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Short Sequence-Paper

Molecular cloning of a full-length cDNA encoding the catalytic subunit of human calmodulin-dependent protein phosphatase (Calcineurin Aα)

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A complementary DNA for human calcineurin Aα (protein phosphatase-2B), encoding a protein of 521 amino acids, was isolated from a hippocampus library. The deduced human sequence differs from that of mouse in only two amino acids, demonstrating that the structure of this catalytic subunit has been strictly conserved during mammalian evolution. Such high homology is in contrast to that seen for calcineurin Aγ, an isoform that shows only 88% identity between human and mouse (Muramatsu, T. and Kincaid, R.L. (1992) Biochem. Biophys. Res. Commun. 188, 265–271).

Reversible protein phosphorylation, one of the major mechanisms of signal integration in cells, depends on balancing the activities of protein kinases and phosphatases. Although the biological roles of protein phosphatases are not completely established, information about their regulation and function has increased enormously over the past five years (reviewed in Ref. 1). The recent discovery that the immunosuppressants cyclosporin A and FK 506, via their cognate immunophilins, form complexes with the calmodulin-dependent protein phosphatase (calcineurin, protein phosphatase-2B) and inhibit enzyme activity [2], has highlighted this phosphatase as a key player in T-cell signal transduction [3–5]. Additionally, roles for calcineurin have been suggested in such critical areas as regulation of renal ion fluxes [6], modulation of heat-shock proteins [7] and proliferation of AIDS virus [8].

Calcineurin is composed of catalytic and regulatory subunits (60 and 18 kDa, respectively). In mammals, three distinct genes for the catalytic (A) subunit have been characterized, each of which can undergo alternative splicing to yield additional variants [9–16]. Although mRNA for all three genes appears to be expressed in most tissues, two isoforms (Aα and Aβ) are found in high amounts in the brain, with α being the predominant form [17], whereas another isoform (Aγ) is enriched in testis [11]. Full-length cDNAs for human Aβ [10] and Aγ [16] have been cloned, but only partial sequence is available for Aα [12]. Further progress in understanding the role of calcineurin in human physiology requires knowledge of the structure and regulation of the human enzyme. Here we present the complete coding sequence of the α isoform (calcineurin Aα) and characterize 447 nucleotides (nt) of an extended 5′ untranslated region (UTR).

Hippocampus exhibits the highest amounts of calcineurin Aα in mouse [12] and rat [18]. A human hippocampus cDNA library (Stratagene) was screened with a probe that was generated by polymerase chain reaction of the mouse calcineurin Aα cDNA, nt 1–248 [12], and labelled with ^{32}P by primer extension. Prehybridization, hybridization and subsequent washing of filters were performed essentially as described [11]. The insert-containing portions of phage DNA from positive clones were excised in vivo as phagemids in *Escherichia coli* and purified using the alkaline lysis procedure. DNA sequencing was carried out on both strands by the dideoxynucleotide chain termination method [19], using Sequenase (USB).

Out of approx. 400 000 plaques that were screened, 4 positive clones were obtained. The clone having the

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largest insert, HHA-1, contained an open reading frame (ORF) coding for a protein of 521 amino acids, a 5′ UTR of 147 nt and a 3′ UTR of 563 nt that included a region of polyadenylylation preceded by a consensus recognition site, TATAAA (Fig. 1). The deduced sequence of HHA-1 differed from that of mouse cal-

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                                -147   CCAGCTCAGAGCCTAGACCTCCAGCCG AGCGGTTTGCAGCCGCGGGCGGCGGCGGCG  -91
GCGGCGGCGTTGAGTGTCTGGCCCGCCGGT CCGGTCGGGGTGTGCAGTCGGACGGACGAG CAGCGCGTCGCTGTCCTCCGGCAGCTGGAG   -1

ATGTCCGAGCCCAAGGCAATTGATCCCAAG TTGTCGACGACCGACAGGGTGGTGAAAGCT GTTCCATTTCCTCCAAGTCACCGGCTTACA   90
M  S  E  P  K  A  I  D  P  K   L  S  T  T  D  R  V  V  K  A   V  P  F  P  P  S  H  R  L  T    30

