Isolation and expression of a novel chick G-protein cDNA coding for a $G\alpha_{13}$ protein with a $G\alpha_o$ N-terminus

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We have cloned cDNAs coding for G-protein α subunits from a chick brain cDNA library. Based on sequence similarity to G-protein α subunits from other eukaryotes, one clone was designated $G\alpha_{13}$. A second clone, $G\alpha_{13-0}$, was identical to the $G\alpha_{13}$ clone over 932 bases on the 3' end. The 5' end of $G\alpha_{13-0}$, however, contained an alternative sequence in which the first 45 amino acids coded for are 100% identical to the conserved N-terminus of $G\alpha_o$ from species such as rat, mouse, human, bovine and

hamster. Both clones were found to be expressed in all tissues studied. The unusual α_o - α_{13} -like G-protein chimera, $G\alpha_{13-0}$, was found to be expressed at significantly lower levels than $G\alpha_{13}$. *In vitro* transcription and translation of the $G\alpha_{13-0}$ cDNA clone gave a protein of approx. 41 kDa which stably bound guanosine 5'-[γ -thio]triphosphate. $G\alpha_{13-0}$ appears to be the first G-protein α subunit cloned which contains ends that are homologous to two different α subunit isoforms, $G\alpha_o$ and $G\alpha_{13}$.

INTRODUCTION

G-proteins couple a variety of extracellular signals to intracellular effectors and second messenger production. G-proteins are heterotrimers consisting of α , β and γ subunits. Receptor stimulation results in the activation of G-proteins by promoting the exchange of bound GDP for GTP followed by the dissociation of the α -subunit-GTP complex from the $\beta\gamma$ subunits (Birnbaumer, 1990; Spiegel et al., 1992). The dissociated α and $\beta\gamma$ subunits have both been shown to interact with a variety of effectors (Neer and Clapham, 1988).

G-proteins have been grouped into a number of families of α subunits and multiple isoforms of β and γ subunits (Jones and Reed, 1987; Strathmann and Simon, 1990; Simon et al., 1991; Spiegel et al., 1992). At present four isoforms of the β subunit have been cloned (Gao et al., 1987; Amatruda et al., 1988; von Weizsacker et al., 1992) and at least five forms of the γ subunit have been demonstrated (Gautam et al., 1990; Fisher and Aronson, 1992). $\beta\gamma$ subunits have an effect on second messenger production, such as the inhibition or stimulation of adenylate cyclase activity (Tang and Gilman, 1991) and the stimulation of muscarinic K^+ channels (Logothetis et al., 1987). More recently, $\beta\gamma$ subunits have been shown to play a direct role in the β -adrenergic receptor kinase (β ARK)-mediated desensitization of β_2 adrenergic receptors (Pitcher et al., 1992) and the activation of phospholipase C (Camps et al., 1992).

Based on amino acid sequence similarity, three families of α subunits have been demonstrated, each composed of multiple subtypes (Strathmann and Simon, 1990; Simon et al., 1991). The specific functions of many $G\alpha$ isoforms remain unclear. However, the $G\alpha_s$ protein has been shown to couple receptors to stimulation of adenylate cyclase activity (Gilman, 1984). $G\alpha_o$ has been implicated in coupling receptors to ion channels (Hescheler et al., 1987) and $G\alpha_{11}$ has been shown to couple rod photo-receptors to cyclic GMP phosphodiesterase (Birnbaumer, 1990). $G\alpha_i$ subunits have been implicated in the inhibition of adenylate cyclase (Neer and Clapham, 1988; Birnbaumer, 1990).

The multiplicity of G-protein α subunits has been found to be increased by alternate splicing of exons within the same α subunit gene. For example, the *Drosophila* $G\alpha_o$ gene produces two transcripts coding for proteins containing different N-termini (de Sousa et al., 1989; Yoon et al., 1989). In hamster, mouse, rat and human the gene for $G\alpha_o$ produces two transcripts for proteins containing different C-termini (Hsu et al., 1990; Bertrand et al., 1990; Strathmann et al., 1990; Tsukamoto et al., 1991).

In the present study, two G-protein α_{13} subunits were cloned from a chick brain cDNA library. One of the clones, $G\alpha_{13-0}$, coded for a protein with a $G\alpha_o$ -like N-terminus, but was otherwise identical to the $G\alpha_{13}$ cDNA throughout the rest of the clone. This appears to be the first reported G-protein clone which codes for a protein which is a chimera between two α subunit G-protein subtypes.

