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Biochemistry

Cloning and expression of a cDNA encoding the α subunit of rat p21^{ras} protein farnesyltransferase

(protein isoprenylation/covalent modification/heterodimeric enzymes)

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ABSTRACT **The complete amino acid sequence of the α subunit of heterodimeric p21^{ras} protein farnesyltransferase from rat has been deduced from the sequence of a cloned cDNA. The cDNA encodes a 377-amino acid protein that migrates on NaDodSO$_4$/polyacrylamide gels identically to the α subunit purified from rat brain. When introduced into mammalian cells by transfection, the cDNA for the α subunit produced no immunodetectable protein or farnesyltransferase activity unless the cells were simultaneously transfected with a cDNA encoding β subunit. In light of previous evidence that α subunit forms a heterodimer with at least two different β subunits, current data suggest a mechanism for coordinating amounts of α and β subunits. If an α subunit were stable only as a complex with a β subunit, the number of α subunits would be automatically maintained at a level just sufficient to balance all β subunits, thereby avoiding the potentially toxic overaccumulation of free α subunits.**

A protein farnesyltransferase purified from rat brain attaches farnesyl residues to a cysteine at the fourth position from the COOH terminus of several proteins, including p21^{ras} proteins and nuclear lamins (1). The natural substrates contain the Cys-A-A-Xaa recognition sequence (2), where the A residues are aliphatic and Xaa represents methionine, serine, glutamine, or cysteine (3). *In vitro* the enzyme farnesylates peptides as short as four residues in length that conform to this consensus (1, 3, 4).

The purified farnesyltransferase is an αβ heterodimer (1, 5). The α and β subunits migrate with apparent molecular masses of ≈49 and ≈46 kDa, respectively, upon NaDodSO$_4$/PAGE. The β subunit binds the peptide substrate (6). Role of the α subunit is unassigned, but it is suspected to participate in formation of a stable complex with the substrate farnesyl pyrophosphate (6). Attempts to separate the subunits with retention of catalytic activity have thus far been unsuccessful.

A protein that appears identical to the α subunit of farnesyltransferase has been identified as a component of a rat brain enzyme that transfers geranylgeranyl residues to a protein that terminates in a Cys-A-A-Xaa recognition sequence in which the Xaa is leucine (5). This finding led to the suggestion that both prenyltransferases share a common α subunit but contain different β subunits (5).

The complete amino acid sequence of the β subunit of rat brain farnesyltransferase has recently been deduced from the sequence of a cloned cDNA (7). The protein is 37% identical to the product of the yeast *DPR1/RAM1* gene (7, 8); mutations in this gene interfere with the farnesylation of the mating pheromone a factor and Ras proteins in yeast (9, 10).

When the cDNA encoding the β subunit of rat farnesyltransferase was introduced into cultured mammalian cells by transfection, it failed to produce farnesyltransferase activity unless the cells were simultaneously transfected with a cDNA encoding part of the α subunit (7). The presence of both cDNAs was also necessary for the cells to accumulate large amounts of either of the subunits as determined by immunoblot analysis. It was suggested that each subunit is unstable in the cell unless the other subunit is present. This hypothesis was tentative because the cDNA for the α subunit encoded only part of the protein (7). Sequence of this partial cDNA was not reported in our earlier paper (7).

In the current paper we report the sequence of a cDNA encoding the complete amino acid sequence of the α subunit of rat p21^{ras} protein farnesyltransferase.* As in the earlier study (7), this cDNA did not elicit the production of large amounts of α subunit protein or farnesyltransferase activity unless the cDNA encoding β subunit was cotransfected. We conclude that development of high levels of farnesyltransferase requires the simultaneous presence of both subunits.

