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Evolutionary conservation of antigen recognition: The chicken T-cell receptor β chain

(immunologic repertoire/gene rearrangement/DNA sequence)

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ABSTRACT T cells play important regulatory roles in the immune responses of vertebrates. Antigen-specific T-cell activation involves T-cell receptor (TCR) recognition of a peptide antigen presented by a major histocompatibility complex molecule, and much has been learned about this antigen-recognition process through structural and genetic studies of mammalian TCRs. Although previous studies have demonstrated that avian T cells express cell-surface molecules analogous to the mammalian TCR heterodimers, TCR genes have not been identified in nonmammalian species. We now report the cloning of a cDNA that encodes the β chain of the chicken TCR. Southern blot analysis using this TCRβ cDNA probe demonstrated that the chicken TCRβ locus was clonally rearranged in chicken T-cell lines. TCRβ mRNA was expressed in cells isolated from the thymus but not in cells from the bursa of Fabricius where B cells are generated. Sequence analysis of six additional TCRβ cDNAs suggested the existence of at least two variable (V) region families, three joining (J) elements, and single diversity (D) and constant (C) elements. As in mammals, considerable nucleotide diversity was observed at the junctions of the variable, diversity, and joining elements in chicken TCRβ cDNAs. Genomic Vβ and Jβ elements were also cloned and sequenced. Both elements are flanked by classical heptamer/nonamer recombination signal sequences. Although the chicken and mammalian TCRβ chains displayed only 31% overall amino acid sequence identity, a number of conserved structural features were observed. These data indicate that (*i*) the chicken TCRβ repertoire is generated by combinatorial and junctional diversity and (*ii*) despite divergent evolution at the level of nucleotide sequence, important structural features of the TCRβ polypeptide are conserved between avian and mammalian species.

One of the central features of vertebrate immunity is the ability of T cells to mount a specific cell-mediated response to a foreign antigen (1). The specificity of the T-cell-mediated immune response is determined by highly polymorphic membrane-bound heterodimers expressed exclusively on T cells (2). Each T-cell expresses a unique T-cell receptor (TCR) heterodimer that can react with a specific antigenic peptide bound to a cell-associated major histocompatibility complex molecule (3, 4). Genes that encode each of the four known mammalian TCR polypeptides, α, β, γ, and δ, have been cloned and characterized (for review, see ref. 5). These genes are all comprised of immunoglobulin-like variable (V), diversity (D; in the case of β and δ), and joining (J) gene segments that are somatically joined near constant (C) region sequences (6, 7). Structural similarities between TCR and immunoglobulin molecules are also reflected in the conservation of specific sequences at the amino acid level (8). TCR gene rearrangement occurs in the thymus and the rearranged genes are expressed selectively in T lymphocytes (3, 4). Disulfide-linked heterodimers formed between α and β chains and between γ and δ chains are expressed on the cell surface in association with a cluster of five polypeptides (the CD3 complex), thought to be involved in signal transduction (9). The αβTCR mediates the major histocompatibility complex-restricted antigen recognition of cytotoxic and helper T cells, whereas the function of T cells expressing the γδ heterodimer is largely unknown (10).

As in mammals, chicken T cells are activated in an antigen-specific, major histocompatibility complex-restricted fashion through the TCR (11, 12). At the protein level, TCRs homologous to αβ (designated TCR2) and γδ (designated TCR1) have been characterized and found to be associated with a CD3-like complex (13–15). In addition, chicken T cells express CD4 or CD8 homologues (16). Therefore, at the structural and functional levels, TCRs and associated molecules appear to have evolved prior to the divergence of avian and mammalian species.

