Immunogenetics (2002) 54:520–525
DOI 10.1007/s00251-002-0490-4

ORIGINAL PAPER

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The chicken *CD4* gene has remained conserved in evolution

Received: 6 March 2002 / Revised: 1 July 2002 / Published online: 2 August 2002

Abstract CD4 has a central role in thymocyte differentiation and cell-mediated immunity. We isolated and analyzed chicken *CD4*. The gene spans 11.5 kb and is composed of ten exons. The promoter is TATA-less and similar to the mouse and human CD4 promoters, with two transcription start sites as determined by 5′RACE analysis. In general the introns are short, although the 5′untranslated region includes a large intron of 5.6 kb containing binding sites of the putative *CD4* silencer. The single-strand conformation polymorphism technique was used to identify a polymorphism to map the gene, which lies on chicken Chromosome 1 in a position showing conserved synteny to mouse and human. This is the first report describing the organization of *CD4* from a non-mammalian species. The structure and localization of chicken *CD4* and many sequence motifs important in its regulation have remained conserved during evolution.

Keywords CD4 · Gene · Evolution

Introduction

CD4 is a transmembrane glycoprotein found on most thymocytes and on a subset of T cells (Parnes 1989). It marks differentiation of thymocytes through positive and negative selection together with CD8. CD4 participates in cell-mediated immunity by associating with MHC class II and mediating signals for antigen recognition. In addition, CD4 is part of the receptor complex for the human immunodeficiency virus (Kwong et al. 1988).

The gene encoding CD4 is composed of ten exons in human and mouse (Gorman et al. 1987; Maddon et al. 1987). Human *CD4* has been mapped to Chromosome (Chr) 12, to a region of conserved synteny on mouse Chr 6 (Field et al. 1987; Kozbor et al. 1986). In both the species, the promoter region of *CD4* does not have a TATA sequence for the activation and initiation of transcription, but it is replaced by an initiator-like (Inr-like) sequence (Salmon et al. 1993; Smale and Baltimore 1989). The activation of the *CD4* promoter also requires several factors, which differ depending on the developmental stage of the cell (Sarafova and Siu 2000). These include the c-Myb transcription factor essential for the expression of CD4 after positive selection of thymocytes (Adlam et al. 1997; Allen et al. 1999; Siu et al. 1992). The Myc-associated zinc finger protein MAZ is able to bend the DNA helix to favor the building of the initiation complex for transcription of *CD4* (Duncan et al. 1995; Sarafova and Siu 2000). Furthermore, an Ets family member Elf-1 binds to the Ets site in the promoter region that is also a binding site of a novel unknown factor (Sarafova and Siu 1999).

During T-cell development, the transcriptional activity of *CD4* is controlled by at least two enhancers (Adlam et al. 1997), with the proximal enhancer 6.5 kb and 13 kb upstream from the *CD4* in human and mouse, respectively (Blum et al. 1993; Sawada and Littman 1991, 1993). An enhancer has also been reported in the 3′ flanking region of mouse *CD4* (Adlam et al. 1997). The *CD4* distal enhancer, situated upstream of the lymphocyte activation gene-3 (*Lag-3*), has also been suggested to increase the promoter activity of *Lag-3* (Bruniquel et al. 1997).

Expression of *CD4* is repressed by the activity of the *CD4* silencer (Ellmeier et al. 1999). The silencer nega-

Nucleotide sequence data reported are available in the EMBL database under the accession number AJ401223

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tively regulates *CD4* transcription in CD4/CD8 double-negative cells during early thymocyte development and in CD8-positive cells, but is inactive in CD4-expressing cells (Donda et al. 1996; Duncan et al. 1996; Sawada et al. 1994; Siu et al. 1994). In human and mouse *CD4*, a large intron in the 5′UTR region contains the silencer region (Duncan et al. 1996; Ellmeier et al. 1999). Three transcription factors have been described that bind to this region and downregulate *CD4* expression. One of these is the Hairy/Enhancer of Split homologue Hes-1, which is a target of the lin12/Notch signaling pathway (Kim and Siu 1998). Notch in turn has been implicated in the control of the choice between CD4 and CD8 T-cell differentiation (Kim and Siu 1998). The other transcription factors binding to this region are Myb and the silencer-associated factor SAF, a novel member of the helix-turn-helix factor family (Kim and Siu 1999).

In this paper we describe the genomic organization of the first non-mammalian *CD4* from the chicken and show its localization on Chr 1 in a position syntenic to mouse and human.