MATERIALS AND METHODS

General Methods. Methods for DNA sequencing and blot hybridization of total cellular RNA and poly(A)$^+$ RNA are described elsewhere (7). Polyclonal antibodies IgG-Y533 and IgG-X287 directed against synthetic peptides derived from the amino acid sequence of tryptic peptides isolated from α and β subunits, respectively, of purified rat farnesyltransferase were prepared as described (5, 6). Immunoblot analysis was done as described (7).

cDNA Cloning. The polymerase chain reaction (PCR) (11), done on rat genomic DNA (7), was used to obtain the specific DNA sequence that encodes a tryptic peptide derived from α subunit of purified rat farnesyltransferase (Table 1). Primers for PCR were based on the NH_2- and COOH-terminal sequences of peptide 2 (Table 1) and included the degenerate codons shown in Fig. 1. The primers were end-labeled with [γ-^{32}P]ATP. The amplified DNA fragment was eluted from a 12% acrylamide gel and sequenced by the method of Maxam and Gilbert (12). The nucleotide sequence was then used to synthesize a 38-mer oligonucleotide probe (see legend to Fig. 1) for cDNA library screening. Random hexamer-primed rat brain and oligo(dT)-primed KNRK-cell cDNA libraries were constructed in λgt10 as described (7) by using a kit from Invitrogen (San Diego). (The rat brain library was provided by Stefan Andersson, University of Texas Southwestern Medical Center at Dallas.) Approximately 1 × 10^6 plaques of the rat brain library were screened. Duplicate filters were hybridized in 6× standard saline citrate (SSC) (1× SSC = 150 mM NaCl/15 mM sodium citrate, pH 7) with 1 × 10^6 cpm per ml of ^{32}P-labeled probe (see above). One positive clone, λRB-17, with an insert of 1.4 kilobases (kb) was identified and plaque purified. Phage DNA from a plate lysate was sub-

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*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M81225).

Table 1. Sequence of tryptic peptides from α subunit of rat farnesyltransferase

Peptide	Amino acid sequence	Amino acid position in rat cDNA sequence
1	*RAEWADIDPVPQNDGPSPVVQIIYSK* D E	68–94
2	DAIELNAANYTVWHFR	122–137
3	HFVISNTTGYSD*H*R*R* RAV	241–255
4	VLVEWLK	174–180
5	LVPHNESAWNYLK	269–281
6	LWDNELQYVDQL*L*K	215–228

Sequences were obtained from HPLC-purified tryptic peptides isolated from the α subunit of purified rat farnesyltransferase, as described (6). Each peptide represents a pure species from a single HPLC peak. Asterisks denote ambiguous residues for which assignments could not be made or were considered tentative in the original peptide sequence analysis. The amino acid sequences of all six peptides were found in the cDNA clone (Fig. 3), except for the differences indicated below the peptide sequence.

cloned into bacteriophage M13 and pBluescript vectors for DNA restriction mapping and sequencing (13).

An M13 probe corresponding to the 5′ end of clone λRB-17 was used to screen the KNRK-cell library. Replicate filters were hybridized in 50% (vol/vol) formamide containing the probe at 1×10^6 cpm per ml. Positive clones were analyzed by PCR, and the clone with the longest insert (λKNRK-3) (Fig. 2) was purified and subcloned for analysis. A 5′ rapid amplification of cDNA end procedure (14) was used to extend the 5′ end of clone λKNRK-3 (Fig. 2). An M13 probe derived from the amplification product (Race-5′) was then used to screen a rat testis library (purchased from Clontech), yielding clone λRTH (Fig. 2), which extended to nucleotide position 53 (see Fig. 3).

To obtain the extreme 5′ end of the cDNA, a primer-extension λgt10 library was constructed from rat testis poly(A)$^+$ RNA. First-strand synthesis was primed with an oligonucleotide corresponding to a sequence near the 5′ end of Race-5′ (nucleotides 138–172 in Fig. 3) using Maloney murine leukemia virus reverse transcriptase. After incubation at 37°C for 1 hr, the reaction was heated at 70°C for 5 min. Five units of thermostable r*Tth* transcriptase (Perkin–Elmer) was then added, and the reaction was continued at 70°C for 30 min. After second-strand synthesis, the cDNAs were ligated to an *Eco*RI/*Not* I linker. Excess linkers were removed by a Centricon 100 microconcentrator (Amicon). Approximately 5×10^5 plaques were screened with a ^{32}P-labeled probe corresponding to nucleotides 54–104 (Fig. 3),

