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MOLECULAR CLONING OF CHICKEN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II MOLECULES

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ABSTRACT: *The chicken major histocompatibility complex (MHC), the B complex, is beginning to be analyzed at the DNA level. Inbred lines of chickens have been reported to possess 3~5 MHC class II genes. To further analyze the molecular structure of the chicken MHC class II genes, cDNA clones coding for chicken MHC class II (B-L) β chain molecules were isolated from chicken spleen and liver. Tissue-specific transcription of B-L β genes was studied by Northern blot analysis. A high level of expression was detected for spleen poly(A)$^+$ RNA whereas a faint signal was detected for liver poly(A)$^+$ RNA. Twenty-nine cDNA clones were isolated from the spleen and eight cDNA clones were isolated from the liver. Based on restriction maps, most clones could be clustered into one family of genes. Four cDNA clones were sequenced (S7, S10 and S19 from the spleen and L1, which was identical to S19, from the liver). Complete amino acid sequences of B-L β chain molecules were predicted from the nucleotide sequences of the cDNA clones. Although both the nature and the location of the conserved residues were similar in chicken and mammalian sequences, some species-specific differences were found, suggesting that the structures of the B-L molecules are similar, but not identical to their mammalian counterparts.*

Key words: *major histocompatibility complex, B-L β chain genes, tissue-specific transcription, cDNA library, sequence homology.*

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INTRODUCTION

The major histocompatibility complex (MHC) is a group of genes encoding molecules that provide the context for the recognition of foreign antigens by T lymphocytes. The MHC class II genes encode cell-surface glycoproteins that serve as restricted elements for the recognition of antigens by T helper cells. Class II molecules are composed of two glycoproteins of 30~34 kd (α chain) and 28~29 kd (β chain), noncovalently associated at the surface of B cells and cells of the myeloid lineage (macrophages and dendritic cells).

The MHC of the chicken was originally described by Briles *et al.* (1950) as a blood group locus and was named the B locus. Over 10 years later, Schierman and Nordskog (1961) found that MHC antigens were encoded in the B locus. It is becoming increasingly apparent that the chicken MHC is very similar in most respects to mammalian MHCs. Most of the immunologically related phenomena or functions which are known for H-2 alleles are now known for the B complex (Crone and Simonsen, 1987). The chicken MHC, the B complex, encompasses three classes of loci: B-F (class I), B-L (class II), and B-G (class IV). The MHC class II genes encode cell-surface glycoproteins that serve as restriction elements for the recognition of antigens by T helper cells. In contrast to the extensive knowledge of the genetic organization of the MHC in mouse and man, information about the number and arrangement of loci in the MHC of the chicken is only recently emerging.

Several clones containing chicken B-L genes have been isolated (Behar *et al.*, 1988, Bourlet *et al.*, 1988, Guilemot *et al.*, 1988, and Xu *et al.*, 1989). Initial isolation of chicken class II β genomic clones was carried out by hybridizing with a human class II MHC probe under low stringency conditions. The chicken class II β genes encoding specific domains showed 62~66% nucleotide homologies with the genes of corresponding domains from humans. The domains are the expected size (92~93 amino acids) and have the intrachain disulfide loop that is typical of the immunoglobulin supergene family (Boulet *et al.*, 1988). Subsequent isolations from libraries of other haplotypes (B^{12} and B^{6}) were done by screening with chicken class II (B-L) β exons (Guillemot *et al.*, 1988, Xu *et al.*, .1989). Sequence homologies among MHC class II β domains of chickens from different haplotypes range from 90~99% (Xu *et al.*, 1989). A transcribed B-L gene of the B^{12} haplotype was sequenced (Zoorob *et al.*, 1990). The 5′ flanking region contains transcription-controlling sequence motifs analogous to those known in mammals.

To elucidate additional information about the molecular structure of the B complex, we isolated and characterized cDNA clones for B-L β genes. Thirty-seven clones were restriction mapped, and four were sequenced for B-L β chain genes from a chicken of the B^{6} haplotype. Intraspecies comparison of the longest clone to other known B-L β sequences and phylogenetic comparison to their mammalian counterparts are presented.