Materials and methods

Genomic cloning and sequencing

A genomic DNA library was made from chicken red blood cells (strain CB, MHC haplotype B12) in λ phage vector (Stratagene, La Jolla, Calif., USA) following the manufacturers procedures. The library was transformed into *Escherichia coli XLI blue MRA P2* bacteria by standard methods (Maniatis et al. 1982). Plaques were transferred onto Protran BA 85 filters (Schleicher and Schuell, London, UK) and hybridized according to methods described earlier (Bumstead et al. 1987). The total *CD4* cDNA (EMBL accession number Y12012; Koskinen et al. 1999) was labeled with [α-^{32}P]dATP by Ready-to-go kit (Pharmacia, Uppsala, Sweden) and used as a probe. A plaque that gave a strong signal was picked in 0.5 ml SM buffer containing 25 µl chloroform. It was re-screened and the positive clone RK1 was amplified on the plate according to standard methods (Maniatis et al. 1982). The specificity of the RK1 clone was confirmed by PCR with *CD4* cDNA-specific primers.

To obtain the templates for sequencing, PCR was performed directly from the amplified phage clone using 1U/50 µl Taq polymerase (Dynazyme, Finnzymes, Espoo, Finland) with *CD4* cDNA-specific primers. Intron-specific primers were then designed based on the sequence obtained. The following primers were used (nucleotide numbering is according to the *CD4* cDNA). 5'UTR: 5′-TACAGCAGGTGACAAACACG-3′ (sense nt 21–40), 5′-GGAATGCTGTTGGTACCAAGG-3′ (antisense nt 101–81), 5′-CTCTTCACTATTGCAAACTG-3′ (antisense nt 166–147).

Leader/I Ig domain: 5′-AGAGGTGTGGAGCAGTGGTG-3′ (sense nt 147–166), 5′-AAAAGCTTATAATAGTAGAA-3′ (antisense nt 356–337), 5′-TTTGCGGTCATCTTGGTTCT-3′ (sense nt 176–195), 5′-ATGCACAGGTGTAGATGCCAGCG-3′ (antisense nt 479–457).

Ig domains I/II: 5′-TGTAGTCAGTATCTCACTGC-3′ (sense nt 490–509), 5′-GTTCTGATTAATCGATGGAG-3′ (antisense nt 772–753).

Ig domains II/III: 5′-CTAAAGCAACTGAAGGCTAT-3′ (sense nt 695–714), 5′-CAGCTGGCATTTGTACTGCC-3′ (antisense nt 1108–1089).

Ig domains III/IV: 5′-AATCTTGTCATGGCGTCTGA-3′ (sense nt 850–869), 5′-TTGTCTTCCACGAGGTGACA-3′ (antisense nt 1371–1352).

The DNA-encoding transmembrane and cytoplasmic regions were obtained from genomic DNA by PCR with the following primers: 5′-GGTGAACGTCAGTGCTCCAG-3′ (sense nt 1321–1340), 5′-CTGACATGTCTTCTTTTCCA-3′ (antisense nt 1582–1563), 5′-GGGCAAGAAGGATGGCACAA-3′ (sense nt 1527–1546), 5′-AGAGTCCCTCAGTCTGCAAA-3′ (antisense nt 1627–1608),

5′-AATAATTATTGGAGCCAGT-3′ (sense nt 1441–1459), 5′-TCATTATAGAGCTGGGGCTC-3′ (antisense nt 1824–1805).

The conditions for PCR varied according to the requirements for different primers and expected length of the product. The samples were directly sequenced using automated cycle sequencing (ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer, Norwalk, Conn., USA) using an ABI 373A DNA Sequencer (Applied Biosystems, Foster City, Calif., USA). Sequence data were analyzed by the GCG (Genetics Computer Group, Madison, Wis., USA) and Transfac database (Hannover, Germany) programs.

Determination of the transcription start site

The transcription start site was determined using a modified 5′RACE system, version 2.0 (Life Technologies, Paisley, UK). Briefly, total RNA was prepared from thymus of a 6-week-old chicken according to the instructions of the TRIzol reagent (Life Technologies). The first-strand cDNA synthesis was primed from total RNA using a *CD4* cDNA-specific antisense primer 5′-GCTCAAGTCTGACACCTTCA-3′ (nt 448–429) with reverse transcriptase. A homopolymeric C tail was added to the 3′ end of the cDNA. PCR amplification was carried out with a gene-specific antisense primer 5′-AAAAGCTTATAATAGTAGAA-3′ (nt 356–337) from a region of the *CD4* gene corresponding to the beginning of the N-terminal Ig domain and an anchor primer provided in the kit. For nested PCR, the gene-specific antisense primer was 5′-CCCAGCTGCAGAACCAAGAT-3′ (nt 204–185). The products from the nested PCR reaction were sequenced with the primer 5′-CCCAGCTGCAGAACCAAGAT-3′ (antisense nt 204–185) directly without cloning. Before sequencing the specificity of the DNA was checked by a PCR with the nested and cDNA-specific primer 5′-TACAGCAGGTGACAAACACG-3′ (sense nt 21–40) located inside of the studied region.

