J. Fish. Sci. Technol. 8(2), 56-64, 2005

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Cloning and Characterization of Two Distinct CD3 Genes from Olive Flounder *Paralichthys olivaceus*

Mu Chan Kim[1] and Chan Il Park*

Department of Marine Biology & Aquaculture, Gyeongsang National University, Tongyeong 650-160, Korea

[1]*Department of Marine Environmental Engineering, Gyeongsang National University, Tongyeong 650-160, Korea*

Two distinct CD3 homologue genes, CD3γ/δ and CDε, were isolated from a olive flounder leukocyte cDNA library and a BAC library. CD3γ/δ consisted of 961 bp encoding 178 amino acid residues, and CD3ε consisted of 1006 bp encoding 164 amino acid residues. When compared with other known CD3 peptide sequences, the most conserved region of the two olive flounder CD3 chain peptides are the cytoplasmic domain and the least conserved are the extracellular domain. A phylogenetic analysis based on the deduced amino acid sequence grouped the two olive flounder CD3 sequences with CD3ε and CD3γ/δ, respectively. The olive flounder CD3 cluster (consisting of CD3ε and CD3γ/δ) spans only 10.4 kb. The CD3ε and CD3γ/δ genes are oppositely transcribed only 3.8 kb apart. Both olive flounder CD3 genes have five exons. The two olive flounder CD3 genes were predominantly expressed in PBLs, kidney, spleen, and gills.

Key words: Olive flounder, CD3, ITAM, T cell receptor, Teleost

Introduction

Mammalian T lymphocytes use a multicomponent cell surface receptor to recognize antigenic peptides associated with major histocompatibility complex (MHC) class I or class II molecules (Clevers et al., 1988a). The T cell receptor (TCR) complex is composed of an αβ or γδ heterodimer of TCR and associated chains (γ, δ, ε and ζ chains) of the CD3 in mammals (Klausner et al., 1990; Huppa and Ploegh, 1997). It has been demonstrated that all six polypeptides of the TCR-CD3 complex are necessary for surface-membrane expression and function (Buferne et al., 1992; Geisler, 1992; Manolios et al., 1991; Rubin et al., 1994; Sussman et al., 1988). Moreover, after TCR ligation by Ag, superantigen, or monoclonal antibodies (mAbs), the cell surface expression of TCR-CD3 is down-modulated. Down-modulation results from increased receptor internalization and degradation (Boyer et al., 1991; Krangel et al., 1987; Krangel, 1987; Niedergang et al., 1997). Internalization of cell surface receptors requires the presence of a particular sequence, which is the immunoreceptor tyrosine-based activation motif (ITAM) (Wange and Samelson, 1996).

T cell recognition, T cell function and the T cell signal transduction pathway are also important activate T cells in non-mammals (Pasquier et al., 1995). However, whereas mammals have three CD3 genes (γ, δ, and ε) (Wange and Samelson, 1996), birds have only CD3γ/δ and CD3ε genes to generate a signal transduction (Bernot and Auffray, 1991; Göbel and Dangy, 2000). CD3γ/δ was designated as it shares equal homology with both mammalian CD3γ and CD3δ (Bernot and Auffray, 1991; Dzialo and Cooper, 1997). Thus, it is important to determine whether the interaction between the antigen recognition unit and the common CD3-signal transmission unit has changed during vertebrate evolution. Calculations based on sequence divergence further demonstrated that human and mouse CD3γ and CD3δ originated from a gene duplication, which occurred about 230 million years ago (Krissansen et al., 1987). The TCR-CD3 complex has been characterized in a number of higher vertebrate phyla (Charlemagne et al., 1998; Göbel and Dangy, 2000). In contrast, difficulties in cloning fish CD3 homologues have limited their

*Corresponding author: vinus96@hanmail.net

analysis and so far, only two fish CD3 genes have been identified in Sterlet and olive flounder (Boris et al., 2000; Park et al., 2001). Moreover, non-mammalian CD3 homologues have been identified only in Xenopus, Sterlet and chicken (Dzialo and Cooper, 1997; Boris et al., 2000; Göbel and Fluri, 1997). Phylogenetic comparisons of the CD3 extracellular domains reveal low overall conservation apart from the cysteines involved in the intrachain disulfide bond and the CXXC motif. In contrast, the C terminus of the CD3 cytoplasmic domains are highly conserved throughout different vertebrate classes.

The aim of this study is the complete sequencing and physical linkage of the entire lower vertebrate CD3 cluster from a cDNA library and a BAC genomic DNA library. The number of CD3 chains in the TCR-CD3 complex of a teleost would provide important information for the understanding of the fish immune system and T cell evolution.

Materials and Methods

Cloning and sequencing of cDNAs

Two distinct CD3 cDNAs were identified from the analysis of expressed sequence tags (ESTs) of olive flounder kidney stimulated with a Con A/PMA cDNA library. Kidney was taken from a single olive flounder and stimulated with Con A/PMA as previously described by Nam et al. (2003). cDNA clones were sequenced using ThermoSequenase (Amersham) with M13 forward and M13 reverse primers (Table 1) and an automated DNA sequencer LC4200 (Li-Cor). A phylogenetic analysis was done as previously reported (Bertram et al., 1996).

The determined nucleotide and deduced amino acid sequences, and multiple sequence alignments were analyzed by GENETYX ver. 8.0 (SDC Software Development).

