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Characterization of Murine CD70, the Ligand of the TNF Receptor Family Member CD27

Kiki Tesselaar,* Loes A. Gravestein,† Gijs M. W. van Schijndel,* Jannie Borst,† and René A. W. van Lier²*

Human CD70 (CD27 ligand) is a type II transmembrane glycoprotein belonging to the TNF family. The protein is not expressed on resting lymphocytes, but is rapidly induced on these cells after cellular activation. Importantly, interaction of CD70 with its receptor CD27 gives a costimulatory signal for lymphocyte activation. Whereas CD27 has been molecularly characterized in the mouse, murine CD70 (mCD70) was undefined until now. Here, we describe the cDNA cloning and initial characterization of mCD70 and the determination of its gene structure. mCD70 is a polypeptide of 195 amino acids that has 62% homology with its human counterpart. In analogy to human CD70, mCD70 transcript levels are strongly but transiently up-regulated during lymphocyte activation, which is in line with a role for the CD27-CD70 receptor pair early in the immune response. In accordance with the comitogenic activity of mCD27-specific mAb, recombinant mCD70 potently costimulates T cell proliferation. Finally, the mCD70 gene consists of three exons spanning ~4 kb of DNA and is localized on chromosome 17. The Journal of Immunology, 1997, 159: 4959–4965.

Members of the TNF receptor family are key regulators of cellular immune reactions as they control proliferation, differentiation, and, importantly, apoptosis of activated immune cells. CD27, a member of this group of receptors, is expressed on the majority of mature T cells and on memory-type B cells. Ligation of CD27, either through agonistic mAb or recombinant ligand (CD70), generates a strong costimulatory signal for T cell proliferation (1, 2). Importantly, T cell activation induces a marked up-regulation of CD70 membrane expression, especially on unprimed (CD45RA⁺) T lymphocytes (3, 4). Furthermore, because CD27 cross-linking preferentially costimulates the proliferation of unprimed T cells (5, 6), a role for CD27-CD70 interactions early in immune reactions has been postulated (7).

The CD70 ligand CD70 is a type II transmembrane glycoprotein that is expressed on activated, but not on resting, T and B cells (2, 8, 9). In agreement with a role for CD27-CD70 interactions during discrete stages of the immune response, expression of CD70 in vivo appears to be very restricted (10, 11). The limited expression pattern appears to be related to the fact that cellular activation signals, such as Ag, costimulatory molecules, and cytokines, carefully tune the level of CD70 expression on stimulated lymphocytes (11, 12). T and B cells that express CD70 in vivo have a primed phenotype compared with CD70⁻ cells. For instance, circulating CD70⁺ T cells are considerably enriched for IFN-γ-secreting T cells (12), whereas production of Ig in response to T cell-derived helper signals is largely confined to CD70⁻ B cells (13). Interestingly, for some members of the TNF family, e.g., OX40, CD40, and CD30 ligands (14–18), their function seems not to be restricted to their roles as ligands, but these proteins apparently also have a signaling potential. Cross-linking of OX40 ligand induces the proliferation and differentiation of murine splenic B cells (14), whereas CD40 transfectants can costimulate the activation of CD4⁺ cells (16). Enhancement of T cell proliferation upon cross-linking with CD70 mAb indicates the possibility of signal transduction via CD70 (9).

Recently, murine CD27 (mCD27) has been cloned, and its expression pattern and function have been analyzed (19). In accordance with studies performed in the human system, mCD27 is expressed on mature T cells, and cross-linking of mCD27 enhances T cell proliferation (20). However, contrary to human thymocytes in which CD27 is expressed on single positive (CD4⁺ or CD8⁺) thymocytes (21) and is inducible on CD4⁺CD8⁺ cells, mCD27 is already expressed in immature CD4⁻CD8⁻CD25⁺ thymocytes. Since it was previously found that, next to activated thymocytes, thymic epithelial cells express CD70 (10), it was inferred that CD27-CD70 interactions may play a role in early T cell development. Indeed, inhibition of CD27 function on immature CD4⁻CD8⁻CD25⁺ thymocytes in vivo impedes generation of the CD4⁺CD8⁻ thymocyte pool (22).