Linkage analysis

Genetic mapping was carried out by single-strand conformational polymorphism (SSCP) using a panel of 52 backcross progeny from the East Lansing mapping reference population (Bumstead 1998; Bumstead et al. 1987). The reference population comprises progeny of a cross between inbred UCD-003 White Leghorn hens with an F1 male derived from a cross between UCD-003 and a Jungle Fowl of line (JL) UCD-001, and currently contains more than 1,000 mapped loci (Groenen et al. 2000). For SSCP analysis, primers 5′-ATGCCAGCTGGAGATCAACGG-3′ (sense nt 1099–1119) and 5′-ATTGTCTTCCACGAGGTGACAGT-3′ (antisense nt 1372–1350) were used to amplify a 391-bp product by PCR. The product was ethanol precipitated, resuspended in loading buffer (98% formamide, 2% EDTA) and denatured at 90°C for 5 min. The non-denaturing polyacrylamide SSCP gel (0.5× monomer solution of SEQUAGEL MD, National Diagnostics, Atlanta, Ga., USA) was run at 8 W for 5 h at 25°C and silver stained using standard conditions (Maniatis et al. 1982).

Two-point linkage analysis was performed to compare the result with data for other loci mapped in this population using MAPMANAGER software (kindly provided by K. Manly, Roswell Park Cancer Institute, Buffalo, N.Y., USA). A LOD score (Z) value of 3.0 (P=0.001) in two-point analysis was set as the significance threshold for linkage and local genetic order was determined by minimizing the number of recombinations. Map distances were calculated with the Haldane map function (cM).

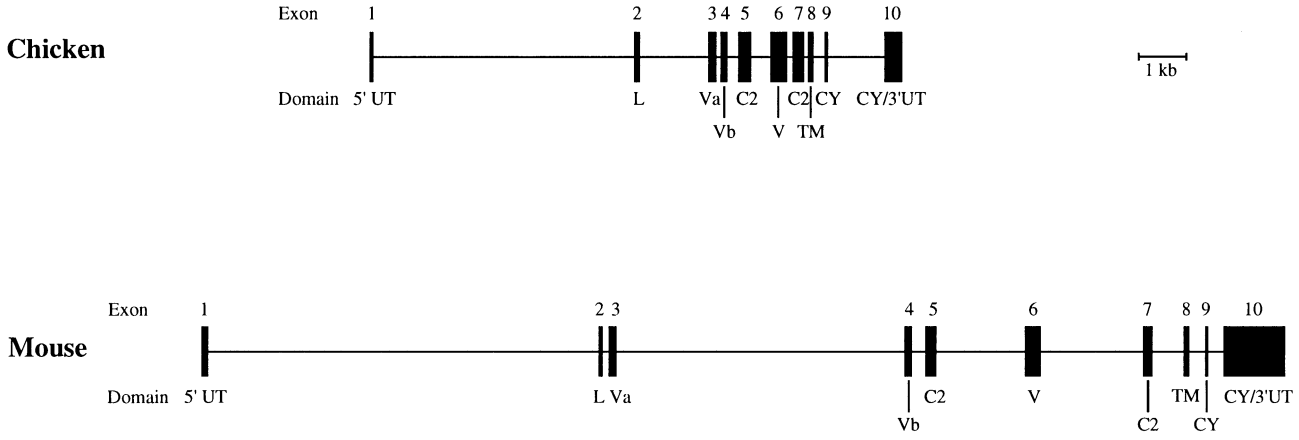


Fig. 1 Genomic organization of chicken and mouse *CD4*. The exons are numbered and shown in *closed boxes* separated by introns. The protein domains are indicated below the exons

Results and discussion

Cloning of chicken *CD4*

We have recently cloned chicken *CD4* cDNA and shown that the structural features of the protein have remained conserved (Koskinen et al. 1999). In this report we analyze the *CD4* gene to further investigate this immunologically essential antigen. To determine the genomic organization of chicken *CD4* a genomic phage library from chicken red blood cells was screened by hybridization with a CD4-specific cDNA probe, resulting in the isolation of a 15-kb clone. The nucleotide sequences of the extracellular portion of the chicken *CD4* transcript and of its 5′UTR were obtained from this clone. The transmembrane and cytoplasmic parts of the gene were sequenced from the PCR product of genomic DNA.