Cloning and sequencing of gene cluster

An olive flounder genomic BAC clones were screened for a CD3 cluster by using a specific PCR-derived probes from olive flounder CD3γ/δ and CD3ε cDNAs. These probes were also used for a Southern blot hybridization analysis. The oligonucleotide sequences of the PCR primers used in this study are given in Table 1. High-density replica filters were hybridized with these probes as previously described (Katagiri et al., 2000). The olive flounder CD3 genomic BAC clones were used for Southern blot hybridization and genomic organization analysis of the CD3 cluster. Approximately 5μg of BAC DNA was digested with *Eco*RI, *Hin*dIII, and *Pst*I. Southern blot hybridization was conducted as described previously (Hirono et al., 2000). The positive bands from Southern blot hybridization were ligated into pUC119 vector plasmid and transformed to JM109.

Table 1. The oligonucleotide primer sets used in the present study

Primer name	Sequence of oligonucleotide primer
M13 forward	5'-CACGACGTTGTAAAACGAC-3'
M13 reverse	5'-GGATAACAATTTCACACAGG-3'
CD3GD BAC-F	5'-GCTGTCTACCTTATCGCGTC-3'
CD3GD BAC-R	5'-GCTGCTAGAACCACGCCAC-3'
CD3E BAC-F	5'-GCTACTTTCTCACATCTGCTC-3'
CD3E BAC-R	5'-CATGGATGTCCGGGCAGTCATC-3'
CD3GD RT-F	5'-CTCAGAAGACAGAGAAGTGC-3'
CD3GD RT-R	5'-TGCATCACACGCTGCACATC-3'
CD3E RT-F	5'-CCAAAGGCTTGTATAGATGTG-3'
CD3E RT-R	5'-CACATTTGAGATTAGCAGTGTA-3'
Beta actin-F	5'-TTTCCCTCCATTGTTGGTCG-3'
Beta actin-R	5'-GCGACTCTCAGCTCGTTGTA-3'

Tissue distribution of olive flounder CD3 genes transcript

Total RNA (50 ng) from the brain, heart, intestine, kidney, liver and spleen were reverse-transcribed into cDNA using an AMV Reverse Transcriptase First-Strand cDNA synthesis kit (Life sciences). PCR was performed on the resulting cDNA using the CD3GD

RT-F/CD3GD RT-R and CD3E RT-F/CD3E RT-R specific primer sets (Table 1). β-Actin was amplified as a control using the Beta-actin-F and Beta-actin-R primers (Table 1). The PCR mixtures were denatured at 94°C for 2 min and then subjected to 25 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 1 min. The products were visualized by separation on a 1.5% agarose gel.

Results

Identification of the CD3 cDNAs from olive flounder

Two distinct CD3 homologues, designated CD3γ/δ and CDε, were cloned. CD3γ/δ consisted of 961 bp encoding 178 amino acid residues, and CDε consisted of 1006 bp encoding 164 amino acid residues. The CD3γ/δ amino acid sequence alignment indicates conservation of the four cysteine residues involved in disulfide bonds, the glutamic acid residue following the two cysteines (CXXCXE motif), and the ITAM, all of which are thought to be important characteristics of CD3 chains (Fig. 1). The predicted amino acid sequence of olive flounder CD3ε was a putative type I membrane protein containing a 22-amino acid signal peptide, a 67-amino acid extracellular domain,

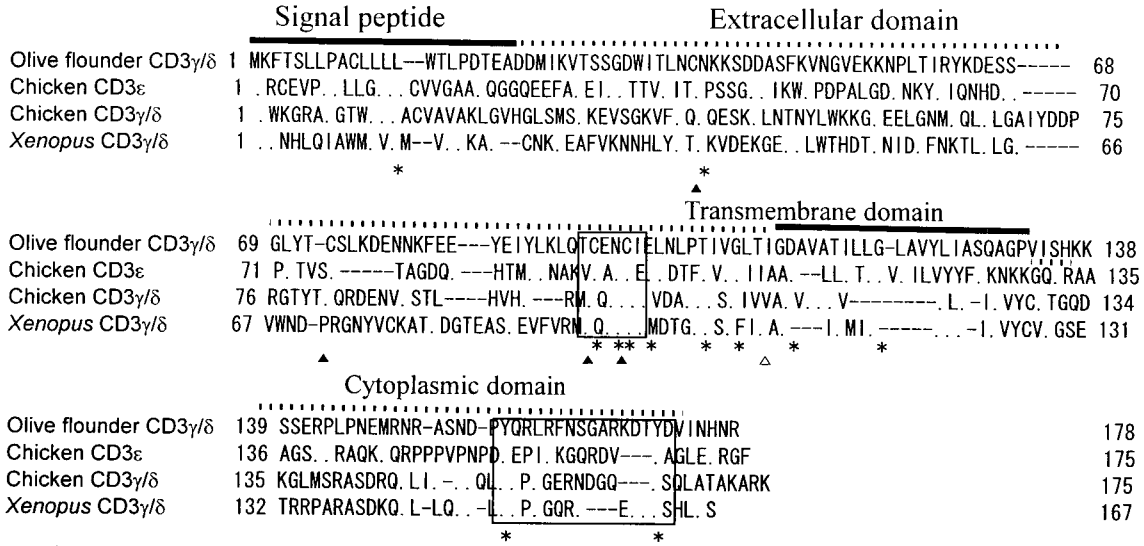

Fig. 1. Amino acid sequence alignment of CD3s. Sequences were obtained from DDBJ/EMBL/GenBank. Amino acids identical to the olive flounder (*Paralichthys olivaceus*) CD3 are shown by dots. The positions of cysteine residues are indicated by closed triangles and a single negatively charged aspartic acid residue is indicated by opened triangle. The conserved CXXC motifs and ITAMs are boxed. Residues that are identical in all sequences are shown as asterisks. Gaps (*dashes*) have been placed to maximize the identity.