To further elucidate the role of the interaction between CD27 and CD70 in vivo, it was mandatory to characterize mCD70. Herein we describe the cloning and characterization of the mCD70 cDNA, the determination of the gene structure, and the chromosomal localization of the mCD70 gene. We also show that, in analogy to its human homologue, mCD70 is an activation Ag that is rapidly but transiently up-regulated after lymphocyte activation. Finally, the functional characteristics of mCD70 are described.

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_sequences have been deposited in the GenBank/EMBL Sequence Database under accession no. Y13636 (cDNA), Y13637 (exons 1 and 2), and Y13638 (exon 3).

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Materials and Methods

Monoclonal Abs and receptor-Fc proteins

CLB-T11.1/1, CLB-T11.2, HIK27 (all CD2 mAbs), CLB-CD4/1/4, CLB-
19/1, CLB-CD3/4E, anti-THLA class II (E1), and CLB-Fc gral (1CD6)
were produced at the Central Laboratory of The Netherlands Red Cross
Blood Transfusion Service (Amsterdam, The Netherlands). Thy-1.2 (clone
53-2.1) and anti-I-A^* (clone AMS-32.1) were purchased from PharMingen
(San Diego, CA). Recombinant receptor-Fc proteins were gifts from D. R.
Goodwin, Immunex (Seattle, WA).

Cells and cell culture

771 is an MCF 1233 murine leukemia virus-transformed B cell line derived
from a tumor of a C57Bl/10 mouse neonatally inoculated with this virus
strain (23). ARHO (Armenian hamster fibroblasts) and human CD70
(hCD70)-transfected 373 cells (373-hCD70, also a gift from D. R.
Goodwin) have been described previously (6, 20). For T cell purification, murine
lymph node single cell suspensions were passed over nylon wool and de-
pleted with rat anti-MHC class II, anti-rat IgG magnetic beads, and goat
anti-mouse Ig magnetic beads (Advanced Magnetics, Cambridge, MA).
Human PBMC were isolated from buffy coats of healthy donors by Ficol-
Hlpaque density centrifugation, and subsequently, T cells were purified by
negative immunoselection with CD19, CD16, CD14, and MHC class II
mAbs and goat anti-mouse Ig magnetic beads (Dynal, Oslo, Norway).
The purity of the resulting populations was tested by immunofluorescence
using Thy-1.2 (murine) and CD3 (human) mAbs and exceeded 90%.

Flow cytometry

Cells were incubated with receptor-Fc proteins (2 μg/ml) in binding buffer
( PBS containing 0.5% BSA and 5% normal goat serum) for 30 min at 4°C,
washed, and stained with phycoerythrin (PE)-conjugated goat anti-human
IgG Fc Fab'2 (Immunotech, Marseille, France). Subsequently, cells were
analyzed on a FACScan flow cytometer (Becton Dickinson, Mountain
View, CA).

Biochemical analysis

Immunoprecipitation was performed as described previously (24). Briefly,
cells were labeled with [3H] (Amersham Co., Aylesbury, U.K.) by the glu-
coneogenesis method and lysed in 1% Nonidet P-40 buffer contain-
ting 10 mM triethanolamine-HCl (pH 7.8), 150 mM NaCl, 5 mM
EDTA, 1 mM PMSF, 20 μg/ml ovomucoid trypsin inhibitor, 1 mM
N0-
p-tosyl-L-lysine chloromethyl ketone, and 20 μg/ml leupeptin. After cen-
trifugation, supernatants were precleared with pooled human serum
(5%). Protein G-Sepharose (Pharmacia, Uppsala, Sweden), followed by
inclusion of cDNA solution (10 μg/ml) and protein G-Sepharose.
Residual Ret-Fc was removed by incubation with protein G-Sepharose.
CD70 was subsequently precipitated with murine or human CD27-Fc,
and complexes were adsorbed onto protein G-Sepharose, eluted, elecro-
phoretically separated by 5 to 15% SDS-PAGE, and visualized by
autoradiography.