MATERIALS AND METHODS

Isolation and sequencing of cDNA clones

A cDNA library, from chick brain 17 days *in ovo*, constructed in the λ -ZAP vector (Stratagene), was kindly provided by Kunio Takeyasu (University of Virginia School of Medicine, Charlottesville, VA, U.S.A.). The library was screened using a fragment from the C-terminus of bovine $G\alpha_{11}$ (*SmaI*-*XbaI* fragment) as a probe (Michel et al., 1986), kindly provided by Eva J. Neer. The probe was radiolabelled with [α - ^{32} P]dCTP by the random primer method (Feinberg and Vogelstein, 1984). The hybridization and washing conditions used to screen the library were as described (Church and Gilbert, 1984); filters were hybridized with the probe at 55 °C in 1% BSA, 7% SDS, 0.5 M NaH_2PO_4 , pH 7.2, 1 mM EDTA and 200 $\mu\text{g}/\text{ml}$ salmon sperm DNA. The filters were washed three times at 55 °C in 0.5% BSA, 5% SDS, 40 mM NaH_2PO_4 and 1 mM EDTA, followed by three washes at 55 °C in 1% SDS, 40 mM NaH_2PO_4 and 1 mM EDTA.

Two different G-protein clones were plaque-purified and excised from λ -ZAP to generate the cDNAs within the

Abbreviations used: RT, reverse transcriptase; GTP[S], guanosine 5'-[γ -thio]triphosphate.

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The nucleotide sequence data reported have been deposited in the GenBank, EMBL and DDBJ Nucleotide Sequence Databases under accession nos. L24550 and L24551.

pBluescript vector as described (Stratagene). These constructs were completely sequenced on both strands by the dideoxy chain termination method (Sanger et al., 1977) using a combination of subcloned restriction fragments and synthetic oligonucleotide primers (Sequenase; United States Biochemical Corp.). A Sequenase 7-deaza-dGTP sequencing kit was used to resolve specific sequencing anomalies (United States Biochemical Corp.).

RNAase protection analysis of G-protein expression

Total RNA was isolated by guanidinium/CsCl centrifugation (Sambrook et al., 1989). RNAase protection assays were performed as described (Ausubel et al., 1987). The 5'-terminal *PvuII* fragments of the $G\alpha_{13}$ and $G\alpha_{13-0}$ cDNAs were subcloned into pBluescript vector and linearized with *BamHI* to generate RNA probes of the sizes shown in Figure 3. Antisense RNA labelled with [α - ^{32} P]UTP was generated from each clone using the T7 RNA polymerase promoters on the pBluescript vector. The probes synthesized from $G\alpha_{13}$ and $G\alpha_{13-0}$ were 376 and 321 nucleotides long respectively and contained an additional 59 nucleotides of pBluescript vector on the 3' end of each transcript. In experiments to determine the specificity of RNAase protection by the riboprobes, full-length sense mRNAs were synthesized in the absence of radioactive nucleotides from the $G\alpha_{13}$ and $G\alpha_{13-0}$ cDNAs to use as control mRNAs (see Figure 4a). Following hybridization of the probes to total RNA or *in vitro* synthesized mRNA, the samples were treated with ribonuclease and analysed by PAGE on 6% gels containing urea followed by autoradiography.

PCR analysis of $G\alpha_{13-0}$ mRNA from chick tissues

Total RNA (1 μ g) from each tissue was reverse-transcribed (Innis et al., 1990) using a 21 bp oligonucleotide primer (TTGCTGTAGACAACCACTTTA; primer c in Figure 6a) which was antisense to a region common to both $G\alpha_{13}$ and $G\alpha_{13-0}$. The resulting cDNAs were amplified by PCR as described (Innis et al., 1990) using a mixture of oligonucleotide primers specific for $G\alpha_{13}$ (GCGGTGGAACGGAGCAAAATG; primer b, Figure 6a) and $G\alpha_{13-0}$ (CTGAAGGAGGACGGCAT-CAGC; primer a, Figure 6a) and primer c. A second set of RNA samples which had been incubated in the absence of reverse transcriptase (RT) was amplified by PCR under the same conditions. The PCR products were electrophoresed on a 2.6% agarose gel and Southern transferred to a Zetaprobe nylon membrane (Bio-Rad) and probed with a ^{32} P-labelled oligonucleotide (TCGTGAATAATCTTCATC; primer p, Figure 6a) which was complementary to both $G\alpha_{13}$ and $G\alpha_{13-0}$. The probe was end-labelled with T4 polynucleotide kinase and [γ - ^{32} P]ATP as described (Sambrook et al., 1989). The filter was prehybridized in $6 \times$ SSC ($1 \times$ SSC = 0.15 M NaCl and 0.015 M sodium citrate), $5 \times$ Denhardt's solution, 20 mM NaH_2PO_4 and 500 μ g/ml salmon sperm DNA at 25 °C and hybridized to the probe in 0.4% SDS, $6 \times$ SSC, 20 mM NaH_2PO_4 and 500 μ g/ml salmon sperm DNA for 18 h at 37 °C. It was then washed in $6 \times$ SSC and 0.1% SDS at 25 °C followed by three washes at 37 °C, dried and subjected to autoradiography.