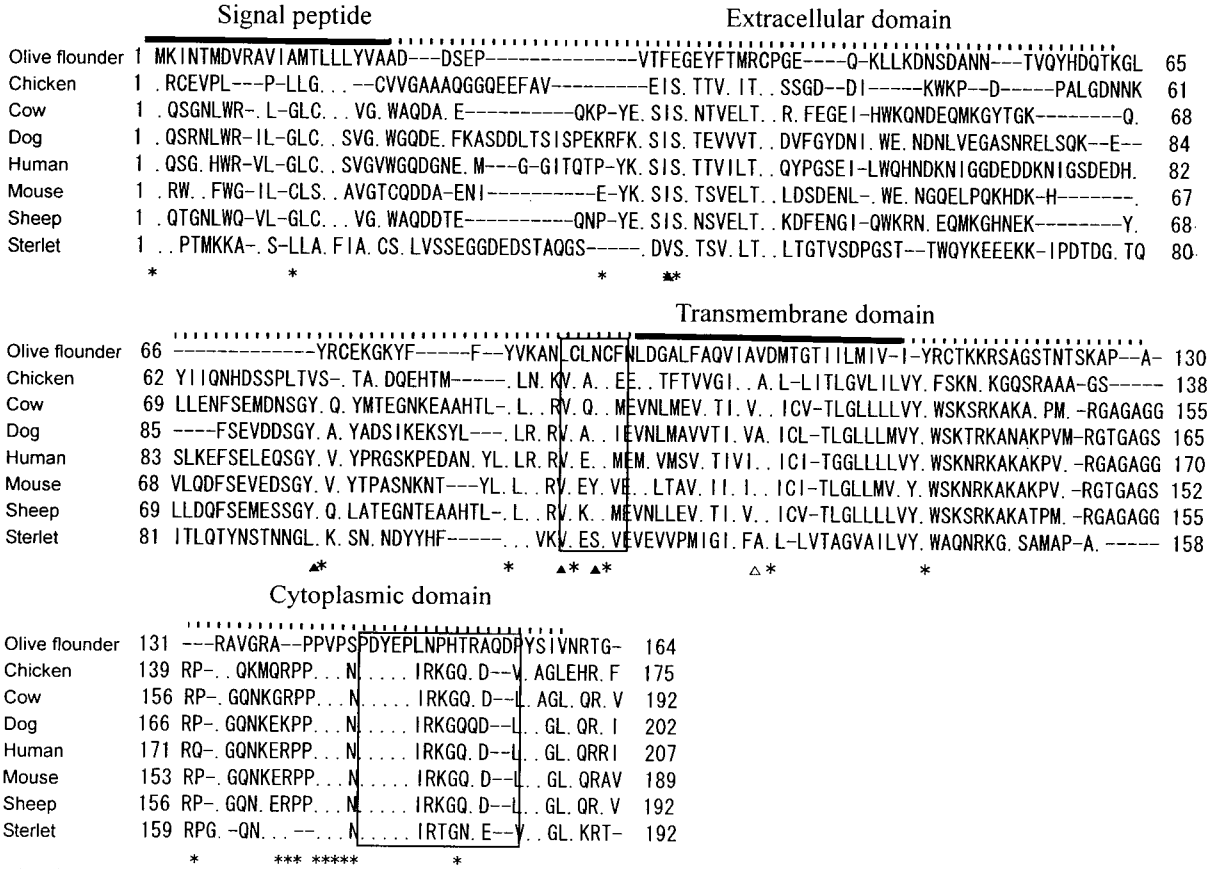

Fig. 2. Amino acid sequence alignment of CD3ε peptides. Sequences were obtained from DDBJ/EMBL/GenBank. Amino acids identical to the olive flounder (*Paralichthys olivaceus*) CD3ε are shown by dots. The positions of cysteine residues are indicated by closed triangles and a single negatively charged aspartic acid residue is indicated by opened triangle. The conserved CXXC motifs and ITAMs are boxed. Residues that are identical in all sequences are shown as asterisks. Gaps (*dashes*) have been placed to maximize the identity.

a 24-amino acid transmembrane, and 51-amino acid cytoplasmic domain (Fig. 2). Like CD3ε sequences in other species examined so far, the olive flounder CD3ε sequence contains four cysteines and a single negatively charged asparagine residue at position 100 within the transmembrane domain. These residues are believed to play a role in stabilization of the TCR-CD3 complex. Olive flounder CD3ε contains two potential tyrosine phosphorylation sites within the ITAM at positions 144 and 157 (Fig. 2).

A phylogenetic tree based on the amino acid sequence alignment indicates that the olive flounder CD3ε is located between the Sterlet CD3ε as shown by Boris et al. (2000) and olive flounder CD3γ/δ as shown by Park et al. (2001) (Fig. 3).

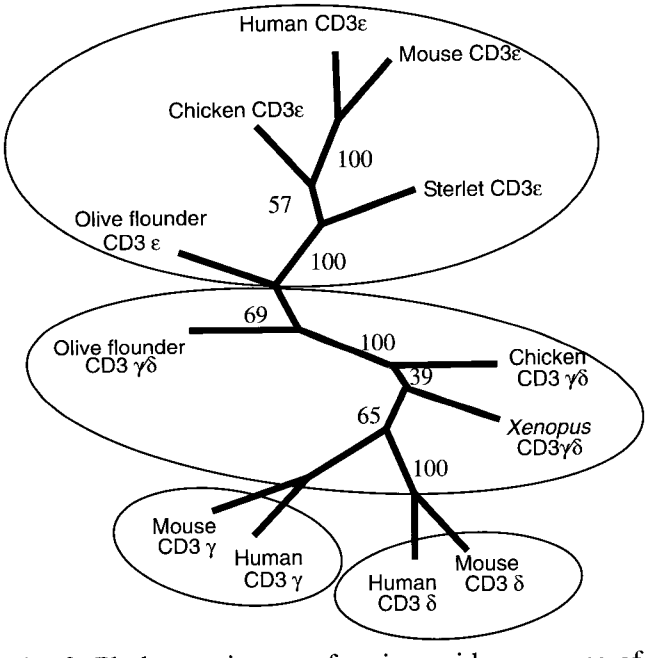

Fig. 3. Phylogenetic tree of amino acid sequences of CD3ε, CD3γ, CD3δ, and CD3γ/δ. The phylogeny of CD3s was estimated by the neighbor-joining method of clustering in the PHYLIP program.