Structural analysis of avian TCRs is of potential interest for two reasons. (*i*) Phylogenetic comparisons at the nucleotide level may provide insights into the evolutionary history of antigen recognition and may reveal structural and regulatory motifs that have been conserved along divergent pathways of evolution. (*ii*) Unlike mammals, birds generate diversity in the immunoglobulin heavy and light chains primarily by gene conversion (17–19). Thus it was of interest to determine whether birds also utilize gene conversion to create diversity in the TCR loci. The studies in this report begin to address these questions by describing the cloning and structural characterization of a chicken TCRβ cDNA.‡

MATERIALS AND METHODS

DNA Libraries. A chicken thymus cDNA library constructed from 2-week-old-chicken thymus RNA by using λgt10 as a vector (20) was kindly contributed by D. Watson (National Cancer Institute, Frederick, MD). A chicken genomic DNA library, kindly provided by K. Conklin (University of Minnesota), was prepared by partial *Sau*3A digestion of erythrocyte DNA from an outbred White Leghorn chicken and cloned into λ-FIX (Stratagene).

DNA Probes and Library Screening. Eleven fragments of human and mouse TCRβ genes were pooled and used in the initial screening of a chicken thymus cDNA library. A murine Cβ cDNA, 86T5 (8), and murine Dβ1, Jβ1, Dβ2, and Jβ2 genomic clones (21, 22) were kindly provided by M. Davis

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Abbreviations: TCR, T-cell receptor; V, variable; D, diversity; J, joining; C, constant.

‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M37798–M37806).

(Stanford University, Stanford, CA). A human J-Cβ cDNA, JUR-β2 (23), and five human VDJCβ cDNAs, 4D1, 12A1, L17, 262, and HUT (24) were also included in the screening. Approximately 200 ng of the pooled fragments was labeled with [α-^{32}P]dCTP and [α-^{32}P]dTTP by nick-translation. Nick-translation was performed using a DNase I concentration of 0.33 μg/ml to generate a probe uniformly labeled at cytidines and thymidines that had a mean size of ≈100 base pairs (bp).

Nitrocellulose blots of primary and secondary platings of the chicken thymus cDNA library were prehybridized at 42°C in 50% (vol/vol) formamide/5× SSC/1× Denhardt's solution/25 mM sodium phosphate, pH 6.5/torula RNA (250 μg/ml). (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; 1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin.) Hybridizations were carried out for 16–20 hr under identical conditions except for the addition of 10% (wt/vol) dextran sulfate and labeled probe at 3×10^6 dpm/ml. After hybridization, blots were rinsed twice at room temperature in 2× SSC/0.1% SDS and then washed 20 min in 2× SSC/0.1% SDS at 56°C. Blots were air-dried and exposed to x-ray film for 8–20 hr at −70°C with intensifying screens. Rapid isolation of λgt10 inserts by the polymerase chain reaction for ^{32}P labeling by random priming was performed as described (25). Isolation of genomic clones and additional cDNA clones was performed using end-labeled oligonucleotides or the insert from cDNA 1340 radiolabeled by random priming (25, 26).

DNA Sequencing. Double-stranded DNA sequencing was performed using a Sequenase kit according to the supplier's protocols (United States Biochemical). Oligonucleotide primers specific for the SP6 and T7 promoter sites of pGEM-3Z and pGEM-7Zf(+) as well as 17- to 20-mer synthetic oligonucleotide primers specific to sequences internal to the chicken TCRβ locus were used to sequence both strands. The DNASTAR software package was used for sequence data analysis.

Southern and Northern Blot Hybridization. DNA and RNA preparation from avian lymphoid cells, electrophoresis, and hybridization methods have been described (27). Southern and Northern blots were probed with cDNA inserts that were released from the plasmid vector by *Eco*RI digestion and labeled by random priming.

Cell Lines. Marek disease virus-induced T-cell lines, CU-16 and CU-24, were kindly provided by K. A. Schat (Cornell University, ref. 28). The Rev-T-induced B-cell lines 30LI and DT40 were courtesy of E. Humphries (25, 27). The UG9 cell line was a gift of L. Schierman (University of Georgia). The MSB-1 and UG9 cell lines were grown and passaged as described (16, 26).

