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Immunology

Molecular cloning of a membrane-associated human FK506- and rapamycin-binding protein, FKBP-13

(rotamase/T-cell activation/mast cell)

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ABSTRACT **The 12-kDa FK506-binding protein (FKBP-12) is a cytosolic receptor for the immunosuppressants FK506 and rapamycin. Here we report the molecular cloning and subcellular localization of a 13-kDa FKBP (FKBP-13), which has a 21-amino acid signal peptide and appears to be membrane-associated. Although no internal hydrophobic region, and thus no transmembrane domain, is apparent within the 120 amino acids of mature FKBP-13, a potential endoplasmic reticulum retention sequence (Arg-Thr-Glu-Leu) is found at its C terminus. FKBP-13 has 51% nucleotide sequence identity and 43% amino acid sequence identity to FKBP-12; the N-terminal sequences are divergent, but the 92-amino acid C-terminal sequence of FKBP-13 has 46 identical and 20 related residues when compared with FKBP-12. The conserved residues that comprise the drug binding site and rotamase active site of FKBP-12 are completely conserved in FKBP-13. Therefore, the three-dimensional structures of FKBP-12 and the FKBP-12/FK506 complex are likely to be excellent models of the corresponding FKBP-13 structure.**

FK506, rapamycin, and cyclosporin A are immunosuppressive agents recently shown to inhibit signal-transduction pathways following binding to cytoplasmic receptors termed immunophilins (1). The complexes of FK506 bound to an FK506-binding protein (FKBP) and cyclosporin A bound to a cyclophilin apparently interfere with cytoplasmic signaling pathways leading to transcription in the T cell (2, 3) and exocytosis in the mast cell (4). Rapamycin bound to an FKBP (possibly the same FKBP associated with FK506) inhibits a lymphokine-dependent signaling pathway in the T cell that results in proliferation.

The predominant FK506- and rapamycin-binding protein in human T cells is the 12-kDa cytosolic receptor FKBP (hereafter referred to as FKBP-12) (5, 6). Although FKBP-12 catalyzes the interconversion of cis and trans rotamers of peptidyl-prolyl amide bonds of peptides, inhibition of this rotamase activity was shown to be an insufficient requirement for mediating the actions of FK506 and rapamycin in T cells (3, 7) and in mast cells (4). Recent studies show that the same is true of the rotamase activity of human cyclophilin (8), which is the predominant cyclosporin A-binding protein in human T cells (9). FKBP-12 has been shown to be the protein responsible for mediating the potent antibiotic actions of rapamycin in yeast. Rapamycin-resistant strains of *Saccharomyces cerevisiae* are produced following deletion of the yeast FKBP-12 gene, and rapamycin sensitivity is returned following transfection of either yeast or human FKBP-12 (10). Similar findings can be inferred from the studies of cyclophilin in two lower eukaryotes (11).

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Recently, several FK506- and rapamycin-binding proteins have been identified from calf thymus and human T cells by using FK506 and rapamycin affinity matrices (12). Here we report the molecular cloning of one of these¶, a 13-kDa FK506- and rapamycin-binding protein hereafter referred to as FKBP-13. Human FKBP-13 has a high degree of sequence similarity to FKBP-12, allowing a structural analysis based on the solution structure of human FKBP-12 (13) and the crystal structure of human FKBP-12/FK506 complex (14).

MATERIALS AND METHODS

Determination of the N-Terminal Sequence of FKBP-13. Homogenized calf thymus (Arlene & Sons, Hopkinton, MA) extract (200 ml) was passed through ≈0.1 ml of a rapamycin affinity matrix, and the matrix was washed as described (12). The matrix was then incubated with 250 μl of a methanolic solution of rapamycin (10 mg/ml) at 4°C. The eluate was electrophoresed in a 12% polyacrylamide gel. The proteins were electroblotted onto a poly(vinylidene difluoride) membrane (Millipore) (12), and the M_r ≈ 15,000 band was visualized by Coomassie blue staining, excised, and submitted for Edman degradation microsequencing (15).