The organization of chicken *CD4*

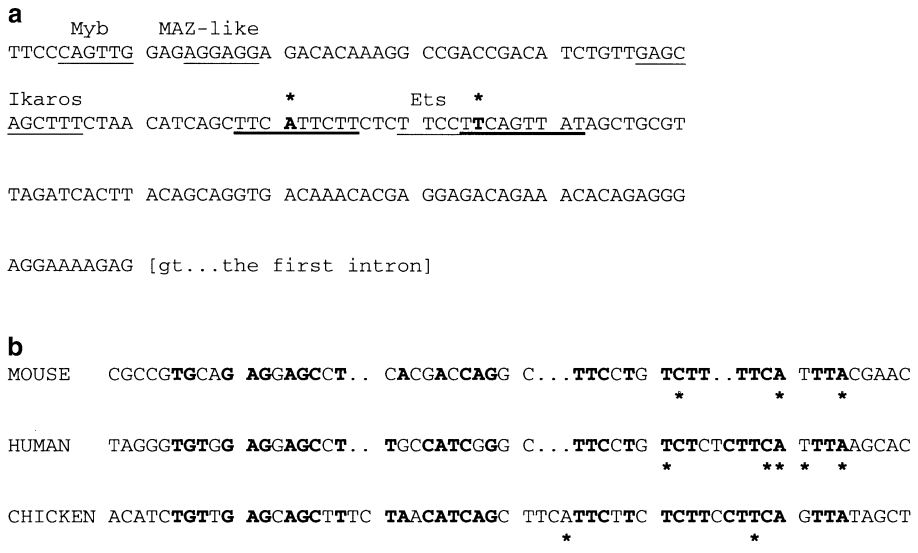
Chicken *CD4* spans a total of 11.5 kb and is composed of ten exons (Fig. 1). The nine introns are spliced almost identically to the corresponding human and mouse introns separating the coding regions of the four extracellular Ig domains of CD4. The N-terminal domain of human and mouse is split by a large intron (Littman and Gettner 1987; Maddon et al. 1987). This intron is also present in chicken but it is only 89 bp long. Other Ig domains and the transmembrane region are encoded by single exons, and the cytoplasmic domain by two exons as in mammals. The gene is more compact than its mammalian equivalents, as are most chicken genes, and its GC content is relatively low, being 44%, in contrast to GC-rich chicken genes such as the β_2-microglobulin gene (Riegert et al. 1996).

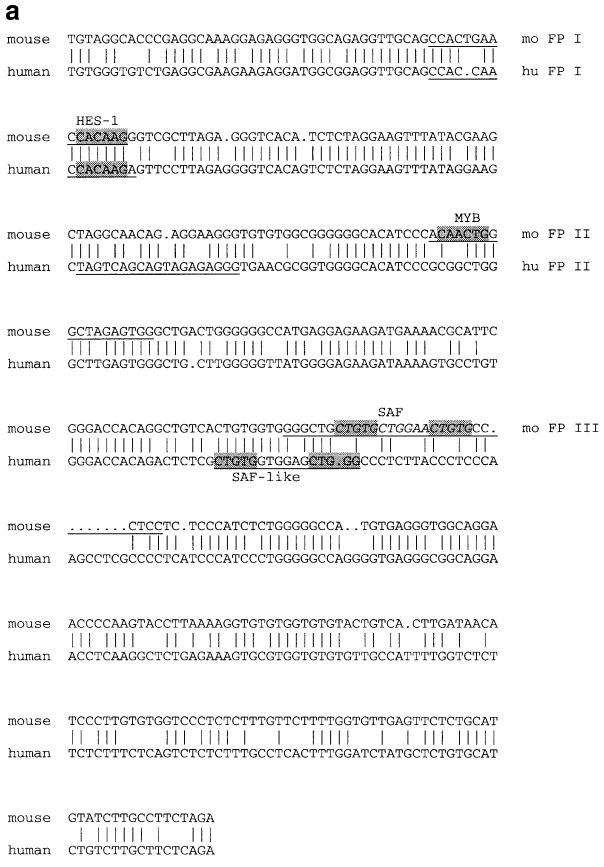
The donor/acceptor sites for splicing of introns follow the gt/ag rule (Breathnach and Chambon 1981). The exact localization of the exon/intron boundaries and the length of introns can be obtained from the EMBL nucleotide database with accession number AJ401223. Splicing for introns 1–7 is of the common type one splicing, whereas for intron 8 it is of the rare type zero and for intron 9 type two, as in mouse and human. In general the introns are much shorter than the corresponding mammalian introns and comprise in total 3.8 kb in the protein coding region. In the 5′UTR there is an intron of 5.6 kb 71 bp upstream of the first methionine of the leader sequence. A similar large intron of 10.4 kb and 8.6 kb, which contains important regulatory elements, has earlier been demonstrated in human and mouse *CD4*, respectively (Donda et al. 1996; Gorman et al. 1987).

