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Biochimica et Biophysica Acta 1281 (1996) 125–128

BBA Biochimica et Biophysica Acta

Short sequence-paper

Human $G_{\alpha q}$: cDNA and tissue distribution [1]

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Received 15 December 1995; revised 8 February 1996; accepted 14 February 1996

Abstract

$G_{\alpha q}$, a member of the G_q family of heterotrimeric G proteins, transduces signals from several G protein-coupled receptors that stimulate membrane phosphoinositide hydrolysis. In order to further define the role of $G_{\alpha q}$ in the function of G protein-coupled receptors, we have cloned the cDNA encoding human $G_{\alpha q}$ from a prostate cDNA library. Human $G_{\alpha q}$ exhibits high homology with its mouse homolog – 94% similarity at the nucleotide level, and 99% similarity at the amino acid level. Northern hybridization data indicate high expression of $G_{\alpha q}$ mRNA in organs of the human reproductive system including ovary, prostate, and testis.

Keywords: $G_{\alpha q}$ protein; Gene expression; Nucleotide sequence; cDNA; Tissue distribution; (Human)

The heterotrimeric guanine nucleotide binding proteins called G proteins consist of α, β, and γ-subunits and transduce signals from agonist-activated receptors to a variety of effectors such as adenylyl cyclase, phospholipase C-β (PLC-β), and ion channels. Several genes encoding G protein α-subunits have been isolated. These G protein α-subunits have been classified based on sequence homology into four subfamilies: G_s, G_i, G_q, and G_{12}. These subfamilies include at least 20 distinct α-subunits; in addition to the existence of multiple genes for Gα-subunits, genes for five distinct β-subunits and eight different γ-subunits have also been isolated [1–3].

The focus of our laboratory is to understand molecular interactions between G protein-coupled receptors (such as substance P and α_1-adrenergic) and G proteins of the G_q subfamily. The G_q subfamily includes α_q, α_{11}, α_{14}, and α_{16}. These Gα-subunits are insensitive to pertussis-toxin, and have been shown to activate PLC-β isozymes that catalyze the hydrolysis of membrane phosphoinositides (PI) into inositol trisphosphate and diacylglycerol [4,5]. Thus, G proteins of the G_q family mediate signal transduction through G protein-coupled receptors linked to PI-hydrolysis. Some of the receptors that have already been shown to interact with the G_q family are α_1-adrenergic [6], endothelin [7], m_1-muscarinic acetylcholine [8], thyrotropin-releasing hormone [9], and thromboxane A_2 receptors [10]. In a recent study, we showed that a mixture of $G_{\alpha q}$ and $G_{\alpha 11}$, purified from bovine liver, converts partially purified and reconstituted substance P receptor (SPR) into a high affinity state for the agonist substance P [11]. In order to further characterize functional interactions between SPR and individually purified recombinant $G_{\alpha q}$ and $G_{\alpha 11}$, we cloned the cDNA of human $G_{\alpha q}$ from a prostate cDNA library. Our results indicate that human $G_{\alpha q}$ is highly homologous to mouse $G_{\alpha q}$, and we find relatively high expression of $G_{\alpha q}$ mRNA in organs of the human reproductive system including ovary, prostate, and testis.

Human prostate mRNA (Clontech, Palo Alto, CA) was converted into cDNA using reverse transcriptase following the manufacturer's protocol (Superscript, Gibco, Gaithersburg, MD). A 150 bp human $G_{\alpha q}$ probe was synthesized by PCR (GeneAmp® Kit, Perkin Elmer, Norwalk, CT) using the prostate cDNA as a template and degenerate oligonucleotide primers derived from the published sequence of mouse $G_{\alpha q}$ [12]. For PCR reaction, the following conditions were used: denature for 30 s at 95°C, anneal for 30 s at 47°C, and extension for 30 s at 72°C. The 150

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[1] The sequence data reported in this paper have been submitted to the GenBank under the accession number U43083.

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PII S0005-2736(96)00039-9

bp PCR product was labeled with digoxigenin (DIG) (Boehringer Mannheim, Indianapolis, IN) utilizing Klenow enzyme, and used as a probe to screen a human prostate λgt10 cDNA library according to the manufacturer's protocol (Clontech, Palo Alto, CA). Eight positive clones were identified, with one clone containing a full length cDNA of $G_{\alpha q}$; DNA sequencing was performed using the fmole® DNA sequencing system (Promega Corporation, Madison, WI).

