ASSESSMENT OF CHICKEN AND TURKEY MHC REGION HOMOLOGY

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INTRODUCTION

In contrast to the well studied human and murine MHCs, very little is known about the genomic organization of non-mammalian MHCs. Although some information is already available for a number of avian MHC systems, chicken MHC is the most studied. The B locus spans 92 kb on a microchromosome, namely chromosome 16. This system contains 19 genes, and is about 20-fold smaller than the human MHC (Kaufman *et al*., 1999). Within the B locus there are two loci containing the classical class I (B-F) and class II (B-L) β genes homologous to human MHC (Kaufman *et al*., 1999 ; MHCSC, 1999). Other B complex genes homologous to genes found in the human MHC are Tapasin, Ring3, DMα, DMß, TAP1, TAP2, and C4. Chicken MHC also contains the B-G (class IV) gene, so far identified only in this species. Within the B locus there is only one gene homologous to the human class III, the complement component C4. Skjødt and co-workers (1985) showed that no recombination occurs between chicken class I and class II regions.

Several studies have already established the striking genetic and functional differences between chicken and mammalian MHC. Features such as small size, small number of introns, together with the established existence of specific associations between the B complex and resistance to pathogens, makes this species a valuable model for further studies in this matter. At the same time, we have learnt that the genome organization of the MHC system between avian species varies regardless their taxonomic distances. Therefore, it is apparent that we cannot infer the genome organization of one avian species from the information we have collected from other species.

Only limited information is available about the organization of the turkey MHC. Four class II β genotypes representing four independent class II phenotypes have been identified, by hybridizing chicken class II probes to turkey DNA (Emara *et al*., 1992 ; 1993). The possibility to hybridize turkey DNA with chicken MHC probes at high stringency let us infer the existence of high sequence homology between the two species at least with regard to class II β genes.

MATERIAL AND METHODS

Primers specific for classical MHC class I and class II genes, and the genes TAP1, and Tapasin were derived from chicken, pheasant, and quail sequences deposited in GenBank. Conserved regions were determined by aligning the sequences using the program MegAlign of the software package DNASTAR. Genomic DNA was isolated from turkey blood cells by standard methods (Sambrook *et al*., 1989). Total RNA was isolated from peripheral blood mononuclear cells (PBMCs) based on the guanidinium thiocyanate method (Birren *et al*., 1998 ; Chomczynski and Sacchi, 1987). The total RNA was reverse transcribed into cDNA using SuperScript II (GIBCO BRL). The genomic DNA was used as template in PCRs with the primers for TAP1 and Tapasin. cDNA was used as template in PCRs with the primers for

MHC class I, and MHC class II genes. The DNA sequence was determined using dye-terminator chemistry (BigDye Terminator, ABI) and M13 reverse and T7 primers on an automated fluorescence DNA sequencing machine (Applied Biosystems 3700).

Table 1. Primer sequences derived from MHC gene specific sequences in chicken, their location in the chicken genes and the length of the expected PCR product

MHC class I		
Forward	5'-CGCCGTGTGCGGGGCGGC-3'	exon 1
Reverse	5'-CGAGGATGTCACAGCCGTACATC-3'	exon 3
expected product length from cDNA		368 bp
MHC class II		
Forward	5'-GGGGGCCGTGCTGGTGGCAC-3'	exon1
Reverse	5'-CCGTCAGCAGCTTGCTCCTGCC-3'	exon4
expected product length from cDNA		655 bp
TAP 1		
Forward	5'-CGAAGAGCCCACAGCCTTC-3'	exon 5
Reverse	5'-ACCGTCAGCACTGGGGACC-3'	exon 6
expected product length from genomic DNA		428 bp
Tapasin		
Forward	5'-GGGACACAGTGATGGACAGC-3'	exon 5
Reverse	5'-GTAGAGCCAACGGATGAGGC-3'	exon 6
expected product length from genomic DNA		473 bp

The collected raw data were analysed using the Geospiza Sequencing Analysis software. Chromatograms were generated from the raw sequence data using the base-calling program PHRED (Ewing and Green, 1998 ; Ewing *et al*., 1998). Sequence quality information was obtained by the program PHRAP : http://bozeman.mbt.washington.edu/phrap.docs/phrap.htmp. Database searches (EMBL, Genbank) were carried out using BLAST (Altschoul *et al*., 1990) to ascertain the homology of the turkey sequences with MHC gene sequences in other species.

RESULTS AND DISCUSSION

The genomic and cDNA clones were amplified with the consensus sequence primers. PCR products of the expected size were thus sequenced. The BLAST results with the highest homologies for the turkey sequences amplified with the MHC class I, class II, TAP1, and Tapasin primers, are shown in table 2.

Table 2 shows the alignment scores and associated e-values for homology of the various sequences with sequences in existing databases. The e-values are based on the extreme value distribution and correspond to the probability of obtaining a sequence with higher homology simply by chance. The alignment of two random sequences is expected to have an e-value near

1 and 10^{-3} is generally used as a critical value to indicate a statistically significant level of homology based on a single comparison of sequences. Clearly, the e-values in this study indicate a highly significant level of homology for all sequences evaluated. For instance, values for MHC class II (e-175, 0.0 respectively) being not different from "0", in terms of the precision of the computing system employed by BLAST indicate the maximum discernable level of homology.

Table 2. Results of the BLAST searches with turkey sequences

Gene	Acc #	Score	e-value	Homology
				%
Tapasin	AJ004999.1	309	1e-81	95
	AJ005072.1	214	6e-53	96
TAP1	AL023516.1	153	7e-35	91
MHC class I	AB005527.1	307	4e-81	85
	Z54359.1	139	1e-30	86
MHC class II	AJ224348.1	694	0.0	93
	M87655.1	620	e-175	90

The study of turkey MHC will let us shed light on the genetic organization of a system, which is very important for animal health and production. The raise of pathogenic strains resistant to antibiotics and the need to tackle pathogens with stronger and stronger vaccines sometimes harmful for the host, are well known problems. Recently, we have also seen the American and European public opinion slowly but steadily shifting towards animal products without antibiotics or any kind of drug residues. All these issues lead us to pay more attention to prevention. Within this context, molecular genetics and immunogenetics are of highly valuable help. Identification of chromosomal regions, if not the actual genes, responsible for resistance/disease to specific diseases would have a great impact on industry. In the long term, the possibility to select animals carrying favourable alleles by a simple and not intrusive DNA test, will reduce loss of income due to death, depopulation, and missed opportunities to sell at the market price. Moreover, selecting animals with a stronger immune response might reduce the need of vaccines and medication. In other words, we will reduce the level of residue in animal products meeting in this way public opinion demand.

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