Tissue-Specific Transcription of B-L β Genes

The chicken MHC class II β chain probes were a gift of C. Auffray. He and his colleagues (Boulet *et al.*, 1988) isolated the p14 clone by cross-hydridization

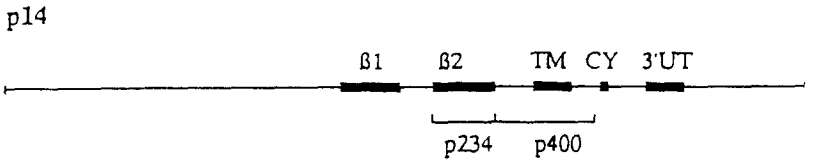


Figure 1. Chicken class II probes p14, p234, and p400 (from Xu *et al.*, 1989). The p14 probe is the 3.2 kb *Hind*III fragment. The p234 probe is 234 bp and the p400 probe is 400 bp. The heavy bars represent the $\beta 1$, $\beta 2$, transmembrane (TM), and cytoplasmic exons.

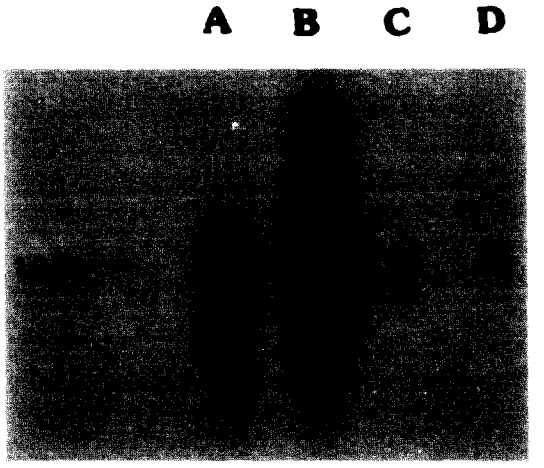


Figure 2. Tissue-specific transcription of the B-L β genes (from Sung *et al.*, 1989). Northern blot analysis was performed using the B-L $\beta 2$ domain probe, p234, RNAs tested are: A, 20 μg of total RNA from spleen: B, 4 μg of poly(A)$^+$ RNA from spleen; C, 20 μg of total RNA from liver; and D, 4 μg of poly(A)$^+$ RNA from liver.

at low stringency conditions, using a probe derived from an HLA-DQ β cDNA clone. A diagram of the isolated genomic clone is shown in Figure 1. The whole clone (3.2 kb *Hind*III fragment) has been designated p14. Digestion of p14 with *Pst*I yielded a $\beta 2$-specific probe (p234) and a transmembrane (TM) specific probe (p400). The p234 was used as a probe to study expression of the B-L β genes by Northern blot analysis of poly(A)$^+$ RNA extracted from the spleen and liver of a chicken of the B^6 haplotype (Figure 2).

A single band was detected in both spleen and liver poly(A)$^+$ RNA. The size of those poly(A)$^+$ RNAs that hybridized to the probe was approximately 1.2 kb, which is sufficiently long to encode a protein of 28~29 kilodaltons (B-L β chain). A high level of expression seen in spleen, one of the lymphoid organs, reflects the fact that this organ contains a large number of B cells on which the class II molecules are expressed. A faint signal detected in liver is probably due to the presence of macrophage-like cells expressing B-L antigens.

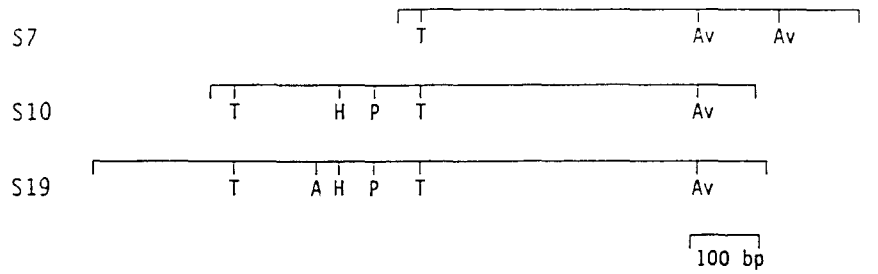


Figure 3. Restriction maps of spleen cDNA clones, S19, S10 and S7 (from Sung *et al.*, in press). Sites for restriction endonucleases are indicated at T (*TaqI*), A (*ApaI*), H (*HinfI*), P (*PstI*), and Av (*AvaI*).

