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The human genome encodes at least three non-allellic G proteins with α_i-type subunits

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The amino acid sequence and composition of α-subunits of signal transducing G proteins of the same kind appear to vary by no more than 2% from species to species. Here we isolated a human liver cDNA using an oligonucleotide complementary to the sequences encoding the pertussis toxin (PTX) ADP-ribosylation site of the α-subunit of the rat brain G protein called G_i. Its open reading frame characterizes it as an α_i-type cDNA – as opposed to α_o-type – but predicts an amino acid composition that differs by 7% and 14%, respectively, from two other human α_i-type molecules. Together with human brain α_i (type-1) and human monocyte α_i (type-2), the new human liver α_i cDNA (type-3) forms part of a family of α_i molecules. Type-3 α_i cDNA hybridizes to a $\sim$ 3.6 kilobase long mRNA and type-2 α_i cDNA hybridizes to an mRNA species of $\sim$ 2.7 kilobases. This indicates that the human genome has at least three non-allellic genes encoding non-α_o-type PTX substrates and provides structural evidence for the hypothesis that distinct effector systems are regulated by similar but nevertheless distinct PTX substrates.

Pertussis toxin; G protein; Adenylyl cyclase; Phospholipase C; Phospholipase A_2; K^+ channel

1. INTRODUCTION

Pertussis toxin (PTX) substrates migrating on SDS-polyacrylamide (SDS-PAGE) gels with apparent M_r values of 40000 to 41000 have been purified from various sources [1–8] and cDNAs encoding these substrates have been cloned from bovine [5], rat [6] and human [9] brain, as well as from mouse macrophages [10], and a human monocyte cell line [11]. PTX substrates are the α-subunits of PTX-sensitive signal transducing G proteins called generically G_i (or N_i). Analysis of the deduced amino acid sequences of the cloned α_i-type subunits shows that of bovine brain (354 amino acids) to be identical to that of human brain (type-1 α_i) but to differ by as much as 45 amino acids from those of rat brain, mouse macrophages and human monocytes, which in turn are very similar, differing by at most 8 amino acids out of 355 (type-2 α_i). We now report the predicted amino acid sequence of most (~80%) of a third human (type-3) α_i molecule (354 amino acids long), that differs by at least 23 amino acids from type-1 α_i and by at least 49 amino acids from type-2 α_i.

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2. MATERIALS AND METHODS

Reagents, enzymes, bacterial strains and the human liver cDNA library in λgt11 were as in [12,13]. Screening of the library was done according to a plaque amplification technique [14] as described in [15] using as probe the synthetic

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oligonucleotide: 3′-AAACT ACGGC AGTGA CTGCA GTAGT AGTTC TTGTT GGACT TCCTG ACACC GGAGA AG-5′, which is complementary to the nucleotide sequence encoding amino acids 337–355 of rat brain α_i [6].

Positive clones were plaque-purified and the longest, λa/ptx-39 (~2.2 kb) was subcloned into M13 mp18 and sequenced by the dideoxynucleotide method of Sanger et al. [16] using as first primer the universal M13 primer and then 22-mers synthesized on the basis of information obtained from previous sequencings.

Isolation of RNA and blot hybridization analysis of electrophoretically separated RNA were reformed as in [15] using Biotrans nylon membranes (ICN). Pre-hybridization was for 3 h at 42°C in 0.1% polyvinyl pyrrolidone, 0.1% bovine serum albumin and 0.1% Ficoll (5 × Denhardt's); 0.75 M NaCl and 0.075 M sodium citrate (5 × SSC); 50 mM sodium phosphate, pH 6.5, 0.1% SDS, 50% formamide and 75 μg/ml

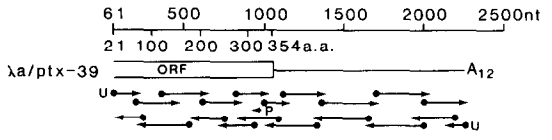

Fig.1. Diagram of λa/ptx-39 cDNA clone isolated from a human liver cDNA library. Open box, open reading frame (ORF); line, 3′-untranslated region. Scales, upper in nucleotides (nt) and lower in amino acids (aa). Arrows indicate sequencing strategy; ←—● P, sequence obtained with the selection primer used to screen the cDNA library; U ●—→ and ←—● U, sequences obtained with the universal M_{13} primers; other arrows, sequences obtained with newly synthesized primers. Note that numbering begins with nt 61 (aa 21).