Isolation of mCD70 cDNA

A λ g11 cDNA library was prepared using the Superscript choice system for
cDNA synthesis (Life Technologies, Gaithersburg, MD) and a λ g11/-
EcoRI ClaI-treated vector kit (Strategene, La Jolla, CA) according to the
manufacturer’s instructions. 771 poly(A)^* RNA was isolated using a oligo-
(dT)-cellulose column (Poly(A) Quik mRNA Isolation Kit, Stratagene)
and used for first-strand cDNA synthesis with oligo(dT)12-18 primers.
Only cDNAs > 500 bp were ligated in the g11 arms. Some 10^4 plagues
were hybridized with a 32P-labeled cDNA of hCD70 (nucleotides 298–
741) (2) encoding the extracellular part of hCD70. Hybridization was per-
formed for 16 h at 65°C. Filters were washed for 15 min in 2 X SSC at
50°C, 3 X SSC subsequently at 50, 55, and 62°C. Positive plaques were purified, and
the inserts were subcloned in the EcoRI site of pcDNA3 (Invitrogen,
San Diego, CA).

Northern blot analysis

A Northern blot containing 2 μg of g11/771 poly(A)^* RNA was prepared
following standard procedures, and a mouse multiple-tissue Northern blot,
also containing approximately 2 μg of poly(A)^* RNA, was obtained from
Clonetech (Palo Alto, CA). The blots were hybridized with the 32P-
labeled mCD70 cDNA.

Semiquantitative RT-PCR analysis

Single-cell suspensions of splenocytes and thymocytes (1 × 10^6/ml) were
stimulated with Con A (5 μg/ml) and recombinant human IL-2 (10 ng/ml).

After the indicated time of culture, cells (2 × 10^6) were collected, and
RNA was isolated with RNAzol (CinnaBiotech Laboratories, Inc.,
Frisco, TX). Single-strand cDNA was prepared in a 20-μl reaction
solution with 500 ng of oligo(dT)12-18 and 100 U of SuperScript II (Life
Technologies). A 25 μl PCR reaction (50 mM KCl, 2 mM MgCl2, 10 mM
Tris-HCl (pH 9.0), 200 mM each dNTP, 0.1% Triton X-100, 200 μM each
primer, 125 U of Taq DNA polymerase (Promega, Madison, WI), and 0.8
μl of cDNA solution) amplifying CD70 and hypoxanthine phosphoribo-
ynucleotransferase (HPRT) was set up for the cDNA derived from each sample.
The following primers were used: CD70: + strand primer, 5' - GGATCC
CGGGAGAAGGTGCCGCCC-3'; – strand primer, 5' - CAAGGCGCATCT
CATTAGAATCT-3'; and HPRT: + strand primer, 5' - TATGGAAGAC
TAGCAAGCACCTTG-3'; – strand primer, 5' - GACACAAAAT
AATTTCAAATCTGTA-3'. The + strand primers were fluorescently
labeled (CD70: 5' fluor, 6-FAM, HPRT, 5' fluor, 6-FAM; Perkin-Elmer,
Foster City, CA). To overcome the difference in fluorescence intensity
between the two labels, a mixture of unlabeled (180 nM) and fluoro-
chrome-labeled (20 nM) CD70 – strand primer was used. PCR products
were resolved on a 5.2% acryl amide gel and analyzed on an ABI 373
automated DNA sequencer (Applied Biosystems, Foster City, CA) with
GeneScan Analysis Software (Perkin-Elmer).