Isolation of and Restriction Mapping of cDNA Clones from B-L β Genes

The initial cDNA library screening with p234 probe (Figure 1) of the spleen library (~18,000 plaques) and the liver library (~26,000 plaques) detected 30 and 11 positive clones, respectively. On secondary and tertiary screenings, a total of 29 hybridizing clones (S1-S30, with S6 missing) was obtained from the spleen cDNA library, and a total of 8 clones (L1, L4, L5, L7, L8, L10 and L11) was obtained from the liver cDNA library (Sung *et al.*, in press).

The restriction map of each of the 29 spleen cDNA clones was determined. Although the length of the cDNAs varied, there were three general patterns of restriction sites (Figure 3). Twenty-six of the spleen cDNA clones showed a pattern similar to S10, two of the clones showed a pattern similar to S10, and one clone was unique, S7. The largest member of each spleen cDNA family was chosen for sequencing (S7, S10, and S19). The restriction map of all eight liver cDNA clones was the same as the map of the S19 spleen family (data not shown). The largest liver clone, L1, was chosen for sequencing.

Nucleotide Sequence of cDNA Clones Encoding The B-L β Chain.

The complete nucleotide sequences and the predicted amino acid sequences of three spleen cDNA clones (S19, S10 and S7) and one liver cDNA clone (L1) encoding B-L β chains were determined (Sung *et al.*, in press). Data for the largest of these clones, S19, are shown in Figures 4 and 5. The S19 cDNA contained a 5′ untranslated region of six nucleotides, and open reading frame of 789 nucleotides, and a 3′ untranslated region of 143 bp followed by a poly A tail, with a total length of 956 bp. The open reading frame started with the initiation codon ATG and was terminated by the stop codon TAG. The sequence shows the typical MHC class II β chain structure. The open reading frame encodes the leader peptide of 31 residues, most of which are hydrophobic amino acids, and the entire β chain sequence of 232 residues, with β1, β2, transmembrane, and cytoplasmic domains. The conserved TGC codons for Cys, which form the disulfide bridges of the β1 and β2 domains are present. The N-linked glycosylaton site (Asn, X, Ser/Thr) is also found at residues 14-16 (Asn 14, Gly 15, and Thr 16) in the β1 domain.