Binding of guanosine 5'-[γ -thio]triphosphate (GTP[S]) to *in vitro*-translated $G\alpha_{13-0}$ and $G\alpha_{13}$ proteins

Full-length mRNA was transcribed from the $G\alpha_{13-0}$ and $G\alpha_{13}$ cDNAs in pBluescript as described for the generation of RNAase

protection probes with the addition of 0.5 mM m⁷G(5')ppp(5')G (Pharmacia). The mRNA (2 μ g) was translated into protein using a rabbit reticulocyte lysate system (Promega) containing 25 μ Ci of [^{35} S]methionine (Amersham) as described (Sambrook et al., 1989). Tryptic proteolysis of the ^{35} S-labelled proteins was performed as described (Winslow et al., 1986; Denker et al., 1992). A 7 μ l sample of each translation reaction was incubated in the presence or absence of 100 μ M GTP[S] followed by incubation with 20 pmol of trypsin at 30 °C for 30 min. The products were analysed on 11% polyacrylamide/SDS gels which were then soaked in 16% sodium salicylate and 1% glycerol for 30 min, dried and subjected to autoradiography.

RESULTS

Isolation of chick G-protein α subunits

A chick brain cDNA library was screened for G-protein α subunits using a bovine $G\alpha_{11}$ cDNA fragment as a probe (Michel

1	GGCGGCGCGCCGCCCGCGCGCCATGGGCTCCAGCGCTGAGCGCGGAGGACAAAGCGCGC	60
	M G C T L S A E D K A A	
61	GTGGAACGGAGCAAAATGATCGACCGCAACTTACGGGAGGACGGGAGAGGCGGCAAG	120
	V E R S K M I D R N L R E D G E K A A K	
121	GAGGTGAAGCTGCTGCTGCTGGGTCTGGGAGTCTGCGAAGAGCACCCTGTAAGCAG	180
	E V K L L L L G A G E S G K S T I V K Q	
181	ATGAAGATTATTCACGAGGACGGCTACTCGGAGGAAGATCCAAACAATATAAAGTGTT	240
	M K I I H E D G Y S E E E C K Q Y K V V	
241	GTCTACAGCAATACTATCCAGTCCATCATTTGCCATCATAAGAGCCATGGGAAGGCTAAAG	300
	V Y S N T I Q S I I A I I R A M G R L K	
301	ATAGACTTTGGGGAAGTTGCCAGAGCAGATGCGCGCGAGCTGTTGCTGTTGGCTGGA	360
	I D F G E V A R A D D A R Q L F V L A G	
361	AGTGCAGAAGAGGGGCTGATCAGCGGCGAGTGGCTGAGTATCAAGCGGCTGTGGCGG	420
	S A E E G V M T A E L A G V I K R L W R	
421	GATGCTGGGGTGCAGGCGCTGCTCAGCAGATGACGAGTACGAGTGAAGAGTCCGCGC	480
	D A G V Q A C F S R S R E Y Q L N D S A	
481	TCATATTACCTGAAGACTTGGATAGATATCCAGCGAGCTACATTCGAGCTCAGCAG	540
	S Y Y L N D L D R I S Q P T Y I P T Q Q	
541	GACGTGCTGCGCACCAGGGTGAAAACAGCGGCATGCTGAGAGCAGACTTCACCTTCAAG	600
	V L A E D E E M N R M H E S M K L F D S	
601	GACCTCTACTTCAAGATGTTGATGCTGGGAGCGGCTCGAGCGGAGTACAGTGGATC	660
	D L Y F K M F D V G G Q R S E R K K W I	
661	CACGTCTCGAAGGCGCTCAGAGCGATCATCTCTGTGCGGCTCAGCGATTATGAOCTT	720
	H C F E G V T A I I F C V A L S D Y D L	
721	GTCTTGGCGAGGATGAAGAAATGAACCGGATGCGAGAGCATGAACCTGTTTACAGC	780
	V L A E D E E M N R M H E S M K L F D S	
781	ATCTGAACAACAATGGTTTCACAGACGCTCATCTTCTTTCTGAAACAAGAGGAC	840
	I C N N K W F T D T S I I L F L N K K D	
841	CTGTTTGGGAAAGATTAAGAAGAGCGCTCATCTGCTACCGGAGTACACAGCGC	900
	L F E E K I K K S P L T I C Y P E Y T G	
901	TCCACACGTCACGAGGAGGCGGCTGCTACATCCAGTGTGCTTGGAGGAGCTGAACCGG	960
	S N T Y E E A A A Y I Q C Q F E D L N R	
961	AGCAAGACACCAAGAGATCTACACGCACTTCACTGCGGACCGGACCAAGAACCTG	1020
	R K D T K E I Y T H F T C A T D T K N V	
1021	CAGTTCTGCTTTCACGCGGCTGACGCGGCTCATTAAGAACAACCTGAAGGAGTGTGGG	1080
	Q F V F D A V T D V I I K N N L K E C G	
1081	CTCTACTGAAGGTAAGCGGAGCGCTCGGCGGCTGCGGCGGCGGCGGCGGCTGCTGCTC	1140
	L Y	
1141	CCTCTGACGCTCACTGCTCTCCCTGCACTTCTCAGGAGCGCCCTGGGAGGAGCAGACT	1200
1201	TTGCTGCGCTGCGGAGCGGCGCATGAGCGGACCGGGGCTGAAAGCAGCATGCAGA	1260
1261	ATCGTCCGCTTCTTAGCACAACCTTCTGATTAGGACCTTTGAATTGGTAGGGGCTGT	1320
1321	TGAGGTTGGCTCGGCGCAGAACGGCCCTCGTGGGA	1356