RESULTS

Cloning of a Chicken TCRβ cDNA. To maximize the probability of detecting a chicken TCRβ cDNA, a pool of 11 TCRβ gene fragments was used to screen a chicken thymus cDNA library at low stringency. This pool included a mouse Cβ cDNA, two mouse genomic Dβ fragments, two mouse genomic Jβ fragments, one human J-Cβ cDNA, and five human VDJCβ cDNAs. By using these pooled fragments as a probe, several putative TCRβ-positive clones were isolated. The insert from one λgt10 clone, cDNA 1340, was found to hybridize to thymic RNA but not bursal RNA. This insert was sequenced (Fig. 1), and a comparison of the predicted amino acid sequence with consensus mammalian TCRβ gene segment sequences (29) revealed a number of compelling similarities (see Fig. 4 for more complete comparison). Within the 5′ portion of the clone, several residues match the position and identity of mammalian Vβ residues thought to be important in maintaining the structural integrity of the TCR. These include the invariant cysteine at position 92 and the invariant or highly conserved tyrosine, serine, aspartic acid, and phenylalanine residues at positions 90, 87, 86, and 65, respectively. In addition, four amino acid residues on the C-terminal side of the Vβ-homologous region correspond to highly conserved or invariant mammalian Jβ residues. The locations of the conserved residues enabled us to predict boundaries between the putative TCRβ gene segments and suggested the presence of a Dβ element between the potential Vβ and Jβ gene segments. Based on the amino acid residue landmarks, cDNA 1340 appears to be truncated at the 5′ end within the putative Vβ gene segment. The C-terminal end of the open reading frame in cDNA 1340 displayed 35% overall amino acid identity with a consensus sequence for the mammalian Cβ region.

cDNA 1340 Fulfills Basic Predictions for a TCR Gene. By virtue of its predicted amino acid sequence, cDNA 1340 resembles the mammalian TCRβ gene. Mammalian TCRβ genes are composed of distinct germ-line gene segments that are rearranged and expressed during T-cell ontogeny in the thymus to create a TCRβ repertoire. To test whether the gene encoding cDNA 1340 shared these features, cDNA 1340 was radiolabeled and used to probe Northern and Southern blots and to rescreen the thymus cDNA library to identify additional clones.

A Northern blot of total cellular RNA from bursal and thymic cells and from two chicken T-cell lines and two chicken B-cell lines was probed with cDNA 1340 (Fig. 2*A*). The cDNA 1340 insert hybridized to a 1.3-kilobase mRNA species from thymocytes and the T-cell lines UG9 and CU-24, but not to RNA from the bursa or the two B-cell lines. UG9 and CU-24 are chicken

FIG. 1. Nucleotide and predicted amino acid sequences of cDNA 1340. Amino acid residues are numbered according to Kabat *et al.* (29) for mammalian TCRβ and underlined residues correspond to invariant mammalian Vβ and Jβ residues (30). Predicted borders between Vβ and Dβ, between Dβ and Jβ, and between Jβ and Cβ are indicated above the nucleotide sequence (><). The predicted Dβ region (N-D-N) includes putative N-nucleotides (N). The predicted polyadenylation signal (underlined) in the 3′ nontranslated region, although atypical, has been reported in other eukaryotic genes.

