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Genes for two homologous G-protein α subunits map to different human chromosomes

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Summary. Signal transduction across biological membranes is modulated by a family of related GTP-binding proteins termed G proteins. These G proteins have a heterotrimeric structure composed of α, β, and γ subunits. The α subunits of the G proteins bind GTP and appear to determine the biochemical specificity of the protein. We have recently cloned and characterized cDNA encoding two G-protein α subunits, α_i and α_h. The former is a substrate for ADP-ribosylation by pertussis toxin. The protein corresponding to α_h has not yet been identified. These cDNAs encode proteins, which demonstrate 90% sequence identity to one another and also show marked similarity to other G proteins. The present studies were designed to determine whether the genes for these related proteins are clustered on a single human chromosome. Genomic DNA isolated from a panel of mouse–human hybrid cell lines was analyzed by hybridization to cDNAs for α_i and α_h. Based on the distribution patterns of α_i and α_h in cell hybrids, the gene for α_i was assigned to human chromosome 7, and the gene for α_h assigned to chromosome 12. These data suggest that the G-protein gene family may be distributed over at least two human chromosomes.

Introduction

Transmembrane signaling by a variety of hormone receptors as well as by retinal rhodopsin is mediated by a set of guanine nucleotide binding proteins (G proteins), which have a heterotrimeric structure composed of α, β, and γ subunits. The distinctive features of the G proteins are conferred by the nucleotide-binding α subunit (Smigel et al. 1984; Neer 1987).

The G proteins that mediate hormone responses can be divided into two broad categories according to their interaction with the bacterial toxins from *Vibrio cholera* and *Bordetella pertussis*. Those G proteins, whose primary function is to stimulate adenylate cyclase, are substrates for ADP-ribosylation by cholera toxin (Gill and Nereb 1978), while those involved in hormonal inhibition of adenylate cyclase and in regulation of other plasma membrane enzymes are substrates for pertussis toxin (Ui et al. 1984).

The recent successes of many laboratories in cloning cDNAs representing the G proteins have revealed an unexpected complexity with the identification of nine distinct but very similar α subunits (Bray et al. 1986; Itoh et al. 1986; Lochrie et al. 1985; Mattera et al. 1986; Medynski et al. 1985; Michel et al. 1986; Nukada et al. 1986a, b; Robishaw et al. 1986a, b; Sullivan et al. 1986; Yatsunami and Khorana 1985). There are at least two genes for the retinal G protein, transducin, one of which is expressed in rods and the other in cones (Lerea et al. 1986). The α subunit of the G protein that mediates hormonal stimulation of adenylate cyclase (α_s) exists in four forms, which may be the result of differential splicing of mRNA (Bray et al. 1986; Robishaw et al. 1986a). Analysis of the proteins that are substrates for pertussis toxin has also revealed heterogeneity. We have purified two such proteins from bovine brain (Neer et al. 1984). A 41-kilodalton G protein, which is the predominant substrate for ADP-ribosylation by pertussis toxin in brain and other tissues, is involved in inhibition of adenylate cyclase and in other cell functions (Ui et al. 1984) and termed α_i or α_{41}. Another G-protein α subunit of 39 kilodaltons (termed α_{39} or α_o) is especially abundant in the central nervous system, but its function is not yet known. In addition, there is a 40-kilodalton pertussis toxin substrate in bovine brain that has not yet been fully characterized (Neer et al. 1984). We have recently cloned and characterized cDNA for α_i (α_{41}) and have identified cDNA that encodes an extremely similar putative G protein, α_h (Michel et al. 1986). Southern blot analysis of bovine genomic DNA shows that the α_h gene is distinct from the one that encodes α_i. The protein that corresponds to α_h has not yet been identified, but mRNA corresponding to it is present in many cell types (Lee and Neer, unpublished). Southern blot analysis of human genomic DNA showed that the cDNAs for α_i and α_h recognize sequences in the human genome that are distinct from each other. We wished to determine whether the genes for these similar proteins, which are closely related structurally (and probably functionally), are clustered on a single human chromosome, or whether they are found on different chromosomes. We report here that the genes for each of these α proteins were found on different chromosomes.

