Easy (Promega Corporation, Wisconsin, U.S.A.). The nucleotide sequences were determined by the dideoxychain termination method using a ‘CEQ Dye terminator Cycle Sequencing Kit’ (Beckman Coulter, Inc., California, U.S.A.) and suitable primers. Sequence analysis was performed with an automated sequencer (CEQ 2000 DNA analysis system, Beckman Coulter, Inc.). Comparison of deduced amino acid sequences was performed using the programs ‘Search homology’, ‘Multiple alignment’, and ‘UPGMA’ of GENETYX version 10.1 (Software Development Co., Ltd, Tokyo, Japan) computer software.

GENBANK ACCESSION NUMBERS

New sequences described in this report have been deposited in GenBank under the accession numbers AF318188–AF318190.

III. Results

RT-PCR ANALYSIS

In a previous study four different classical MHC class I lineages Sal-MHCIA*-A, -B, -C and -D were detected in rainbow trout [19]. In the present study lineage-specific RT-PCR assays were established (see Materials and Methods). These assays were based on primers that were expected to bind to all of the rainbow trout MHC class I sequences reported for each respective lineage. Lineage definitions are discussed below.

Total RNA from the blood of 60 rainbow trout was tested with Sal-MHCIA lineage-specific primer combinations following reverse transcription. A number of the RT-PCR fragments (25) were sequenced (see below) and two additional lineages, Sal-MHCIA*-E and -F, were detected. For these new lineages specific RT-PCRs were established as well. The RT-PCR results are summarised in Table 1. The results indicated that in 41 of the 60 fish at least two different MHC class I sequences were present, and for one Donaldson fish three different fragments were amplified. The main difference between the two rainbow trout strains was that sequences belonging to lineage A were very common in the Nikko strain but were not detected in the Donaldson strain. In
addition, the C lineage was absent in the Nikko strain fish and the E lineage was absent in the Donaldson strain fish.

In Table 1 the lineages Sal-MHCia*-G, -H, and -K are indicated between brackets. These lineages were discovered in other rainbow trout strains during the course of this study. Sequences belonging to these lineages would probably be amplified by the RT-PCR systems used, but they were not detected in the Nikko and Donaldson strains.

SEQUENCE ANALYSIS

The 25 amplified and sequenced RT-PCR fragments derived from 13 fish are shown in Table 1. For each fragment the complete sequences from at least three clones were determined to exclude PCR mistakes, the exact number depending on the quality of the sequence information obtained and whether the fragment contained one or two Onmy-UBA sequences. The sequences
detected were Onmy-UBA*401, -501, -0501, -701, -1401, and -4901. The sequences Onmy-UBA*401, -701 and -4901 were present in both the Nikko and Donaldson strains. Onmy-UBA*401, -501 and -701 had previously been detected in other rainbow strains [19]. The sequences Onmy-UBA*501 and -1401 were recently submitted to the GenBank by Shum et al. [23] (GenBank AF296362 and AF296371). The sequence Onmy-UBA*4901 has not been previously published. The new sequences were numbered from 4901 downwards, since Shum et al. [23] recently submitted Onmy-UBA sequences numbered through Onmy-UBA*1601 to the GenBank database, and it was necessary to avoid giving the same designations. It is unfortunate that, more or less simultaneously, sequences were designated as Onmy-UBA*-101 to -701 [19] while others designated different Onmy-UBA sequences as Onmy-UBA*0101 to -0701 [23] (Genbank AF091785, AF296359–AF296364).

In Fig. 1 the deduced amino acid sequences of the extracellular domains of the different alleles detected in the present study are compared, with a description of features typical of classical MHC class I molecules.

HOMOLOGY COMPARISON

The new sequences obtained were placed into lineages based on similarities in a1, a2 and a3 domains as done by Aoyagi et al. [19]. Sequences were placed into the same Sal-MHCIA* lineage when (i) the a1 and a2 domains each had over 67% amino acid identity with the bulk of the other lineage members and were grouped together by UPGMA tree analysis, and (ii) the a3 domains had a similar length.