PCR Cloning of a Fragment of the FKBP-13 cDNA. Based on the 40-amino acid N-terminal sequence, two PCR primers were synthesized: 5′-GA(A/G)-GG(G/A/T/C)-AA(A/G)-(A/C)G(G/A/T/C)-AA(A/G)-(C/T)T-3′ and 5′-CC-(G/A/T/C)GT-(A/G)TA-(A/G)TG-CAT-(A/G)TG-3′. By using these two primers and cDNA prepared from 100 ng of human Jurkat T-cell total RNA (see below), a 90-base-pair (bp) fragment was amplified, subcloned into pBluescript (Stratagene) by blunt-end ligation, and sequenced (Sequenase system, United States Biochemical).

Cloning and Sequencing of the Full-Length cDNA Encoding FKBP-13. A human colon carcinoma cDNA library in λgt11 was screened for FKBP-13 cDNA, using the 90-bp fragment as a hybridization probe as described (16). Filters were hybridized at 42°C in 50% (vol/vol) formamide/5× standard saline citrate (SSC)/5× Denhardt's solution/50 mM sodium phosphate, pH 6.5/0.1% SDS and washed at 24°C in 2× SSC/0.1% SDS for 30 min and at 65°C in 1× SSC/0.1% SDS for 1 hr. From a total of 2×10^5 phage plaques, 3 positive clones were obtained. The 0.6-kilobase (kb) *Eco*RI insert was subcloned into pBluescript, and double-strand sequencing was performed. The University of Wisconsin Genetics Computer Group programs (17) were used to search for homologous sequences and align the sequences of FKBP-12 and FKBP-13.

Abbreviations: FKBP, FK506-binding protein; ER, endoplasmic reticulum.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M65128).

Northern Blot Analysis. Total RNA was prepared from Jurkat T cells and JY B cells by the guanidinium method (18). Poly(A)$^+$ RNA was enriched by oligo(dT)-cellulose chromatography. RNA was electrophoresed in a 2.2 M formaldehyde/1.6% agarose gel, transferred to nitrocellulose, and hybridized with random-primed FKBP-13 cDNA (18) in 50% formamide/5× SSC/5× Denhardt's solution/50 mM potassium phosphate, pH 7.4, containing 50 μg of salmon sperm DNA per ml at 42°C for 16–20 hr. The low-stringency wash conditions were 2× SSC/0.1% SDS for 15–30 min at room temperature, followed by 0.5× SSC/0.1% SDS for 15–30 min at room temperature. The high-stringency wash was followed by 0.1× SSC/0.1% SDS at 50°C for 60 min.

Functional Determination of the Gene Product from FKBP-13 cDNA. FKBP-13 RNA was transcribed from *Bam*HI-linearized FKBP-13 cDNA by T7 RNA polymerase (Stratagene) in the presence of G(5′)ppp(5′)G. RNA was translated *in vitro* by a rabbit reticulocyte lysate system in the presence of [^{35}S]methionine, and the translation was repeated in the presence of canine pancreatic microsomal membranes (Promega). These products were electrophoresed in an SDS/14% polyacrylamide gel. The products of both translation reactions were incubated with 20 μl of rapamycin affinity matrix overnight at 4°C. After the matrix was washed, the bound proteins were eluted with a methanolic solution of rapamycin (10 mg/ml) at 4°C for 2 hr (12). The supernatants were electrophoresed, and the gels were dried at 80°C for 1 hr and autoradiographed at −70°C on Kodak XAR-5 film.

Assay for FKBP-13 Secretion. Jurkat T cells (2.5×10^6) were labeled with 5 mCi (185 MBq) of [^{35}S]methionine for 2 hr in methionine-free medium, washed, and cultured in 5 ml of RPMI 1640 with 5% fetal bovine serum for either 2 or 5 hr. The cell lysate and culture medium were separately incubated with the rapamycin affinity matrix as described above. These samples were analyzed by SDS/PAGE and autoradiography as described above.