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Materials and methods

cDNA probes for α_i and α_h

The cDNAs encoding α_i and α_h were isolated from a bovine pituitary library as described by Michel et al. (1986). The probes used were purified fragments of the total cDNA, which

contained only sequences coding for the α proteins. The probes were radiolabeled with ^{32}P by nick translation using ^{32}P-dCTP to give a specific activity of $3 \times 10^8 - 8 \times 10^8$ cpm/μg.

Southern blot hybridization analysis

DNA was prepared from the various cell lines listed in Table 1 and digested with EcoRI. The DNA fragments were separated by electrophoresis on agarose gels and transferred onto nitrocellulose (Schleicher and Schuell) as described by Southern (Southern 1979; Maniatis et al. 1982). Hybridization was carried out at 42°C for 18 h. The filters were rinsed twice in 2×SSC, 0.1% SDS at room temperature and then washed in 0.2×SSC, 0.1% SDS at 45°C for 30 min before analysis by radioautography.

The 37 cell hybrids used in this study include 15 unrelated human cell lines and 4 mouse cell lines (Sakaguchi and Shows 1982; Shows et al. 1984). The hybrids were characterized by chromosome analysis and mapped enzyme markers, and partly by mapped DNA probes (Shows 1983; Shows et al. 1978, 1982). The location of α_h and α_i was determined by scoring the presence (+) or absence (−) of certain human bands in the hybrids on the blots. Concordant hybrids have either retained or lost α_h or α_i, together with a specific human chromosome. Discordant hybrids either retained the α_h or α_i, but not a specific chromosome or the reverse. The percentage of discordancy indicates the degree of discordant segregation for a marker and a chromosome. A 0% discordancy is the basis for chromosome assignment.

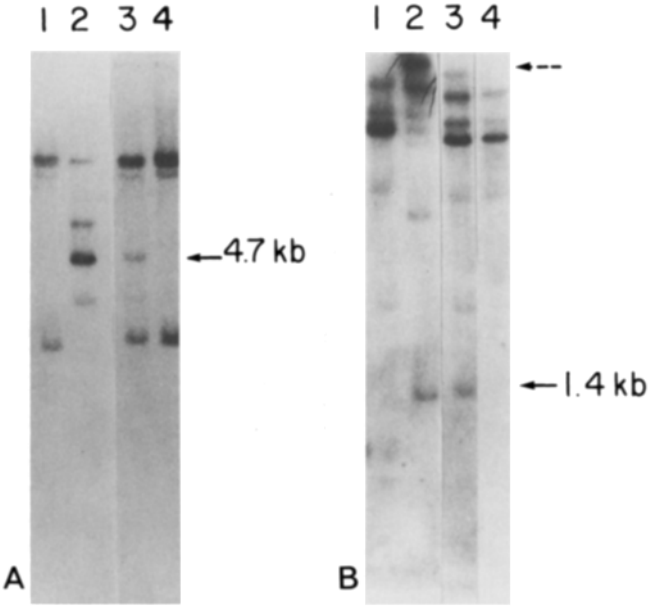

Fig. 1A, B. Southern blot analysis of EcoRI-digested genomic DNA from mouse–human hybrid cell lines. The experimental procedure is described in the text. **A** Experiments with α_i cDNA; **B** experiments with α_h cDNA. In each panel, *lane 1* contains mouse DNA; *lane 2* contains human DNA; *lane 3* is a representative lane containing DNA from a mouse–human hybrid that was scored as positive for the α subunit cDNA; *lane 4* is a representative negative lane. The *solid arrows* indicate the human bands used in scoring. The *dashed arrow* in **B** indicates a large DNA fragment that was recognized specifically by α_h cDNA but could not be used for scoring, as discussed in the text