Transient and stable expression of mCD70

COS-7 or ARHO cells were transfected with mCD70 cDNA using Lipo-
fectamine (Life Technologies). Transiently transfected cells were used
on day 3 after transfection. For stable transfection, ARHO cells (4 × 10^5)
were transfected with 20 μg of plasmid DNA, and cells were selected with
G418 (Life Technologies) at 500 μg/ml in culture medium. Stable trans-
fectants were tested for mCD70 expression by FACS analysis and
immunoprecipitation.

Proliferation assay

Proliferation assays were performed as described previously (6, 20). In
short, purified murine T cells (10^5/well) were stimulated suboptimally
with Con A (2 μg/ml) and cultured with cells transiently transfected
with mCD70 or mock transfected (5 × 10^5 cells/well). [3H]thymidine incorpo-
ration was measured after 3 days of culture. Human 7 cells were stimulated with
a combination of three anti-CD2 mAbs in the presence of the differen-
t transfants. [3H]thymidine incorporation was measured after 5 days of culture.

Isolation of genomic clones

Genomic clones were custom-isolated by Genome Systems, Inc. (St. Louis,
MO). For this, ES cell-derived genomic DNA (mouse strain 129Sv) was
cloned using the bacteriophage P1 cloning system (25). A PCR-based
screening strategy, using the mCD70-specific primers (+ strand primer,
5'-CTGCGCTGCGGCCATCTGACCT-3'; – strand primer, 5' - CAAGGG
CATATCCGACTAATCTGTA-3') was used to isolate positive clones (25).
Positive clones (Plate 264, Genome Systems (GS) control No.
12354; plate 289, GS control No. 12355; plate 317, GS control No. 12356)
were identified, and plasmid DNA was prepared according to the
manufacturer’s recommendations.

Sequence analysis and genomic structure determination

PCR products and plasmid was sequenced with a series of synthetic
oligonucleotide primers and either the fmol DNA Sequencing System (Pro-
mega) or a DNA sequencing kit (dye terminator cycle sequencing ready
reaction, Perkin-Elmer). Dye-labeled sequences were analyzed on an ABI
373 automated DNA sequencer (Applied Biosystems). The same primers
were used to determine the sizes of the introns by PCR. Sequences have
been deposited in the GenBank/EMBL Sequence Data Base (accession no.
Y13636 (cDNA), Y13637 (exons 1 and 2), and Y13638 (exon 3)).
Sequences were analyzed with PC/Gene (IntelliGenetics, Inc., Geneva,
Switzerland).

Fluorescence in situ hybridization

A fluorescence in situ hybridization experiment was performed by Genome
Systems. DNA from a P1 clone containing the mCD70 gene (GS control
No. 12354) was labeled with digoxigenin dUTP by nick translation. La-
tered probe was combined with sheared mouse DNA and hybridized to
normal metaphase chromosomes derived from mouse embryo fibroblasts
in a solution containing 50% formamide, 10% dextran sulfate, and 2 ×
SSC. Specific hybridization signals were detected by incubating the hy-
bridized slides in fluoresceinlabeled anti-digoxigenin Abs followed by coun-
tering with DAPI (4',6-diamidino-2-phenylindole). The initial identifi-
cation of the chromosome specifically labeled with the probe was based
The predicted amino acid sequence of the mouse CD70 protein was capable to bind hCD70 as expressed on transfected 771 cells. The library was screened with hCD70 cDNA as a probe. Of 106 plaques, eight positive clones were isolated. Using λ gt11-specific primers, the insert sizes of the different clones were determined, and the nucleotide sequence of the largest insert was analyzed. The insert contained an open reading frame of 595 nucleotides with the first ATG adjacent to a Kozak initiation sequence, 80 nucleotides of 3' untranslated region, and 176 nucleotides of 5' untranslated region (Fig. 2). Specific binding of mCD27-Fc to ARHO cells stably transfected with mCD70 cDNA (AR-mCD70, Fig. 3) confirmed the integrity of the isolated cDNA.

