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Human G_i protein α-subunit: deduction of amino acid structure from a cloned cDNA

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The amino acid sequence of the α-subunit of G_i, the human adenylate cyclase inhibiting GTP-binding protein, has been deduced from the nucleotide sequence of a DNA clone complementary to $G_{i\alpha}$ mRNA from differentiated U937 cells. The cDNA encodes a polypeptide of 355 amino acids (M_r 40456). The amino acid sequence homology between human $G_{i\alpha}$ and rat, murine, and bovine $G_{i\alpha}$ is 98.6, 97.7 and 87.9% respectively. Differentiation of the U937 cells from monoblasts to monocyte-like cells resulted in a 3-fold increase in $G_{i\alpha}$ mRNA as well as a 3.6-fold increase in the 41 kDa pertussis toxin substrate presumed to be $G_{i\alpha}$. Thus, increased levels of this G-protein are associated with monocyte differentiation and appear to be regulated transcriptionally.

Adenylate cyclase-inhibiting G-protein; cDNA cloning; Nucleotide sequence; Differentiation

1. INTRODUCTION

Hormonal inhibition of adenylate cyclase is mediated by a distinct guanine nucleotide-binding-protein, G_i [1]. G_i is one member of a family of related GTP-binding proteins that are involved in transmembrane signalling. Other members include: G_s which mediates activation of adenylate cyclase [2], transducin which couples rhodopsin to a cGMP phosphodiesterase [3], and G_o whose function is unknown [4]. G-proteins are composed of three subunits: α, β and γ. β- and γ-subunits appear to be common to all G proteins [5] with the exception of the γ-subunit of transducin [6]. Specificity in structure and function of G-proteins is apparently determined in the unique GTP-binding α-subunits.

The α-subunit of G_i is a 41-kDa protein that is ADP-ribosylated by pertussis toxin resulting in a loss of hormonal and GTP-dependent inhibition of adenylate cyclase. We report here the cloning of a cDNA encoding the α-subunit of human G_i. This clone was derived from the U937 human monocyte-like cell line which had been differentiated with dibutyryl cyclic AMP. The differentiated U937 cells acquire many of the morphological and functional activities of mature monocytes [7]. The complete amino acid sequence of human $G_{i\alpha}$ is compared to rat, murine and bovine $G_{i\alpha}$ subunits. Northern blot analysis demonstrated transcriptional activation of $G_{i\alpha}$ mRNA upon differentiation of U937 cells to monocyte-like characteristics.

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2. MATERIALS AND METHODS

Poly(A)$^+$ RNA was isolated [8] from U937 cells differentiated to a monocyte-like cell type with dibutyryl cyclic AMP [7] and used in the construction of a cDNA library [9] in the cloning vector λgt11.

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A 39-base oligodeoxyribonucleotide probe (5′-GAAGATGATGGCGGTCACACCCTCGA-AGCAGTGGATCCA-3′) was synthesized based on the amino acid sequence of bovine $G_{i\alpha}$ (amino acids 210–223 [10]), $G_{s\alpha}$ (amino acids 234–247 [11]) and transducin (amino acids 207–220 [12]).

Approx. 4×10^5 recombinant plaques from a human monocyte λgt10 library (Clontech Laboratories, Palo Alto, CA) were screened by plaque hybridization [13] with the oligodeoxyribonucleotide probe labeled with ^{32}P at the 5′-end. Hybridization was carried out at 43°C in a solution containing 6 × SSC (1 × SSC = 0.15 M NaCl/15 mM sodium citrate, pH 7), 1 × Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll), 0.05% sodium pyrophosphate and 20 μg/ml yeast tRNA. Filters were washed at 55°C in 6 × SSC/0.1% sodium pyrophosphate and subject to autoradiography. One positive clone was isolated (1050 bp) and found to be a partial cDNA clone for $G_{i\alpha}$ by DNA sequencing. A 617 bp *Eco*RI–*Bgl*II fragment encoding amino acids 115–340 of $G_{i\alpha}$ was isolated from this cDNA clone, labeled with ^{32}P [13] and used to screen approx. 2.5×10^5 plaques from the differentiated U937 λgt11 cDNA library. Hybridization was performed at 32°C in a solution containing 50% (v/v) formamide, 3 × SSC, 5 × Denhardt's, 50 mM sodium phosphate (pH 6.8), 0.1% SDS and 200 μg/ml heat-denatured salmon sperm DNA. Filters were washed in 6 × SSC at 42°C and subject to autoradiography. Positive clones giving duplicate signals in the screening procedure were purified, their inserts excised with *Eco*RI and analyzed by Southern blotting [14]. Insert DNA