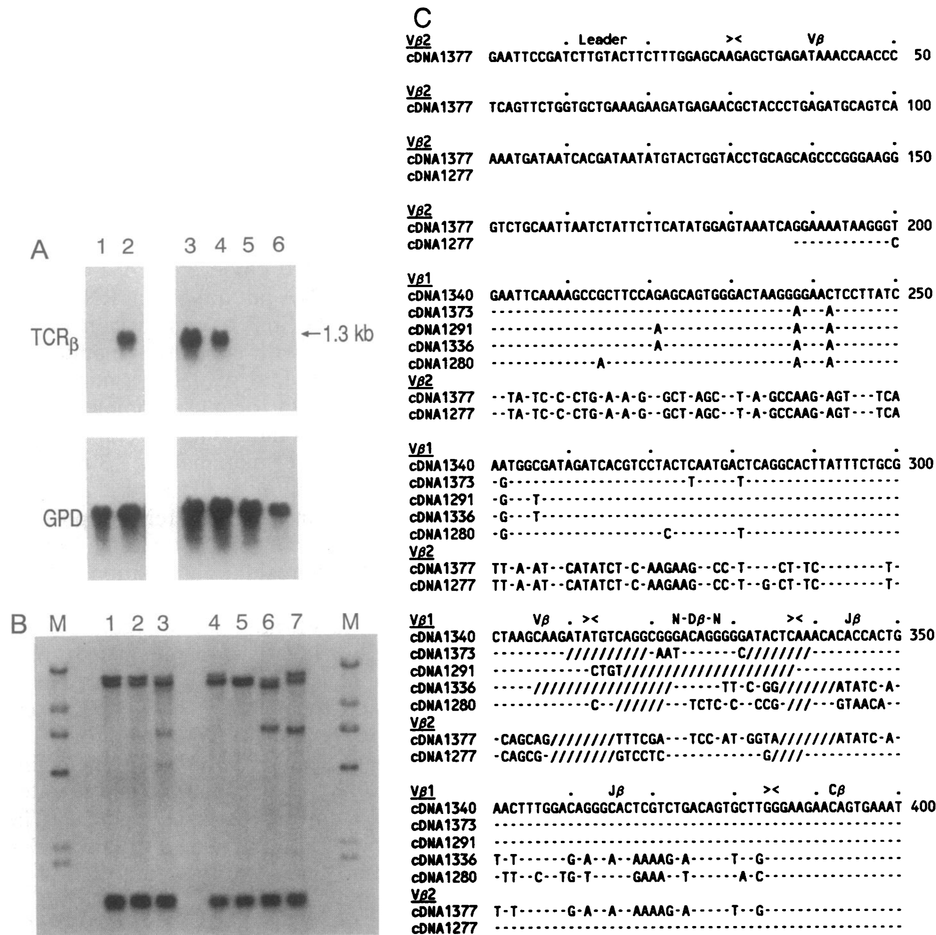

FIG. 2. Analysis of rearrangement and expression patterns of the chicken TCRβ gene (*A* and *B*) and nucleotide sequence of additional TCRβ cDNA clones (*C*). (*A*) Northern blot containing total cellular RNA from bursal (lane 1) and thymic (lane 2) cells, T-cell lines MSB-I (lane 3) and CU-24 (lane 4), and B cell lines 30LI (lane 5) and DT40 (lane 6) hybridized sequentially with a radiolabeled probe from cDNA 1340 (TCRβ; *Upper*) and a probe specific for glyceraldehyde-3-phosphate dehydrogenase (GPD; *Lower*). (*B*) Southern blot of DNA from erythrocytes as a source of germ-line tissue (lane 1), bursal (lane 2) and thymic (lane 3) cells, and T-cell lines MSB-I (lane 4), UG9 (lane 5), CU-16 (lane 6), and CU-24 (lane 7) hybridized with the radiolabeled insert from cDNA 1340. *Hin*dIII-digested λ DNA was used for size markers (lanes M). (*C*) Additional cDNAs detected by hybridization with the radiolabeled insert from cDNA 1340 were subcloned and sequenced. Nucleotide sequence identity to the top line of the sequence is indicated (–) and spacers (/) were introduced to maximize homology among the clones. Borders between gene segments are as in Fig. 1. Two apparent Vβ families were found. Vβ segments related to the 1340 Vβ are classified as Vβ1 genes, whereas Vβ segments unrelated to the 1340 Vβ, but similar to each other, are classified as Vβ2. The C regions of all cDNAs were identical except for cDNA 1336, which has a cytidine at position 608 (data not shown).