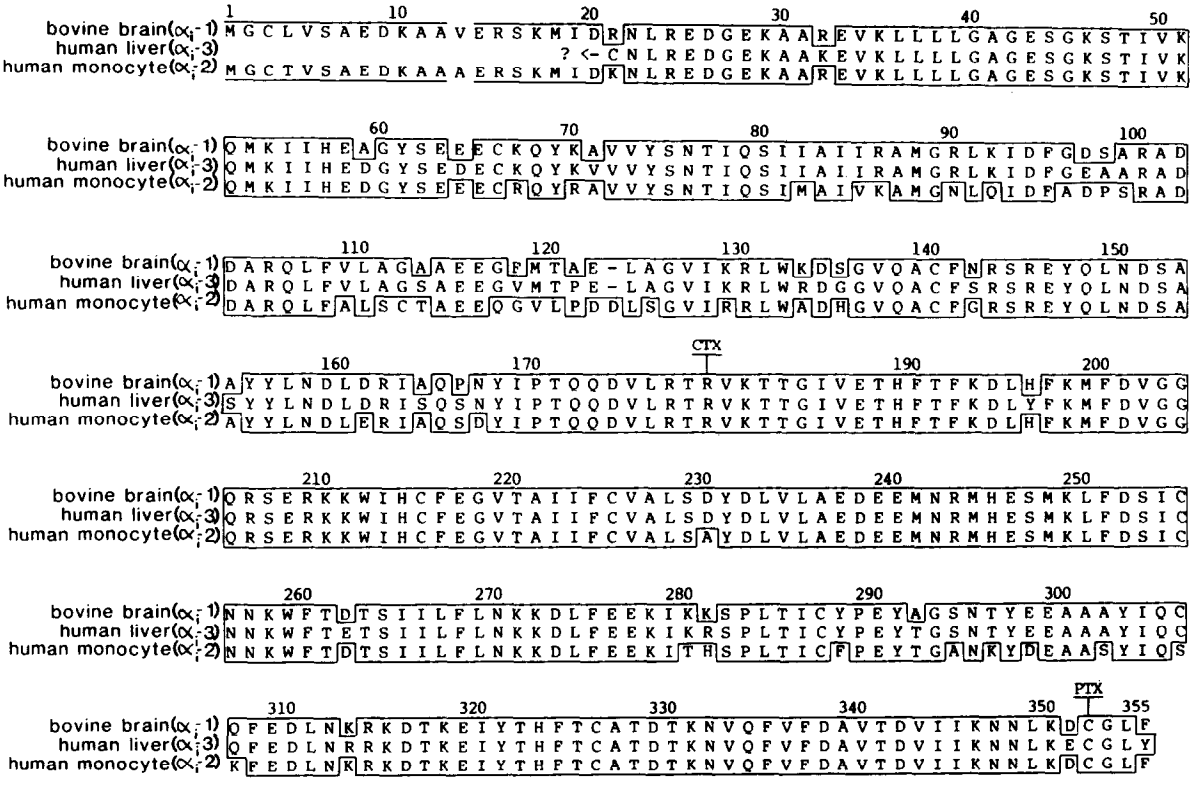

Fig.2. Amino acid sequence (single letter notation) deduced for the human α_i-3 from the nucleotide sequence of the λa/ptx-39 cDNA clone (middle line) and comparison to the amino acid sequences of bovine (= human) brain α_i-1 [5] (top line) and human monocyte α_i-2 [6] (bottom line). Identical amino acids are enclosed by solid lines. PTX and CTX indicate, respectively, the putative sites of ADP-ribosylation by PTX (Cys in position 352) and cholera toxin (Arg in position 179).

yeast tRNA. Hybridization was for 15 h at 42°C in the same solution plus 10% dextran sulfate and 10^6 cpm/ml of nick-translated and boiled cDNA probes. After hybridization the membranes were washed sequentially with 6 × SSC at 4°C for 20 min, 2 × SSC plus 0.1% SDS at 22°C for 20 min and with 0.1 × SSC plus 0.1% SDS at 22°C for 20 min.

3. RESULTS

Complete sequencing of both strands of the longest cDNA insert, out of 56 initial positive recombinant clones, revealed it to be composed of 2203 base-pairs. It has an open reading frame (ORF) of 1002 nucleotides, encoding 334 amino acids, followed by a stop codon (TGA) and a 3′-untranslated region of 1198 nucleotides, including a 12 nucleotide long poly(A) tail. Fig.1 presents the sequencing strategy and a schematic representation of the cDNA. Fig.2 presents the amino acid composition of the protein deduced from the sequence of the newly cloned cDNA (type-3 human α_i), as well as a comparison to the amino acid sequences of bovine or human brain α_i (type-1 α_i) obtained by Nukuda et al. [5] and Bray