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      10         20         30         40         50         60         70         80         90         100        110        120
CCGGCAGTCC CGAGTGCTTC CCGCAGAGGG CTGGTGGTGG GAGCGGAGTG GAGTCGGGCG GGGCCGAAGC CGGGCCGTGG GCGTAGATGG GGGCCGGGCG GCGGCGGAGC GGCGGAACGC GGG ATG GGC TGC
                                                                                                                                    M  G  C

   138                153                168                183                198                213                228
ACC GTG AGC GCC GAG GAC AAG GCG GCG GCC GAG CGC TCT AAG ATG ATC GAC AAG AAC CTG CGG GAG GAC GGA GAG AAG GCG GCG CGG GAG GTG AAG TTG CTG CTG TTG
T  V  S  A  E  D  K  A  A  A  E  R  S  K  M  I  D  K  N  L  R  E  D  G  E  K  A  A  R  E  V  K  L  L  L  L

243                258                273                288                303                318                333                348
GGT GCT GGG GAG TCA GGG AAG AGC ACC ATC GTC AAG CAG ATG AAG ATC ATC CAC GAG GAT GGC TAC TCC GAG GAG GAA TGC CGG CAG TAC CGG GCG GTT GTC TAC AGC
G  A  G  E  S  G  K  S  T  I  V  K  Q  M  K  I  I  H  E  D  G  Y  S  E  E  E  C  R  Q  Y  R  A  V  V  Y  S

           363                378                393                408                423                438                453
AAC ACC ATC CAG TCC ATC ATG GCC ATT GTC AAA GCC ATG GGA AAC CTG CAG ATC GAC TTT GCC GAC CCC TCC AGA GCG GAC GAC GCC AGG CAG CTA TTT GCA CTG TCC
N  T  I  Q  S  I  M  A  I  V  K  A  M  G  N  L  Q  I  D  F  A  D  P  S  R  A  D  D  A  R  Q  L  F  A  L  S

      468                483                498                513                528                543                558
TGC ACC GCC GAG GAG CAA GGC GTG CTC CCT GAT GAC CTG TCC GGC GTC ATC CGG AGG CTC TGG GCT GAC CAT GGT GTG CAG GCC TGC TTT GGC CGC TCA AGG GAA TAC
C  T  A  E  E  Q  G  V  L  P  D  D  L  S  G  V  I  R  R  L  W  A  D  H  G  V  Q  A  C  F  G  R  S  R  E  Y

      573                588                603                618                633                648                663
CAG CTC AAC GAC TCA GCT GCC TAC TAC CTG AAC GAC CTG GAG CGT ATT GCA CAG AGT GAC TAC ATC CCC ACA CAG CAA GAT GTG CTA CGG ACC CGC GTA AAG ACC ACG
Q  L  N  D  S  A  A  Y  Y  L  N  D  L  E  R  I  A  Q  S  D  Y  I  P  T  Q  Q  D  V  L  R  T  R  V  K  T  T

   678                693                708                723                738                753                768
GGG ATC GTG GAG ACA CAC TTC ACC TTC AAG GAC CTA CAC TTC AAG ATG TTT GAT GTG GGT GGT CAG CGG TCT GAG CGG AAG AAG TGG ATC CAC TGC TTT GAG GGC GTC
G  I  V  E  T  H  F  T  F  K  D  L  H  F  K  M  F  D  V  G  G  Q  R  S  E  R  K  K  W  I  H  C  F  E  G  V

783                798                813                828                843                858                873                888
ACA GCC ATC ATC TTC TGC GTA GCC TTG AGC GCC TAT GAC TTG GTG CTA GCT GAG GAC GAG GAG ATG AAC CGC ATG CAT GAG AGC ATG AAG CTA TTC GAT AGC ATC TGC
T  A  I  I  F  C  V  A  L  S  A  Y  D  L  V  L  A  E  D  E  E  M  N  R  M  H  E  S  M  K  L  F  D  S  I  C