Fig. 1 shows the nucleotide and deduced amino acid sequences of human $G_{\alpha q}$. A comparison of these sequences with the published sequences of mouse $G_{\alpha q}$ [12] reveals high sequence similarity. At the nucleotide level, human $G_{\alpha q}$ and mouse $G_{\alpha q}$ are 94% identical (1016 bp out of 1077 bp); at the amino acid level, the deduced amino acid sequence of human $G_{\alpha q}$ and mouse $G_{\alpha q}$ are 99% identical (355 identical amino acids out of 359). Human $G_{\alpha 11}$ has been cloned [13] and shares 89% identity at the amino acid level (321 identical amino acids out of 359) with human $G_{\alpha q}$ (see Fig. 2).

To determine tissue distribution of $G_{\alpha q}$, a Northern blot containing 2 μg each of poly(A)$^+$ RNA from different human tissues was purchased from Clontech (Palo Alto, CA). An 800 bp PCR-generated fragment from the coding sequence of human $G_{\alpha q}$ was labeled with DIG and used as a probe for Northern analysis. The blot was prehybridized in ExpressHyb Solution (Clontech, Palo Alto, CA) at 68°C for 30 min, then hybridized with the DIG-labeled probe in the same solution at 68°C for 1 h. The membrane was washed in 2 × SSC, 0.1% SDS at room temperature for 30 min, followed by a wash with 0.1 × SSC, 0.1% SDS at 68°C for 30 min. The washed blot was developed using chemiluminescence substrate Lumi-Phos 530 according to the manufacturer's protocol (Boehringer Mannheim, Indianapolis, IN).

Results of the Northern hybridization are shown in Fig.

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                                   CGGCGGCCCAGACTATCCGCTCCCACCGCGCCCCCGGCCC  -181
ACCTGGTGGCCCCGGCCTGGCCGCCGCCCCCGCGCTGTGCCCGGGAGCTCGTCCCGGACG  -121
CGCGACCGGGCGGCGGGGGCTCGGCGGCCACCGCTGCCTCAAGGGAGCGAGGCGGGAGGG   -61
TGTGTGTGCGCGGCTGTGAGCAGGGGTGCCGGCGGGCTGCAGCGGAGGCACTTTGGAAGA    -1