Results

Figure 1A, B shows representative positive and negative lanes for Southern blots probed with α_i and α_h cDNA. Lane 1 in each panel contains mouse DNA; lane 2 contains human DNA; lanes 3–4 contain DNA from mouse–human hybrid cells. The human band used for scoring is indicated with a solid arrow. The two cDNA probes recognize different human EcoRI fragments. This observation confirms our earlier finding that the cDNAs recognize different restriction enzyme fragments in bovine genomic DNA. The predominant band recognized by α_i cDNA is a 4.7-kilobase (kb) fragment while α_h recognized a unique 1.4-kb fragment. There is some cross-hybridization of α_h cDNA with the 4.7-kb fragment, but α_i cDNA does not hybridize with the 1.4-kb DNA. In addition, the α_h cDNA hybridizes with a very large DNA fragment (dashed arrow). However, we were not able to score this band for two reasons: first, this band was hard to distinguish in lanes where digestion of DNA was not optimal; second, comparison of different human cell lines revealed a polymorphism in the high-molecular-weight fragments. It was clear, however, that the large-molecular-weight fragment segregated differently from the small one. This observation suggests that there may be another gene for α_h on a different chromosome.

Table 1 summarizes data obtained on 37 independent mouse–human hybrids. Some of these were analyzed on two or more separate Southern blots. The α_h mapped to human chromosome 12, and α_i mapped to human chromosome 7. In each case, the percentage discordancy was 0. The hybrid JSR-17S with the 7/9 translocation 7pter→7q22::9p24→9pter, and no intact chromosome 7 had a positive score for the human α_i band. This would localize α_i to the pter→q22 region of human chromosome 7.

Discussion

The signal-transducing G-protein family is composed of at least nine distinct, but extremely similar, α subunits (Bray et al. 1986; Itoh et al. 1986; Lochrie et al. 1985; Mattera et al. 1986; Medynski et al. 1985; Michel et al. 1986; Nukada et al. 1986a, b; Robishaw et al. 1986a, b; Sullivan et al. 1986; Yatsunami and Khorana 1985). These studies represent the first human chromosomal assignment of any members of the G-protein gene family. Using the bovine cDNAs for α_i and α_h, these two α subunits were mapped to human chromosomes 7 and 12, respectively. However, there may be more than one gene for both α_i and α_h. Analysis of bovine genomic DNA with nonoverlapping probes derived from the α_i cDNA suggested that there were at least two, and perhaps three, genes for α_i. The polymorphism described above in the α_h restriction fragments suggests that there may be more than one α_h gene. It is possible that each of these forms is located on a different chromosome.

The striking sequence similarities among the G proteins suggest that they may have arisen from successive duplications of a common ancestor gene, and therefore that they might be clustered on a single chromosome. However, our results show clearly that the genes for α_i and α_h are found on different human chromosomes. The division of the G-protein α subunit genes between at least two chromosomes suggests that their

Table 1. Segregation of cDNA probes α_h and α_i with human chromosomes in EcoRI-digested human–mouse cell hybrid DNA (+ or − indicate presence or absence of DNA fragments characteristic of α_i or α_h; t indicates a chromosomal translocation, which is defined in the last column)