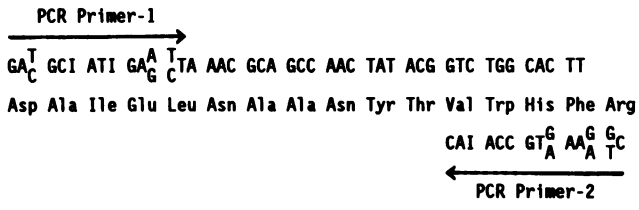

FIG. 1. Use of PCR to generate a specific cDNA probe corresponding to amino acid sequence of peptide 2 from rat farnesyltransferase α subunit. Primer 1 and primer 2 were used in the PCR with rat genomic DNA to obtain the nucleotide sequence encoding peptide 2 as described. The nucleotide sequence 5′-ATIGAGTTAAACG-CAGCCAACTATACGGTCTGGCACTT-3′ was used as a probe to screen a rat brain cDNA library.

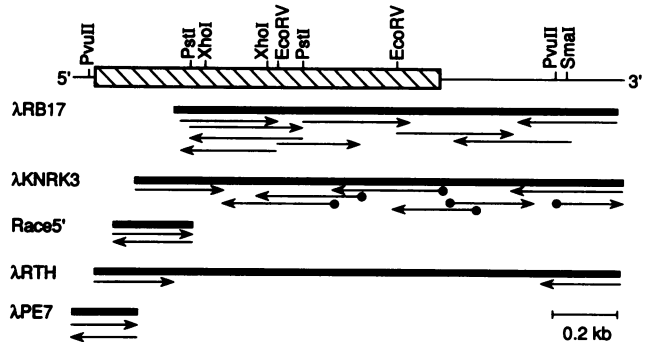

FIG. 2. Partial restriction endonuclease map and sequencing strategy for cDNAs encoding rat farnesyltransferase α subunit. A schematic diagram of the mRNA for the farnesyltransferase α subunit is shown at top. Restriction endonuclease sites in cDNAs used for sequencing are indicated. cDNAs used for sequencing were obtained as described. Arrows denote direction and extent of sequencing for each cDNA. Solid circles indicate sequences obtained with internal oligonucleotide primers. The 5′-untranslated and coding regions were sequenced on both strands. Approximately 90% of the 3′-untranslated region was sequenced on both strands. The 5′ ends of cDNA clones λRB-17, λKNRK-3, Race-5′, λRTH, and λPE-7 correspond to nucleotide positions 345, 210, 112, 53, and 1, respectively (see Fig. 3).

which was obtained from the λRTH sequence. Twenty-five positive clones were identified. Phage DNA was prepared from plate lysates, and the insert from one of the longest clones, λPE-7 (Fig. 2), was subcloned for sequencing (13).

Expression Vectors. Expression vectors for the α subunit of rat farnesyltransferase were constructed in pCMV5, a plasmid that contains the promoter-enhancer region of the major immediate early gene of human cytomegalovirus (15). A *Pvu* II fragment containing 20 base pairs (bp) of the 5′-untranslated region and the entire coding region was excised from clone λRTH-B and ligated into *Sma* I-digested pCMV5 in both orientations. Plasmid λRTH-B is identical to λRTH (Fig. 2), except for the presence of an intron in the 5′-untranslated region at nucleotide position 39, upstream of the *Pvu* II site at position 37–42. The resulting plasmids, designated pFT-α (correct orientation) and pFT-αrev (reverse orientation), were characterized by restriction mapping. pCMV5-based-expression vectors for β subunit of rat farnesyltransferase are designated pFT-β1 (correct orientation) and pFT-β1rev (reverse orientation) and are described elsewhere (7).

DNA Transfection. Monolayers of human embryonic kidney 293 cells were transfected with 3 μg of the indicated plasmid plus 1 μg of pVA (a plasmid encoding adenovirus VA RNA$_1$) (16) as described (7). On day 4 after transfection, cells were harvested for measurement of farnesyltransferase activity as described (7).