           903                918                933                948                963                978                993
AAC AAC AAG TGG TTC ACA GAC ACG TCC ATC ATC CTC TTC CTC AAC AAG AAG GAC CTG TTT GAG GAG AAG ATC ACA CAC AGT CCC CTG ACC ATC TGC TTC CCT GAG TAC
N  N  K  W  F  T  D  T  S  I  I  L  F  L  N  K  K  D  L  F  E  E  K  I  T  H  S  P  L  T  I  C  F  P  E  Y

         1008               1023               1038               1053               1068               1083               1098
ACA GGG GCC AAC AAA TAT GAT GAG GCA GCC AGC TAC ATC CAG AGT AAG TTT GAG  GAC CTG AAT AAG CGC AAA GAC ACC AAG GAG ATC TAC ACG CAC TTC ACG TGC GCC
T  G  A  N  K  Y  D  E  A  A  S  Y  I  Q  S  K  F  E   D  L  N  K  R  K  D  T  K  E  I  Y  T  H  F  T  C  A

      1113               1128               1143               1158               1173               1188       1201       1211       1221
ACC GAC ACC AAG AAC GTG CAG TTC GTG TTT GAC GCC GTC ACC GAT GTC ATC ATC  AAG AAC AAC CTG AAG GAC TGC GGC CTC TTC TGA GGGGCAGCGG GGCCTGGCGG GATGGGCCAC
T  D  T  K  N  V  Q  F  V  F  D  A  V  T  D  V  I  I   K  N  N  L  K  D  C  G  L  F

      1231       1241       1251       1261       1271       1281       1291       1301       1311       1321       1331       1341       1351       1361
CGCCGAATTT GTACCCCCCA ACCCCTGAGG AAGATGGGGG CAAGAAGATC ACGCTCCCCG CCTGTTCCCC CGCCGCTTTT CTCCTCTTTC CTCTCTTTGT TCTCAGCTCC CCCTGTCCCC TCAGCTCCAA ACGTAGGGGA

      1371       1381       1391       1401       1411       1421       1431       1441       1451       1461       1471       1481       1491       1501
GGGGTTCGCA CAGGCCTCCC TGTTTGAAGC CTGCCCTTGT CTGAGATGCT GGTAATGGCC ATGGTACCCC CTTCTGGGCA TCTGTTCTGG TTTTTAACCA TTGTCTTGTT CTGTGATGAG GGGAGGGGGG CACATGCTGA

      1511       1521       1531       1541       1551       1561       1571       1581       1591       1601       1611       1621       1631       1641
GTCTCCCAAG GCTGCGTCTG GAGGGGCCCC TGCTTCTCCA GCCTGGACCC CCAGCTTTGC CCAACACCAG CCCCTGCCCC AGCCCAAGTC CAAATGTTTA CGGGAGCCTC CTGCCCAGTC CCCCAACCCC AGCCGCTCGG