The promoter region

The isolated genomic clone also contained the promoter region of chicken *CD4*. This is similar to the mammalian *CD4* promoter in lacking the TATA sequence and in containing two potential transcription start sites, which are 147 bp and 161 bp upstream of the first methionine as detected by 5′RACE analysis (Salmon et al. 1993; Fig. 2A). These sites are surrounded by Inr (5′-$P_YP_YCAP_YP_YP_YP_YP_Y$-3′) or Inr-like sequences, suggesting that the Inr sequence acts in the initiation of transcription of chicken *CD4*, as in many TATA-less promoters (Sarafova and Siu 1999; Siu et al. 1992; Fig. 2A). Moreover, this region has 63% and 59% identity to the respective human and mouse sequences before and around their transcription start sites, while the average identity of the promoter region is under 40% for both species (Fig. 2B).

The chicken *CD4* promoter contains potential transcription factor binding sites that are important in the transcriptional activity of mouse *CD4* (Sarafova and Siu 2000; Fig. 2). One of these sites is for the nuclear oncoprotein Myb required for the expression of mouse *CD4* (Siu et al. 1992). In mouse, three nucleotides downstream from the Myb binding site there is a site to bind the Myc-associated zinc finger protein MAZ (Sarafova and Siu 2000). MAZ is thought to induce a bend in the DNA helix affecting the transcriptional activity (Sarafova and Siu 1999). In the chicken *CD4* promoter, a potential binding site for Myb is found in an antisense orientation at position –151 to –156 from the first intron (Siu et al. 1992). In chicken, also at exactly at three nucleotides distance





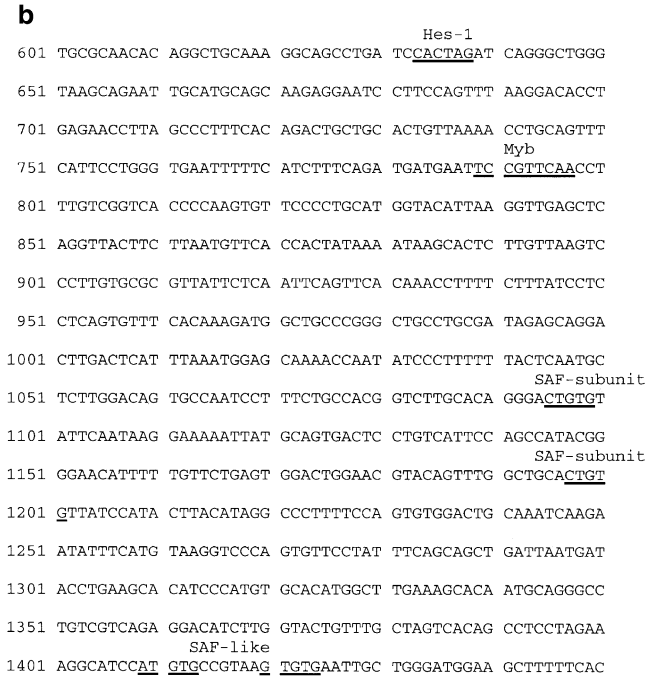


Fig. 2 **a** The promoter region of chicken *CD4*. The transcription start sites are in *bold* and marked with an *asterisk*. The Inr and Inr-like sequences are *underlined in bold*. The potential transcription factor binding sites are *underlined* and named above the sequence. **b** The alignment of the promoter regions of the chicken, human, and mouse *CD4* genes. Gaps were introduced to sequences to allow maximal alignment. The transcription start sites are marked with *asterisks*. The nucleotides identical to the corresponding nucleotide in the chicken sequence are in *bold*

Fig. 3 **a** The alignment of mouse and human CD4 silencer regions. The DNase I hypersensitivity (*FP*) sites are *underlined* and marked to the right. The potential transcription factor binding sites are in *grey* and named above or under the sequence. **b** The potential silencer region of chicken *CD4*. The potential binding sites for Hes-1, Myb, and SAF subunits are marked and *underlined*. The sequence is numbered starting from the beginning of the first intron

from the potential Myb-binding site, there is a sequence AGGAGG that corresponds to the MAZ consensus binding site [GGG(T/A)GG] with one mismatch (Sarafova and Siu 2000). In addition, an Elf-1 transcription factor has been shown to bind next to the mouse transcription start site, where sequence TCC is the key element for binding (Blum et al. 1993). In chicken, a potential Ets family binding site is found between the two transcription start sites, overlapping the Inr-like sequence (Fig. 2A). In addition, in the chicken there is a potential Ikaros transcription factor binding site between the MAZ and Elf-1 sites close to the site for an unknown factor binding to mouse *CD4* promoter (Sarafova and Siu 2000).