RESULTS

To obtain a specific probe with which to screen a cDNA library, we determined the amino acid sequences of several tryptic peptides from the purified rat brain farnesyltransferase α subunit (Table 1). Degenerate oligonucleotides encoding the 5′ and 3′ ends of one peptide (peptide 2) were used as primers in a PCR using rat genomic DNA (Fig. 1). The sequence of the amplified product was used as a probe to screen a random hexanucleotide-primed rat brain cDNA library cloned in λgt10. This procedure yielded λRB-17 (Fig. 2), which extended from a poly(A) tract up to nucleotide position 345 (nucleotide positions refer to final sequence of mRNA as shown in Fig. 3).

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                                                                                5' GCGGGCCGCGGAGGGGGCGGGGCTCC ACCACCACCTCAGCTGCGGACGGAGGCGAG   56

    ATGGCGGCCACTGAGGGGGTCGGGGAATCT GCGCCAGGCGGTGAGCCGGGACAGCCAGAG CAGCCGCCGCCCCCGCCTCCTCCGCCGCCA GCACAGCAGCCGCAGGAAGAAGAGATGGCG   176
  1 MetAlaAlaThrGluGlyValGlyGluSer AlaProGlyGlyGluProGlyGlnProGlu GlnProProProProProProProPro AlaGlnGlnProGlnGluGluGluMetAla

    GCCGAGGCCGGGGAAGCAGCGGCGTCCCCT ATGGACGACGGGTTTCTGAGCCTGGACTCG CCCACCTATGTCTTGTACAGGGACAGGGCA GAGTGGGCTGACATAGACCCAGTGCCCCAG   296
 41 AlaGluAlaGlyGluAlaAlaAlaSerPro MetAspAspGlyPheLeuSerLeuAspSer ProThrTyrValLeuTyrArgAspArgAla GluTrpAlaAspIleAspProValProGln

    AATGATGGCCCCAGTCCAGTGGTCCAGATC ATCTACAGTGAAAAGTTTAGAGACGTCTAT GATTACTTCCGAGCTGTTCTGCAGCGCGAT GAAAGAAGCGAACGAGCCTTTAAGCTCACT   416
 81 AsnAspGlyProSerProValValGlnIle IleTyrSerGluLysPheArgAspValTyr AspTyrPheArgAlaValLeuGlnArgAsp GluArgSerGluArgAlaPheLysLeuThr

    CGAGATGCTATTGAGTTAAACGCAGCCAAC TATACGGTGTGGCATTTTCGGAGAGTTCTC TTGAGGTCGCTTCAGAAGGATCTGCAAGAA GAAATGAACTACATCATCGCAATAATTGAG   536
121 ArgAspAlaIleGluLeuAsnAlaAlaAsn TyrThrValTrpHisPheArgArgValLeu LeuArgSerLeuGlnLysAspLeuGlnGlu GluMetAsnTyrIleIleAlaIleIleGlu

    GAACAGCCCAAAAACTATCAAGTTTGGCAC CATAGGAGAGTATTAGTGGAGTGGCTGAAA GATCCTTCTCAAGAGCTCGAGTTCATCGCC GATATCCTTAATCAGGATGCAAAGAATTAC   656
161 GluGlnProLysAsnTyrGlnValTrpHis HisArgArgValLeuValGluTrpLeuLys AspProSerGlnGluLeuGluPheIleAla AspIleLeuAsnGlnAspAlaLysAsnTyr

    CATGCCTGGCAGCATCGACAGTGGGTCATT CAGGAGTTTCGACTTTGGGATAATGAGCTG CAGTATGTGGACCAGCTTCTCAAAGAGGAT GTGAGAAATAACTCTGTGTGGAACCAAAGA   776
201 HisAlaTrpGlnHisArgGlnTrpValIle GlnGluPheArgLeuTrpAspAsnGluLeu GlnTyrValAspGlnLeuLeuLysGluAsp ValArgAsnAsnSerValTrpAsnGlnArg