Subcellular Fractionation of Jurkat T Cells. Jurkat T cells (2×10^8) were labeled *in vivo* with [^{35}S]methionine as described above. After washing, the cells were incubated in 2 ml of hypotonic buffer (42 mM KCl/10 mM Hepes, pH 7.4/5 mM $MgCl_2$) for 15 min at 4°C. The cells were passed through a 30-gauge needle five times. The extract was centrifuged at $200 \times g$ for 10 min at 4°C to pellet the nuclei. The supernatant was centrifuged at $10,000 \times g$ for 10 min to collect the heavy membrane fraction and then at $150,000 \times g$ for 90 min to collect the light membrane fraction. The remaining supernatant was collected as the cytoplasmic fraction. The unfractionated cells, nuclei, and the heavy and light membrane fractions were lysed for 30 min at 4°C in 1 ml of lysis buffer [1% (wt/vol) Nonidet P-40/10 mM Tris, pH 7.4/0.15 M NaCl/1 mM EDTA/5 mM 2-mercaptoethanol/1% bovine serum albumin/2 mM phenylmethanesulfonyl fluoride/50 mM NaF]. Cellular debris was removed by centrifugation at $16,000 \times g$ for 15 min at 4°C. These fractions were analyzed with a rapamycin affinity matrix as described above.

RESULTS

Isolation of the Human FKBP-13 cDNA. By use of a rapamycin affinity matrix, a protein ($M_r \approx 15,000$ in a 12% polyacrylamide gel) was isolated from bovine thymus tissue extracts (12), and the N-terminal amino acid sequence TGTEGKRKLQIGVKKRVDHCPIKSRKGDVLHMHYTGKLED was determined. Based on this 40-amino acid N-terminal sequence, two degenerate oligonucleotide primers were synthesized for subsequent PCRs. A 90-bp PCR product was generated, subcloned, sequenced, and used to screen a λgt11 cDNA library from a human colon carcinoma cell line. From three positive clones, the same 0.6-kb insert was isolated. This insert was subcloned into pBluescript plasmid and sequenced (Fig. 1). The 570-bp sequence of this cDNA contains an open reading frame of 423 nucleotides that begins at an ATG translation initiation codon and ends at a TAA termination codon. The open reading frame is flanked by 37 bp of 5′ untranslated sequence and 110 bp of 3′ untranslated sequence. The poly(A) tail begins at nucleotide 551. The N-terminal 40 amino acids of the protein from bovine thymus match the predicted amino acid sequence from nucleotide 101, except that the third amino acid of human FKBP-13 is alanine, while in bovine FKBP-13 it is threonine. The first 21 amino acids encoded by the open reading frame include 13 hydrophobic residues and may constitute a signal peptide. The terminal 4 amino acids, Arg-Thr-Glu-Leu, may be an ER retention sequence (19). No internal hydrophobic region that could serve as a transmembrane segment is present. The mature protein of 120 amino acids has a predicted molecular mass of 13.2 kDa.

Homology Between FKBP-13 and FKBP-12. A computer search of both protein and cDNA data bases (GenBank, November 1990) revealed that this 13-kDa protein sequence is highly homologous only with FKBP-12, differing substantially from any other proteins or protein segments. Overall, FKBP-13 has 51% nucleotide sequence identity and 43% amino acid sequence identity with FKBP-12 (Fig. 2). The region of highest homology begins with Ser-25 of FKBP-13 and with Pro-17 of FKBP-12 and ends at the C terminus of FKBP-12, leaving the last five amino acids of FKBP-13 unmatched. Of the C-terminal 92 amino acids of FKBP-13, 46 are identical to, and 20 are conservative replacements of, their counterparts in FKBP-12. The N-terminal portions of these proteins show little homology. Neither human FKBP-12 nor human FKBP-13 has sequence similarity with any cyclophilin. As previously reported for FKBP-12 (20), if one allows for a single deletion at position 338, from a cryptic sequence from the pathogenic bacterium *Neisseria meningitidis*, FKBP-13 and an open reading frame from that bacterium share 49% sequence identity in their C-terminal regions (data not shown).