Internal silencer

The first intron of mammalian *CD4* contains a silencer that downregulates *CD4* transcription at different stages of T-cell development and possibly influences T-cell fate

decisions via lin12/Notch signaling (Donda et al. 1996; Duncan et al. 1996; Ellmeier et al. 1999; Kim and Siu 1999; Sawada et al. 1994; Siu et al. 1994). The end point of this pathway is the basic-helix-loop-helix protein Hes-1 that also binds to the mammalian CD4 silencer and represses its promoter and enhancer function (Kim and Siu 1998). The binding site is an N box (consensus sequence CACNAG) (Kim and Siu 1998).

The nucleotide sequence of the *CD4* silencer has been described in several species (Donda et al. 1996; Sawada et al. 1994). In the mouse *CD4* silencer, there are three DNase I hypersensitivity footprint sites (FP), but in human only two FP sites have been found (Duncan et al. 1996; Sawada et al. 1994). The FP I site is highly conserved between human and mouse and contains an N box CACAAG for binding of HES-1 (Kim and Siu 1998; Sawada et al. 1994; Fig. 3A). The FP I region in mouse has 67% identity to the chicken sequence from nt 624 to 638 (as counted from the first intra-intronic nt), while the average identity of the gene is below 40%. In chicken there is an N box CACTAG for potential binding of HES-1, and we suggest that the silencer region of chicken CD4 starts at this point (Fig. 3B).

In the context of mouse *CD4* silencer, the transcription factor c-Myb has been reported to have a role as a negative regulator and to bind to the FP II site (Allen et al. 2001). The corresponding region in human does not contain a c-Myb binding site. In chicken a potential v-Myb site (a truncated form of c-Myb) is observed at 150 nt downstream from the possible HES-1 binding site (Fig. 3B).

In mouse, the third FP site has a binding site for the silencer-associated factor SAF, a member of the helix-turn-helix factor family (Kim and Siu 1999). The SAF site is a 16-base stretch with two CTGTG repeats separated by 6 bases. A similar region that contains two A/GTGTG repeats (nt 1409–1424) separated by 6 bases is also found in the putative silencer of chicken *CD4* (Fig. 3B). In addition, there are two single CTGTG subunits even closer to the potential Myb binding site. It has been reported that mutation in either of the SAF repeats affected the SAF binding in EMSA (electrophoretic mobility shift assay) competition analyses, but changes of the first nt of the subunits were not tested (Kim and Siu 1999).

Taken together, the data presented here and those obtained for the conserved function of CD4 imply a similar silencer activity for chickens as in mammals. Further analyses including functional studies of the putative chicken silencer region in the transgenic mouse model are needed to clarify its role in the regulation of CD4 expression.

Although the degree of sequence identity is low between the chicken and mammalian *CD4* gene, the structural and regulatory element conservation for potential functional action has been kept relatively unchanged. The chicken *CD4* gene is different enough from the human and mouse *CD4* to make comparisons and recognize conserved regions, whereas the sequence between human and the mouse *CD4* can be too similar to find separate regions. In addition, generally the chicken genome may have sufficient relatedness to and divergence from the human genome to reliably find the regulatory regions corresponding to those in the human sequence.