    CACTTCGTCATTTCTAATACCACTGGCTAC AGTGATCGCGCTGTGTTGGAGAGAGAAGTC CAATATACTCTGGAAATGATCAAATTAGTG CCACACAATGAGAGTGCGTGGAACTACTTG   896
241 HisPheValIleSerAsnThrThrGlyTyr SerAspArgAlaValLeuGluArgGluVal GlnTyrThrLeuGluMetIleLysLeuVal ProHisAsnGluSerAlaTrpAsnTyrLeu

    AAAGGGATTTTGCAGGACCGTGGTCTTTCC AGATACCCTAATCTATTAAACCAGTTGCTT GATTTACAACCAAGTCACAGCTCCCCCTAC CTAATTGCCTTTCTTGTGGATATCTATGAA  1016
281 LysGlyIleLeuGlnAspArgGlyLeuSer ArgTyrProAsnLeuLeuAsnGlnLeuLeu AspLeuGlnProSerHisSerSerProTyr LeuIleAlaPheLeuValAspIleTyrGlu

    GACATGCTGGAAAACCAGTGTGACAACAAG GAGGACATTCTTAATAAAGCACTAGAGTTA TGTGAGATTCTAGCTAAAGAAAAGGACACT ATAAGAAAGGAATATTGGAGATATATTGGA  1136
321 AspMetLeuGluAsnGlnCysAspAsnLys GluAspIleLeuAsnLysAlaLeuGluLeu CysGluIleLeuAlaLysGluLysAspThr IleArgLysGluTyrTrpArgTyrIleGly

    CGGTCCCTCCAGAGTAAACACAGCAGAGAA AGTGACATACCGGCGAGTGTATAGCAGCAA GAGCGGCTGGAAGAAGTGGACAATGCTTTC TAAGGCCTCTTATTCGGGAGTGTAGAGCGG  1256
361 ArgSerLeuGlnSerLysHisSerArgGlu SerAspIleProAlaSerValEnd

    TTAGAGCGGTCATCTCATGCCTGTGAGCTA ACGTTGTCCAGGTGCTGTTTCTAACAAGAA CTAAGGATGACTCCTGTGTCTGACGCTGTT CAGACTAGCTAAGAGTCGATTTCCTAAAGC  1376
    AAGGTCATTGGAGGGGAGGGTGAAGAAAAC TTTCCCGTAAAGGAACTACTGCTTTTGTAG TCTTCCCAACATTTAATCCAATCCTGTAGA ATCAGCATCTCCTGAAAGACATGGTGCACG  1496
    GCTGTGTGCTGGGCGTGGCTAAGGGCAGCT GTGTCATGGGTTTGCAGTCATGGGAACCTC GGAGCGCTGCCCGGGACTGCATTGATGATT AGGGCTGCTGGCCTCACCCACAGGATCTTG  1616
    CTATCACTGTAACCAACTAATGCCAAAAGA AAGGTTTTATAATAAAATCACATTATCTAC AAACAAAAAAAAAAAAAAAAAAAAA 3'                                  1701
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FIG. 3. Nucleotide and predicted amino acid sequences of the cDNA encoding rat farnesyltransferase α subunit. Nucleotide positions are numbered on right; amino acid residues are numbered on left. Amino acid residue 1 is the putative initiator methionine. A single underline indicates a stretch of nine contiguous prolines. The potential polyadenylylation signal is double-underlined.

The 5′ end of the mRNA encoding α subunit contains a sequence extremely rich in G+C base pairs (76% G+C from nucleotides 71–205 and 90% G+C from nucleotides 116–145). Multiple attempts to traverse this region by primer extension with reverse transcriptase gave products that terminated prematurely or that encoded unspliced introns. To obtain the 5′ end of the mRNA, we resorted to the multiple strategies described earlier in text. These methods yielded the various cDNAs shown in Fig. 2. A composite of these cDNA sequences was used to generate the overall sequence of the mRNA (Fig. 3).