Hybrid	α_h	α_i	Human chromosomes																							Translocations
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
ATR-13	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	t	5/X
DUA-3BSAGA	−	+	−	+	−	−	−	−	+	+	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	
DUA-5BSAGA	−	−	−	−	+	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	−	−	+	−	−	
DUM-13	+	+	+	+	+	−	+	+	+	−	−	+	+	+	−	+	t	+	+	+	+	+	+	+	t	X/15 15/X
EXR-5CSAz	+	+	+	+	+	+	+	+	+	+	+	+	t	+	+	+	+	−	+	+	+	+	+	+	+	X/11
GAR-1	+	−	−	−	+	−	+	−	−	+	−	+	−	+	−	+	+	+	−	−	−	+	−	−	+	
ICL-15	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−	
JSR-14	+	−	−	+	+	+	+	+	−	−	−	−	−	+	+	−	−	−	+	−	−	+	+	−	+	
JSR-17S	+	+	+	+	+	−	+	−	t	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7/9
NSL-9	+	−	−	−	−	−	+	−	−	+	t	+	−	+	+	+	+	+	+	−	−	+	+	+	−	17/9
NSL-16	+	+	−	−	+	+	+	−	+	+	t	+	−	+	−	+	+	+	+	+	−	+	+	−	−	17/9
REW-7	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
REW-8D	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	+	+	+	+	
REX-11BSAgB	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	−	−	−	−	−	
REX-11BSHF	−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	t	t	22/X
SIR-8	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	
TSL-1	−	−	−	−	+	+	−	−	−	−	+	+	+	−	+	+	−	+	+	+	−	+	+	−	−	
TSL-2	+	−	−	+	t	−	+	+	−	−	−	+	−	+	−	−	−	−	t	+	−	+	+	−	+	17/3 3/17
VTL-6	−	+	−	+	−	−	−	+	+	+	−	+	+	−	−	−	+	−	+	−	+	+	+	+	−	
VTL-8	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+	+	+	−	
VTL-17	−	+	−	−	−	−	+	−	+	−	−	+	+	−	+	+	−	−	+	−	−	+	+	−	−	
WIL-2	+	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	
WIL-2CSAZ	+	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	−	−	+	−	−	−	+	+	−	
WIL-5	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	−	−	+	+	−	−	+	−	+	
WIL-6	−	+	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+	
WIL-7	−	−	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	
WIL-8	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
WIL-8X	+	+	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	+	
WIL-13	−	−	−	−	+	−	+	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	+	−	
WIL-15	+	+	−	+	+	+	−	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	−	+	
XER-11	+	+	+	−	+	+	+	+	+	+	+	+	t	+	−	−	+	+	+	+	+	+	+	+	t	11/X X/11
XOL-6	+	+	t	−	−	−	+	+	+	−	−	+	+	+	−	+	−	−	+	−	+	+	−	+	t	1/X
XOL-9	+	−	t	+	+	+	−	+	−	−	−	−	−	+	−	−	+	−	+	+	+	−	+	+	+	X/1
XTR-2	+	−	−	−	t	−	+	−	−	+	−	+	−	+	+	+	−	−	−	+	−	+	+	−	t	3/X
XTR-3BSAgB	+	−	−	−	t	−	−	−	−	−	+	t	−	+	−	−	−	−	−	−	−	+	+	−	t	3/X 10q−
XTR-3CSAZK	+	−	−	+	−	−	−	−	−	+	−	+	−	+	−	−	−	−	+	−	−	+	+	−	−	
XTR-22	−	−	−	+	t	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	+	X/3

Total number of concordant and discordant hybrids and the percentage discordancy for α_i and α_h

α_h	Chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Concordant no. of hybrids	22	21	21	20	24	21	21	23	18	23	15	37	18	19	23	22	22	20	20	25	23	19	19
Discordant no. of hybrids	13	16	12	17	13	16	15	14	17	13	20	0	19	18	13	15	14	15	17	12	14	17	11
Discordancy (%)	37	43	36	46	35	43	42	38	49	36	57	0	51	49	36	41	39	46	46	32	38	47	37
α_i	Chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Concordant no. of hybrids	29	26	19	25	25	26	37	22	23	23	28	23	24	27	24	26	23	21	29	22	16	22	16
Discordant no. of hybrids	7	12	14	13	13	12	0	16	13	14	8	15	14	11	13	12	14	17	9	16	22	15	15
Discordancy (%)	19	32	42	34	34	32	0	42	36	38	22	39	37	29	35	32	38	45	24	42	58	41	48

coordinate expression may be regulated by *trans*-acting factors rather than by a common *cis* regulatory mechanism.

The chromosomal location of the G-protein β and γ subunits is not known. There appears to be less sequence heterogeneity among the β and γ subunits (Fong et al. 1986). It is likely that there are fewer genes for these subunits, and that these genes may show a chromosomal distribution distinct from the G-protein α subunits.

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