Thus, the mCD70 cDNA encodes a polypeptide of 195 amino acids with a predicted molecular mass of 21 kDa that, like human CD70 and other TNF family members, has a type II membrane orientation. Three potential N-glycosylation sites are on a gray background.

RESULTS AND DISCUSSION

Identification of a CD70-expressing murine cell line

CD70 and its receptor CD27 are members of the TNF and TNF-R families that are defined on the basis of structural homology. Between species, receptors and ligands generally display relative low percentages of homology at the amino acid level. However, tertiary structure appears to be well conserved, since, in general, receptors and ligands cross-react across species (26–28). We set out to identify cells expressing mCD70 using a mCD27-Fc recombinant protein as a probe. As shown in Figure 1, the mCD27-Fc protein was capable to bind hCD70 as expressed on transfected mouse fibroblasts (3T3-hCD70). After screening a variety of murine T and B cell lines, a murine leukemia virus-induced B cell lymphoma line (771) was found to specifically bind mCD27-Fc. Perhaps not surprisingly, 771 cells were also capable to bind hCD27-Fc (Fig. 1). The 771 cell line was considered a good candidate to isolate the mCD70 cDNA.

Cloning of the mCD70 cDNA

To clone the mCD70 cDNA, a λ gt11 cDNA library was prepared from poly(A)+ RNA of the CD70-expressing B cell lymphoma line 771. The library was screened with hCD70 cDNA as a probe. Of 106 plaques, eight positive clones were isolated. Using λ gt11-specific primers, the insert sizes of the different clones were determined, and the nucleotide sequence of the largest insert was analyzed. The insert contained an open reading frame of 595 nucleotides with the first ATG adjacent to a Kozak initiation sequence, 80 nucleotides of 3' untranslated region, and 176 nucleotides of 5' untranslated region (Fig. 2). Specific binding of mCD27-Fc to ARHO cells stably transfected with mCD70 cDNA (AR-mCD70, Fig. 3) confirmed the integrity of the isolated cDNA.

Thus, the mCD70 cDNA encodes a polypeptide of 195 amino acids with a predicted molecular mass of 21 kDa that, like human CD70 and other TNF family members, has a type II membrane orientation. Three potential N-glycosylation sites are on a gray background.

FIGURE 1. Human and murine CD27-Fc proteins detect hCD70 and bind to a murine B cell line. FACS analysis of 3T3-hCD70 (3T3 cells transfected with hCD70 cDNA) and the murine B cell lymphoma line 771 was performed. Cells were incubated with hCD27-Fc protein (dashed curve), mCD27-Fc protein (solid curve), or control Fc protein (dotted curve; 2 μg/ml) and stained with PE-labeled goat anti-human IgG Fc.

FIGURE 2. Nucleotide sequence and predicted amino acid sequence of mCD70 cDNA. Amino acids comprising the predicted transmembrane region are doubly underlined; potential N-linked glycosylation sites are on a gray background.
located on the lower part of the trimer. Comparison of mCD70 with hCD70 revealed an overall homology of 62% (Fig. 4). The amino acids in presumed β-strands showed 84% homology, whereas the amino acids implied in ligand-receptor interaction showed 68% homology. All cysteines involved in disulfide bond formation in the hCD70 monomer are preserved in mCD70. Furthermore, one additional cysteine is found in the extracellular part of the protein. The intracellular parts of the proteins have a homology of only 33%. For TNF family members with known signaling potential, relatively high conservation of the intracellular part is found (CD30 ligand, 67%; CD40 ligand, 81%; OX40 ligand, 50%) (27, 30, 31). These observations do not argue in favor of the idea that the intracellular part of CD70 plays a dominant role in signaling processes.