Characterization of CD3 cluster sequence from olive flounder

A BAC library was screened with an olive flounder CD3 cDNA fragments and two positive clones were isolated. The olive flounder CD3γ/δ gene is approximately 2.8 kb long and consists of five exons and four introns (Fig. 4). The olive flounder CD3ε is encoded on five exons separated by four introns (Fig. 5). Typical intron splice motifs occur at the 5'(gt) and 3'(ag) ends of each intron. The structure and exon patterns of the olive flounder CD3 cluster are more similar to those in the chicken CD3 cluster (Göbel and Dangy, 2000) than to those in the human CD3 cluster (Clevers et al., 1988b) (Fig. 6). The size of the olive flounder CD3 cluster (10.4 kb) is smaller than that of the human CD3 cluster (50 kb). The CD3γ/δ and CD3ε genes are oppositely transcribed and separated by a distance of only 3.8 kb, contrast with a distance of 22 kb in humans (Fig. 6).

CD3ε is expressed in PBLs, kidney, spleen, and gills

Expression of the two CD3 genes in the tissues of olive flounder was detected by RT-PCR. Olive flounder CD3ε transcripts were expressed predominantly in the PBLs, kidney, spleen, and gills but not in liver, brain, or muscle after 25 cycles of PCR (Fig. 7). The patterns of detection of CD3γ/δ were same as those of CD3ε in different tissues.

Discussion

CD3 expression in mammals occurs early in lymphoid development and in mature T cells. This paper is the first to describe an entire fish CD3 cluster. The predicted amino acid sequences of olive flounder CD3s were more homologous to the predicted amino acid sequences of other vertebrate CD3s in the cytoplasmic domain than in the extracellular domain. The two olive flounder CD3s extracellular and cytoplasmic domains are shorter than those in human, mouse, and chicken. However, cysteine residues in the extracellular domain, a single negatively charged aspartic acid residue within the transmembrane domain, and ITAM, which are important characteristics of the CD3 chain, are well conserved. Most CD3 chains conserve a CXXC motif in the extracellular domain. The ITAM participates in intracellular signaling by CD3 (Reth, 1989) or in regulation of the assembly and surface expression of the TCR-CD3 complex (Mallabiabarrena et al., 1992). The amino acid sequence of ITAM in the mammalian (Clevers et al., 1988a) and chicken (Göbel and Fluri, 1997) CD3s is YXPL/IX$_7$YSXL, whereas, it is YXRLX$_{10}$YDVI in olive flounder CD3γ/δ (Fig. 1) and YXPLX$_9$YAGV in olive flounder CD3ε (Fig. 2). It is unknown whether the spacing of the variant amino acid and substituted valine residue of the ITAM are required for receptor signaling transduction. However, the strict adherence to this pattern in all known the mammalian and the non-mammalian ITAMs during evolution may indicate that_it allowed the recruitment of a more specific kinase to enhance signal transduction. Alternatively, TCR-mediated signal transduction in fish might favor a binding site that is slightly different from there in the mammalian and non-mammalian