Sequence data for the other clones (S7, S10, and L1) are not shown. The length

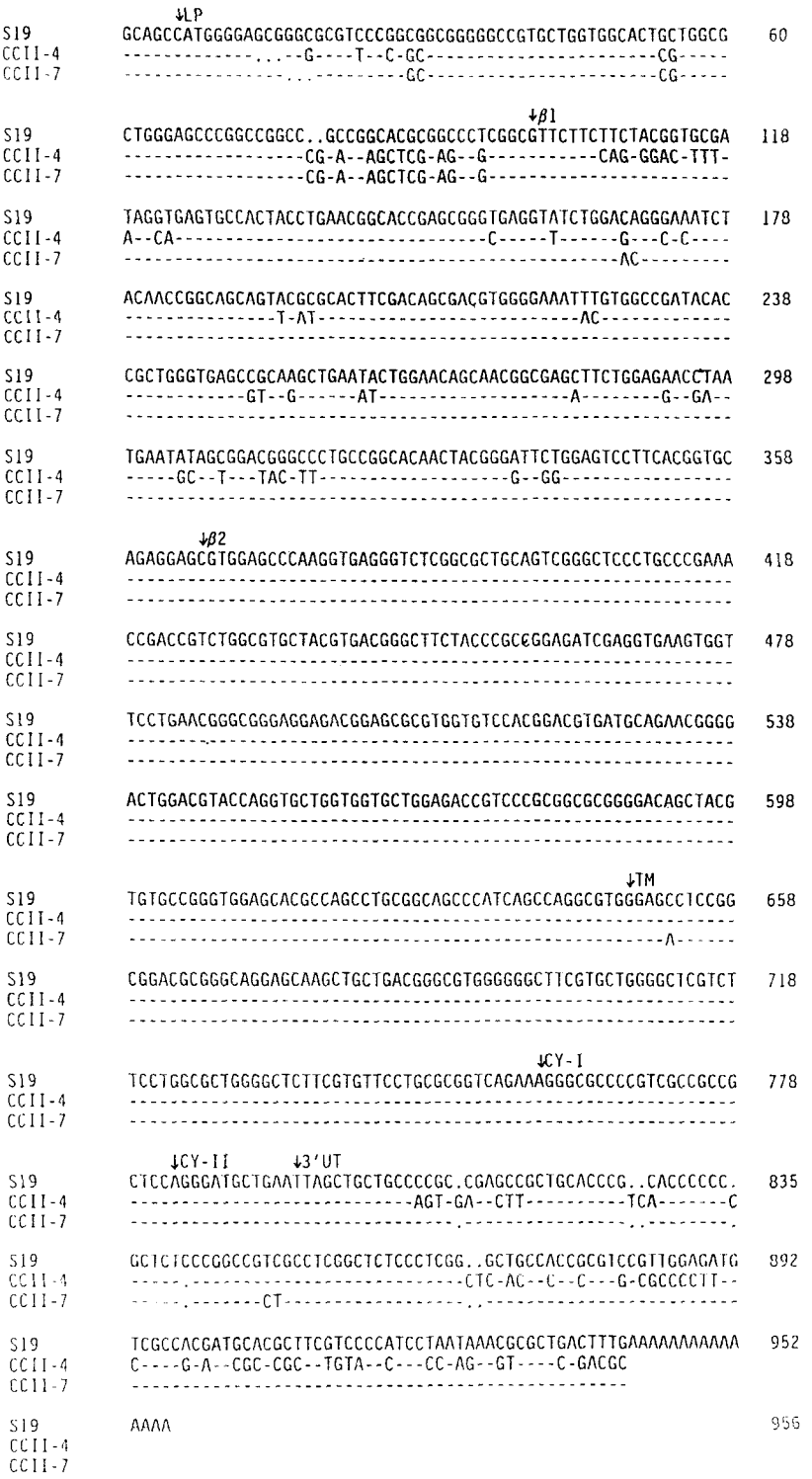
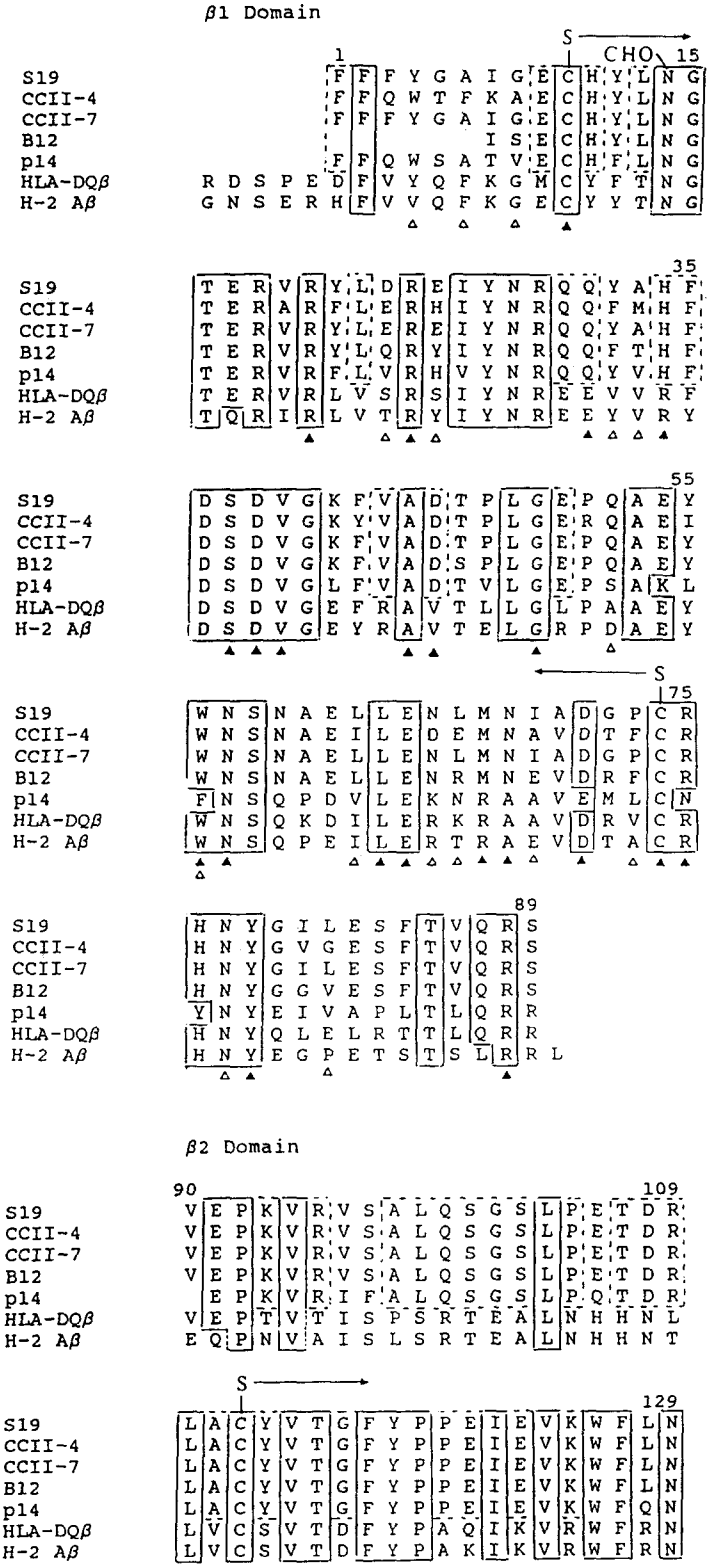


