

GENE 09111

Cloning of the gene encoding the mouse homologue of the human calcium signal-modulating ligand*

(Cyclophilin; immunophilin; cyclosporin; calcineurin; T-cell activation; thymus; lymphocyte; T-cell receptor)

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SUMMARY

A cDNA clone, representing the mouse homologue of the recently described gene encoding the human calcium signal-modulating ligand, was isolated from a mouse thymus library. This clone exhibits extensive conservation of the primary nucleotide and deduced amino-acid sequences that, when considered with a similar secondary protein structure, transcript size and distribution of expression, suggests a similarity in function.

T-cell activation in response to antigenic stimulation results from initial engagement of the T-cell receptor (TCR) and later transcriptional upregulation events (Weiss and Littman, 1994). The coupling of surface recognition and nuclear response is mediated through an increase of intracellular calcium which activates calcineurin, a calcium/calmodulin-regulated phosphatase (Clipstone and Crabtree, 1992), which in turn activates and translocates into the nucleus DNA binding proteins, such as NF-AT (Jain et al., 1993). Immunophilins, especially the *cis*-peptidyl-prolyl isomerases cyclophilin and

FKBP, are among the cellular regulators of calcineurin (Siekierka et al., 1989; Harding et al., 1989). The immunosuppressant drugs CsA and FK506 bind cyclophilin and FKBP, respectively, strongly blocking the antigen-receptor-mediated T-cell activation (Crabtree and Clipstone, 1994). CAML is a recently described natural ligand for human cyclophilin B in lymphocytes that is likely involved in the mobilization of calcium as a result of TCR/CD3 complex activation (Bram and Crabtree, 1994). Whether a mouse homologue of this molecule exists, which would be useful in determining the exact role of this novel cyclophilin-binding protein in T-cell activation, was unknown. We report here the cloning of the mouse homologue of the hCAML.

During an immunoscreening of a human intestine cDNA library, constructed in the vector λ gt11, with a monoclonal antibody that recognizes a protein present on the surface of human intestinal epithelial cells, a clone was isolated that contained a 360-bp *EcoRI-HindIII* insert. This clone was later shown to be a false positive; however, its sequence proved to be identical to that of hCAML. This 360-bp *EcoRI-HindIII* fragment was used as probe to screen, by Southern hybridization, a mouse thymus cDNA library in λ ZAP (Stratagene, La Jolla, CA,

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*On request, the authors will supply detailed experimental evidence for the conclusions reached in this Brief Note.

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Abbreviations: aa, amino acid(s); bp, base pair(s); CAML, calcium signal-modulating ligand; CsA, cyclosporin A; kb, kilobase(s) or 1000 bp; FK506, immunosuppressant drug; FKBP, FK506-binding protein; h, human; hCAML, gene encoding hCAML; m, mouse; mCaml, gene encoding mCAML; ORF, open reading frame; TCR, T-cell antigen receptor; TM, transmembrane (domain).

USA) with the result that a 1373-bp clone was identified and sequenced. An ORF extends from an A³⁵TG to a stop codon at nt position 917 leaving 456 bp of 3'-untranslated sequence. This ORF codes for 294 aa (32514 Da) (Fig. 1). Searches in the DNA and protein databases revealed no significant homologies, except to the recently cloned hCAML. Alignments of the DNA (data not shown) and deduced aa sequences (Fig. 1) of the human and mouse CAML show marked conservation: 81% homology for the nt sequences and 91% homology for the deduced aa sequences. Most of the aa differences between the two proteins are concentrated in four regions (A to D) which account for about 65% of the sequence divergence.

Sequence conservation between hCAML and the mouse homologue suggests conservation of function which is further supported by analysis of the predicted secondary structure of the protein. As described for hCAML, the C terminus of the mouse protein is predicted to contain three TM regions (TM1–3). Their locations are virtually identical in the human and mouse molecules with only a single conservative and a single nonconservative aa discrepancy in the T2 region. These data suggest that the mouse homologue is also an integral membrane protein.

The mCaml expression in various tissues was studied by hybridization of a mouse organ Northern blot

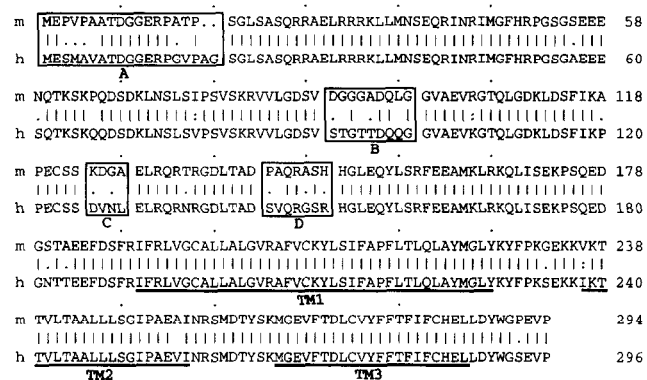


Fig. 1. Alignment of the deduced aa sequences of mouse and human CAML. Alignment-caused deletions are indicated by periods. The aa sequence of the mCAML was predicted from the nt sequence of the insert in clone pMTD (GenBank accession No. U18242). Regions in which aa differences are concentrated are boxed and designated A–D. Regions with TM protein characteristics, based upon hydrophobicity plots (data not shown), are underlined and labelled TM1–3. Highly conserved aa substitutions are designated by colons (:) and moderately conservative substitutions by dots (.). Alignments were performed with the program BESTFIT of the Genetics Computer Group (Madison, WI, USA).

(Clontech), with poly (A)⁺-enriched mRNA from heart, brain, spleen, lung, liver, skeletal muscle, kidneys and testis. This blot was probed with a 560-bp *EcoRI-SacII* fragment from pMTD. A 1.4-kb hybridizing band, corresponding to the insert size in pMTD, was observed in all tissues examined (data not shown) with the highest level of message found in the testis, kidney, liver and brain. Striping and reprobing with a β -actin cDNA showed equivalent amounts of mRNA in all lanes. This wide distribution of tissue expression, similar to that of hCAML, suggests a more generalized role of this molecule beyond that described in T cells (Bram and Crabtree, 1994). Thus, the cloning of the mCaml will be important in the use of the mouse as a model system in the study of the mechanisms of calcium-mediated pathways of cellular activation.

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