GCAAAAGAAGTGTTTGATAATGATGGAAAA CCTCGTGTGGATATCTTAAAGGCGCATCTT ATGAAGGAGGGAAGGCTGGAAGAGAGTGTT  180
A  K  E  V  F  D  N  D  G  K   P  R  V  D  I  L  K  A  H  L   M  K  E  G  R  L  E  E  S  V    60

GCATTGAGAATAATAACAGAGGGTGCATCA ATTCTTCGACAGGAAAAAAATTTGCTGGAT ATTGATGCGCCAGTCACTGTTTGTGGGGAC  270
A  L  R  I  I  T  E  G  A  S   I  L  R  Q  E  K  N  L  L  D   I  D  A  P  V  T  V  C  G  D    90

ATTCATGGACAATTCTTTGATTTGATGAAG CTCTTTGAAGTCGGGGGATCTCCTGCCAAC ACTCGCTACCTCTTCTTAGGGGACTATGTT  360
I  H  G  Q  F  F  D  L  M  K   L  F  E  V  G  G  S  P  A  N   T  R  Y  L  F  L  G  D  Y  V    120

GACAGAGGGTACTTCAGTATTGAATGTGTG CTGTATTTGTGGGCCTTGAAAATTCTCTAC CCCAAAACACTGTTTTTACTTCGTGGAAAT  450
D  R  G  Y  F  S  I  E  C  V   L  Y  L  W  A  L  K  I  L  Y   P  K  T  L  F  L  L  R  G  N    150

CATGAATGTAGACATCTAACAGAGTATTTC ACATTTAAACAAGAATGTAAAATAAAGTAT TCAGAACGAGTATATGATGCCTGTATGGAT  540
H  E  C  R  H  L  T  E  Y  F   T  F  K  Q  E  C  K  I  K  Y   S  E  R  V  Y  D  A  C  M  D    180

GCCTTTGACTGCCTTCCCCTGGCTGCCCTG ATGAACCAACAGTTCCTGTGTGTGCATGGT GGTTTGTCTCCAGAGATTAACACTTTAGAT  630
A  F  D  C  L  P  L  A  A  L   M  N  Q  Q  F  L  C  V  H  G   G  L  S  P  E  I  N  T  L  D    210

GATATCAGAAAATTAGACCGATTCAAAGAA CCACCTGCATATGGACCTATGTGTGATATC CTGTGGTCAGACCCCCTGGAAGATTTTGGA  720
D  I  R  K  L  D  R  F  K  E   P  P  A  Y  G  P  M  C  D  I   L  W  S  D  P  L  E  D  F  G    240

AATGAGAAGACTCAGGAACATTTCACTCAC AACACAGTCAGGGGGTGTTCATACTTCTAC AGTTACCCGGCTGTATGTGAATTCTTACAG  810
N  E  K  T  Q  E  H  F  T  H   N  T  V  R  G  C  S  Y  F  Y   S  Y  P  A  V  C  E  F  L  Q    270
                                                                                 *

CACAATAACTTGTTATCTATACTCCGAGCC CACGAAGCCCAAGATGCAGGGTACCGCATG TACAGGAAAAGCCAAACAACAGGCTTCCCT  900
H  N  N  L  L  S  I  L  R  A   H  E  A  Q  D  A  G  Y  R  M   Y  R  K  S  Q  T  T  G  F  P    300

TCTCTAATTACAATTTTTTCAGCACCAAAT TACTTAGATGTATACAATAACAAAGCTGCA GTATTGAAGTATGAGAACAATGTTATGAAT  990
S  L  I  T  I  F  S  A  P  N   Y  L  D  V  Y  N  N  K  A  A   V  L  K  Y  E  N  N  V  M  N    330