A simplified form of the UPGMA tree analysis for the a1 and a2 domains, in which homologous sequences are indicated as groups instead of individual sequences, is shown in Fig. 2. In addition to salmonid sequences several cyprinid MHC class I sequences were compared, in order to show conservation of lineages between evolutionarily distant fish. The figure legend describes which sequences were compared. Sequences within the a1 and a2 homology groups, indicated with Roman numerals, share greater than 67% amino acid identities with the bulk of the other members in the group. With this definition six different a1 groups and two different a2 groups were established for rainbow trout. The a1 homology group V was divided into Va and Vb, as the UPGMA analysis clustered them close together, but the cyprinid sequence Brre-UAA*01 showed greater than 67% amino acid identity with only a few of the members of group Va. The a3 region was divided into three different groups, each of them with a different length of the C-terminus: I has no extension, II has a short extension, and III a long extension (compare Fig. 1 and Table 2). The C-termini of the a3 domains were based on the expected intron-exon borders, in comparison with the sequences Onmy-UBA*401, -501, and -701 for which these borders had been determined (unpublished data).

The sequences compared in Fig. 2 that encompass the a1, a2 and a3 domains are schematically compared in Table 2. The Roman numbers in Table 2 refer to those in Fig. 2. By lineage definition the sequence Onmy-UBA*501 can be placed into a new lineage Sal-MHCIA*E, and Onmy-UBA*4901 can be placed into a new lineage Sal-MHCIA*F. Table 2a shows the Sal-MHCIA
Fig. 1. Predicted amino acid sequences of the extracellular domains of the Onmy-UBA*-1401, -401, -501, -701, -0501, and -4901 molecules detected in Nikko and Donaldson strain rainbow trout. The sequences Onmy-UBA*-401, -501 and -701 have been already described by Aoyagi et al. [19] (GenBank AF287487, AF287488, AF287492) and the sequence Onmy-UBA*-0501 and -1401 by Shum et al. [23] (GenBank AF296362 and AF296371). Alignment was performed by computer software and modified by hand. Numbering of amino acids was performed for the Onmy-UBA*-1401 sequence, starting from the α1 domain. Dashes indicate identical amino acids; asterisks indicate gaps in the sequence; filled circles indicate the conserved positions believed to interact with antigenic peptide termini in mammals (Madden [32]); P indicates positions believed to interact with the antigenic peptide and determine its specificity in mammals (Matsumura et al. [33]); C indicates conserved cysteine residues. The box at position 86 indicates a conserved N-glycosylation site.
$\alpha_1$

\begin{align*}
\text{I} & \quad (36\text{rt}, 6\text{bt}, 1\text{as}, 1\text{ps}; \leq 0.145) \\
\text{II} & \quad (4\text{rt}; \leq 0.012) \\
\text{III} & \quad (3\text{rt}; \leq 0.006) \\
\text{VI} & \quad (2\text{rt}, 2\text{bt}, 1\text{ca}; \leq 0.145) \\
\text{Va} & \quad (5\text{rt}, 1\text{bt}; \leq 0.166) \\
\text{Vb} & \quad (1\text{zb}) \\
\text{IV} & \quad (8\text{rt}, 1\text{bt}; \leq 0.052) \\
\text{VII} & \quad (1\text{zb})
\end{align*}

$\alpha_2$

\begin{align*}
\text{I} & \quad (38\text{rt}, 9\text{bt}, 1\text{as}, 1\text{ps}; \leq 0.217) \\
\text{II} & \quad (17\text{rt}, 1\text{bt}, 1\text{ca}, 2\text{zb}; \leq 0.181)
\end{align*}

Fig. 2. UPGMA analysis. The amino acid sequences of the $\alpha_1$ and $\alpha_2$ regions of published rainbow trout (rt) MHC class Ia molecules and those of several other fish [brown trout (bt), Atlantic salmon (as), pink salmon (ps), common carp (ca) and zebrafish (zb)] are compared. Amino acid substitutions per site is indicated on the horizontal bars and is also represented by bar length. Division into $\alpha_1$ and $\alpha_2$ regions is in agreement with Fig. 1. The clustering of the individual sequences is shown in a simplified manner by depicting only the homology groups, indicated with Roman numerals. The number of sequences included in each group, as well as the amino acid substitutions per site between group members, is indicated between parentheses. Compared are: the rainbow trout sequences Onmy-UBA$^\text{101–701}$ by Aoyagi et al. [19] (GenBank AF287483–AF287492), Onmy-UBA$^\text{4801–4901}$ (this report; GenBank AF318188–AF318190) and Onmy-UBA$^\text{0101–1601}$ by Shum et al. [23] (GenBank AF091785, AF296359–AF296373, AJ007847). Also the partial rainbow trout sequences Onmy-A$^\ast$- and Onmy-UA$^\ast$- reported by Miller et al. [17] (GenBank AF104523–4529 and AF104579–4582), Onmy-UA$^\ast$-, Onmy-UAA$^\ast$-, Onmy-UCA$^\ast$-, and Onmy-UBA$^\ast$- reported by Hansen et al. [30] (GenBank AF002171–2179 and AF115518–5528), the brown trout sequences Satr-UBA$^\text{0101–1001}$ by Shum et al. [23] (GenBank AF091785, AF296359–AF296373, AJ007847), the Atlantic salmon sequence Sasa-p30 by Grimholt et al. [34] (GenBank L07606), the pink salmon sequence Ongo-UA-(92H) by Katagiri et al. [35] (GenBank D58386), the common carp sequence Cyca-UA$^\ast$01 by van Erp et al. [31] (GenBank X91015), and the zebrafish sequences Brre-UAA$^\ast$01 and Brre-UBA$^\ast$01 by Takeuchi et al. [36] (GenBank Z46776 and Z46777).