The mRNA encodes a protein of 377 amino acids (Fig. 3) with a calculated M_r of 44,053. The most remarkable feature is a string of nine consecutive proline residues near the NH_2 terminus (underlined in Fig. 3), the codons of which account for much of the extreme G+C richness of this region. The mRNA encodes all six peptides for which amino acid sequences were obtained (Table 1). The only discrepancies occur at positions assigned tentatively when the trace amounts of protein were sequenced. The protein shows no significant homology to any sequences contained in available protein data banks.

When introduced into human kidney 293 cells by transfection, the cDNA produced a protein the molecular weight of which was indistinguishable on immunoblots from that of the α subunit purified from rat brain (compare lane 2 with lanes 1 and 3 in Fig. 4*A*). The accumulation of detectable amounts

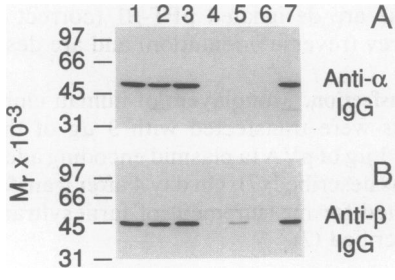

FIG. 4. Immunoblot analysis of α and β subunits of rat protein farnesyltransferase expressed in transfected 293 cells. Samples were subjected to $NaDodSO_4$/10% PAGE and transferred to nitrocellulose. Filters were incubated with either rabbit anti-α subunit IgG-Y533 at 1 μg/ml (*A*) or rabbit anti-β subunit IgG-X287 at 5 μg/ml (*B*) and then incubated with ^{125}I-labeled goat anti-rabbit IgG (1×10^6 cpm/ml). Lanes: 1 and 3, 20 μg of partially purified Mono Q fraction of rat brain farnesyltransferase (1); 2 and 4–7, 20 μg of cytosol from 293 cells (7) transfected with the following plasmids: pFT-α plus pFT-β1 (lanes 2 and 7), pFT-α plus pFT-β1rev (lane 4), pFT-αrev plus pFT-β1 (lane 5), and pFT-αrev plus pFT-β1rev (lane 6). Filters were exposed to Kodak XAR-5 film for 48 hr (*A*) or 16 hr (*B*) at −70°C. Molecular weight markers are indicated. Plasmids pFT-αrev and pFT-β1rev contain cDNAs inserted in the reverse (noncoding) orientation as described.

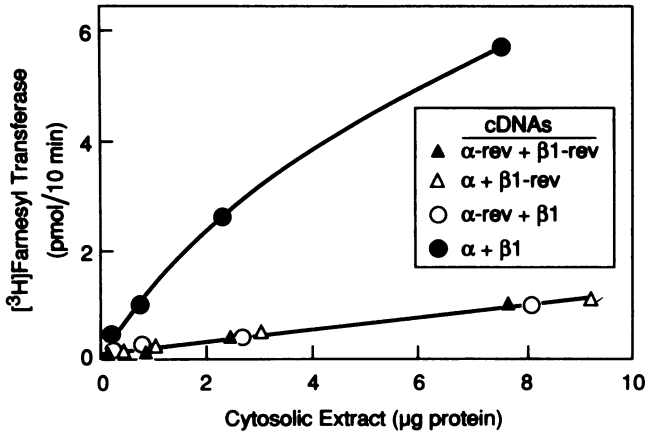

FIG. 5. Farnesyltransferase activity of cytosolic extracts from 293 cells transfected with cDNAs encoding α and β subunits of rat protein farnesyltransferase in correct or reverse (rev) orientations. Cells were transfected with 3 μg of the indicated plasmid plus 1 μg of pVA as described. Each assay contained, in a final volume of 25 μl, the indicated amount of cytosolic extract (7), 50 mM Tris·HCl (pH 7.5), 50 μM $ZnCl_2$, 20 mM KCl, 3 mM $MgCl_2$, 1 mM dithiothreitol, 0.4% (vol/vol) octyl β-glucopyranoside, 40 μM $p21^{H\text{-}ras}$, and 15 pmol of [^{3}H]farnesyl pyrophosphate (15,335 dpm/pmol). Assay tubes were incubated at 37°C for 10 min, after which the amount of [^{3}H]farnesyl attached to $p21^{H\text{-}ras}$ was measured. Each value is the average of duplicate incubations.