Figure 4. Alignment of the coding sequences of the S19, the CCII-4, and the CCII-7 genes (from Sung *et al.*, in press). Dashes denote sequences identical to the S19 sequences. Arrows indicate domain boundaries. Sequences of the CCII-4 and the CCII-7 genes are from Xu *et al.*, (1989).


β1 Domain
S
CHO
1
15
S19
CCII-4
CCII-7
B12
p14
HLA-DQβ
H-2 Aβ
35
55
75
89
β2 Domain
90
109
129

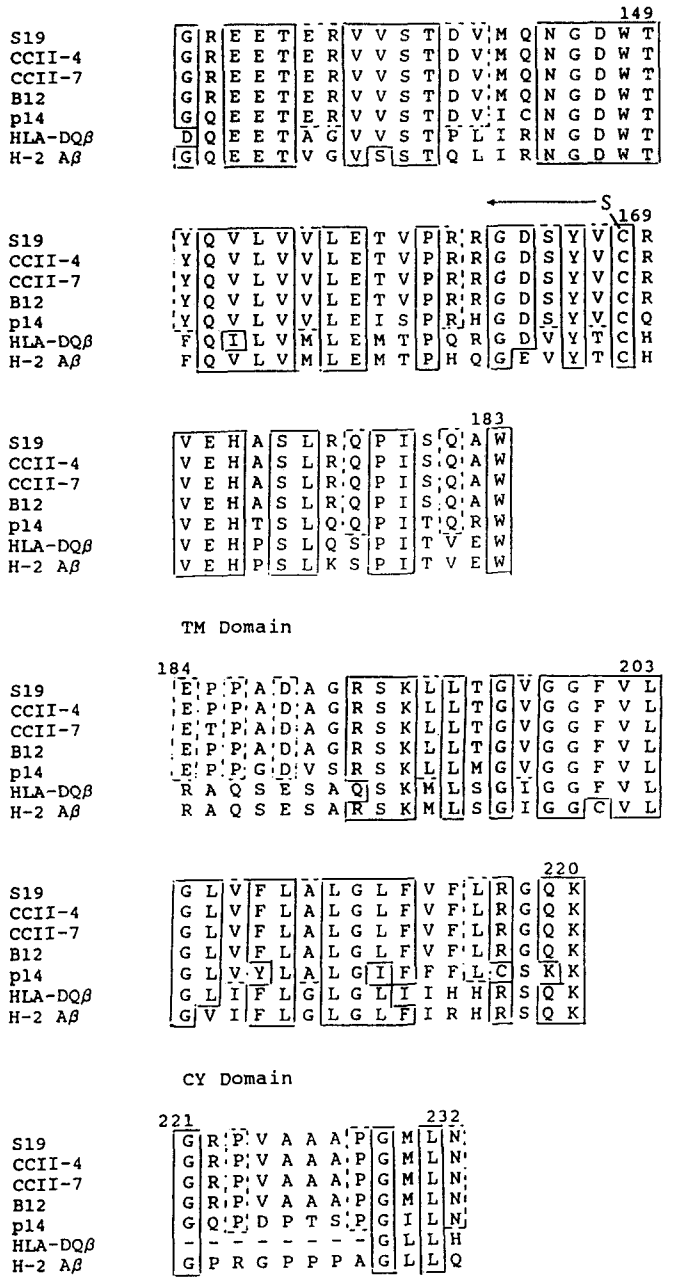


Figure 5. Comparison of the predicted amino acid sequences of class II β chaing (from Sung *et al.*, in press). The conserved regions are boxed by solid lines. The conserved regions between chickens are boxed by dashed lines. The disulfide bridges and the carbohydrate attachment site (CHO) are shown. Open triangles in the β1 domain denote residues pointing towards the antigen recognition site based on a hypothetical model of class II MHC structure (Brown *et al.*, 1988). Solid triangles denote conserved amino acids in the β1 domain whose positions are also conserved in the class I α2 domain. The CCII-4 and CCII-7 sequences are from Xu *et al.* (1989), the B12 from Zoorob *et al.* (1990) p14 from Boulet *et al.* (1988), HLA0DQβ from Larhammar *et al.* (1982) and H-2 Aβ from Malissen *et al.* (1983).

of the S10 cDNA was 767 bp. The S10 sequence lacked the leader peptide and the first 30 amino acid residues of the $\beta 1$ domain. The nucleotide sequence of the S10 cDNA showed only one nucleotide difference compared to the S19 cDNA. This difference is responsible for loss of an *Apa*I site in S10 (Figure 3). The size of the S7 cDNA was 677 bp. The S7 sequence lacked the leader peptide, the $\beta 1$ domain, and the first 24 amino acid residues of the $\beta 2$ domain. Up to 432 bp, the S7 sequence was identical to the S19 sequence. After that point, the S7 cDNA had a totally different sequence at the 3′ end which may be the result of a cloning artifact. The L1 (liver) cDNA was 941 bp long, with a sequence identical to that of the S19 cDNA.

Because the vast majority of the clones seem to belong to one family and the three deviating clones have only minor differences, it may be that the minor family clone sequence differences are artifactual. The nucleotide substitution of ^{314}G in S19 to T in S10 would cause an amino acid difference from Gly to Val comparing the amino acid sequences of other class II β chain molecules (Figure 5). The Gly 72 residue is one of the most polymorphic residues. It is possible, however, that this single nucleotide change is due to an artifact obtained during cDNA cloning. The one base change is in a GGGCCC stretch, where the reverse transcriptase or DNA polymerase are more likely to make a mismatch during first or second strand cDNA synthesis. Therefore, it may be that this highly inbred line, selected for homozygosity at the MHC, possesses only one major family of class II genes. The sequencing data did not strongly substantiate the case for multiple families of class II β genes being transcribed in this line.

Comparison of the B-L β Chain Sequences from the B^6 Haplotype.