Biochemical analysis of mCD70

To further characterize mCD70, biochemical analysis was performed. The predicted molecular mass (21 kDa) of nonglycosylated mCD70 was confirmed by in vitro transcription and translation of the cDNA clone (results not shown). Upon immunoprecipitation with mCD27-Fc or hCD27-Fc from cell surface iodinated 771 cell lysates, a protein of 29 kDa was isolated (Fig. 5A), which is in good agreement with the reported molecular mass of hCD70 (24). The presence of N-linked carbohydrates, which may (partially) explain the difference between the predicted and the observed molecular mass of mCD70, was shown by N-glycanase sensitivity of the protein (data not shown). Under nonreducing conditions mCD70 had a molecular mass of approximately 85 kDa, presumably representing the trimeric form of the protein (Fig. 5B). Since interchain disulfide binding has not been reported for other TNF-related ligands, it is unclear whether the trimer does contain such interchain bonds or whether it forms a noncovalently linked, SDS-stable, trimeric structure when the intrachain bonds are intact (32). Finally, biochemical analysis of AR-mCD70 confirmed that the molecular mass of recombinant mCD70 was identical with that of endogenous mCD70 (Fig. 5C).

Function of mCD70

Costimulation for T cell proliferation and cytokine production is one of the most prominent functional consequences of the interaction between CD27 and CD70 (2, 6, 9). We prepared cells transiently transfected with mCD70 cDNA, analyzed them for mCD70 expression by FACS analysis (data not shown), and tested their...
The different transient mCD70 (black bar, 12% mCD70+; striped bar, 3% mCD70+) or mock (white bar) transfected COS-7 cells (5 x 10⁴). [¹H]Thymidine incorporation was measured after 3 days of culture. Human (10⁵) T cells were stimulated with a combination of three CD2 mAbs, and [¹H]Thymidine incorporation was measured after 5 days of culture. Shown is one representative experiment of two performed (means of triplicate cultures).

FIGURE 6. Murine CD70 costimulates T cell proliferation. Murine (10⁵) T cells were stimulated with Con A (2 μg/ml) in the presence of different transient mCD70 (black bar, 12% mCD70+; striped bar, 3% mCD70+) or mock (white bar) transfected COS-7 cells (5 x 10⁴). [¹H]Thymidine incorporation was measured after 3 days of culture. Human (10⁵) T cells were stimulated with a combination of three CD2 mAbs, and [¹H]Thymidine incorporation was measured after 5 days of culture. Shown is one representative experiment of two performed (means of triplicate cultures).

FIGURE 7. RT-PCR analysis of mCD70 mRNA expression. Splenocytes and thymocytes were stimulated with Con A (5 μg/ml) and recombinant human IL-2 (10 ng/ml). Cells (2 x 10⁶) were collected at different time points after stimulation, mRNA levels of mCD70 and HPRT were assessed by RT-PCR. Relative amounts of mCD70 mRNA (mCD70/HPRT) are shown.

ability to costimulate either murine or human T cells. Addition of mCD70 transfectants to murine T cells that had been suboptimally stimulated with Con A led to a profound increase in proliferation depending on the expression level of the protein (Fig. 6). Furthermore, mCD70 transfectants were able to costimulate human T cell proliferation, showing the lack of species specificity on a functional level.

Regulation of CD70 expression

To determine the tissue distribution of the mCD70 mRNA, Northern blots with 2 μg of poly(A)+ 771 RNA and poly(A)+ RNA from multiple tissues (heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis) were hybridized with 32P-labeled mCD70 cDNA. No specific signal could be detected after 3 wk of autoradiography, indicating very low levels of mCD70 mRNA expression (data not shown). Therefore, the kinetics of mCD70 mRNA expression were analyzed by RT-PCR. Splenocytes and thymocytes were isolated and stimulated with Con A and IL-2, total RNA was isolated at the indicated times of culture, and RT-PCR was performed. As shown in Figure 7, only low levels of mCD70 mRNA were detected in freshly isolated splenocytes and thymocytes. However, shortly after stimulation of either splenocytes or thymocytes, a significant transient increase in mCD70 mRNA expression was seen. For splenocytes, the level of mCD70 mRNA peaked after 8 h, while the highest level in thymocytes occurred after 1 day. This transient expression of mCD70 mRNA indicates that only recently activated cells will express the CD70 protein. These observations in the murine system correspond well with the observation that in human PBL only small percentages of CD70+ cells can be found, which have a primed phenotype (12, 13). Future usage of mCD70-specific mAb will allow the systematic analysis of CD70 expression in secondary lymphoid organs during ongoing immune responses. This information will be essential to understand the exact localization and function of CD27-CD70 interactions in vivo.