FKBP-13 Is Transcribed at Approximately Equal Levels in T Cells and B Cells. To investigate the expression of the gene encoding FKBP-13 in lymphocytes, Northern blot analyses were performed using the FKBP-13 cDNA as a probe (Fig. 3). With high-stringency wash conditions, a predominant 0.6-kb band hybridized to the probe. The 0.6-kb band was almost equally abundant in RNA preparations from T and B lymphocytes. The intensity of the ethidium bromide-stained rRNA was similar in the two non-poly(A)-selected RNA preparations, indicating that similar amounts of total RNA were loaded onto the gel. When Northern blotting was performed using low-stringency wash conditions (data not shown), several weak bands were detected in both the total and poly(A)$^+$ RNA preparations, including a 1.6-kb that may be the FKBP-12 mRNA cross-hybridized to the FKBP-13 cDNA probe.

***In Vitro* Transcription and Translation of FKBP-13 cDNA.** To demonstrate that the first 21 amino acids encoded by the open reading frame of the FKBP-13 cDNA constitute a signal peptide that can be cleaved by a signal peptidase, the mRNA of FKBP-13 was synthesized *in vitro* by T7 RNA polymerase and then expressed in an *in vitro* reticulocyte translation system. In the presence of canine pancreatic microsomal membranes, which contain signal peptidase activity, a mature protein was synthesized that migrated as a $M_r \approx 15,000$ band in an SDS/14% polyacrylamide gel (Fig. 4*A*, lane 2); in the absence of the microsomal membranes a precursor protein was detected as a $M_r \approx 17,000$ band (lane 1). The cleavage was almost complete within a 1-hr incubation. Both the precursor and the mature form of FKBP-13 bound to FK506 and rapamycin affinity matrices and could be eluted with the respective drug (data not shown).

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1                                   GGCCGGGGTT GACTCCGGGG GCGCGGCGAG GAGAGAC    37

38   ATG AGG CTG AGC TGG TTC CGG GTC CTG ACA GTA CTG TCC ATC TGC CTG AGC    88
     Met Arg Leu Ser Trp Phe Arg Val Leu Thr Val Leu Ser Ile Cys Leu Ser
     -21                                      -10
                     ↓
89   GCC GTG GCC AGC ACG GGG GCC GAG GGC AAA AGG AAG CTG CAG ATC GGG GTC   139
     Ala Val Ala Ser Thr Gly Ala Glu Gly Lys Arg Lys Leu Gln Ile Gly Val
                    -1  +1                                    10

140  AAG AAG CGG GTG GAC CAC TGT CCC ATC AAA TCG CGC AAA GGG GAT GTC CTG   190
     Lys Lys Arg Val Asp His Cys Pro Ile Lys Ser Arg Lys Gly Asp Val Leu
                              20                                    30

191  CAC ATG CAC TAC ACG GGG AAG CTG GAA GAT GGG ACA GAG TTT GAC AGC AGC   241
     His Met His Tyr Thr Gly Lys Leu Glu Asp Gly Thr Glu Phe Asp Ser Ser
                                        40

242  CTG CCC CAG AAC CAG CCC TTT GTC TTC TCC CTT GGC ACA GGC CAG GTC ATC   292
     Leu Pro Gln Asn Gln Pro Phe Val Phe Ser Leu Gly Thr Gly Gln Val Ile
               50                                    60

293  AAG GGC TGG GAC CAG GGG CTG CTG GGG ATG TGT GAG GGG GAA AAG CGC AAG   343
     Lys Gly Trp Asp Gln Gly Leu Leu Gly Met Cys Glu Gly Glu Lys Arg Lys
                         70                                    80

344  CTG GTG ATC CCA TCC GAG CTA GGG TAT GGA GAG CGG GGA GCT CCC CCA AAG   394
     Leu Val Ile Pro Ser Glu Leu Gly Tyr Gly Glu Arg Gly Ala Pro Pro Lys
                                   90

395  ATT CCA GGC GGT GCA ACC CTG GTG TTC GAG GTG GAG CTG CTC AAA ATA GAG   445
     Ile Pro Gly Gly Ala Thr Leu Val Phe Glu Val Glu Leu Leu Lys Ile Glu
         100                               110