ATGACTCTGGAGTCCATCATGGCGTGCTGCCTGAGCGAGGAGGCCAAGGAAGCCCGGCGG    60
M  T  L  E  S  I  M  A  C  C  L  S  E  E  A  K  E  A  R  R    (20)
ATCAACGACGAGATCGAGCGGCAGCTCCGCAGGGACAAGCGGGACGCCCGCCGGGAGCTC   120
I  N  D  E  I  E  R  Q  L  R  R  D  K  R  D  A  R  R  E  L    (40)
AAGCTGCTGCTGCTCGGGACAGGAGAGAGTGGCAAGAGTACGTTTATCAAGCAGATGAGA   180
K  L  L  L  L  G  T  G  E  S  G  K  S  T  F  I  K  Q  M  R    (60)
ATCATCCATGGGTCAGGATACTCTGATGAAGATAAAAGGGGCTTCACCAAGCTGGTGTAT   240
I  I  H  G  S  G  Y  S  D  E  D  K  R  G  F  T  K  L  V  Y    (80)
CAGAACATCTTCACGGCCATGCAGGCCATGATCAGAGCCATGGACACACTCAAGATCCCA   300
Q  N  I  F  T  A  M  Q  A  M  I  R  A  M  D  T  L  K  I  P   (100)
TACAAGTATGAGCACAATAAGGCTCATGCACAATTAGTTCGAGAAGTTGATGTGGAGAAG   360
Y  K  Y  E  H  N  K  A  H  A  Q  L  V  R  E  V  D  V  E  K   (120)
GTGTCTGCTTTTGAGAATCCATATGTAGATGCAATAAAGAGTTTATGGAATGATCCTGGA   420
V  S  A  F  E  N  P  Y  V  D  A  I  K  S  L  W  N  D  P  G   (140)
ATCCAGGAATGCTATGATAGACGACGAGAATATCAATTATCTGACTCTACCAAATACTAT   480
I  Q  E  C  Y  D  R  R  R  E  Y  Q  L  S  D  S  T  K  Y  Y   (160)
CTTAATGACTTGGACCGCGTAGCTGACCCTGCCTACCTGCCTACGCAACAAGATGTGCTT   540
L  N  D  L  D  R  V  A  D  P  A  Y  L  P  T  Q  Q  D  V  L   (180)
AGAGTTCGAGTCCCCACCACAGGGATCATCGAATACCCCTTTGACTTACAAAGTGTCATT   600
R  V  R  V  P  T  T  G  I  I  E  Y  P  F  D  L  Q  S  V  I   (200)
TTCAGAATGGTCGATGTAGGGGGCCAAAGGTCAGAGAGAAGAAAATGGATACACTGCTTT   660
F  R  M  V  D  V  G  G  Q  R  S  E  R  R  K  W  I  H  C  F   (220)
GAAAATGTCACCTCTATCATGTTTCTAGTAGCGCTTAGTGAATATGATCAAGTTCTGGTG   720
E  N  V  T  S  I  M  F  L  V  A  L  S  E  Y  D  Q  V  L  V   (240)
GAGTCAGACAATGAGAACCGAATGGAGGAAAGCAAGGCTCTCTTTAGAACAATTATCACA   780
E  S  D  N  E  N  R  M  E  E  S  K  A  L  F  R  T  I  I  T   (260)
TACCCCTGGTTCCAGAACTCCTCGGTTATTCTGTTCTTAAACAAGAAAGATCTTCTAGAG   840
Y  P  W  F  Q  N  S  S  V  I  L  F  L  N  K  K  D  L  L  E   (280)
GAGAAAATCATGTATTCCCATCTAGTCGACTACTTCCCAGAATATGATGGACCCCAGAGA   900
E  K  I  M  Y  S  H  L  V  D  Y  F  P  E  Y  D  G  P  Q  R   (300)
GATGCCCAGGCAGCCCGAGAATTCATTCTGAAGATGTTCGTGGACCTGAACCCAGACAGT   960
D  A  Q  A  A  R  E  F  I  L  K  M  F  V  D  L  N  P  D  S   (320)
GACAAAATTAACTACTCCCACTTCACGTGCGCCACAGACACCGAGAATATCCGCTTTGTC  1020
D  K  I  N  Y  S  H  F  T  C  A  T  D  T  E  N  I  R  F  V   (340)
TTTGCTGCCGTCAAGGACACCATCCTCCAGTTGAACCTGAAGGAGTACAATCTGGTCTAA  1080
F  A  A  V  K  D  T  I  L  Q  L  N  L  K  E  Y  N  L  V  *   (359)

TTGTGCCTGCTAGACACCCGCCCTGCCCTTCCCTGGTGGGCTATTGAAGATACACAAGAG  1140
GGACTGTATTTCTGTGGAAAACAATTTGCATAATACTAATTTATTGCCGTCCTGGACTCT  1200
GTGTGAGCGTGTCCACAGAGTTTGAGTAAATATTATGATTTTATTTAAACTATTCCAGAG  1260
GAAAAACAGAGGATGCTGAAGTACAGTCCCAGCACATTTCCTCTCTATCTTTTTTTAGGC  1320
AAACCTTGTGACTCAGTGTATTTTAAATTCTCAGTCATGCACTCACAAAGATAAGACTTG  1380
TTTCTTTCTGTCTCTCTCTCTTTTTCTTTTCTATGGAGCAAAACAAAGCTGATTTCCCTT  1440
TTTTTCTTCCCCCGCTAATTCATACCTCCCTCCTGATGTT                       1480
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Fig. 1. Nucleotide and deduced amino acid sequences of the cDNA encoding human $G_{\alpha q}$. The nucleotide number is indicated on the right and the amino acid number is in parentheses. Positive numbering starts from the putative translation initiation codon ATG. The stop codon is indicated by an asterisk.

3. Of the tested tissues, $G_{\alpha q}$ expresses highest in ovary, prostate, testis, and colon. Furthermore, each tissue exhibits two major $G_{\alpha q}$ transcripts of approximately 6 and 8 kb sizes. The significance of multiple $G_{\alpha q}$ transcripts is not clear; however, these results are in agreement with multiple (at least three major) $G_{\alpha q}$ transcripts seen in various mouse tissues [12].