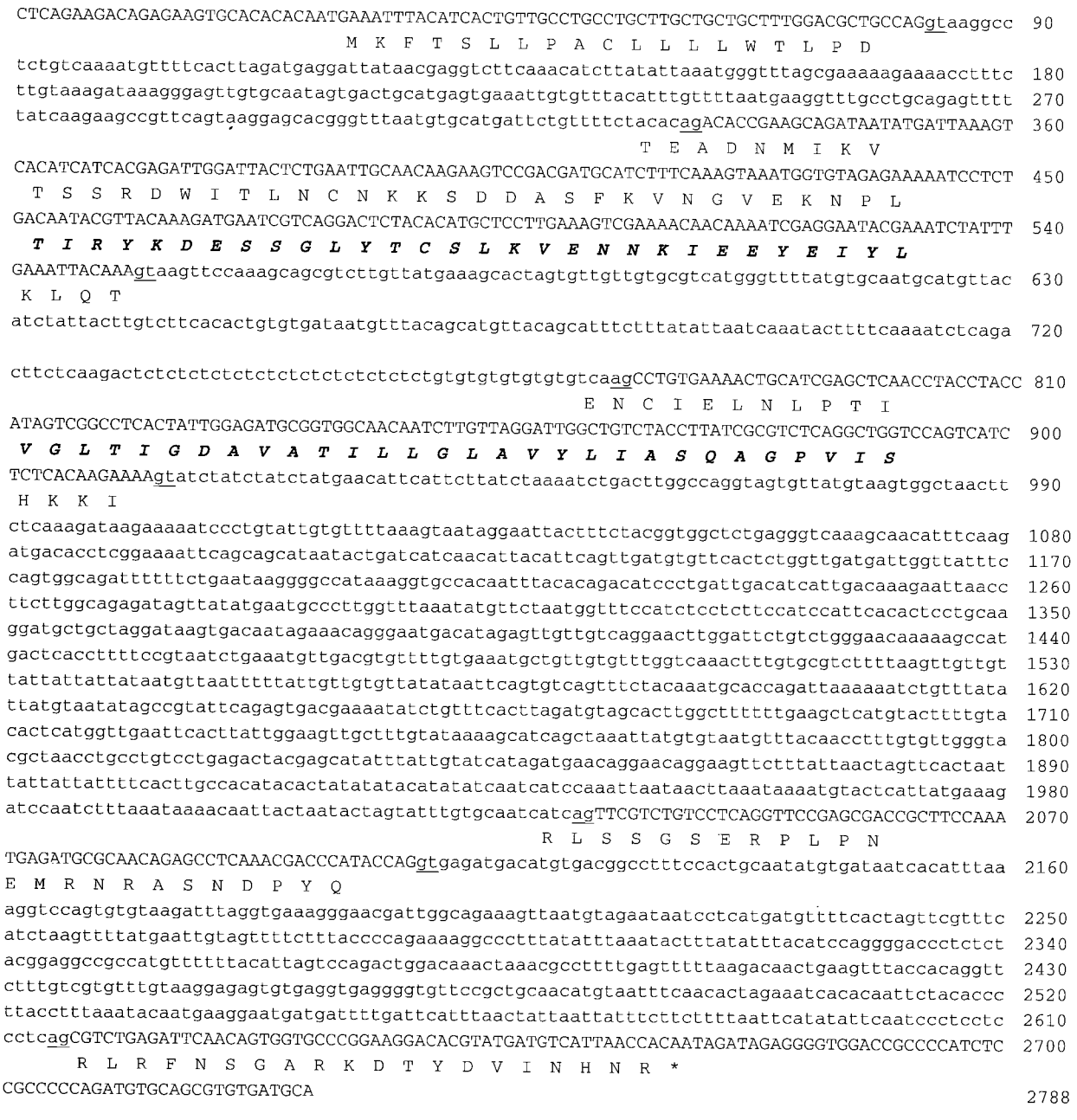

Fig. 4. Nucleotide and deduced amino acid sequences of the olive flounder (*Paralichthys olivaceus*) CD3γ/δ gene. The 5'(gt) and 3'(ag) ends of each intron are underlined.

CD3s. The CD3ε amino acid sequence of the Sterlet (Boris et al., 2000), which is a more primitive fish (a chondrostei) than the olive flounder, also has an ITAM, which has significant identity to those of other vertebrates. The numbers of exons in vertebrate CD3s do not_reveal any evolutionary patterns (Fig. 6). Indeed, with CD3 sequence available for only one fish species (Sterlet), it is too early to speculate about CD3 evolution. In the phylogenetic analysis, the olive flounder CD3γ/δ is closer to non-mammalian CD3γ/δs and the olive flounder CD3ε is closer to other CD3εs (Fig. 3).