446  CGA CGA ACT GAG CTG TAACCAGACT GGGAGGGGCA GGGGAGAGGC CCCCATCAGG ACC   503
     Arg Arg Thr Glu Leu
                    120
504  AGACTGT TCCAAAAAAA AAAAAACAAA AAACAAACAAA AAAACACTTA AAAAAAAAAA AAA   564

565  AAAAAA                                                             569
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FIG. 1. cDNA and predicted protein sequence of cloned human FKBP-13. Arrow denotes the signal peptide cleavage site. The N-terminal sequence obtained from bovine thymus FKBP-13 by protein microsequencing is underlined. A possible polyadenylylation signal (ACAAA) is shown in boldface (nucleotides 537–541). Italicized amino acids represent the putative endoplasmic reticulum (ER) retention sequence.

Subcellular Localization of FKBP-13. To determine whether FKBP-13 is a secreted protein, [^{35}S]methionine-labeled Jurkat T cells were incubated for 2 and 5 hr in nonradioactive medium. The cells were pelleted, and the culture supernatant was incubated with the rapamycin affinity matrix. No radioactive protein bands were detected by autoradiography from the affinity matrix eluates (Fig. 4*B*, lanes 1 and 2). Neither FKBP-13 nor FKBP-12 was secreted into the culture medium even after 5 hr of incubation. The cell pellet contained both FKBP-12 and FKBP-13 (lane 3). In these experiments, because the matrix was not eluted by drug, more protein bands were observed with SDS/PAGE compared with Fig. 4*A*. Several other protein bands seen in these gels might be additional FKBP family members, as previously reported (12).

To localize FKBP-13, Jurkat T cells were labeled with [^{35}S]methionine, lysed in hypotonic solution, and fractionated by centrifugation into cytosolic, crude nuclear, heavy membrane, and light membrane fractions (Fig. 4*A*). The FKBPs were extracted from these fractions and from the whole cell lysate by FK506 affinity matrices. FKBP-13

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                                    ↓
FKBP-13    MRLSWFRVLTVLSICLSAVASTGAEGKRKL   9

FKBP-12                              MG     2


FKBP-13    QIGVKKRVDHCPIKSRKGDVLHMHYTGKLE  39
                                 |    |||| ||
FKBP-12    VQVETISPGDGRTFPKRGQTCVVHYTGMLE  32


FKBP-13    DGTEFDSSLPQNQPFVFSLGTGNVIKGWDN  69
           ||  ||||   | || | ||   || ||
FKBP-12    DGKKFDSSRDRNKPFKFMLGKQEVIRGWEE  62


FKBP-13    GLLGMCEGEKRKLVIPSELGYGERGAPPKI  99
           |   |  |   || |     ||  | |  |
FKBP-12    GVAQMSVGQRAKLTISPDYAYGATGHPGII  92


FKBP-13    PGGATLVFEVELLKIERRTEL           120
           |  ||||| ||||| |
FKBP-12    PPHATLVFDVELLKLE                108
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FIG. 2. Protein sequence alignment of human FKBP-13 and FKBP-12. Vertical bars denote identity. Arrow denotes the signal peptide cleavage site of FKBP-13.

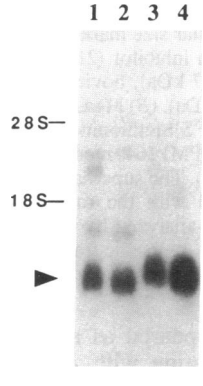

FIG. 3. Northern blot analysis of FKBP-13 mRNA. Total RNA and poly(A)$^+$ RNA from human Jurkat T cells and human JY B cells were electrophoresed in a 1.6% agarose gel, blotted to nitrocellulose membrane, and hybridized with the FKBP-13 cDNA probe. Lanes: 1, Jurkat total RNA; 2, Jurkat poly(A)$^+$ RNA; 3, JY total RNA; 4, JY poly(A)$^+$ RNA. Arrowhead indicates FKBP-13 mRNA. Positions of 28S and 18S rRNA are shown.