$TCR2^+$ cell lines that are believed to express the chicken homologue of the mammalian αβTCR (14).

To determine whether the gene encoding cDNA 1340 undergoes tissue-specific rearrangement, a Southern blot of bursal and thymic DNA was probed with cDNA 1340 (Fig. 2*B*). Only the germ-line pattern of hybridization is observed in DNA from bursal lymphocytes. In contrast, several new bands were detected in thymocyte DNA, and the intensity of at least the largest germ-line band was diminished. Non-germ-line bands were also observed in all four of the chicken T-cell lines tested. Thus, the locus appears to undergo multiple distinct rearrangements in the T-cell lineage. That such rearrangements are clonal is indicated by the presence of only one or sometimes two non-germ-line bands within each of the T-cell lines.

Finally, if cDNA 1340 represents a chicken TCRβ cDNA, there should be multiple clones in a thymic cDNA library that are related but not identical to cDNA 1340. This was confirmed by rescreening the cDNA library using cDNA 1340 as a probe. Six additional distinct cDNA clones were identified, subcloned, and sequenced (Fig. 2*C*). All clones shared the same 3′ Cβ sequence. However, the 5′ regions of all seven clones were distinct. Two distinct Vβ families appear to be represented. Four clones contained Vβ segments that were highly homologous to the Vβ region of cDNA 1340. These Vβ segments were collectively designated the Vβ1 family. The 5′ end of each of these clones was located at the same position as the 5′ end of cDNA 1340 as a result of a conserved *Eco*RI site within the Vβ region of this Vβ family. The other two clones encoded nearly identical Vβ segments that were only 40% identical to the cDNA 1340 Vβ segment at the nucleotide level. These two Vβ segments have been grouped together as the Vβ2 family. From these sequences, it is impossible to determine whether differences between V elements within each of the two families identify unique germ-line Vβ segments or whether they represent allelic polymorphisms or modifications of a single germ-line Vβ element as observed in the chicken immunoglobulin light chain gene (17). However, preliminary Southern blot analyses indicated the presence of multiple members of each of the two families in the germ line (data not shown).

The exact size, sequence, and number of putative Dβ regions are difficult to estimate from this group of cDNAs. cDNA clones 1340 and 1277 share a 12-bp sequence within the predicted Dβ region. Subsets of this sequence appear in all of the other clones except cDNA 1291. If the Dβ is about the same size as mammalian D regions (12–14 bp; refs. 22 and 31), there appear to be considerable deletions and N-nucleotide additions at the ends of the recombining elements. Finally, three distinct Jβ segments are represented in the group of seven cDNAs. Therefore, unlike the chicken immunoglobulin loci, which contain only single V and J elements, the TCRβ locus appears to utilize combinatorial diversity in creating a repertoire.

Genomic TCR Vβ and Jβ Gene Segments. To define the nature of the chicken TCR recombination signal sequences and transcription promoter regions, radiolabeled oligonucleotide probes specific for the cDNA 1340 Vβ and Jβ elements were used to screen a chicken genomic library. $V\beta1^+$ and $J\beta^+$ fragments were subcloned from two nonlinked phage clones and sequenced (Fig. 3). As expected for recombination-competent TCRβ gene segments, both elements are flanked by heptamer and nonamer recombination signal sequences. The length of the spacer region between heptamer and nonamer, 22 bp for Vβ and 12 bp for Jβ, matches that found between signal sequences of mammalian Vβ and Jβ elements. A decanucleotide sequence found within the promoter region of mammalian TCRβ V regions (32) was also found in the chicken Vβ promoter region (Fig. 3*A*).