Figure 1 Nucleotide and deduced amino acid sequences of chick $G\alpha_{13}$ and $G\alpha_{13-0}$ cDNAs

The complete nucleotide and deduced amino acid sequences of $G\alpha_{13}$ are shown (bottom two rows of four). Nucleotide differences between $G\alpha_{13-0}$ and $G\alpha_{13}$ are shown above the nucleotide sequence of $G\alpha_{13}$. These differences all reside within the first 137 nucleotides of $G\alpha_{13-0}$. — indicates the beginning and end of the $G\alpha_{13-0}$ nucleotide sequence. Amino acid differences between $G\alpha_{13-0}$ and $G\alpha_{13}$ are shown on the top row.

1	50
Ro	.....ER..L....A.E K..K...IS. ..D.....
i3	MGCTLSAEDK AAVERSKMID RNLREDGEKA AKEVKLLLG AGESGKSTIV
3o	.....ER..L....A.E K..K...IS. ..D.....
51	100
Ro	..... F.G.DV.... P..... LA..V...DT .GVEY.DKE.
i3	KQMKIHHEDG YSEEECKQYK VVVSNTIQS IIAIRAMGR LKIDPGEVAR
3o	.....
101	150
Ro	KA.SKMVCV VSRM.DTEPF S...LSAMM. ..G.S.I.E. .N.....
i3	ADARQLFVL AGSAEE GVM TAEAGVIKR LWRDAGVQAC FSRSEYQLN
3o	.....
151	200
Ro	...K...DS. ...GAAD.Q. .E..I..... ..N.H.RL.
i3	DSASYLNDL DRISQPTYIP TQQDVLRTRV KTTGIVETHF TPKDLYFKMF
3o	.....
201	250
Ro	.....D... ..G... ..Q..H...T T.....LM.
i3	DVGQQRSEK KWIHCPEGV T AIIFCVASLD YDLVLAEDDE MNRMHESMKL
3o	.....
251	300
Ro	.....F. I. ....G.... ..F... ..P.....D.
i3	FDSICNNKMF TDTSIILPLN KKDLPFEKIK KSPLTICYPE YTGSNITYEEA
3o	.....
301	350
Ro	.....T...S KN.SPN... ..C.M..... N.I.V..... ..I..A...R
i3	AAIYQCQFED LNRKDTKEI YTHFTCATDT KNVQVFDAV TDVIKNNLK
3o	.....
351	
Ro	G....
i3	ECGLY
3o	.....

Figure 2 Comparison of amino acid sequences of chick $G\alpha_{13}$ and $G\alpha_{13-o}$ and rat $G\alpha_o$

The complete deduced amino acid sequence for $G\alpha_{13}$ is shown in the middle row (i3). . indicates deduced amino acids which are identical to those of $G\alpha_{13}$ for $G\alpha_{13-o}$ (3o) and rat $G\alpha_o$ (Ro) (Jones and Reed, 1987). Amino acids which are different from the $G\alpha_{13}$ sequence are indicated for each clone.

Figure 3 Restriction sites within the G-protein cDNAs and regions used as RNAase protection probes

Thick lines denote the coding region for each cDNA, with the 5' to 3' direction from left to right. Untranslated regions are shown as thin lines. The restriction sites for *AclI* (A), *BamHI* (B), *PstI* (P) and *PvuII* (Pv) are shown. The regions used as RNAase protection probes for each clone are shown on the 5' ends as lines labelled with their respective nucleotide lengths.

et al., 1986). Screening of 270000 recombinant plaques gave 29 positive clones which were plaque-purified. Two clones containing open reading frames for full-length G-protein α subunits were sequenced and characterized. Based on the comparison of its amino acid sequence to those of α subunits from other species, one clone was designated chick $G\alpha_{13}$. The designation of this

Figure 4 RNAase protection analysis of $G\alpha_{13-o}$ expression in brain

(a) Specificity of the probes for $G\alpha_{13}$ and $G\alpha_{13-o}$ mRNA. Radiolabelled antisense $G\alpha_{13}$ riboprobe was untreated (lane 1) or hybridized to 5 ng of *in vitro*-synthesized sense $G\alpha_{13}$ mRNA (lane 2), $G\alpha_{13-o}$ mRNA (lane 3) or tRNA (lane 4) (10 μ g) as indicated and subjected to RNAase protection analysis. (b) Total brain RNA (100 μ g) (lane 2) or tRNA (100 μ g) (lane 3) was hybridized to a radiolabelled 321 bp $G\alpha_{13-o}$ antisense riboprobe, subjected to RNAase protection analysis and electrophoresed on an acrylamide/urea denaturing gel as described in the Materials and methods section. Lane 1 contains the 380 bp $G\alpha_{13-o}$ RNA probe untreated with ribonuclease. The size of the untreated riboprobe (probe) and the fragments protected from ribonuclease by $G\alpha_{13}$ mRNA (α_{13}) and $G\alpha_{13-o}$ mRNA (α_{13-o}) are shown in bp on the left of each panel. DNA sequencing reactions run as standards in each experiment (not shown) were used to determine the fragment sizes.