      1651       1661       1671       1681       1691       1701
AGGCCCCAAA GGAAAAAGCA CAAGAAGCGT GAGACGCCAC CATTCCTGGA AACCACAGTC C
```

Fig.1. Nucleotide sequence of cDNA clone pJD43 encoding the α-subunit of human G_i. Numbering of the nucleotide sequence begins at the first nucleotide and proceeds in the 5′- to 3′-direction. The open reading frame begins at nucleotide 124 and extends 1065 bp until a termination codon starting at nucleotide 1189.

from clones reacting with a cDNA fragment from the human monocyte $G_{i\alpha}$ clone (containing largely 3′-noncoding sequence) were ligated into pUC18. Clones containing large inserts (>1 kb) were sequenced directly in pUC18 using the dideoxy chain termination method [15,16] and complementary oligodeoxyribonucleotide primers.

Northern blot analysis was performed by the method of Thomas [17] using nylon membranes (Zeta Bind, AMF Cuno) and poly(A)$^+$ RNA from U937 and differentiated U937 cells isolated by the method of Chirgwin [8] and passaged one time over oligo(dT)-cellulose (Collaborative Research, type III).

3. RESULTS AND DISCUSSION

Twenty-six positive clones were obtained from screening of the λgt11 U937 differentiated cDNA library with a partial $G_{i\alpha}$ coding region specific cDNA fragment isolated from a λgt10 human monocyte cDNA library. Six clones which reacted with a 3′-noncoding region portion of this cDNA on Southern blot analysis were sequenced. Fig.1 shows the nucleotide sequence of one of these clones, pJD43, containing the largest cDNA insert (1702 bp). Sequencing reactions were performed using 21-mer oligonucleotide primers. Confirming reactions were done by sequencing of both strands of the cDNA insert and sequencing of other overlapping $G_{i\alpha}$ cDNA clones.

Clone pJD43 contains 123 bp of 5′-noncoding sequence followed by an open reading frame of 1065 bp encoding a polypeptide of 355 amino acids (including an initiator methionine), with a calculated molecular mass of 40455 Da. The 3′-noncoding region (513 bp) is devoid of any polyadenylation signals, AATAAA [18]. Sequenc-

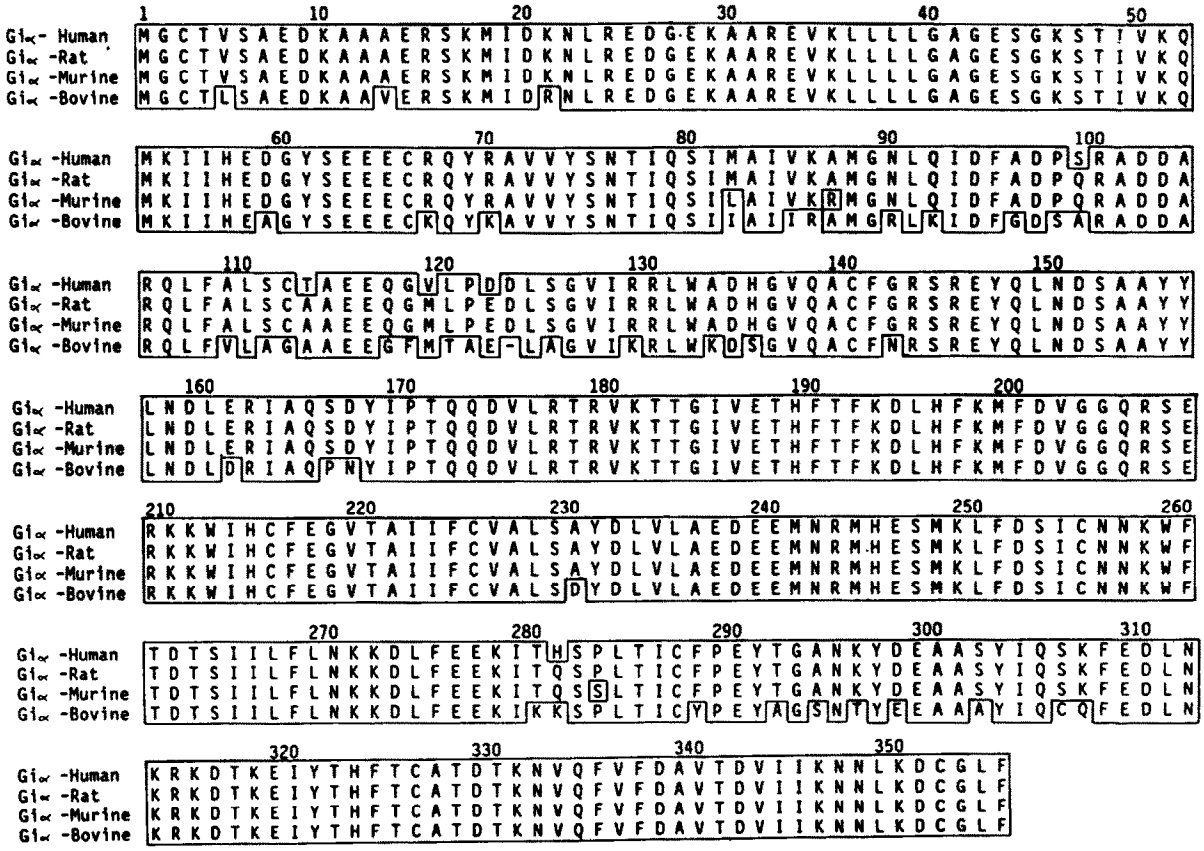

Fig.2. Comparison of amino acid sequences of α-subunits of G_i. The amino acid sequences (single letter notation) of human (pJD43), rat [23], murine [19] and bovine [10] $G_{i\alpha}$ subunits are shown. Sets of identical residues are indicated by solid lines.

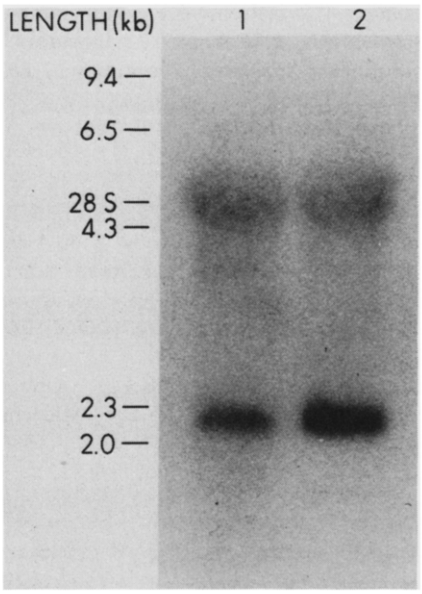

Fig.3. RNA blot analysis. Poly(A)$^+$ RNA (2.5 μg) isolated from U937 (lane 1) and dibutyryl cyclic AMP-differentiated U937 (lane 2) cells hybridized with human $G_{i\alpha}$-subunit cDNA probe pJD43. The size markers are *Hin*dIII-digested λDNA and *Hae*III digested ϕX174 DNA subjected to the same glyoxylation procedure as the poly(A)$^+$ RNA.