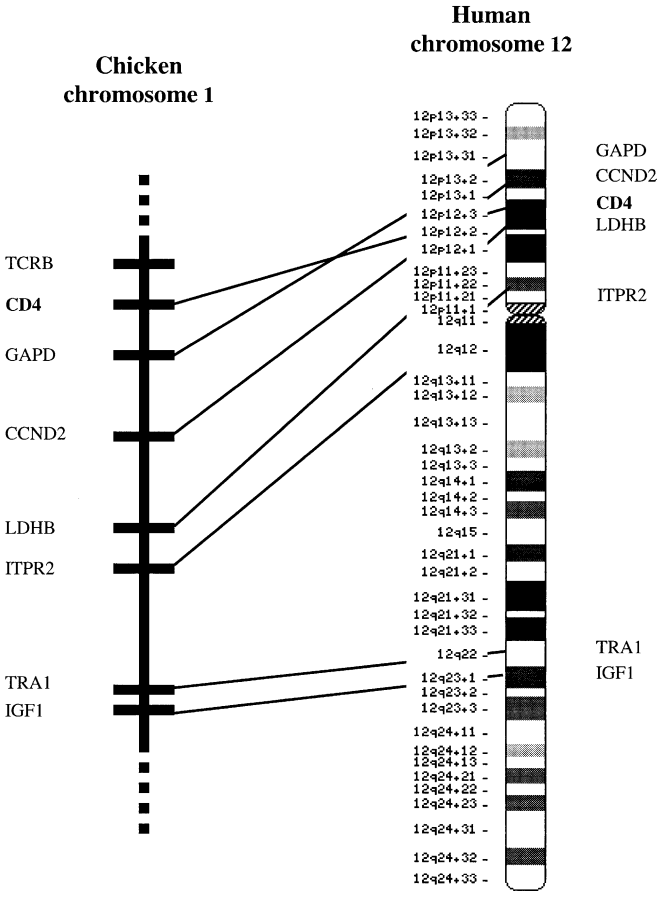


Fig. 4 The relative positions of genes flanking *CD4* in chickens and in humans. The region of chicken Chr 1 shown has been inverted to allow alignment with the human chromosome. The flanking gene *TCRB* lies on human Chr 7. Data are derived from the human and chicken genome databases at www.ncbi.nlm.nih.gov and www.ri.bbsrc.ac.uk, respectively

Chicken *CD4* maps to Chr 1

To determine the chromosomal localization of chicken *CD4* we carried out a segregation analysis using a progeny of the East Lansing reference population (Bumstead et al. 1987). An SSCP was detected for a 391-bp product amplified using primers designed from the *CD4* sequence. This product gave rise to two bands in the White Leghorn parents and three bands in the JL×WL parent of this population when analyzed on a non-denaturing SSCP gel (data not shown). The segregation pattern was scored for a panel of 52 offspring and compared with those of other loci mapped in this population. Strong linkage was observed between *CD4* and *GAPDH* (glyceraldehyde phosphate dehydrogenase, LOD 9.3) and the microsatellite marker locus ADL 0252 (LOD 6.0), placing *CD4* between these loci on chicken Chr 1, in a linkage group that shows conserved synteny with regions on human Chr 12 and mouse Chr 6 (Fig. 4).

Concluding remarks

We have determined the genomic organization of the first non-mammalian *CD4* from the chicken. The basic structure of the gene is similar to respective human and mouse genes, with many conserved motifs for potential regulation of transcription. Chicken *CD4* is localized to Chr 1 immediately adjacent to *GAPDH* in a region of conserved synteny with human and mice, confirming that the gene is the orthologue of mammalian *CD4*.

Acknowledgements We thank Paavo Toivanen, Mikael Skurnik, Ari Hinkkanen, Jari Jalava, and Riikka Lautamäki for helpful discussions, Marjo Hakkarainen, Anne Peippo, Ann Sofie Hakulinen, and Katariina Koivusalo for the excellent technical assistance, and Timo Kattelus and Teuvo Virtanen for advice on computing. This work was supported by the Academy of Finland, Cancer Foundation, the Danish National Science Research Council (grant 9401634), Emil Aaltonen Foundation, EVO financing of the Turku University Central Hospital, Finnish Cultural Foundation/Southwestern Finland, The Hede Nielsen Foundation/Denmark, Human Frontier Science Program (grant RG 366/96), Paulo Foundation, Research and Science Foundation of Farmos, and Turku University Foundation.

References

Adlam M, Duncan DD, Ng DK, Siu G (1997) Positive selection induces CD4 promoter and enhancer function. Int Immunol 9:877–887

Allen III RD, Bender TP, Siu G (1999) c-Myb is essential for early T cell development. Genes Dev 13:1073–1078

Allen RD, Kim HK, Sarafova SD, Siu G (2001) Negative regulation of CD4 gene expression by a hes-1-c-myb complex. Mol Cell Biol 21:3071–3082

Blum MD, Wong GT, Higgins KM, Sunshine MJ, Lacy E (1993) Reconstitution of the subclass-specific expression of CD4 in thymocytes and peripheral T cells of transgenic mice: identification of a human CD4 enhancer. J Exp Med 177:1343–1358

Breathnach R, Chambon P (1981) Organization and expression of eucaryotic split genes coding for proteins. Annu Rev Biochem 50:349–383

Bruniquel D, Borie N, Triebel F (1997) Genomic organization of the human Lag-3/CD4 locus. Immunogenetics 47:96–98

Bumstead N (1998) Genomic mapping of resistance to Marek's disease. Avian Pathol 27:S78–S81

Bumstead N, Greenwood NM, Messer LI (1987) Use of ev loci as a measure of inbreeding in domestic fowls. Br Poult Sci 28:717–725