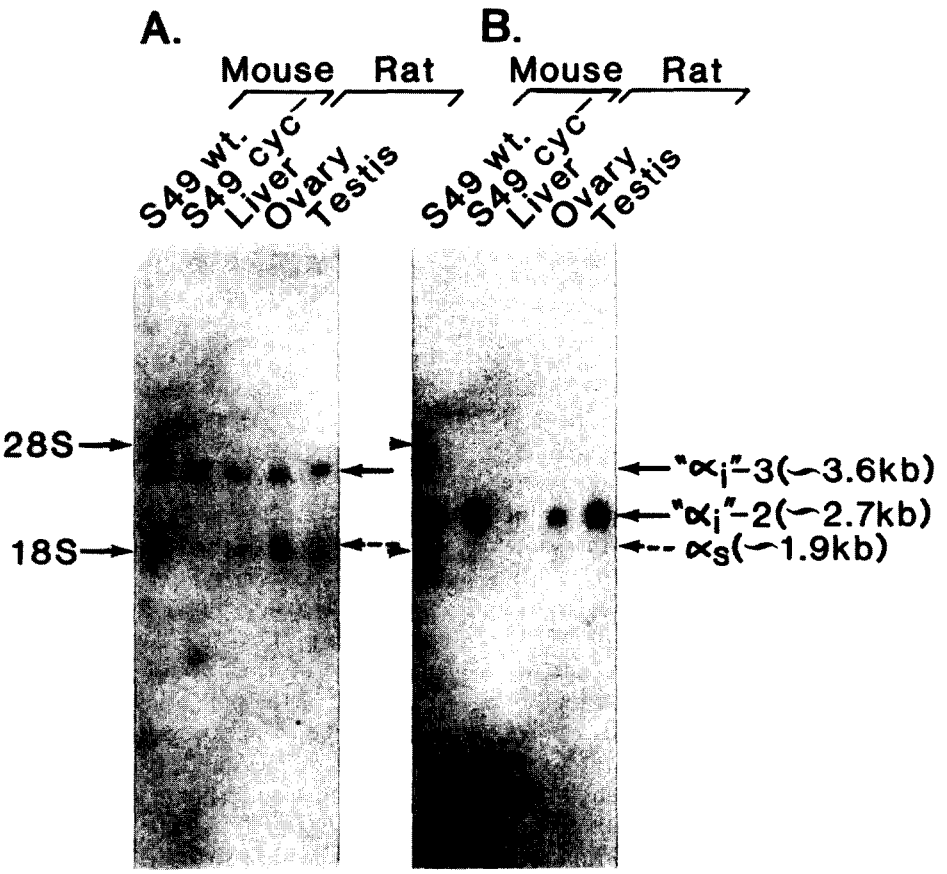

Fig.3. Hybridization blot (Northern) analysis of RNA fractions isolated from the indicated cells or tissues. Each lane received 25 μg of total RNA. (A) Nylon membranes were probed with type-3 α_i labeled with ^{32}P by nick-translation to a specific activity of 1.15×10^6 cpm/fmol; autoradiography, 49 h at −70°C. (B) The same membrane probed for panel A was immersed for 30 min in a boiling solution containing 18 mM NaCl, 1 mM sodium phosphate, pH 7.4, 0.1 mM EDTA and 0.1% SDS and reprobed with murine α_i-2 cDNA (subcloned in pUC-3) labeled to a specific activity of 0.26×10^6 cpm/fmol; autoradiography, 15 h at −70°C. The concentration of activity of the hybridization solutions was 10^6 cpm/ml in both cases. The composition of the hybridization solutions, the washing conditions and other details are given in the text. The migration of α_s mRNA [15] is indicated by the broken arrows.

et al. [9], and of human monocyte α_i (type-2 human α_i) obtained by Didsbury et al. [11]. Further cloning attempts led to isolation of two additional, yet shorter, cDNAs starting, respectively, 12 and 61 amino acids further into the same open reading frame of λgt a/ptx-39.

Fig.3 shows that by blot hybridization analysis the new type-3 human α_i cDNA (panel A) and the murine type-2 α_i cDNA (panel B) recognize RNA species of different sizes: α_i-3 RNA is ~3.6 kilobases long while α_i-2 RNA is ~2.7 kilobases long. Fig.3 also shows that even though RNAs coding for type-2 [9] and type-3 α_i are present in at least four tissues or cells (murine lymphoma cells, liver, testis and ovary), their absolute and relative abundance varies widely. For example, for the samples examined, the α_i-2 RNA is significantly lower for liver than lymphoma, ovary or testis, and all type-3 α_i RNAs are much less abundant than α_i-2 RNAs. (Note that the time of exposure for panel B was 3-times less than that for panel A, and that the specific activity of the α_i-3 probe was 4.4-times higher than that of the α_i-2 probe.)