Evolutionary Conservation of TCRβ Structure. The subcloning of a germ-line Vβ1 gene segment enabled us to compare the chicken TCRβ gene with a mammalian TCRβ gene over the entire coding region. Sequence comparisons were performed at both the nucleotide and amino acid levels. At the nucleotide level, the chicken and mammalian TCRβ sequences were 47–51% identical, depending on the species compared. Thus considerable sequence heterogeneity has occurred at the TCRβ locus between avian and mammalian species. This suggests that much of the conservation within the coding region occurs as a result of evolutionary selection at the protein level. In Fig. 4, the amino acid sequence of the genomic Vβ1 clone was substituted for the truncated Vβ1 sequence of cDNA 1340. The amino acid sequence of the composite chicken TCRβ polypeptide was then compared with a consensus amino acid sequence of a composite mammalian TCRβ polypeptide comprised of the consensus Vβ sequence from the mammalian Vβ1 subgroup (30), and Jβ and Cβ consensus sequences compiled by Kabat *et al.* (29). No significant homology was found in the leader exons (data not shown). As a result of the high degree of variability in the CDR3 domain, no consensus Dβ amino acid sequence has been reported within mammalian cDNAs.

Overall, the chicken Vβ1 amino acid sequence had only 22% amino acid identity with the mammalian Vβ1 subgroup consensus sequence. However, the Vβ segments of the mammalian Vβ1 subgroup share 11 invariant or very highly conserved (>93%) residues (underlined in Fig. 4). In the chicken Vβ1 family, 9 of these 11 residues are conserved, including the cysteine residues at positions 23 and 92 that can form intrachain disulfide bonds (35). In addition, the chicken Vβ1 family has the conserved arginine residue at position 64 and the aspartic acid residue at position 86 proposed by Schiffer *et al.* (30) to form a salt bridge characteristic of the mammalian Vβ1 subgroup. The chicken Vβ2 family was found to be most homologous to the mammalian Vβ2 subgroup (refs. 30 and 33; 46% amino acid identity). The chicken Vβ2 family has a tyrosine residue at position 65 that is invariantly found in the mammalian Vβ2 subgroup.

The chicken Jβ amino acid sequences are overall 47% identical to the mammalian consensus Jβ (29). The amino acid differences were primarily localized at the N terminus of the Jβ element, which is encoded by nucleic acid sequences susceptible to exonuclease degradation during TCR rearrangement. Five of the six invariant residues in the Jβ regions of mammals have been conserved in all three of the avian Jβ regions that we have identified.

The chicken Cβ region shares 35% identity with the mammalian consensus Cβ at the amino acid level. Important residues including the cysteines at positions 147 and 212, which are believed to form an intrachain disulfide bond, and the cysteine at 247, which may form a disulfide bond with a corresponding cysteine on the TCRα chain (35), have been conserved in the chicken sequence. The predicted chicken transmembrane domain (residues 267–286) is structurally conserved and retains the lysine at position 271. This charged residue has been reported to be required for the association of the mammalian TCRαβ heterodimer with the CD3 complex (34). Three leucine residues have also been conserved within the transmembrane domain at spaced intervals that suggest that the TCRβ chain may form an amphipathic helix through the membrane. The chicken and mammalian cytoplasmic domains bear little resemblance except for the lysine residues that presumably constitute the stop-transfer signal associated with transmembrane domains. This is consistent with the proposal that signal transduction is mediated through the CD3 complex and not the cytoplasmic tail of the receptor molecule (9).

DISCUSSION

This report describes the cloning of TCR β chain genes in the chicken. A cDNA clone was isolated by low-stringency hybridization with a pool of mammalian TCRβ gene fragments. By using nick-translation to generate a probe of high specific activity consisting of small random fragments, we were able to obtain cross-hybridization despite the low levels of nucleotide sequence identity between the mammalian probes and the chicken TCRβ cDNA. As described for mammalian TCRs, the chicken TCRβ gene was found to be rearranged in T-cell lines but not in B-cell lines. Similarly, the gene is expressed in thymic tissue and T-cell lines, but not in the bursa of Fabricus or B-cell lines. Structural analysis of additional cDNAs suggests that the chicken TCRβ locus contains at least two V families, one D region, three J elements, and a single C region.