Donda A, Schulz M, Bürki K, De Libero G, Uematsu Y (1996) Identification and characterization of a human CD4 silencer. Eur J Immunol 26:493–500

Duncan DD, Stupakoff A, Hendrick SM, Marcu KB, Siu G (1995) A myc-associated zinc finger protein binding site is one of four important functional regions in the CD4 promoter. Mol Cell Biol 15:3179–3186

Duncan DD, Adlam M, Siu G (1996) Asymmetric redundancy in CD4 silencer function. Immunity 4:301–311

Ellmeier W, Sawada S, Littman DR (1999) The regulation of CD4 and CD8 coreceptor gene expression during T cell development. Annu Rev Immunol 17:523–554

Field EH, Tourvielle B, D'eustachio P, Parnes JR (1987) The gene encoding the mouse T cell differentiation antigen L3T4 is located on chromosome 6. J Immunol 138:1968–1970

Gorman SD, Tourveille B, Parnes JR (1987) Structure of the mouse gene encoding CD4 and an unusual transcript in brain. Proc Natl Acad Sci U S A 84:7644–7648

Groenen MA, Cheng HH, Bumstead N, Benkel BF, Elwood Briles W, Burke T, Burt DW, Crittenden LB, Dodgson J, Hillel J, Lamont S, Ponce de Leon A, Soller M, Takahashi H, Vignal A (2000) A consensus linkage map of the chicken genome. Genome Res 10:137–147

Kim HK, Siu G (1998) The notch pathway intermediate HES-1 silences CD4 gene expression. Mol Cell Biol 18:7166–7177

Kim WS, Siu G (1999) Subclass-specific nuclear localization of a novel CD4 silencer binding factor. J Exp Med 190:281–291

Koskinen R, Lamminmäki U, Tregaskes CA, Salomonsen J, Young JR, Vainio O (1999) Cloning and modeling of the first nonmammalian CD4. J Immunol 162:4115–4121

Kozbor D, Finan J, Nowell PC, Croce CM (1986) The gene encoding the T4 antigen maps to human chromosome 12. J Immunol 136:1141–1143

Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA (1988) Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393:648–659

Littman DR, Gettner SN (1987) Unusual intron in the immunoglobulin domain of the newly isolated murine CD4 (L3T4) gene. Nature 325:453–455

Maddon PJ, Molineaux SM, Maddon DE, Zimmerman KA, Godfrey M, Alt FW, Chess L, Axel R (1987) Structure and expression of the human and mouse T4 genes. Proc Natl Acad Sci USA 84:9155–9159

Maniatis TE, Fritsch F, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Parnes JR (1989) Molecular biology and function of CD4 and CD8. Adv Immunol 44:265–311

Riegert P, Andersen R, Bumstead N, Dohring C, Dominguez-Steglich M, Engberg J, Salomonsen J, Schmid M, Schwager J, Skjodt K, Kaufman J (1996) The chicken beta 2-microglobulin gene is located on a non-major histocompatibility complex microchromosome: a small, G+C-rich gene with X and Y boxes in the promoter. Proc Natl Acad Sci USA 93:1243–1248

Salmon P, Giovane A, Wasylyk B, Klatzmann D (1993) Characterization of the human CD4 gene promoter: transcription from the CD4 gene core promoter is tissue-specific and is activated by Ets proteins. Proc Natl Acad Sci USA 90:7739–7743

Sarafova S, Siu G (1999) A potential role for Elf-1 in CD4 promoter function. J Biol Chem 274:16126–16134

Sarafova S, Siu G (2000) Precise arrangement of factor-binding sites is required for murine CD4 promoter function. Nucleic Acid Res 28:2664–2671

Sawada S, Littman DR (1991) Identification and characterization of a T-cell-specific enhancer adjacent to the murine CD4 gene. Mol Cell Biol 11:5506–5515

Sawada S, Littman DR (1993) A heterodimer of HEB and an E12-related protein interacts with the CD4 enhancer and regulates its activity in T-cell lines. Mol Cell Biol 13:5620–5628

Sawada S, Scarborough JD, Killeen N, Littman DR (1994) A lineage-specific transcriptional silencer regulates CD4 gene expression during T lymphocyte development. Cell 77:917–929

Siu G, Wurster AL, Lipsick JS, Hedrick SM (1992) Expression of the CD4 gene requires a myb transcription factor. Mol Cell Biol 12:1592–1604

Siu G, Wurster AL, Duncan DD, Soliman TM, Hedrick SM (1994) A transcriptional silencer controls the developmental expression of the CD4 gene. EMBO J 13:3570–3579

Smale ST, Baltimore D (1989) The "Initiator" as a transcription control element Cell 57:103–113