The coding sequences of the $\beta 1$, $\beta 2$, transmembrane, and cytoplasmic domains of the CCII-4 and CCII-7 genomic clones, coding for B-L β chain molecules isolated from sperm DNA of a B^6 chicken (Xu *et al.*, 1989) were compared to the sequences of the S19 cDNA clone (Figure 4). The S19 had similar but not exactly the same sequence as the two genomic clones. The S19 sequence shared very high homology (99.6%) with the CCII-7 sequence. Only three nucleotide differences existed between the S19 and the CCII-7 clone, two of them in the $\beta 1$ domain and one in the transmembrane domain. Although a change of ^{168}C to A caused an amino acid difference: ^{166}GAC (Asp)→GAA(Glu), the acidic nature remains the same. The change of ^{169}A to C was a silent substitution. The change of ^{652}C to A in the transmembrane domain, however, caused a change in amino acid: ^{652}CCT (Pro)→ACT (Thr). Sequences for the $\beta 2$ and cytoplasmic domain of S19 cDNA and the CCII-7 gene were the same. The S19 sequence shared relatively less homology (93.8%) with the CCII-4 sequence. All forty-three nucleotide differences were found in the $\beta 1$ domain, reflecting the extreme polymorphism of the class II $\beta 1$ region. Sequences for the $\beta 2$, transmembrane, and cytoplasmic domains of S19 cDNA were identical to those of CCII-4.

Considering that the genomic library from which the CCII-4 and the CCII-7 genes were obtained was only 86% complete (Xu *et al.*, 1989), it is possible that there are other genomic genes with sequences the same as the cDNAs. Even though the chickens of the B^6 haplotype are highly inbred (>99%), it cannot be

ruled out that the chickens used for the genomic library and for the cDNA library might have had small differences in the B-L region. Alternatively, the genes in sperm might be different from those in spleen and liver.

Interspecies Comparison of MHC Class II β Chain amino acid Sequences

Shown in Figure 5 is an alignment of the amino acid sequences of class II chains from three species: chicken (S19, CCII-4, CCII-7, B12, and p14), human (HLA-DQβ, Larhammar *et al.*, 1982), and mouse (H-2 Aβ, Malissen *et al.*, 1983). The B12 sequence is from a cDNA of the B^{12} haplotype (Zoorob *et al.*, 1990). The p14 sequence is from a genomic clone of unknown haplotype (Boulet *et al.*, 1988). These seven protein sequences show identity in 89 out of 232 residues (38%). The percentage sequence identity among the individual domains is summarized in Table 1. Differences in amino acid sequence conservation are found among individual domains. In the β1 domain (residues 1-89), there are several highly conserved regions. The disulfide bridge (C10 and C74), the carbohydrate attachment site (N14), the charge pair ($R20^{+}D38^{-}$), NGTER (residues 14-18), FDSDVG (residues 35-40), and CRHNY (residues 74-78) are conserved. Many species-specific residues which are conserved only in chicken genes are also found. The charge pair ($R67^{+}E71^{-}$ in p14, $R67^{+}D71^{-}$ in HLA-DQβ and H-2 Aβ) has disappeared in S19, CCII-4, CCII-7, and B12 due to a R67→M67 mutation which may cause a structural change of the class II antigen. It suggests that the secondary structure of the chicken MHC β chain may not be identical to that of the mouse and the human MHC β chains. In addition to the conserved regions, the β1 domain contains highly polymorphic regions as well. Four major regions in the β1 domain that contain polymorphic differences (residues 3 to 8, 21 to 33, 59 to 73, and 79 to 84) are situated between clusters of highly conserved regions. The size of the β1 exon is conserved among the chicken sequences (89 amino acids). HLA-

Table 1. Sequence similarities of nucleotides and amino acids among class II β chain domains[a].