The homology groups to which the individual sequences belong can be found in Table 2, except for the single domain sequences of Miller et al. [17] and the two-domain sequences of Hansen et al. [30]. For the sequences not depicted in Table 2 the following groupings were made: the $\alpha_1$ domain sequences Onmy-A$^\ast$-1, -2, -3, -4, -5, -6 and -7 to $\alpha_1$ group I, the $\alpha_2$ domain sequences Onmy-A$^\ast$-1, -2, and -3 to $\alpha_2$ group I and Onmy-UA$^\ast$1 to $\alpha_2$ group II. The $\alpha_1$ domains of Onmy-UA$^\ast$-b13, -K19, -b4.10 and Onmy-UCA*KD2.11 belong to $\alpha_1$ group I, the $\alpha_1$ domains of Onmy-UA$^\ast$A4.10, Onmy-UAA$^\ast$KD4.5 and -SP1.3 belong to $\alpha_1$ group II, the $\alpha_1$ domains of Onmy-UA$^\ast$A4.3 and Onmy-UCA*KD2.9 belong to $\alpha_1$ group III, the $\alpha_1$ domains of Onmy-UA$^\ast$-B3, -K18, -A5.1 and Onmy-UAA$^\ast$Spu3.1 belong to $\alpha_1$ group IV and the $\alpha_1$ domains of Onmy-UA$^\ast$A4.11 and Onmy-UAA$^\ast$KD1.5 belong to $\alpha_1$ group V; whereas the $\alpha_2$ domains of Onmy-UA$^\ast$b13, K19, -A4.10, -B3, -K18, -A4.11, Onmy-UAA$^\ast$KD4.5, -SP1.3, -KD1.5 and Onmy-UAA$^\ast$Spu3.1 belong to $\alpha_2$ group I, and the $\alpha_2$ domains of Onmy-UA$^\ast$b4.10, -A4.3, -A5.1, Onmy-UCA*KD2.9 and -KD2.11 belong to $\alpha_2$ group II.
lineages detected in the Nikko and Donaldson strains in Japan, 2b shows lineages detected only in other rainbow trout strains, 2c shows brown trout lineages for which no rainbow trout sequences were found and 2d shows several cyprinid sequences. The lineages Sal-MHClA*A–K can be distinguished when comparing all rainbow trout sequences, and the lineages Sal-MHClA*A–O when comparing all salmonid sequences.

The $a_3$ domains of the cyprinid sequences are not indicated in Table 2 (despite being known for Cyca-UA*01 and Brre-UBA*01) since they are very

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**Table 2. Organisation of the full length classical MHC class I sequences detected in salmonids into lineages**

<table>
<thead>
<tr>
<th>Lineage</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$a_3$</th>
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<tbody>
<tr>
<td>a1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sal-MHClA*+</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The Roman numerals for the $a_1$ and $a_2$ domains refer to the homology groups indicated in Fig. 2. The sequences of the $a_3$ groups I, II and III have no extension at the C-terminus, a short extension and a long extension respectively. (a) Sal-MHClA lineages detected in the present study, (b) Sal-MHClA lineages detected in other rainbow trout, (c) Sal-MHClA lineages detected only in brown trout and (d) cyprinid sequences. The salmonid sequences are organised into lineages Sal-MHClA*A–O based on having their extracellular domains placed into the same groups. The sequences detected in this study are underlined.
different from the salmonid sequences [19]. Table 2 clearly shows a mosaic structure of domains, some which are shared between cyprinid and salmonid MHC class I sequences.