Our finding of relatively high expression of $G_{\alpha q}$ mRNA in ovary, prostate, and testis is interesting. Since $G_{\alpha q}$ mediates PI-hydrolysis, a biochemical response involved in cell proliferation [14], it is conceivable that this G protein plays a role in normal/abnormal growth of these organs. In this connection, it is important to note that constitutively active mutants of $G_{\alpha q}$ have been shown to cause cell transformation [15,16], and oncogenic mutations in other G proteins have been noted in various human cancers [17–19].

In conclusion, we have isolated the cDNA encoding $G_{\alpha q}$ from a human prostate cDNA library, and demonstrate that this Gα-subunit is relatively abundant in ovary, prostate, and testis. The availability of human $G_{\alpha q}$ cDNA should now make it possible to purify this G protein in sufficient quantities for functional interactions with substance P and other receptors.

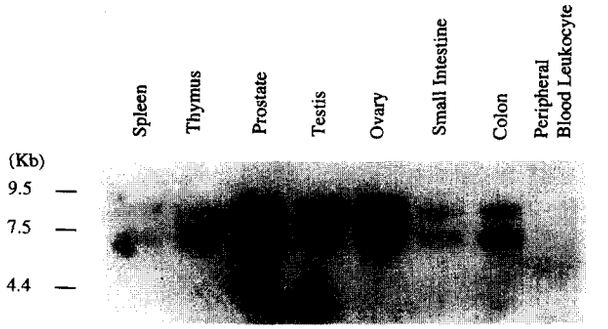

Fig. 3. Northern blot analysis of $G_{\alpha q}$ mRNA in various human tissues. The blot (purchased from Clontech) contains 2 μg poly(A)$^+$ RNA from the listed human tissues. This blot was probed with an 800 bp, DIG-labeled, PCR-generated probe from the coding sequence of human $G_{\alpha q}$ cDNA. The blot was developed using chemiluminescence substrate Lumi-Phos 530 (Boehringer Mannheim).

```
human Gαq   MTLESIMACCLSEEAKEARRINDEIERQLRRDKRDARRELKLLLLGTGES
mouse Gαq   ----------------------------HV--------------------
human Gα11  -----M------D-V--SK---A---KQL---------------------

human Gαq   GKSTFIKQMRIIHGSGYSDEDKRGFTKLVYQNIFTAMQAMIRAMDTLKIP
mouse Gαq   ------------------------------------------------
human Gα11  --------------A---E------------------------E----L

human Gαq   YKYEHNKAHAQLVREVDVEKVSAFENPYVDAIKSLWNDPGIQECYDRRRE
mouse Gαq   ------------------------------------------------
human Gα11  ----Q-----L-I--------TT--HQ--S---T--E------------

human Gαq   YQLSDSTKYYLNDLDRVADPAYLPTQQDVLRVRVPTTGIIEYPFDLQSVI
mouse Gαq   --------------------S-----------------------------
human Gα11  ------A----T-V--I-TLG------------------------ENI-

human Gαq   FRMVDVGGQRSERRKWIHCFENVTSIMFLVALSEYDQVLVESDNENRMEE
mouse Gαq   ------------------------------------------------
human Gα11  ------------------------------------------------

human Gαq   SKALFRTIITYPWFQNSSVILFLNKKDLLEEKIMYSHLVDYFPEYDGPQR
mouse Gαq   ------------------------------------------------
human Gα11  ------------------------------D--L----------F-----

human Gαq   DAQAAREFILKMFVDLNPDSDKINYSHFTCATDTENIRFVFAAVKDTILQ
mouse Gαq   ----------------------I-------------------------
human Gα11  EP--------------------------------------------------

human Gαq   LNLKEYNLV
mouse Gαq   ---------
human Gα11  ---------
```

Fig. 2. Alignment of deduced amino acid sequence of human $G_{\alpha q}$, mouse $G_{\alpha q}$ [12], and human $G_{\alpha 11}$ [13].

This research was supported in part by funds from the American Cancer Society Institutional Research Grant (ACS-IRG 158I) and from the American Lung Association (North Carolina Affiliate) grant to M.M.K. We thank Mary Carnell for secretarial assistance.

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