ATCAGGCAATTCAACTGTTCTCCTCATCCA TACTGGCTTCCAAATTTCATGGATGTTTTT ACTTGGTCCCTTCCATTTGTTGGGGAAAAA 1080
I  R  Q  F  N  C  S  P  H  P   Y  W  L  P  N  F  M  D  V  F   T  W  S  L  P  F  V  G  E  K    360

GTGACTGAGATGCTGGTAAATGTCCTCAAC ATCTGCTCAGATGATGAACTAGGGTCAGAA GAAGATGGATTTGATGGTGCAACAGCTGCA 1170
V  T  E  M  L  V  N  V  L  N   I  C  S  D  D  E  L  G  S  E   E  D  G  F  D  G  A  T  A  A    390

GCCCGGAAAGAGGTGATAAGGAACAAGATC CGAGCAATAGGCAAAATGGCCAGAGTGTTC TCAGTGCTCAGAGAAGAGAGTGAGAGTGTG 1260
A  R  K  E  V  I  R  N  K  I   R  A  I  G  K  M  A  R  V  F   S  V  L  R  E  E  S  E  S  V    420

CTGACGCTGAAAGGCTTGACCCCAACTGGC ATGCTCCCCAGCGGAGTACTTTCTGGAGGG AAGCAAACCCTGCAAAGCGCTACTGTTGAG 1350
L  T  L  K  G  L  T  P  T  G   M  L  P  S  G  V  L  S  G  G   K  Q  T  L  Q  S  A  T  V  E    450

GCTATTGAGGCTGATGAAGCTATCAAAGGA TTTTCACCACAACATAAGATCACTAGCTTC GAGGAAGCCAAGGGCTTAGACCGAATTAAT 1440
A  I  E  A  D  E  A  I  K  G   F  S  P  Q  H  K  I  T  S  F   E  E  A  K  G  L  D  R  I  N    480

GAGAGGATGCCGCCTCGCAGAGATGCCATG CCCTCTGACGCCAACCTTAACTCCATCAAC AAGGCTCTCACCTCAGAGACTAACGGCACG 1530
E  R  M  P  P  R  R  D  A  M   P  S  D  A  N  L  N  S  I  N   K  A  L  T  S  E  T  N  G  T    510
                                                                        *

GACAGCAATGGCAGTAATAGCAGCAATATT CAGTGACCACTTCCTGTTCACATTTTTTTT TTTTTTTTTTTTTTTTTTTTTTGAGCTG 1620
D  S  N  G  S  N  S  S  N  I   Q  .     521

CGGGGCATGATGGGGATTGCTGCATATCAG CAGTTGGATGTTCTTGCCTCTGACAGTAGC TTATTTGCTCTGGGGGCCAGGAATTGGATT 1710
CAGTTTACACTATCATTAAAAAAAGMGGGAG AGAGATAATAAACTATATTTTGGTGGGGAT GGTGATTAAACACCTCTTTTGGGTATGCCT 1800
TTTAAAAATGCTTATAGMGAAAAAAAATTT TAAAAAAAGAAAGCTAATGCTAGTATATAC TGCAATGTTAGGGGAATGAACATGTTTTCC 1890
TACTGCATTGGGGACTTCTAGATAGGTTAA TGAAAGGCCTTTTATTCTGTTACTGGACAT GAAAACTTTGTCTAATTTCTTACTCTATTG 1980
TACGTTTACAGTCGCAGCACTAAAAATGGA TGACATCAAACATTTTTAACAAAATGATGA TGTACAAACTAAGGACTATTTATTGATAAT 2070
GTTTTGCTACTCTTGTCAGACAATGGCTAT AAACTGAATTAGGCAGTCTTAAAAAAAAA     2129
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Fig. 1. Nucleotide and deduced amino-acid sequences for HHA-1, a cDNA for the catalytic subunit of calcineurin from human hippocampus (Calcineurin Aα). The nucleotide and deduced amino-acid sequences of HHA-1 are presented, with positions in the 5′ UTR shown as negative numbers. The putative polyadenylylation recognition site, TATAAA, is underlined. The asterisks indicate the two amino acids that differ from mouse calcineurin Aα [12].