clone as chick $G\alpha_{13}$ was further verified by its comparison with chick $G\alpha_{11}$ and $G\alpha_{12}$, which were also cloned in this study (results not shown). The $G\alpha_{13}$ cDNA was 1356 nucleotides long with 1066 bases coding for a 354-amino-acid protein (Figure 1) which was 98% identical to rat $G\alpha_{13}$ (Jones and Reed, 1987). $G\alpha_{13}$ also contained 34 nucleotides on the 5' untranslated end and 267 nucleotides on the 3' untranslated end.

The second clone, $G\alpha_{13-o}$, was 1072 nucleotides long with an open reading frame of 1066 nucleotides coding for a 354-amino-acid protein with a C-terminus identical to that of chick $G\alpha_{13}$ and a unique N-terminus (Figure 1). $G\alpha_{13-o}$ contained 935 nucleotides on the 3' end which were identical to the 3' sequence of $G\alpha_{13}$. However, the 5' end of $G\alpha_{13-o}$ was different from the 5' end of $G\alpha_{13}$ over the first 137 nucleotides. In this region, 10 of the first 45 amino acids which are coded for in $G\alpha_{13-o}$ were different from those in $G\alpha_{13}$ (Figure 2). These changes resulted in an N-terminus for $G\alpha_{13-o}$ which was 100% identical to the conserved N-terminus of $G\alpha_o$ from the rat (Figure 2), human, mouse and bovine (Jones and Reed, 1987; Strathmann et al., 1990; Tsukamoto et al., 1991). Hence the $G\alpha_{13-o}$ clone codes for a protein with a $G\alpha_o$ N-terminus and a $G\alpha_{13}$ C-terminus.

Tissue-specific expression of chick $G\alpha$ mRNAs

Because of the high degree of similarity between G-protein isoforms, RNAase protection assays were used to measure the expression of the G-protein clones in total RNA from various chick tissues. To test the specificity of the $G\alpha_{13}$ riboprobe for $G\alpha_{13}$ compared with $G\alpha_{13-o}$, full-length sense mRNA was transcribed from the α_{13} and α_{13-o} cDNAs and subjected to RNAase protection using the $G\alpha_{13}$ antisense probe shown in Figure 3. Since the mRNA and probe were both transcribed from their cDNAs in pBluescript, *in vitro*-transcribed $G\alpha_{13}$ mRNA protected 409 nucleotides of the antisense $G\alpha_{13}$ probe (Figure 4a), which, as predicted (Figure 3), corresponds to 376 nucleotides specific for $G\alpha_{13}$ and 33 nucleotides specific for the pBluescript vector. *In vitro*-transcribed $G\alpha_{13-o}$ sense mRNA only protected 183 nucleotides of the $G\alpha_{13}$ antisense probe (Figure 4a),

Figure 5 Tissue distribution of $G\alpha_{13}$ mRNA

Total RNA (15 μ g) from each tissue or 15 μ g of tRNA was subjected to RNAase protection analysis using the $G\alpha_{13}$ riboprobe and electrophoresed on acrylamide/urea denaturing gels as described in the Materials and methods section. Probe untreated with ribonuclease is shown in the left-hand lane.

Figure 6 PCR analysis of $G\alpha_{13-0}$ and $G\alpha_{13}$ mRNA expression in chick tissues

(a) Oligonucleotide primers used in PCR analysis of $G\alpha_{13}$ and $G\alpha_{13-0}$ mRNAs. Total RNA was reverse-transcribed using the antisense oligonucleotide common to $G\alpha_{13}$ and $G\alpha_{13-0}$ (primer c). For the PCR reaction, primer c plus a mixture of a $G\alpha_{13-0}$ -specific primer (primer a) and a $G\alpha_{13}$ -specific primer (primer b) was used. A ^{32} P-labelled oligonucleotide common to both clones (primer p) was used to analyse the PCR products. The sequence of each primer is given in the Materials and methods section. The predicted product sizes from $G\alpha_{13-0}$ mRNA and $G\alpha_{13}$ mRNAs are shown (161 bp and 194 bp respectively). (b) Total RNA (1 μ g) from each tissue was reverse-transcribed (+ RT) or not (- RT), amplified by PCR, resolved on agarose gels, transferred to nylon membranes and analysed using a ^{32} P-labelled probe (primer p) as described in the Materials and methods section. The location of DNA markers (bp) is indicated.

as predicted from the overlapping nucleotide sequence of each clone beginning at the 5' end of their *Pvu*II restriction sites (Figures 1 and 3).