Olive flounder CD3γ/δ and CD3ε each contains five exons. This appears to be the most primitive form of the CD3 gene because the mammalian CD3γ, CD3ε and chicken CD3ε genes have 2 or 3 mini-exons (Clevers et al., 1988b; Göbel and Dangy, 2000). These data suggest that the olive flounder CD3 gene represents an ancestral CD3 form that gave rise to both the CD3γ and CD3δ genes, as suggested that the

```
GCTACTTTCTCACATCTGCTCTATACAATAATAACATATAGAAGCTCAGAACAGAATCAGGGGTCGTCTGATGAAAATCAACACCATGGA 90
                                                                  M  K  I  N  T  M  D
TGTCCGGGCAGTCATCGCCATGACGCTCCTCCTGTATGTAGCTGCCGATGACAGCGAGCCTGgtaaatatctaactgctctgagggtggt 180
 V  R  A  V  I  A  M  T  L  L  L  Y  V  A  A  D  D  S  E  P
ttcgactctgcttcatctttccaagtcatgtattgtcattaggctcatttagatttagaacaacacacattctgatgataatattacatt 270
tcagagaatgtttaacaaaaccgctcgaatttcctgtatttcatttgtgtgctgtatctactgtattgaaaaggtctgtgtttttccatg 360
tgatgcaccagactgaactctaatctttccctcggatttgtgtttcctttgcagtagagtatagatgcattttatttttgatagtttgatg 450
tattttggtatcgttcctgactgttatttgacacgtgtcttagcgtggtgtattacacagtaagacatagaacatatcagtaaccccatg 540
ttgaaatgaatgatttactatattattatattgtattatatacagtgtatatatatatatatatatatatgcgctgcagtaaaatctaaa 630
tcacttccattctgtgtacaaaggtttttttcaatacacaagagtaaatatgattctgatatgaagaggtgtctttgttcaaaaagctaaa 720
acagggattaaaagtagatgtgtctattgctggggtgagatttcggaacaaaacatatacaaaacttaaaatgtttaatttgttaattcc 810
ctaaaaataaattacaaaaactgtgtttgagggctacacatgtaaaagttttaaggcatctagaggcatttgcttctgcttgaacttttg 900
tctgtcaatatatattgtgaagacagttcagactttgtttacactgctgacattgtttacagttgctttacaatatgtgatgatgactga 990
atgaatcaatcaattcaaaaaatgatyttagatatacaaaagggaggtttggtcttggacacccacctcatgcccatataaagaaacgtg 1080
gagtaaggatgttgatgaagtgatgaaatgaagctaaaactcaacatgccatgtgtttttctttttcttcttttttccagTAACATTCGAGG 1170
                                                                                  V  T  F  E
GGGAATATTTCACAATGAGGTGTCCAGGAGAACAGAAATTGTTCAAGGATAATAGTGATGCGAACAATACTGTACAGTACCACGATCAAA 1260
 G  E  Y  F  T  M  R  C  P  G  E  Q  K  L  F  K  D  N  S  D  A  N  N  T  V  Q  Y  H  D  Q
CCAAAGGCTTGTATAGATGTGAAAAGGGAAAATATTTTTTCTACGTGAAAGCAAATTgtgagtaaagggtcttttaatgtctcacctgag 1350
 T  K  G  L  Y  R  C  E  K  G  K  Y  F  F  Y  V  K  A  N
tcaggtgtcctattctgagcaaacacttttgtaactgctgttctttgttccctagTGTGTTTAAATTGTTTCAACCTGGACGGAGCTTTG 1440
                                                          L  C  L  N  C  F  N  L  D  G  A  L
TTCGCTCAGGTCATCGCTGTGGACATGACCGGGACGATAATTCTGATGATAGTCATCTATAGGTGCACCAAGAAACGCTCAGCTGGATCT 1530
 F  A  Q  V  I  A  V  D  M  T  G  T  I  I  L  M  I  V  I  Y  R  C  T  K  K  R  S  A  G  S
ACAAACACCTCAAAAGgtaaagattttacagtttctgtgctggacgttgtttgcatagcttttaacagtcaatggtgcagagtgagaaaa 1620
 T  N  T  S  K
ctttgtgttcatcctgcctggtttatctggaatggacacaaaattaaacagaatttgctttattactgtttacgtgtgtggtttattata 1710
agttttagtgattgaccaaactctttttttttttttacatcgcttatcgactatttcagatgtctcttcagcgattgacacaatcttcaaa 1800
gttcacaacttataaaaatgaaactctgtaattcagctctatgtaagttattgcagcaacacacaagggttgtgctttcacttttggggac 1890
gaattgggaaccagggaagcgatttaatgaatgttgttgagttgtaggtccactgatgtcgtttgactgaggcacatgtaattcacgttt 1980
ttcttgatgcatgctgatttttttaggtgagcttttgcagctgatgtggccctttgaaattctaaattcacctgacgccgaagagtgctga 2070
ctcgccatatagattagattttggctccagggacattttacaaggagctttatcctgatgtaataaaagctgatacaacttgtctgcata 2160
cacagtgaagcacaaagctttttaagtaatatgtgcttggtcacagtattgttgtcattactttgcagCACCTGCTCGTGCAGTAGGCCG 2250
                                                                    A  P  A  R  A  V  G
TGCACCACCGGTCCCATCTCCTGACTATGAGgtaggttttgtttttcgtccaatgtccattaattccttgatgaactcaatgaaagaggg 2340
R  A  P  P  V  P  S  P  D  Y  E
gtgccaagaaaggaaagtgctcaggacacaaccccacagtttctcttaagaacgtttgtgacaccagccacacgctcagatgtctgtcta 2430
cgacacaataaatggtagatgcctggaaatcttctctgatgcttccagttatctgattctttctcaagtgtactgaagagaggacataag 2520
tttggatatgcatgtatctgtagccgtgctctacagatactatattgttacagcctcccataggtgatgaaaaaattcgaaaaaggcatt 2610
tcattagcagcattttgaatgtgaggtgaaaaagtgatcggaaaaagtgaactcagcagagagaaatgtgggtttgcatctgttaataat 2700
gagcttcgatgagagatggaacttctcaagttggcaatttatttgttggaggcagtcccagctgacattgagttcagctccagaattata 2790
gttaaatattttgtcattgtaaataaagtgaaaatagctgaaaattttaaatcattgtccatgggcgagatatttacatacccaaattgc 2880
ccctgagcgctgtgcagagtgatggatgaaagagagagtgctgcacagatgcactgtacgacgatcctcagatgagggcttgacaattgt 2970
ttctatatttcattttaccccatcgacggtgatatgttgtggtttctctcatgtgacaaatgtacttattgtaagtcactttagacaaaa 3060
gcgtctgctaaaggccctaaatgtaaatgtaaatgtgggatgtgtgtgtgaatgggtgtatggcaaaactgtgaagctctttgattggcc 3150
accaagacaagaagagcgctatgtaaacacaccatttaacaataatattgaaaattgtgtattgctttctaaccatcttaaacaaacaac 3240
tatcacacagtttagtggttatatgacactgatccacctaacactgtatatgttgtgttttggcagCCACTGAACCCGCACACTCGCGCC 3330
                                                                    P  L  N  P  H  T  R  A
CAGGACCCTTACTCTATTGTGAACAGGACGGGATAAAGTCACATGACCTGACTGCACCAGGACACTGGtTTAAAGGCTGCACCGACAAAA 3420
 Q  D  P  Y  S  I  V  N  R  T  G  *
GCATTAACACCATGCTCCCAGTTTGTGCAGCGGTGACCTGGAATCTAGTTTGTGGGATTTTCAGTATTTGCAAATTATACTGAAAAACTA 3510
CTTTCCTCTCCAGAAATCATCCCTAAAGAAACCATCAACAGCGTGTATAGATTCAGGGGAATGTTCACATTGCTTTTGTGTGTTGCTGTA 3600
GATTTGTTGCAGTCATTTACAACCTGAGGGTACTTCAGCAGCTCAGCTTATTTGAAACTGACTAATTGTCATGTTCATGATCATGTTTAG 3690
GAAGGAAAATAATTACAAAAATAACATGGAATTGCAAAATTGGTACACTGCTAATCTCAAATGTGTTTATTGTAACAAAAATAAAGAGAG 3780
GTTTTCTCCGGG                                                                              3792
```

Fig. 5. Nucleotide and deduced amino acid sequences of the olive flounder (*Paralichthys olivaceus*) CD3ε gene. The 5'(gt) and 3'(ag) ends of each intron are underlined.

human and mouse CD3γ and CD3δ genes have originated from a gene duplication that occurred about 230 million years ago (Krissansen et al., 1987).