Nucleotides	β1	β2	TM	CY-I	CY-II
S19: CCII-4	83.9	100.0	100.0	100.0	100.0
S19: CCII-7	99.3	100.0	99.1	100.0	100.0
S19: B12	92.0	100.0	99.1	100.0	100.0
S19: p14	72.7	87.6	83.8	58.3	83.3
S19: HLA-DQβ	56.0	64.3	67.6	–	66.7
S19: H-2 Aβ	60.2	62.4	65.8	45.8	66.7
Amino Acids	**β1**	**β2**	**TM**	**CY-I**	**CY-II**
S19: CCII-4	73.0	100.0	100.0	100.0	100.0
S19: CCII-7	98.9	100.0	97.3	100.0	100.0
S19: B12	84.3	100.0	100.0	100.0	100.0
S19: p14	56.2	83.0	73.0	37.5	75.0
S19: HLA-DQβ	50.0	52.1	51.4	–	50.0
S19: H-2 Aβ	48.4	48.9	51.4	40.0	50.0

[a]Data calculated from pairwise sequence alignment by using MicroGenie. Similarities indicated as percentage of matching positions.

DQβ and H-2 Aβ molecules contain five more amino acids in the β1 domain.

In the β2 domain (residues 90-183), two Cys residues (C112 and C168), which are thought to form an intrachain disulfide loop, are conserved. The peptide NGDWT (residues 145-149) is also conserved in the β2 domain. A cluster of chicken-specific sequences ALQSGS (residues 98-103) was found in the β2 domain. The β2 domains of all the compared sequences consist of 94 amino acid residues, except for p14 which has 93 amino acids. The transmembrane region (residues 184-220) contains a conserved stretch of hydrophobic amino acids GGFVLGL (residues 199-205). The size of the transmembrane exon is conserved in all the sequences compared. The cytoplasmic regions (residues 221-232) consist of two exons: CY-I (residues 221-228) and CY-II (residues 229-232), except for HLA-DQβ which is missing the first exon, CY-I. Although the cytoplasmic regions of S19, CCII-4, CCII-7, and B12 are identical, those of p14 show much less homology (37.5% for CY-I and 75% for CY-II).

The β1 domain and the α1 domain of class II molecules are responsible for binding peptides at a single site (Brown *et al.*, 1988). Positions of residues conserved in both MHC class I and MHC class II generally face away from the foreign antigen binding site, while those polymorphic in both classes face into the site. In Figure 5, the conserved residues are marked with solid triangles, and the polymorphic residues with open triangles. Residue 56 is an exception because it is a conserved residues which faces into the putative antigen binding site. Of a total of 22 conserved amino acid residues in mouse and man, 17 residues (77%) are also conserved in the chicken sequences. Five residues not conserved between mammalian and avian species are all conserved among the chicken molecules. Whether these species-specific differences cause significant structural changes in chicken class II molecules is not known yet. A change of Arg 67→ Met 67 will disrupt the charge pair of Arg 67^{+} Asp 71^{-}, which is hypothesized to form a helix-stabilizing salt bridge in mammalian class II molecules. The structure of chicken class II molecules may be similar, therefore, but not identical to their mammalian counterparts.

Shown as open triangles in Figure 5 are the residues forming the proposed antigen binding site in class II molecules, based on the structure of HLA-A2 and an alignment of class II β1 domains to class I α2 domains of mouse and human MHCs. Eighty-one percent of these residues (13 out of 16) are also polymorphic in the chicken sequences. Because these residues are potential contact points for the binding of a processed foreign antigen, substitutions at these positions by site-directed mutagenesis could be performed to study the effects of class II amino acids mutations on peptide binding and the ability of T cells to recognize the class II-peptide complex.

The polymorphic residues are candidates for causing differences in the immune response in chickens of different haplotypes. The chickens used in the present study, strain G-B2 (MHC haplotype B^{6}), are highly inbred (99%) and are congenic at the B complex with strain G-B1 (MHC haplotype B^{13}). These lines differ for several parameters of the immune response (MacCubbin and Schierman, 1986) and disease resistance (Schierman *et al.*, 1977, and Parker and Schierman, 1983, 1987). These two lines have different restriction fragment length polymorphism

(RELP) patterns of *Pvu*II-digested DNA hybridized with a B-L β (Warner *et al.*, 1989) or B-F probe (Chen and Lamont, 1992) suggesting that the B-L β and B-F genes are different between B^6 and B^{13} birds. Use of inbred chicken lines, which have been well-characterized for genetic, immunological, and biochemical traits related to their MHC haplotypes, will be valuable as the fine structure of the chicken MHC is elucidated.

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