To study the expression of $G\alpha_{13-0}$, a $G\alpha_{13-0}$ riboprobe was synthesized which spanned an area common to both $G\alpha_{13-0}$ and $G\alpha_{13}$ and the area unique to $G\alpha_{13-0}$ (Figure 3). The probe therefore should be partially protected by $G\alpha_{13}$ mRNA (183 bp)

Figure 7 Effect of GTP[S] on tryptic digestion of $G\alpha_{13}$ and $G\alpha_{13-0}$ proteins

In vitro-translated $G\alpha_{13}$ (left-hand lanes) and $G\alpha_{13-0}$ proteins (right-hand lanes) were synthesized in the presence of ^{35} S and were either untreated (C) or digested with 20 pmol of trypsin (T) in the absence or presence of 100 μ M GTP[S] (G) and run on acrylamide gels, dried and subjected to autoradiography as described in the Materials and methods section.

and completely protected from ribonuclease by $G\alpha_{13-0}$ mRNA (321 bp). Since $G\alpha_{13-0}$ mRNA was found to be in very low abundance in the tissues examined, 100 μ g of total brain RNA was analysed for the presence of $G\alpha_{13-0}$ mRNA. $G\alpha_{13-0}$ mRNA was detected in brain RNA, as seen by the 321 bp full-length protected $G\alpha_{13-0}$ probe (Figure 4b). The riboprobe was also protected by the 183 bp of $G\alpha_{13}$ mRNA common to both $G\alpha_{13}$ and $G\alpha_{13-0}$ (lower band where indicated). The relative intensity of the $G\alpha_{13}$ and $G\alpha_{13-0}$ bands was determined by video densitometry (Macintosh Image Analysis, IBI). $G\alpha_{13}$ mRNA was found to be expressed at a 60-fold higher level than $G\alpha_{13-0}$ mRNA in chick brain. $G\alpha_{13}$ was detected in 15 μ g samples of total RNA from liver, lung, kidney, atria and ventricles as well as brain (Figure 5).

Because of the low abundance of $G\alpha_{13-0}$ mRNA, PCR amplification of reverse-transcribed RNA was used to determine $G\alpha_{13-0}$ mRNA expression in chick tissues. Since the tissue distribution of $G\alpha_{13}$ mRNA was determined by RNAase protection, $G\alpha_{13}$ was used as a positive PCR control. An antisense oligonucleotide primer, complementary to both $G\alpha_{13}$ and $G\alpha_{13-0}$ mRNAs (primer c, Figure 6a) was used as a primer in the RT step. Primer c and two sense oligonucleotides, one specific for $G\alpha_{13}$ (primer b, Figure 6a) and one specific for $G\alpha_{13-0}$ (primer a, Figure 6a) were used in the PCR amplification step (primer sequences are shown in the Materials and methods section). The predicted PCR product sizes using these oligonucleotides were 194 and 161 bp for $G\alpha_{13}$ and $G\alpha_{13-0}$ respectively. PCR products of these sizes were amplified in RNA samples from kidney, lung, liver, brain and heart, demonstrating the presence of $G\alpha_{13-0}$ as well as $G\alpha_{13}$ mRNAs in these tissues (Figure 6b). When each RNA sample was amplified by PCR without prior addition of RT, no products were detected, indicating that the products generated in the presence of RT were not due to genomic or plasmid DNA contamination. When primers b and c alone were used in the PCR reaction, only the 194 bp fragment was generated. When primers a and c were used, only the 161 bp fragment was generated (results not shown). These data not only demonstrate the expression of both $G\alpha_{13-0}$ and $G\alpha_{13}$ in chick tissues, but further demonstrate the existence of two separate transcripts with a common 3' end and unique 5' ends.

The $G\alpha_{13-0}$ protein binds GTP[S]

To determine if the $G\alpha_{13-0}$ cDNA actually codes for a stable GTP binding protein, the $G\alpha_{13}$ and $G\alpha_{13-0}$ cDNAs were transcribed and translated *in vitro* and the ability of the protein products to bind GTP[S] was determined. GTP[S] binding to α subunits has been shown to result in their protection from trypsin digestion, due to conformational changes. In the absence of GTP[S] or in the presence of GDP or GTP, α subunits have been shown to be cleaved at several tryptic sites. However, trypsin only cleaves GTP[S]-liganded α_0 at lysine-21, with the formation of a 37 kDa peptide, and GTP[S]-liganded α_1 at arginine-21, with the formation of 39 and 25 kDa peptides (Winslow et al., 1986; Denker et al., 1992). In the presence of GTP[S], *in vitro*-translated chick $G\alpha_{13-0}$ and $G\alpha_{13}$ both generated peptides of approx. 39 kDa when treated with trypsin (Figure 7). In the absence of GTP[S] only 18 kDa peptides were formed by trypsin digestion (Figure 7). These results demonstrate that the $G\alpha_{13}$ clone and the unusual $G\alpha_{13-0}$ cDNA clone both translate into stable GTP binding proteins.