Determination of the mCD70 gene structure

To determine the gene structure of the mCD70 gene, P1 clones containing the mCD70 gene were isolated using a PCR-based strategy. Further PCR analysis showed that all the P1 clones contained the entire coding sequence of the mCD70 protein. Therefore, only one of the three derived clones (no. 12354) was used for further analysis. Intron/exon boundaries were determined by sequence analysis and comparison with the mCD70 cDNA sequence. PCR analysis revealed the size of the introns. As shown in Figure 8, the mCD70 gene consists of three exons, spread over approximately 4 kb. The first exon encodes the 5' untranslated region, the intracellular domain, the transmembrane domain, and the first 16 amino acids of the extracellular domain. Exon 2 encodes another 11 amino acids of the extracellular domain, whereas the largest part (127 amino acids) of the extracellular domain is encoded by exon 3. Exon 3 also encodes the 3' untranslated region. Table 1

FIGURE 8. Structure of the mCD70 gene. Schematic representation of the mCD70 gene. The different exons are denoted by boxes. The 5' and 3' untranslated regions are in white, the depicted intracellular part is blocked, the transmembrane region is in black, and the extracellular part is gray.

FIGURE 9. Chromosomal location of the mCD70 gene. Schematic representation of mouse chromosome 17. The location of the mCD70 gene is indicated by an arrow.
shows the exon-intron organization of the mCD70 gene. All exon-intron boundaries were found to obey the GT-AG rule for splice junctions (33). Recently, the gene structures of several TNF family members have been determined (30, 34–36). Although there seems to be no conservation of splice junctions, the genes have a similar organization. In general, the first exon encodes the intracellular and transmembrane domain, while the last exon encodes the largest part of the extracellular region (seven of the eight β-strands and connecting loops). In this respect the mCD70 gene has the same structure.

Chromosomal location of the mCD70 gene

Chromosomal localization of the mCD70 gene was performed by fluorescence in situ hybridization. Metaphase chromosomes were hybridized with a probe containing the exon-intron organization of the mCD70 gene. This resulted in specific labeling of the telomeric region and the middle portion of chromosome 17 was observed. Measurements of 10 specifically hybridized chromosomes 17 demonstrated that the mCD70 gene is located at a position that is 50% of the distance from the heterochromatic-euchromatic boundary to the telomere of chromosome 17, an area that corresponds to the boundary between bands 17C and 17D (Fig. 9).

Concluding remarks

In this report, we describe the properties of mCD70. This member of the TNF family shows both considerable structural and functional homology with its human counterpart. Detailed analysis of expression and function in vivo will now help to elucidate the biologic function of CD27/CD70 interactions in early T cell development (22) and in the regulation of T and B cell responses (12, 13, 37, 38).

References

3. Sugita, K., T. Hirose, D. M. Rothstem, M. Brouwer, P. Baars, and R. A. W. van Lier. 1993. Regulation of CD27 expression and function in vivo will now help to elucidate the biologic function of CD27/CD70 interactions in early T cell development (22) and in the regulation of T and B cell responses (12, 13, 37, 38).

Table 1. Exon-intron organization of the mCD70 gene

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<td>3</td>
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marker for Hodgkin's lymphoma, is a receptor whose ligand defines an emerging superfamily of cytokines with homology to TNF. Cell 73:1349.