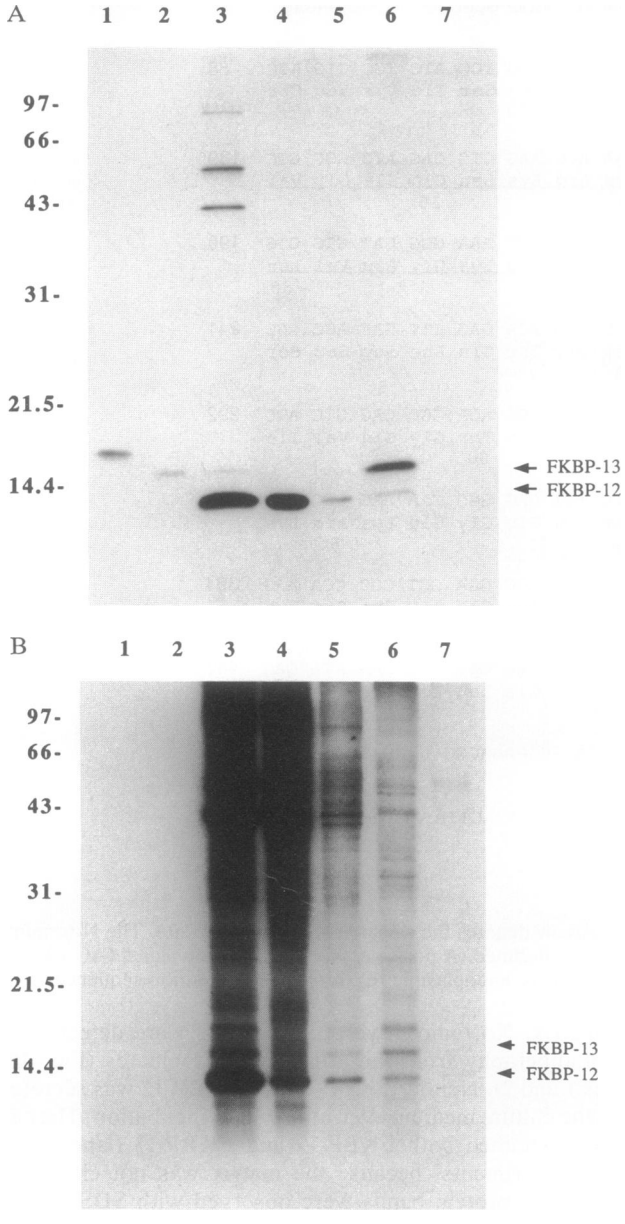

FIG. 4. (*A*) *In vitro* translation of FKBP-13 cDNA (lanes 1 and 2). FKBP-13 cDNA was transcribed by T7 RNA polymerase *in vitro*. Synthetic RNA was translated using reticulocyte lysate and [^{35}S]methionine in the absence (lane 1) or presence (lane 2) of canine pancreatic microsomal membranes. Samples were analyzed by reducing SDS/14% PAGE and autoradiographed. Subcellular localization of FKBP-13 (lanes 3–7). [^{35}S]Methionine-labeled human Jurkat T cells were fractionated by centrifugation; FKBP-13 and FKBP-12 were extracted by an FK506 affinity matrix, eluted by an FK506 solution, and analyzed by reducing SDS/14% PAGE. The gel was dried and autoradiographed. Lanes: 3, whole cell lysate; 4, cytosolic fraction; 5, crude nuclei; 6, heavy membrane; 7, light membrane. Bands corresponding to FKBP-13 and FKBP-12 are indicated. The protein molecular size markers (Bio-Rad) are lysozyme (14.4 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhydrase (31.0 kDa), ovalbumin (42.7 kDa), bovine serum albumin (66.1 kDa), and phosphorylase *b* (97.4 kDa). (*B*) Measurement of FKBP-13 in Jurkat T-cell culture medium. [^{35}S]Methionine-labeled human Jurkat T cells were incubated in 5 ml of RPMI 1640 medium/5% fetal bovine serum for 2 hr (lane 1) or 5 hr (lane 2). The supernatant and the pelleted, fragmented cells were extracted with the rapamycin affinity matrix; the bound protein was directly analyzed by reducing SDS/14% PAGE. Lanes: 3, whole cell lysate; 4, cytosolic fraction; 5, crude nuclei; 6, heavy membrane; 7, light membrane.