Relatively large quantities of olive flounder CD3ε transcript were expressed in PBLs, kidney, spleen, and gills. CD3γ/δ mRNAs were found in the same

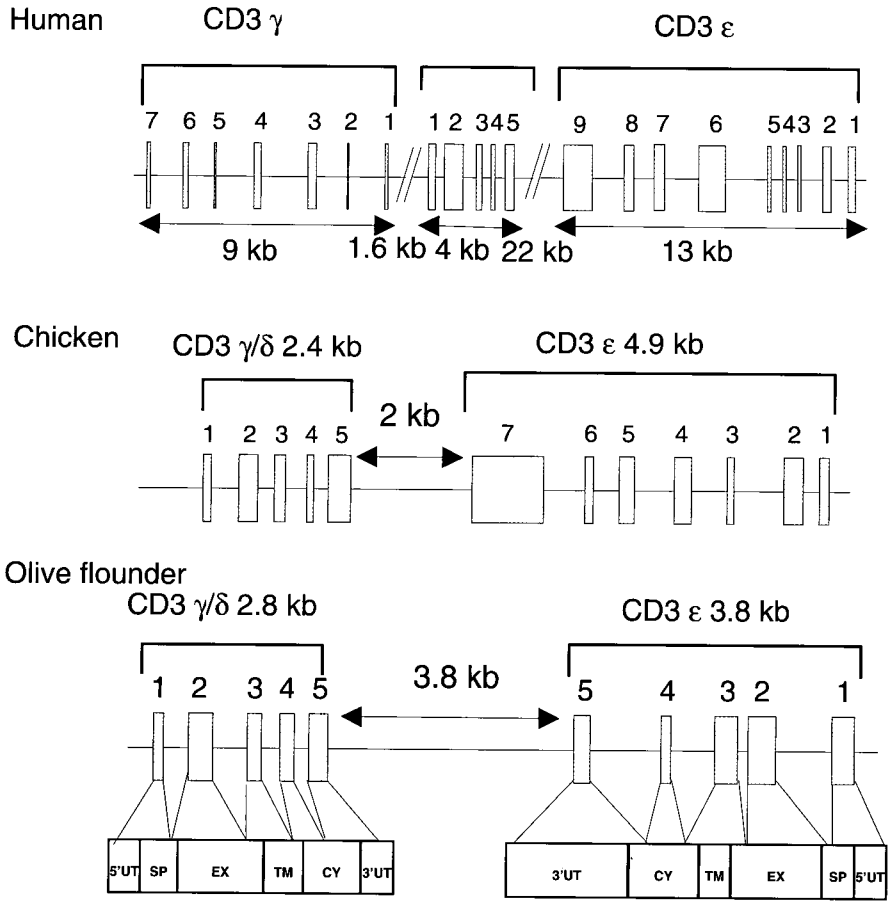

Fig. 6. Comparison of the human, chicken and olive flounder (*Paralichthys olivaceus*) CD3 clusters. Sketch representing the human and chicken CD3 cluster organization. Approximate size of CD3 genes and the intervening sequences are given. Genomic construction of the olive flounder CD3s. Their de-rived mRNAs are drawn to scale as indicated. The genomic DNAs and mRNAs are represented in the top and bottom, respectively. Exons are indicated by closed boxes.

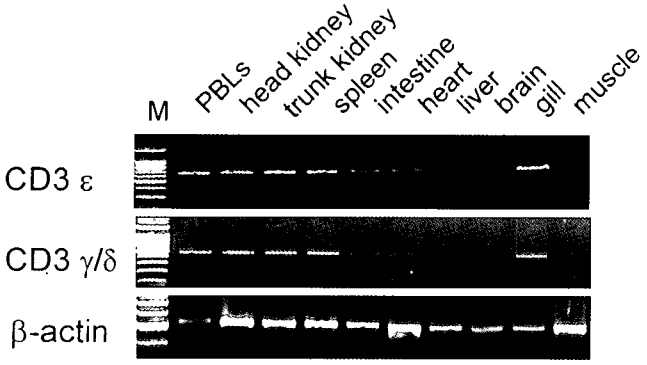

Fig. 7. Expression of CD3 mRNAs levels in various tissues of healthy olive flounder (*Paralichthys olivaceus*) as determined by RT-PCR. β-Actin amplification was included as a control. M; 100-bp ladder marker.

tissues (Fig. 7). These results suggest that the CD3ε and CD3γ/δ genes are very closely related and the two CD3 chains may form a heterodimer.

Further work on primitive fish (chondrostei) may elucidate the duplication of CD3 and stepwise evolution of TCR-CD3 complex. It will be of particular interest to identify the point at which CD3γ/δ was added to the TCR-CD3 complex in lower vertebrates. A probable model for CD3 evolution would predict two successive gene duplications where a single CD3 gene was first duplicated to form CD3γ/δ and CD3ε and a second duplication of CD3γ/δ has finally generated mammalian CD3γ and CD3δ.

Acknowledgements

We thank Drs. Takashi Aoki and Ikuo Hirono, Tokyo University of Marine Science and Technology, for generously accommodating me for the work on

cDNAs and genes cloning in their laboratory.

References

Bernot, A. and C. Auffray. 1991. Primary structure and ontogeny of an avian CD3 transcript. Proc. Natl. Acad. Sci. USA, 88, 2550-2554.

Bertram, E.M., R.G. Wilkinson, B.A. Lee, A.R. Jilbert and I. Kotlarski. 1996. Identification of duck T lymphocytes using an anti-human T cell (CD3) antiserum. Vet. Immunol. Immunopathol., 51, 353-363.

Boris, Y.A., V.G. Sergei, M.N. Alexander, V.M. Ludmila and V.T. Alexander. 2000. CD3ε homologues in the chondrostean fish *Acipenser ruthenus*. Immunogenetics, 51, 1012-1020.

Boyer, C., N. Auphan, F. Luton, J.M. Malburet, M. Barad, J.P. Bizozzero, H. Reggio and A.M. Schmitt-Verhulst. 1991. T cell receptor/CD3 complex internalization following activation of a cytolytic T cell clone: evidence for a protein kinase C-independent staurosporine-sensitive step. Eur. J. Immunol., 21, 1623-1634.