DISCUSSION

We have isolated two G-protein α subunits from a chick brain cDNA library. One clone has been designated chick $G\alpha_{13}$ based on its similarities to G-protein α subunits from other species. The second clone, $G\alpha_{13-0}$, codes for a G-protein which is identical to $G\alpha_{13}$ at the C-terminus and identical to rat $G\alpha_0$ at the N-terminus. $G\alpha_{13}$ and $G\alpha_{13-0}$ were both found to be expressed in all chick tissues studied. $G\alpha_{13-0}$ was found to be expressed at very low levels in comparison to $G\alpha_{13}$. This is consistent with either a low level of $G\alpha_{13-0}$ mRNA expression throughout the tissues examined or localization of $G\alpha_{13-0}$ mRNA to a small number of cells within these tissues. The RNAase protection studies and PCR of reverse-transcribed RNA from chick both clearly demonstrate that the $G\alpha_{13-0}$ cDNA is derived from an mRNA that is expressed in chick. Furthermore, the protein product of the $G\alpha_{13-0}$ cDNA was shown to have the properties expected of a G-protein α subunit, as demonstrated by its apparent molecular mass and its ability to stably bind GTP[S].

The diversity of G-protein α subunits has been shown to be increased by alternative splicing within the same α subunit gene. The G-protein $G\alpha_s$ has been found to exist as four different isoforms resulting from alternative splicing within exon 3 (Kozasa et al., 1988). Alternatively spliced transcripts have been shown to arise from the $G\alpha_0$ gene in hamster (Hsu et al., 1990; Bertrand et al., 1990), *Drosophila* (de Sousa et al., 1989; Yoon et al., 1989), mouse (Strathmann et al., 1990), human and rat (Tsukamoto et al., 1991). The *Drosophila* $G\alpha_0$ gene codes for two $G\alpha_0$ proteins with different N-termini as a result of splicing of alternative first exons. In the chick, $G\alpha_{13}$ and $G\alpha_{13-0}$ differ in their 5' ends over the nucleotides coding for the first 45 amino acids of each clone. The remaining nucleotide sequences of these clones are otherwise identical. The 100% conservation of bases between the 3' ends of $G\alpha_{13}$ and $G\alpha_{13-0}$ supports the hypothesis that both of these transcripts arise from alternative splicing of the same gene. The unique feature of the $G\alpha_{13-0}$ cloned in this study compared with previously described alternatively spliced G-proteins is that chick $G\alpha_{13-0}$ codes for a protein which is actually a chimera of two distinct α subunit isoforms, $G\alpha_0$ and $G\alpha_{13}$.

These studies raise the question of the function of identical G-protein α subunits which differ in their N-terminus. The N-terminus of G-protein α subunits has been shown to be required for their interaction with G-protein $\beta\gamma$ subunits. Substitutions and deletions of specific amino acids on the N-terminus of α

subunits have previously been shown to interfere with the interaction of α subunits with $\beta\gamma$ subunits (Denker et al., 1992). Studies with purified $G\alpha_1$ demonstrated that $G\alpha_1$ is more sensitive to ADP-ribosylation by pertussis toxin than is $G\alpha_0$ (Neer et al., 1984). Since the presence of $\beta\gamma$ is necessary for the ADP-ribosylation of the α subunit, the greater susceptibility of $G\alpha_1$ to ADP-ribosylation by pertussis toxin may be related to a different affinity of $G\alpha_1$ for $\beta\gamma$ in comparison with the affinity of $G\alpha_0$ for $\beta\gamma$. In several systems, $\beta\gamma$ complexes consisting of different isoforms of $\beta\gamma$ have demonstrated an altered interaction with G-protein α subunits and effectors. For example, human placental $\beta_{35}\gamma$ (β_2 isoform) facilitates rhodopsin-catalysed GTP[S] exchange on $G\alpha_i$ with a higher apparent affinity than $\beta\gamma$ from the transducing heterotrimers (Fawzi et al., 1991). Furthermore, bovine brain $\beta\gamma$ is more effective than bovine retinal transducin $\beta\gamma$ in inhibiting isoprenaline-stimulated adenylate cyclase activity (Cerione et al., 1987). The specificity of G-protein-receptor interaction is believed to reside in the C-terminus of the α subunits (Sullivan et al., 1987). For example, the two mammalian $G\alpha_0$ proteins, which differ only in their C-termini, couple Ca^{2+} channel inhibition through different receptors (Kleuss et al., 1991). If differences in the N-termini of different G-protein α subunits effect their specificity of interaction with different $\beta\gamma$ subunits, the differences in the N-termini of $G\alpha_{13}$ and $G\alpha_{13-0}$ may result in their interaction with $\beta\gamma$ subunits having different specificity of function.

We thank Carl J. Schmidt, Joey V. Barnett and Eva J. Neer for advice and critical reading of this manuscript. We thank Kunio Takeyasu for providing the chick brain cDNA library, Eva J. Neer for providing the bovine $G\alpha_{11}$ cDNA clone and Bradley M. Denker for advice on GTP[S] binding analysis. This work was supported by National Institutes of Health Grant HL36014. E.J.K. is a recipient of National Research Service Award no. HL08474 from the National Institutes of Health.