cineurin Aα [12] in only two amino acids (residues 267 and 504), demonstrating that the structure of this protein has been highly conserved during the course of mammalian evolution. By comparison to the other two human catalytic subunit genes, calcineurin Aα is 84% identical to the Aβ gene and 81% identical to Aγ, with greatest similarities in the catalytic domain (HHA-1 residues 71–325). The calmodulin-binding domain (residues 391–414) [9] and autoinhibitory domain (residues 467–490) [20] are also well-conserved (Fig. 2). The nucleotide sequence contains a single *Eco*RI site within the ORF (nt 799), a fact that probably accounts for earlier difficulties in obtaining a full-length cDNA.

Another clone, HHA-2, gave additional information about the region preceding the initiation codon. This cDNA contained a 5′ UTR of 447 nt, as well as 144 nt of the ORF which were identical to HHA-1. Comparison of the 5′ UTR of the human cDNA with the 5′ flanking region of genomic sequence for rat calcineurin Aα [21] shows extreme conservation, with 90% identity in aligned regions (Fig. 3), and may suggest a need to retain some essential aspect of regulation of the mRNA transcript. In the study of the rat gene, the major transcription start site was reported to be at nt −359 from the start codon, corresponding to nt −363 of HHA-2, and a putative 'neuron-specific' promoter element, TCGCCCCCG, was assigned to nt −441 to −433 in the rat sequence (boxed area, Fig. 3). Because this region, which is contained within the SNN consensus sequence, (C/G)TT(C/T)GCC(C/T)C(C/T)GC [22], is also present in transcribed human mRNA, it seems unlikely that it represents a part of the promoter

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α          MSEPKAIDPKLSTTDRVVKAVPFPPSHRLTAKEVFDNDGKPRVDILKAHLMKEGRLEESVAL  62
β  MAAPEPARAAPP.PPPP.PPPGA..........T....SE....L..I....V..N..V....VD.EI..  71
γ              MSGRRFH.......I.......TQ...F....E.-...K..V..N..V.......E...  58

α  RIITEGASILRQEKNLLDIDAPVTVCGDIHGQFFDLMKLFEVGGSPANTRYLFLGDYVDRGYFSIECVLYL 133
β  ...N...A...R..TMIEVE..I.................................................. 142
γ  K..ND..A......TMIEV...I.....................S..................... 129

α  WALKILYPKTLFLLRGNHECRHLTEYFTFKQECKIKYSERVYDACMDAFDCLPLAALMNQQFLCVHGGLSP 204
β  .V......S.................................E...E...S......L.............. 213
γ  .S...NH.................D........R.....Q......ET.........L..........M.. 200

α  EINTLDDIRKLDRFKEPPAYGPMCDILWSDPLEDFGNEKTQEHFTHNTVRGCSYFYSYPAVCEFLQHNNLL 275
β  ..H......R.........F.....L.....S.......S....S...........N.........N.... 284
γ  ..TS..........T....F..V..L.....S..Y.....L..Y.....................N.... 271

α  SILRAHEAQDAGYRMYRKSQTTGFPSLITIFSAPNYLDVYNNKAAVLKYENNVMNIRQFNCSPHPYWLPNF 346
β  ..I..................................................................... 355
γ  ..I.................A..................................................... 342

α  MDVFTWSLPFVGEKVTEMLVNVLNICSDDELGSE-EDGFDGATAAARKEVIRNKIRAIGKMARVFSVLREE 416
β  .......................S.......MT.G..Q...S-......I..................... 425
γ  ............................I.DD.A--E.S.TV-...I.................I..Q. 410
                                                  ************************

α  SESVLTLKGLTPTGMLPSGVLSGGKQTLQSATVEAIEADEAIKGFSPQHKITSFEEAKGLDRINERMPPRR 487
β  ......................A..R.............EK..R....P.R.C..................K 496
γ  ..............T..L.........IET----------..R...L....R.....R............K 469
                                                  ======================