Buferne, M., F. Luton, F. Letourneur, A. Hoeveler, D. Couez, M. Barad, B. Malissen, A.M. Schmitt-Verhulst and C. Boyer. 1992. Role of CD3 delta in surface expression of the TCR/CD3 complex and in activation for killing analyzed with a CD3 delta-negative cytotoxic T lymphocyte variant. J. Immunol., 148, 657-664.

Charlemagne, J., J.S. Fellah, A. De Guerra, F. Kerfourn and S. Partula. 1998. T-cell receptors in ectother-mic vertebrates. Immunol. Rev., 166, 87-102

Clevers, H., B. Alarcon, T. Wileman and C. Terhorst. 1988a. The T cell receptor/CD3 complex: a dyna-mic protein ensemble. Annu. Rev. Immunol., 6, 629-662.

Clevers, H.C., S. Dunlap, T.E. Wileman and C. Terhorst. 1988b. Human CD3-ε gene contains miniexons and is transcribed from a non-TATA promoter. Proc. Natl. Acad. Sci. USA, 85, 8156-8160.

Dzialo, R.C. and M.D. Cooper. 1997. An amphibian CD3 homologue of the mammalian CD3 gamma and delta genes. Eur. J. Immunol., 27, 1640-1647.

Geisler, C. 1992. Failure to synthesize the CD3-gamma chain. Consequences for T cell antigen receptor assembly, processing, and expression. J. Immunol., 148, 2437-2445.

Göbel, T.W. and M. Fluri. 1997. Identification and analysis of the chicken CD3 ε gene. Eur. J. Immunol., 27, 194-198.

Göbel, T.W. and J.P. Dangy. 2000. Evidence for a stepwise evolution of the CD3 family. J. Immunol., 164, 879-883.

Hirono, I., B.H. Nam, T. Kurobe and T. Aoki. 2000. Molecular cloning, characterization, and expression of TNF cDNA and gene from Japanese flounder *Paralichthys olivaceus*. J. Immunol., 165, 4423 -4427.

Huppa, J.B. and H.L. Ploegh. 1997. In vitro translation and assembly of a complete T cell receptor-CD3 complex. J. Exp. Med., 186, 393-403.

Katagiri, T., S. Asakawa, I. Hirono, T. Aoki and N. Shimizu. 2000. Genomic bacterial artificial chromosome library of the Japanese flounder *Paralichthys olivaceus*. Mar. Biotechnol., 2, 571-576.

Klausner, R.D., J. Lippincott-Schwartz and J.S. Bonifacino. 1990. The T cell antigen receptor: insights into organelle biology. Ann. Rev. Cell Biol., 6, 403-431.

Krangel, M.S. 1987. Endocytosis and recycling of the T3-T cell receptor complex. The role of T3 phosphorylation. J. Exp. Med., 165, 1141-1159.

Krangel, M.S., B.E. Bierer, P. Devlin, M. Clabby, J.L. Strominger, J, McLean and Brenner M.B. 1987. T3 glycoprotein is functional although structurally distinct on human T-cell receptor gamma T lymphocytes. Proc. Natl. Acad. Sci. USA, 84, 3817-3821.

Krissansen, G.W., M.J. Owen, P.J. Fink and M.J. Crumpton. 1987. Molecular cloning of the cDNA encoding the T3 gamma subunit of the mouse T3/T cell antigen receptor complex. J. Immunol., 138, 3513-3518.

Mallabiabarrena, A., M. Fresno and B. Alarcón. 1992. An endoplasmic reticulum retention signal in the CD3 ε chain of the T-cell receptor. Nature, 357, 593-596.

Manolios, N., F. Letourneur, J.S. Bonifacino and R.D. Klausner. 1991. Pairwise, cooperative and inhibitory interactions describe the assembly and probable structure of the T-cell antigen receptor. EMBO J., 10, 1643-1651.

Nam, B.H., I. Hirono and T. Aoki. 2003. Bulk isolation of immune response-related genes by expressed sequenced tags of Japanese flounder *Paralichthys olivaceus* leucocytes stimulated with Con A/PMA. Fish Shellfish Immunol., 14, 467-476.

Niedergang, F., A. Dautry-Varsat and A. Alcover. 1997. Peptide antigen or superantigen-induced downergulation of TCRs involves both stimulated and unstimulated receptors. J. Immunol., 159, 1703-1710.

Park C.I., I. Hirono, J. Enomoto, Nam B.H., T. Aoki. 2001. Cloning of Japanese flounder *Paralichthys olivaceus* CD3 cDNA and gene, and analysis of its expression. Immunogenetics, 53, 130-135.

Pasquier, L., M. Courtet and J. Robert. 1995. A Xenopus lymphoid tumor cell line with complete Ig genes rearrangements and T-cell characteristics. Mol. Immunol., 32, 583-593.

Reth, M. 1989. Antigen receptor tail clue. Nature, 338, 383-344.

Rubin, B.J. Arnaud, S. Caspar-Bauguil, F. Conte and Huchenq 1994. A. Biological function of the extracellular domain of the T-cell receptor constant region. Scand. J. Immunol., 39, 517-525.

Sussman, J.J., J.S. Bonifacino, J. Lippincott-Schwartz, A.M. Weissman, T. Saito, R.D. Klausner and J.D. Ashwell. 1988. Failure to synthesize the T cell CD3-zeta chain: structure and function of a partial T cell receptor complex. Cell, 52, 85-95.

Wange, R.L. and L.E. Samelson. 1996. Complex complexes: signaling at the TCR. Immunity, 5, 197-205.

(Received July 2004, Accepted May 2005)