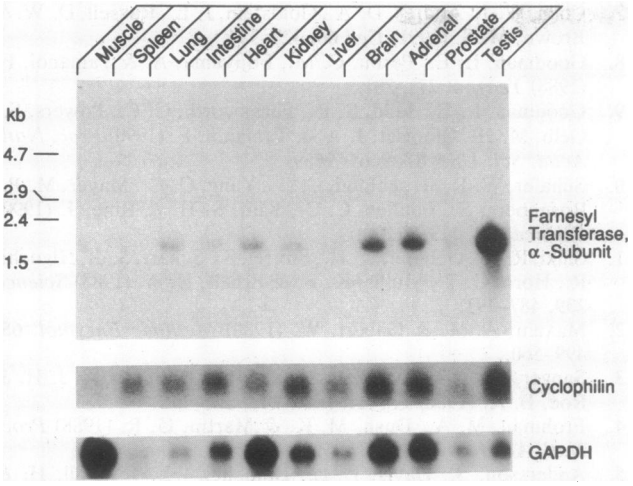

FIG. 6. Tissue distribution of rat farnesyltransferase α-subunit mRNA. Total RNA was isolated from the indicated rat tissues, and an aliquot (30 μg) was subjected to blot analysis as described (7). Hybridization was done at 42°C for 20 hr with a mixture of two single-stranded uniformly ^{32}P-labeled cDNA probes for rat farnesyltransferase α subunit. Each probe was ≈500 nucleotides in length and was used at 2×10^6 cpm/ml. Filter was exposed to film for 2 days at −70°C. As a loading control, the same filter was then reprobed initially with a ^{32}P-labeled 49-mer oligonucleotide corresponding to rat cyclophilin cDNA (2×10^6 cpm/ml) (7) and subsequently with a uniformly ^{32}P-labeled cDNA (≈1.2 kb) for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (4×10^6 cpm/ml) (7). The reprobed filter was exposed for 12 hr at −70°C.

of α subunit required simultaneous transfection with a properly oriented cDNA encoding the β subunit (Fig. 4A). Similarly, the amount of detectable β subunit was increased by transfection with α-subunit cDNA in the correct orientation (Fig. 4B). Transfection with the two cDNAs in the correct orientation was also required to produce significant amounts of p21^{ras} farnesyltransferase activity as measured in cytosolic extracts (Fig. 5).

Northern RNA blot analysis with ^{32}P-labeled probes derived from the α-subunit cDNA revealed a single mRNA of ≈1.75 kb in multiple tissues, including lung, heart, kidney, brain, adrenal, and testis (Fig. 6). The amount of mRNA in testis was several-fold higher than in any other tissue, an observation that was repeated on several occasions. An mRNA of the same size was also observed in two lines of cultured rat cells derived from kidney (KNRK cells) and adrenal medulla (PC12 cells) (Fig. 7).

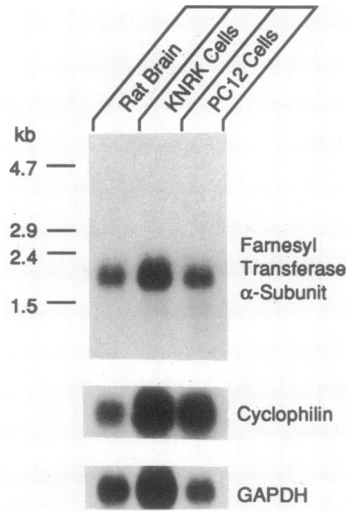

FIG. 7. Blot hybridization of RNA from rat brain, KNRK cells, and PC12 pheochromocytoma cells. An aliquot of poly(A)$^+$ RNA from each sample (10 μg) was subjected to blot analysis, as described in the legend to Fig. 6.