IV. Discussion

The present study investigated MHC class I variability between two rainbow trout strains. The purpose was to investigate the distribution of Onmy-UBA alleles between rainbow trout strains maintained in captivity, and to determine whether Onmy-UBA polymorphism can be used as a genetic marker.

Characterisation of fish strains in order to understand their historical relationships and differences in properties such as disease resistance has long been of interest in aquaculture, resulting in the discovery of a variety of markers including allozymes, DNA-fingerprints and microsatellites [24–27]. For rainbow trout specifically, DNA fingerprinting has been established [28] and a number of microsatellites and allozyme markers have been described [29].

MHC class I polymorphism has been used previously for the characterisation of salmonid populations by Miller et al. [15–18]. They found that for British Columbia coho and chinook populations, the MHC class I variability was similar to the variability found in microsatellites [17]. However, their analysis was based on genomic PCR analysis of single a1 and a2 domains and no distinction between expressed and non-expressed genes could be made. Their PCR system led them to the possibly false assumption that genes with a2 group I and II sequences can be expressed from different loci. A problem when only a1 or a2 domains are analysed is that non-classical MHC class I loci in rainbow trout have domains very similar to those in some Onmy-UBA alleles. Besides the Onmy-UBA locus at least two closely related non-classical MHC class I loci (other than Onmy-UAA [23] which shows only low homology with Onmy-UBA) can be detected, and the a1 domains of these loci may be indistinguishable from some Onmy-UBA sequences (unpublished observations). Therefore, for MHC class I analysis of relatively small populations as maintained in hatcheries, full length gene analysis is preferable, in order to avoid unnecessary complexity due to the existence of closely related non-classical MHC class I sequences. For the screening of large populations, the method of Miller et al., who rapidly analysed short PCR fragments by denaturing gradient gel electrophoresis (DGGE), is certainly the better method of choice; they succeeded in analysing samples from 20 000 coho and chinook salmon [18].

In the present study the Nikko and Donaldson rainbow trout strains maintained in Japan were shown to have distinguishing differences in MHC class I sequence lineages. Specifically, MHC class I sequences belonging to lineages Sal-MHC1a*-A and -E were common in the Nikko strain but not in the Donaldson strain. Although more than 30 classical MHC class I sequences have been described for rainbow trout, probably representing alleles (see below), in the Nikko and Donaldson strains only five and four different sequences were detected respectively. It must be recognised that these results
pertain only to these strains as maintained in one hatchery, and that due to inbreeding practices the Onmy-UBA alleles in fish from other hatcheries may be different. The variability in MHC class I genes is probably representative for rainbow trout strains maintained in Japan, since the breeding practice applied to maintain the strains used in this study is in general use throughout the country.

The definitions used in this study (also in Aoyagi et al. [19]) for classical MHC class I lineages in salmonids were based on similarities of a1, a2 and a3 domains. These distinctions seem to represent natural divisions for the a1 and a2 domains, since all reported classical MHC class I sequences of salmonids fit in easily. The a3 region is more problematic, since we could not find a division for all salmonid a3 regions in which length, homology and UPGMA analysis agree, although these parameters match rather well when a3 domains of only the rainbow trout sequences are compared (not shown). The transmembrane and cytoplasmic regions are not used in this definition, since natural-appearing lineages could not be detected and the functional importance of the variability in this region is questionable. The lineage definition was based on individual domains, rather than on overall homology, since recombination events creating different combinations of a1 and a2 groups seem to have been a major cause of polymorphism. For example, the sequences Onmy-UBA*-101 and -701 have a completely identical a1 region, but a very different a2 region, with differences beginning at the domain border [19]. And for example Onmy-UBA*601 [19] and Onmy-UA*A5·1 [30] have an identical a2 domain, but a very different a1 domain. A probable reason for this pattern is that the intron between the a1 and a2 domains is very large, which would stimulate recombination events. This large intron size has not been proven, but is expected based on the genome analysis of the carp MHC class I sequence Cyca-12 [31] and the fact that several groups, including ours, have been unsuccessful to amplify the complete intron at this location in Onmy-UBA.