ing of one of the overlapping $G_{i\alpha}$ clones reveals a poly(dA) tail 38 bp 3′- to the 3′-terminus of pJD43 (not shown). The amino acid sequence encoded by the long open reading frame of pJD43 was aligned with known amino acid sequences of bovine, murine and rat $G_{i\alpha}$ subunits (fig.2) confirming the identity of pJD43. The amino acid sequence homology between human $G_{i\alpha}$ and rat, murine and bovine $G_{i\alpha}$ was 98.6, 97.7 and 87.9%, respectively.

Four regions presumed to be the site of GTP binding and hydrolysis (amino acids 32–49, 200–206, 222–230 and 265–278 [19]) are conserved in all four $G_{i\alpha}$ subunits. Another region of homology is the carboxy-terminal nonapeptide surrounding the site of ADP-ribosylation by pertussis toxin (Cys-352) [21,22]. Polypeptide structure analysis [20] of the amino acid sequence encoded by pJD43 is identical to rat and murine $G_{i\alpha}$ subunits and similar to bovine $G_{i\alpha}$ which differs from the other $G_{i\alpha}$ subunits in lacking an α-helix turn at amino acids 97–100 and possessing an additional α-to-β turn (amino acids 132–137) and β-turn (amino acids 228–231) (not shown).

The differences in amino acid sequence between human $G_{i\alpha}$ and rat, murine and bovine $G_{i\alpha}$ are likely species specific. There is only one nonconservative substitution when compared with rat $G_{i\alpha}$ (His-281), two compared to murine $G_{i\alpha}$ (Ala-87, His-281) and eight vs bovine $G_{i\alpha}$ (Asp-59, Asn-90, Gln-92, Ala-133, His-135, Asp-167, Thr-280, Lys-296). None of these substitutions occurs in the putative areas of GTP binding and hydrolysis and are unlikely to alter functions of the protein between species.

Northern blot hybridization of poly(A)$^+$ RNA from U937 and dibutyryl cyclic AMP-differentiated U937 cells with pJD43 revealed a 2.1 kb hybridizable mRNA (fig.3). Densitometric scanning of the autoradiogram showed a 3.2-fold increase in the amount of $G_{i\alpha}$ mRNA upon differentiation of U937 cells from monoblastic to monocyte-like cells. $G_{i\alpha}$ subunits from both cell types were radiolabeled by ADP-ribosylation with pertussis toxin and run on SDS-polyacrylamide gels. Densitometry of the 41 kDa labeled pertussis toxin substrate showed there to be a 3.6-fold increase in $G_{i\alpha}$ upon differentiation of U937 cells (not shown) which is in close agreement with the observed increase in $G_{i\alpha}$ mRNA. Thus it appears that regulation of $G_{i\alpha}$ subunit levels in cells is under transcriptional control. The exact relationship between cellular differentiation and functions requiring an increased amount of G_i can now be investigated.

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