Despite extensive divergence of the chicken TCRβ gene at the nucleic acid level, several regions of the amino acid

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A  Chicken genomic Vβ1
                                                                                                                                 mRNA
                                             decamer                                                               Leader        splice
gcatttcttccttcctcaatgtttcctcctcacacaacagagctcctaggtcagtgctggcaccacaggcagatcagctgaagaagataacacttttcccATGTTTCCCTGCTGGTTTCTTACGGCCTTGCTGGCCCATGTAGgtatgta 150
                                                                                                    MetPheProCysTrpPheLeuThrAlaLeuLeuAlaHisValG
                                                                                                    -13      -10                          -1 1

aattttgttctcccttgacaagccttctgctggtgcttcaacaatgtgttcagttgttggcaatgctgcattccccagggctccctggggaggatgagcaatgaatgaggtgaaattttcctcagtctcttactcaaaaagctctgctgg 300

tctacacattttatttttttatttttttcacatttaaaacttcaaccaagggtggtgaagttagtatttaaaaaaaatatatgtaagaaaaaaatactagtagtacacttgaaattgtttttttctgtctcctatctgtcccttcctg 450

                                                                                                                       mRNA
                                                                                                                      splice
agaaatgggacagcgtaagacacgctggcatcccatctattaattcaaggatgtgtctctgtgtctctttcaactcattccattcctcacagcttcttttcttctttccttttctagGCTGGGCTCTTCAGCAAACCCCAGACATGATTG 600
                                                                                                                        lyTrpAlaLeuGlnGlnThrProAspMetIleV
                                                                                                                        2                      10

TCCGACTGGGAGACTCTCTGACTCTGAACTGTTCACACAAAGAGAGTGGAGCCTGGACCATGCTCTGGTACAAGCTGCCAGTGGGGAAGAACGCCACTTTGCAGCTGATTGTTCGTTCAGTGGAAGGTAGCAAAGCAGAGTTTGAGGAAG 750
alArgLeuGlyAspSerLeuThrLeuAsnCysSerHisLysGluSerGlyAlaTrpThrMetLeuTrpTyrLysLeuProValGlyLysAsnAlaThrLeuGlnLeuIleValArgSerValGluGlySerLysAlaGluPheGluGluG
   15 17        20                                30  31A                     40                                   50                               60

                                                                                                                 heptamer                nonamer
AATTCAAAAGCCGCTTCCAAAGCAGTGGGACTAAGGAGAAATCCTTATCAATGGCGATAGATCACGTCCTACTCAATGACTCAGGCACTTATTTCTGCGCTAAGCAAGATAcactgtgacacgaaggctggagcagcacaacgcaaacct 900
luPheLysSerArgPheGlnSerSerGlyThrLysGluLysSerLeuSerMetAlaIleAspHisValLeuLeuAsnAspSerGlyThrTyrPheCysAlaLysGlnAsp
   62A                           70                              80                              90          96

B  Chicken genomic Jβ
                                                                               mRNA
      nonamer           heptamer                                              splice
tggcagacaattctggtacttcttgaaaatactgtgCAAACACACCACTGAACTTTGGACAGGGCACTCGTCTGACAGTGCTTGgtaagtcttctctgta 100
                                       AsnThrProLeuAsnPheGlyGlnGlyThrArgLeuThrValLeu
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FIG. 3. Nucleotide and amino acid sequences of genomic Vβ and Jβ gene segments. Amino acid residues are numbered according to Kabat *et al.* (29). (*A*) Sequence of genomic Vβ1 clone 1397. Amino acid residue 1 is preceded by leader sequence and represents the start of the Vβ1 coding region. The conserved decamer in the promoter region, the mRNA splice donor and splice acceptor signal sequences of the leader and Vβ1 exons, and the heptamer and nonamer recombination signal sequences are underlined. (*B*) Genomic sequence of the Jβ element found in cDNA 1340. Recombination signal sequences and the mRNA splice site of the Jβ gene segment are underlined.