4. DISCUSSION

Bovine and human type-1 α_i cDNAs encode identical 354 amino acid long molecules [5,9]. Rat, mouse and human type-2 α_i molecules encode 355 amino acid long molecules that differ by not more than 8 amino acids [6,10,11]. Assuming that the missing portion of type-3 human α_i cDNA encodes 20 amino acids, as suggested by alignment, the comparison shows that it differs from type-2 human α_i in 49 amino acids and from type-1 α_i in 23 amino acids. Taken together, these data indicate the existence in the same species (human) of at least three classes of PTX substrates of the so-called α_i-type, in addition to the α_o-type [6].

The existence of multiple α_i-type PTX substrates is of special interest because of current uncertainties as to how many of the signal transduction processes sensitive to interference by PTX are mediated by which G protein. When PTX substrates were first isolated [1,2] the only function known to be affected by PTX was receptor-mediated inhibition of adenylyl cyclase [17–19]. As a consequence, even though these G proteins were originally called 'predominant substrate for islet activating protein' [1] and 'putative N_i' [2], they were soon called simply G_i (N_i). Moreover, G proteins with PTX-sensitive α-subunits of M_r between 40000 and 41000 purified subsequently from other tissues (e.g. [3,4,7,8]) were also called G_i. It is now known, however, that PTX affects not only inhibition of adenylyl cyclase but, depending on the system studied, also receptor- and GTP-dependent activation of phospholipases of the C and/or A_2 type [20–24] and activation of K^+ channels [25,26]. Since inhibition of adenylyl cyclase and activation of phospholipases are regulated by receptors independently of each other, this suggests that more than one PTX-sensitive G protein should exist. Indeed immunological studies by Spiegel and collaborators [27–29] identify the existence of at least two types of PTX-sensitive antigens of M_r 40000–41000 in human neutrophils, in that antibody CW6 made against bovine transducin crossreacts well with human erythrocyte and bovine brain M_r 40000 'α_i' but not with the major PTX substrate of human neutrophils [27,28], while anti-transducin peptide antibody AS7 crossreacts well not only with brain but also with neutrophil PTX substrate(s) of M_r 40000–41000 [29]. In agreement with these immunological studies, two distinct non-G_o PTX-sensitive G proteins with α-subunits that migrate with apparent M_r values of 41000 and 40000 and eluting, respectively, before and after G_o from ion-exchange resins have recently been identified in porcine brain [7] and HL-60 cells [8].

The identity of the proteins encoded by the 'G_i'-type cDNAs is a matter for further research. In functional assays purified G_i proteins have been at best poor in inhibiting adenylyl cyclase activity on the basis of their α-subunits [30,31]. However, they are active in other reconstitution assays. Purified G_i from rat brain reconstitutes chemotactic receptor-mediated stimulation of phosphatidylinositol bisphosphate-specific phospholipase C activity in PTX-treated HL-60 cell membranes [32]. This classifies the purified G_is with α-subunits of M_r 41000 by SDS-PAGE as G_ps, i.e., the G proteins responsible for coupling receptor occupancy to activation of phospholipase C activity. α_i-subunits of M_r 41000 are encoded in type-1 α_i cDNA [5,6] and differ in at least 23 of its 354 amino acids from type-3 α_i reported here and in at least 45 amino acids from type-2 α_is. The human erythrocyte 'putative N_i' (α-subunit of M_r 40000

[2,33]) is active in stimulating the opening of K^+ channels [25,26] through its α-subunit [34,35] and has been re-named by us G_k. G_i proteins with α_is of M_r ~40000 were recently also purified from porcine brain [7] and HL-60 cells [34], but their possible functions have not yet been reported on. They might be encoded by either the type-2 or the type-3 cDNAs.

Our finding that there exist at least three closely related but nevertheless distinct non-allelic α-subunits of human origin strongly supports the hypothesis that distinct G protein activities reside on distinct molecules. Further, since only two classes of non-G_o PTX substrates have thus far been purified, the existence of three types of cDNAs indicates that at least one more class of PTX-sensitive G protein has to be sought and purified. Work is currently in progress to determine by amino acid sequencing which of the two types of cDNA for which the protein has not been identified (type-2 or type-3) encodes the α-subunit of G_k. As of now the type-3 human α_i cDNA may be encoding α_k, true α_i, inhibitory to adenylyl cyclase [19], an α_p mediating PTX-sensitive stimulation of phospholipase A_2 activity [23,24], or the α-subunit of a PTX-sensitive signal transducing protein the function of which has yet to be discovered. The same, of course, also applies to the type-2 α_i cDNA, the function of which is also unknown.

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