DISCUSSION

The p21^{ras} protein farnesyltransferase purified from rat brain has a complex reaction mechanism that requires two nonidentical subunits. The enzyme forms a noncovalent complex with farnesyl pyrophosphate that remains stable for long periods unless an acceptor peptide is present, whereupon transfer occurs (6). Crosslinking studies revealed that the β subunit binds the p21^{ras} acceptor (6). The α subunit may play a role in the farnesyl pyrophosphate carrier function. The amino acid sequence of the α subunit does not provide a clue as to its catalytic role. Identity searches of protein data bases failed to reveal significant resemblance of the α subunit to other proteins, except for proteins that contain long stretches of prolines. These proteins include such apparently unrelated proteins as the catalytic subunits of rat and human protein phosphatase 2B, mouse retinoblastoma-associated protein pp105, and *Dictyostelium discoideum* protein-tyrosine kinase 1. The α subunit does not bear significant structural resemblance to known prenyltransferases, such as mammalian farnesyl pyrophosphate synthetase or yeast hexaprenyl pyrophosphate synthetase (17).

Although the cDNA sequence in Fig. 3 does not contain a terminator codon upstream of the first methionine codon, we believe that this methionine represents the true initiator codon because the cDNA produces a protein for which migration is indistinguishable from that of the purified rat brain α subunit on NaDodSO$_4$/polyacrylamide gels (Fig. 4). If our cDNA were incomplete, the initiator methionine must be upstream of the 5′ end of the sequence obtained and, therefore, the protein produced by the cDNA should be at least 2 kDa smaller than the authentic protein. Such a difference should have been detected in the gel electrophoresis experiment of Fig. 4.

The transfection experiments indicate that mammalian cells will not accumulate high levels of either subunit of the farnesyltransferase unless the other subunit is present. This is particularly true for α subunit, the accumulation of which almost completely depended on coexpression of β subunit. It is likely that the α subunit is rapidly degraded unless the β subunit is present. However, until pulse-chase labeling experiments are done, we cannot rule out the possibility of control at the level of mRNA stability or translation.

Present evidence suggests that the α subunit may be shared with another prenyltransferase with a different β subunit that exhibits geranylgeranyltransferase activity (5). If the shared α subunit were stable only as a complex with one of several β subunits, this mechanism would assure that cells maintain only enough α subunits to satisfy all β subunits, thereby avoiding accumulation of excess α subunits, which might be toxic.

Based on genetic arguments, the α subunit of rat brain farnesyltransferase is probably the animal counterpart of the protein encoded by *RAM2* in yeast (7, 9). Mutations in *RAM2*, like those in *DPR1/RAM1*, interfere with farnesylation (9, 10). Inasmuch as yeast DPR1/RAM1 protein is the counterpart of mammalian β subunit (7), it is likely that RAM2 protein is the counterpart of α subunit. Confirmation of this hypothesis awaits publication of the DNA sequence of *RAM2* and its deduced amino acid sequence.

Note Added in Proof. After this manuscript was submitted for publication, Kohl *et al.* (18) reported the cloning of a partial cDNA for bovine farnesyltransferase α subunit. The 329 amino acids encoded by this partial cDNA are 95% identical to the corresponding region in the α subunit of the rat farnesyltransferase described in the

current paper. Comparison of the complete amino acid sequence of rat farnesyltransferase α subunit (377 amino acids) with that of yeast *RAM2* gene product (316 amino acids) (see ref. 19) reveals that the two proteins are 30% identical in the COOH-terminal 211 residues, suggesting that RAM2 is the yeast counterpart of the α subunit of mammalian farnesyltransferase.

The first two authors, W.-J.C. and D.A.A., made equal contributions to this work. We thank our colleagues David Russell, Yuval Reiss, and Miguel Seabra for invaluable discussions; Clive Slaughter, Carolyn Moomaw, and Joan Hsu for peptide sequence analysis; and Jeff Cormier, Gloria Brunschede, Debbie Noble, and Heidi Chamberlain for excellent technical assistance. This research was supported by grants from the National Institutes of Health (HL20948), the Lucille P. Markey Charitable Trust, and the Perot Family Foundation. D.A.A. is the recipient of a postdoctoral fellowship from the Jane Coffin Childs Memorial Fund for Medical Research.

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