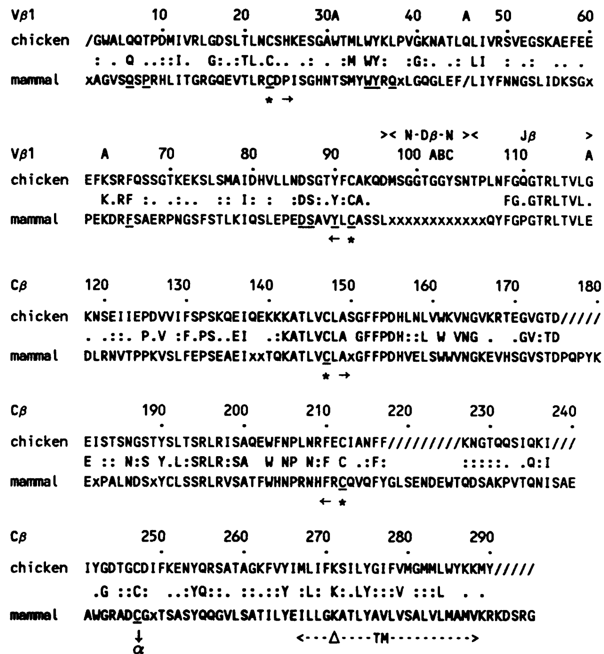

FIG. 4. Amino acid sequence comparison of the chicken TCRβ coding regions and consensus mammalian TCR sequences derived from Kabat *et al.* (29, 30). The upper line represents the amino acid sequence for the chicken TCRβ chain beginning with the Vβ1 exon as defined by genomic Vβ1 clone 1397. Chicken sequence from amino acid 61 to amino acid 290 is derived from TCRβ cDNA 1340. The lower line consists of consensus mammalian TCR Vβ1 (30), Jβ, and Cβ (29) coding sequences. Spacers (/) were introduced to maximize homology and sites lacking consensus residues (×) are indicated. Alignments were made on the basis of the PAM250 amino acid similarity matrix using the AALIGN program (DNASTAR). A positive relationship (:), a 0 value relationship (.), and a negative relationship (blank space), as well as identity between two residues, are depicted on the line between the chicken and mammalian sequences. Residues that are invariant or highly conserved in mammalian Vβ1 genes (30, 33) are underlined. Cysteine residues believed to form intrachain disulfide bonds within the V and C regions are marked with an asterisk (*) and an arrow to indicate direction of bond formation. A cysteine at position 247 (α) is thought to form an interchain disulfide bond with the constant region of TCRα. The predicted mammalian transmembrane domain (TM) is marked and the conserved lysine residue at position 271 (34) is indicated (△).

sequence have been conserved. This suggests that there has been evolutionary selection for certain structural features of the molecule. Amino acid residues thought to be central to the mammalian TCRβ protein structure (35) are found in the chicken TCRβ. These include the two pairs of cysteine residues, one in Vβ and the other in Cβ, that are believed to form intrachain disulfide bonds. The cysteine found in exon 2 of Cβ, which may form the disulfide linkage with the α subunit of the TCR, is also found in the corresponding position in chicken Cβ. Within the putative transmembrane domain, the chicken has retained the invariant lysine thought to mediate the association between the β subunit and the CD3 complex (34). These data confirm, at the nucleotide and amino acid levels, the earlier observations that the chicken TCR molecules are structurally and developmentally similar to the mammalian TCR (13–15). Therefore, the TCR antigen recognition system appears to have predated the evolutionary divergence of birds and mammals and the original structure of the receptor has been largely maintained during the estimated 250–300 million years of divergent evolution.

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