extracted from the whole cell lysate appeared as a $M_r \approx$ 15,000 band (Fig. 4*A*, lane 3), comigrating with *in vitro* synthesized mature FKBP-13 (lane 2). FKBP-13 was enriched in the heavy membrane fraction (lane 6), which included most of the mitochondrial and lysosomal membranes and some microsomal membranes. FKBP-12 was the predominant cytoplasmic protein (lane 4), consistent with previous observations (12). The small amount of FKBP-13 in the cytosol (lane 4) and nuclear fractions (lane 5) and also the small amount of FKBP-12 in nuclear and heavy membrane fractions most likely resulted from cross contamination. Thus, we conclude that FKBP-12 is the major cytoplasmic FKBP and that FKBP-13 is an intracellular membrane-associated FKBP in T cells.

DISCUSSION

Based on N-terminal sequence, a 13-kDa FK506- and rapamycin-binding protein was cloned. The sequence of the cDNA revealed a 141-amino acid protein of which the N-terminal 21 amino acids appeared to be a signal sequence. *In vitro* translation of the FKBP-13 gene demonstrated that the putative signal peptide is a substrate for a signal peptidase *in vitro*. In addition, the gene product was retained on FK506 and rapamycin affinity matrices and subsequently was eluted with the corresponding drug, demonstrating its ability to bind FK506 and rapamycin.

Comparison of the FKBP-13 and FKBP-12 sequences indicated that the C-terminal amino acids of FKBP-13 have identity with FKBP-12; however, the N-terminal region is divergent. All of the conserved residues in all known sequences of FKBP-12 are conserved in FKBP-13. Structural studies of free FKBP-12 (13) and FKBP-12 bound to FK506 (14) have implicated roles for the conserved residues in drug binding and/or rotamase catalysis, suggesting that FKBP-13 has rotamase activity.

FKBP-13 has five additional amino acids before its C terminus. The sequence Arg-Thr-Glu-Leu is suggestive of an ER retention sequence (19), a "comparton" (21). Although a common sequence for an ER comparton in animal cells is Lys-Asp-Glu-Leu (22), His-Thr-Glu-Leu (23) and Arg-Asp-Glu-Leu (24) are also examples of functional ER retention sequences. Moreover, the pancreatic membranes containing the signal peptidase were derived from ER membranes. Thus, an ER signal peptidase can process FKBP-13, consistent with the putative ER localization. Cell fractionation of Jurkat cells indicated that the heavy membrane fraction was enriched with FKBP-13. This fraction possesses most of the mitochondrial and lysosomal membranes as well as some ER. The signal sequence does not have any acidic residues, a trait of mitochondrial signal peptides (25). Also, this signal peptide does not have a mitochondrial signal peptide cleavage motif (arginine at position −2, −3, or −10) and is cleaved on the C-terminal side of serine; most mitochondrial signal peptides are cleaved on the N-terminal side of serine residues (25). Nevertheless, the unequivocal determination of the intracellular localization of FKBP-13 awaits the generation of an antibody and further fractionation experiments.

The presence of a signal sequence for FKBP-13 indicates that it may be localized to a compartment with an oxidizing environment that promotes the formation of disulfide bonds. FKBP-13 has two cysteine residues, Cys-20 and Cys-75, that are not conserved in FKBP-12; they correspond to Gly-12 and Ser-67 in FKBP-12. However, when two cysteine side chains are added onto the x-ray structure of FKBP-12 in place of the side chains of Gly-12 and Ser-67 (Fig. 5), they are found to be within disulfide bond-forming distance. Thus, we postulate that there is a disulfide linkage involving Cys-20 and Cys-75 in the structure of FKBP-13. In fact, this putative disulfide is located at an interesting, unique topological feature of the structure of FKBP-12, a crossing of two loops that connect four strands of a five-stranded antiparallel β-sheet. Loop crossings were thought to be disallowed in