The lineage definition was kept similar to that in Aoyagi et al. [19] in order to be consistent, but in the future lineage definitions may need improvement with respect to the a3 domain. It is fascinating, however, that only three discrete lengths of a3 domains are found for the growing number of salmonid classical MHC class I sequences. The sequences of Atlantic salmon, brown trout, pink salmon and rainbow trout compared in Table 2 have a3 domains with C-termini lengths similar to Onmy-UBA*401 (no extension), Onmy-UBA*501 (6 aa extensions) or Onmy-UBA*701 (11 aa extensions) (Fig. 1) instead of continuous variation between absent and 11 aa extensions. Future research is necessary to determine if there is a functional reason for these discrete length differences, since there are presently no studies on other species that show the same phenomenon.

The primer sets used in this study were developed to distinguish among sequences of the lineages Sal-MHCIA*-A–F, but rainbow trout sequences belonging to the Sal-MHCIA*-G, -H and -K lineages would also have been amplified (as indicated in the Materials and Methods section); therefore those lineages are likely to be absent in the Nikko and Donaldson strain rainbow trout investigated. A few of the primers used in the present study bind upstream or downstream from the reported rainbow trout sequences of
lineages Sal-MHCIa*-I and -J (Table 2), and therefore it cannot be predicted if they would have been amplified.

Since the lineages definitions do not include precise sequences, and the classical MHC class I sequences in salmonid species are highly variable, lineage determination can never be performed by PCR alone. Nevertheless, the lineage-specific PCR system indicated in Table 1 would amplify most of the rainbow trout sequences published and give substantive clues regarding their identity. Exceptions do occur, however, such as the Nikko strain fish for which the partial Onmy-UBA*1401 sequence could be amplified by the primer set pG-LPf/pI-a3/TMr but not by the primer set pG-LPf/pG-3’UTRr (Table 1). As the forward primer binding to the leader peptide region is identical in both primer sets, the lack of amplification by the latter set is probably due to a mutation in the 3’UTR (where the reverse primer should bind). Differences in the 3’UTR have been previously observed: Onmy-UBA*401 [19] and Onmy-UBA*SP3 [30] are identical, except that Onmy-UBA*SP3 has a 274 bp insertion in its 3’UTR.

Expression from the rainbow trout locus Onmy-UBA had previously been shown only for sequences of the lineages Sal-MHCIa*-A, -B, -C, and -D [19]. Since Onmy-UBA is most likely the only classical MHC class I locus in rainbow trout, we gave the new sequence belonging to the Sal-MHCIa*F lineage a name starting with ‘Onmy-UBA’, and followed the Onmy-UBA designation of Shum et al. [23] (Genbank) for the detected sequence belonging to the lineage Sal-MHCIa*E. The fact that in this study only one or two sequences were amplified per individual from 59 fish, despite an intensive search for presence of different sequences, strongly supports the idea that all classical MHC class I sequences are derived from the same locus. However, for one Donaldson strain fish, three sequences belonging to the lineages Sal-MHCIa*-B, -D, and -F were obtained. This might be due to triploidy in this individual since it is not consistent with the other results, but further studies on locus identification of the Sal-MHCIa*F lineage are necessary. Although the allomorph Onmy-UBA*401 has a somewhat different a1 domain, lacking tyrosine at position 59 (Fig. 1; [30]), locus identification and expression analysis suggested that it functions as a classical MHC class I molecule [19].

From the nomenclature for their sequences released to GenBank (Table 2), it appears that Shum et al. [23] share the view that there is most likely only one classical MHC class I locus. However, Miller et al. [17] and Hansen et al. [30] suggested that the different classical MHC class I lineages might be derived from different loci, and designated sequences accordingly [17] or state that the designation is based on lineage rather than on locus [30]. This discussion on loci and lineages is disturbing clear nomenclature shared by different groups (as seen in Table 2), and should change in the future. Despite the confusing nomenclature, all investigators seem to agree on several important aspects of MHC class I molecules in rainbow trout: that they are highly variable and that both their structure and the detection of other genes involved in the MHC class I pathway [30] indicates that the MHC class I molecules play an important role similar to their counterparts in higher
vertebrates. This makes research on trout MHC class I genes an important and promising field.

In conclusion this study employed a convenient system to analyse Onmy-UBA polymorphism in rainbow trout. The Nikko and Donaldson rainbow trout strains appear to have different Onmy-UBA alleles. Future studies will address relationships between Onmy-UBA alleles and differences in disease resistance.

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