α  DAMPSDANLNSINKALTSETNGTDSNGSNSSNIQ*  521
β  ..VQQ.G-F..L.T.HAT.NH..GNHTAQ*       524
γ  .SIYPGGPMK.VTS.HSHAAHRS.QGKKAHS*     502
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Fig. 2. Comparison of the deduced amino-acid sequences of cDNAs corresponding to three human genes for the catalytic subunit of calcineurin. The deduced sequence of HHA-1 (α) is aligned with those of the β (polyproline-containing) gene [10] and the γ (testis-specific) gene [16]; using the conventions for alternatively-spliced isoforms suggested in Ref. 15, these correspond to $PP2B\alpha_1$, $PP2B\beta_2$ and $PP2B\gamma_2$, respectively. Positions of identity between the α sequence and the other cDNAs are indicated by periods, and the cumulative number of residues for each form are indicated on the right. The region underlined with asterisks represents the calmodulin-binding domain [9] and that with the broken double underline corresponds to the autoinhibitory domain [20]. Note added in proof: residues 473 and 474 in the published Aγ sequence [16] are incorrect due to an error in entering the nucleotide sequence; these residues should be *his* and *ala*, rather than *tyr* and *pro*.

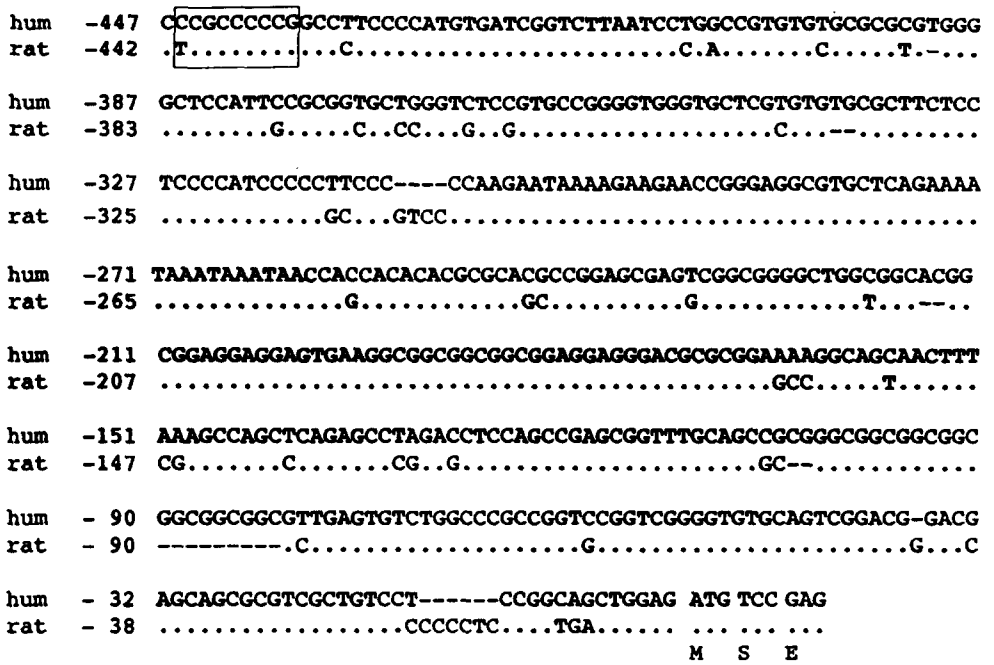

Fig. 3. Comparison of the nucleotide sequences of the 5′ flanking regions of human and rat calcineurin Aα. The 5′ UTR of the cDNA, HHA-2 (hum), is aligned with rat genomic calcineurin Aα 5′ flanking region (rat) [21]. The numbers are presented relative to the first base of the translation start codon, which is set to 1; the first three amino-acid residues (Met, Ser, Glu) also are indicated. Positions of identity between the two sequences are indicated by periods. The putative neuron-specific element [22] is boxed.

per se. Presumably, the extended 5′ UTR found in clone HHA-2 also argues for a transcription start site(s) that must exist further upstream, at least in the human gene.

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