REFERENCES

- Amatruda, T. T., Gautam, N., Fong, H. K. W., Northup, J. K. and Simon, M. I. (1988) *J. Biol. Chem.* **263**, 5008–5011
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) *Current Protocols In Molecular Biology*, vol. 1, pp. 4.7.1–4.7.8, John Wiley and Sons, New York
- Bertrand, P., Sanford, J., Rudolph, U., Codina, J. and Birnbaumer, L. (1990) *J. Biol. Chem.* **265**, 18576–18580
- Birnbaumer, L. (1990) *FASEB J.* **4**, 3178–3188
- Camps, M., Hou, C., Sidiropoulos, D., Stock, J. B., Jakobs, K. H. and Gierschik, P. (1992) *Eur. J. Biochem.* **206**, 821–831
- Cerione, R. A., Gierschik, P., Staniszewski, C., Benovic, J. L., Codina, J., Somers, R., Birnbaumer, L., Spiegel, A. M., Lefkowitz, R. J. and Caron, M. G. (1987) *Biochemistry* **26**, 1485–1491
- Church, G. M. and Gilbert, W. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991–1995
- Denker, B. M., Neer, E. J. and Schmidt, C. J. (1992) *J. Biol. Chem.* **267**, 6272–6277
- de Sousa, S. M., Hoveland, L. L., Yarfitz, S. and Hurley, J. B. (1989) *J. Biol. Chem.* **264**, 18544–18551
- Fawzi, A. B., Fay, D. S., Murphy, E. A., Tamir, H., Erdos, J. J. and Northup, J. K. (1991) *J. Biol. Chem.* **266**, 12194–12200
- Feinberg, A. P. and Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267
- Fisher, K. J. and Aronson, N. N. (1992) *Mol. Cell. Biol.* **12**, 1585–1591
- Gao, B., Gilman, A. G. and Robishaw, J. D. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6122–6125
- Gautam, N., Northup, J., Tamir, H. and Simon, M. I. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7973–7977
- Gilman, A. (1984) *Cell* **86**, 577–579
- Heschler, J., Rosenthal, W., Trautwein, W. and Schultz, G. (1987) *Nature (London)* **325**, 445–447
- Hsu, W. H., Rudolph, U., Sanford, J., Bertrand, P., Olate, J., Nelson, C., Moss, L. G., Boyd, A. E., III, Codina, J. and Birnbaumer, L. (1990) *J. Biol. Chem.* **265**, 11220–11226
- Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (1990) *PCR Protocols: A Guide To Methods And Applications*, pp. 21–27, Harcourt Brace Jovanovich, San Diego
- Jones, D. T. and Reed, R. R. (1987) *J. Biol. Chem.* **262**, 14241–14249
- Kleuss, C., Heschler, J., Ewel, C., Rosenthal, W., Schultz, G. and Wittig, B. (1991) *Nature (London)* **353**, 43–48

- Kozasa, T., Itoh, H., Tsukamoto, T. and Kaziro, Y. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2081–2085
- Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J. and Clapham, D. E. (1987) *Nature (London)* **325**, 321–326
- Michel, T., Winslow, J. W., Smith, J. A., Seidman, J. G. and Neer, E. J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7663–7667
- Neer, E. J. and Clapham, D. E. (1988) *Nature (London)* **333**, 129–134
- Neer, E. J., Lok, J. M. and Wolf, L. G. (1984) *J. Biol. Chem.* **259**, 14222–14229
- Pitcher, J. A., Inglese, J., Higgins, J. B., Arriza, J. L., Casey, P. J., Kim, C., Benovic, J. L., Kwatra, M. M., Caron, M. G. and Lefkowitz, R. J. (1992) *Science* **257**, 1264–1267
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn., pp. 7.19–7.22, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Sanger, F., Nicklen, S. and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463–5467
- Simon, M. I., Strathmann, M. P. and Gautam, N. (1991) *Science* **252**, 802–808
- Spiegel, A. M., Shenker, A. and Weinstein, L. S. (1992) *Endocr. Rev.* **13**, 536–565
- Strathmann, M. and Simon, M. I. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9113–9117
- Strathmann, M., Wilkie, T. M. and Simon, M. I. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6477–6481
- Sullivan, K. A., Miller, R. T., Masters, S. B., Beiderman, B., Heidman, W. and Bourne, H. R. (1987) *Nature (London)* **330**, 758
- Tang, W. J. and Gilman, A. G. (1991) *Science* **254**, 1500–1503
- Tsukamoto, T., Toyama, R., Itoh, H., Kozasa, T., Matsuoka, M. and Kaziro, Y. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2974–2978
- von Weizsacker, E., Strathmann, M. P. and Simon, M. I. (1992) *Biochem. Biophys. Res. Commun.* **183**, 350–356
- Winslow, J. W., Van Amsterdam, J. R. and Neer, E. J. (1986) *J. Biol. Chem.* **261**, 7571–7579
- Yoon, J., Shortridge, E. D., Bloomquist, B. T., Schneuwly, S., Perdew, M. H. and Pak, W. L. (1989) *J. Biol. Chem.* **264**, 18536–18543

Received 20 May 1993/13 August 1993; accepted 18 August 1993