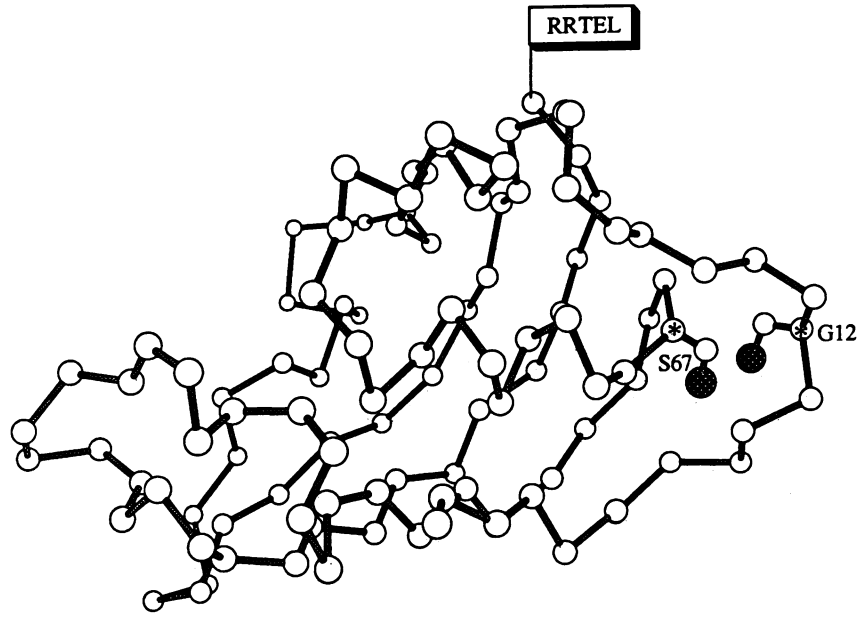

FIG. 5. Structure of human FKBP-12 (α-carbon only) used as a model of the structure of FKBP-13. Gly-12 (G12) and Ser-67 (S67) of FKBP-12, which align with Cys-20 and Cys-75 of FKBP-13, respectively, have been modified to incorporate the thiomethyl side chain of a cysteine residue with the natural stereochemistry. The thiol groups (dark circles) are within disulfide-bonding distance (see text). The putative ER retention sequence (Arg-Arg-Thr-Glu-Leu, RRTEL) is anticipated to project away from the edge of the five-stranded β-sheet (from the C terminus of FKBP-12) to be available for interactions with the receptor for the ER retention signal peptide.

antiparallel β-sheets (26–28), due to difficulties in packing the side chains efficiently, and were previously unobserved. FKBP-12 has a number of backbone–backbone and backbone–side chain hydrogen bonds and van der Waals contacts that stabilize this topology (13). The sequences of FKBPs are not highly conserved around Cys-20, which is part of the divergent N-terminal region, or around Cys-75. Thus, the putative disulfide may be critical for stabilizing this unusual topological feature in FKBP-13.

A number of cyclophilins associated with subcellular organelles have been reported. These include the cyclophilin-like gene *ninaA* of *Drosophila melanogaster* (29, 30). The *ninaA* gene product has an N-terminal signal sequence and a C-terminal hydrophobic domain that may serve as a membrane anchor. Mutations in this gene result in photoreceptor cell dysfunction that in turn may result from improper trafficking of rhodopsin molecules. A yeast cyclophilin with a signal sequence has also been described (31), and recently a human cyclophilin with a signal sequence and with sequence similarity to this yeast cyclophilin has been cloned (32). Perhaps the physiological roles of these immunophilins with a signal sequence, including FKBP-13, are similar and may be related to protein translocation or trafficking in cells.

Recently, IgE receptor-mediated exocytosis in mast cells has been shown to be inhibitable by FK506 (4). Affinity chromatography of lysates from RBL rat basophilic leukemia cells, a rat mast cell line, indicated that FKBP-13 is the predominant FK506- and rapamycin-binding protein in this cell line. Therefore, FKBP-13 warrants further investigation as a possible mediator of the actions of FK506 in mast cells.

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