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# The Gene for the $\alpha_{i1}$ Subunit of Human Guanine Nucleotide Binding Protein Maps near the Cystic Fibrosis Locus

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## Summary

**The gene for the $\alpha_{i1}$ subunit of human guanine nucleotide binding (G) protein was mapped by in situ hybridization to chromosome 7 at band q21. The regional chromosomal location of the human $\alpha_{i1}$ gene was confirmed using human/mouse somatic-cell hybrid lines containing portions of human chromosome 7. Because the $\alpha_{i1}$ gene mapped near the cystic fibrosis locus and because an abnormal G protein might be expected to contribute to the pathophysiology of this disease, the $\alpha_{i1}$ gene was mapped with respect to the cystic fibrosis locus as defined by the Met oncogene and anonymous DNA marker pJ3.11. The location of the $\alpha_{i1}$ gene proved to be distinct from that of the cystic fibrosis locus.**

## Introduction

Guanine nucleotide binding (G) proteins promote the transduction of extracellular signals in cell membranes. G proteins are heterotrimers with subunits designated α, β, and γ. The G-protein α subunit binds and hydrolyzes GTP and appears to determine G-protein specificity for extracellular-receptor and intracellular-effector molecules. Distinct groups of G proteins have been characterized and include proteins that stimulate ($G_s$) and inhibit ($G_i$) adenylate cyclase. Retinal transducin ($G_t$) couples rhodopsin to a cGMP phosphodiesterase. The function of a fourth type of G protein, $G_o$, is as yet unknown (Gilman 1987).

The nucleotide sequences of three closely related α subunits of human $G_i$ proteins have been reported. Each of these three subunits has been designated "$\alpha_i$" on the basis of homology with bovine $\alpha_i$ (Bray et al. 1987; Didsbury et al. 1987; Didsbury and Snyderman 1987). The amino acid sequence predicted by a cDNA clone isolated from a human fetal basal ganglion cDNA library (Bray et al. 1987) was identical to that of bovine $\alpha_i$ (Nukada et al. 1986). A second cDNA clone isolated from a library prepared from promyelocytic cell line HL-60 (Didsbury et al. 1987) was 88% homologous to bovine $\alpha_i$. A third clone isolated from a promyelocyte (U937) cDNA library (Didsbury and Snyderman 1987) was 94% homologous to bovine $\alpha_i$. In accordance with nomenclature introduced for rodent α subunits of G proteins (Jones and Reed 1987), these human $G_i$ subunits will be referred to as $\alpha_{i1}$, $\alpha_{i2}$, and $\alpha_{i3}$, respectively.

Cystic fibrosis is a disease of unknown etiology. Patients with cystic fibrosis were hyporesponsive to β-adrenergic medications (Davis et al. 1980), and their lymphocytes showed a blunted rise in cAMP in response to isoproterenol in vitro. The β-adrenergic receptors of the lymphocytes were qualitatively and quantitatively normal (Davis et al. 1983). On the basis of these observations, it was suggested that abnormal receptor-cyclase coupling was involved in the pathophysiology of cystic fibrosis (Davis et al. 1983).

In the present study, two genomic fragments of the $\alpha_{i1}$ gene of human G protein were identified and cloned. One of the genomic fragments was used to

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map the $\alpha_{i1}$ gene to chromosome 7 at band q21 by in situ hybridization. The location of the $\alpha_{i1}$ gene was confirmed using human/mouse somatic-cell hybrid lines that contained fragments of human chromosome 7. Because the $\alpha_{i1}$ gene mapped near the cystic fibrosis locus and because an abnormal G protein may be involved in this disease, the $\alpha_{i1}$ gene was mapped with respect to Met and pJ3.11, two DNA markers that flank the cystic fibrosis locus.

## Material and Methods

### Human $\alpha_{i1}$ Genomic Fragments

A genomic library was prepared from *Eco*RI-digested, size-fractionated human T-cell DNA cloned into λgt10 (Maniatis et al. 1978). The library was plated and transferred to nitrocellulose filters according to a method described elsewhere (Benton and Davis 1977). An *Xba*I-*Sma*I fragment of bovine $\alpha_i$ cDNA (Michel et al. 1986) was radiolabeled by nick-translation and used to screen the genomic library. Filters were incubated with radiolabeled probe overnight at 42 C and washed twice in 2 × SSC (2 × SSC = 0.3 M NaCl, 30 mM sodium citrate, pH 7.0), 0.1% SDS at room temperature and twice in 0.2 × SSC, 0.1% SDS at 42 C prior to autoradiography. These washed filters were considered to have been subjected to "low-stringency" conditions. Two non-cross-hybridizing clones, GC1 and GC2, were identified. Fragments of GC1 and GC2 were subcloned into pBS (Stratagene, San Diego) and sequenced by the dideoxynucleotide chain-termination method (Sanger et al. 1977) by using commercially available T3 and T7 promoter oligonucleotide primers (Stratagene, San Diego).

### In Situ Hybridization

The regional chromosomal location of the human $\alpha_{i1}$ gene was determined by in situ hybridization of genomic probe GC2 to normal human metaphase chromosomes as described elsewhere (Morton et al. 1984). Chromosomes were stained with quinacrine mustard dihydrochloride and visualized by a combination of incident UV light and transmitted visible light which permitted simultaneous observation of chromosome bands and silver grains.

### Southern Blot Analysis

Southern blots were prepared from *Pst*I-treated DNA of somatic-cell line GM1059 and human/mouse somatic-cell hybrid lines 1CF/KO16 and Rag22-2. Somatic-cell line GM1059 was obtained from the Mutant Cell Repository (Camden, NJ). DNA samples prepared from 1CF/KO16 (Arfin et al. 1983) and Rag22-2 (K.-H. Grzegshik, unpublished data) were provided by L.-C. Tsui (Department of Genetics, Research Institute, The Hospital for Sick Children, Toronto, and Departments of Medical Biophysics and Medical Genetics, University of Toronto). The Southern blots were incubated overnight at 68 C with radiolabeled probe (gc-2) derived from GC2 and, prior to autoradiography, washed twice in 2 × SSC, 0.1% SDS at room temperature, once at 68 C in 2 × SSC, 0.1% SDS and twice at 68 C in 0.2 × SSC.

A Southern blot prepared from *Eco*RI-treated DNA of human/mouse somatic-cell hybrid lines JSR-17S and REW-11 was provided by T. Shows (Roswell Park Memorial Institute, Buffalo). Prior to autoradiography, this filter was incubated overnight at 42 C with radiolabeled gc-2 and washed twice in 2 × SSC, 0.1% SDS at room temperature and twice in 0.2 × SSC, 0.1% SDS at 65 C.

A probe for the Met oncogene locus (White et al. 1985) was obtained from the American Type Culture Collection (Rockville, MD). A probe for the anonymous DNA marker pJ3.11 (Wainwright et al. 1985) was provided by Jorg Schmidtke (Göttingen, West Germany).

## Results and Discussion

In previous studies using Southern blots prepared from human/mouse somatic cell hybrid lines (Neer et al. 1987), a bovine $\alpha_i$ cDNA probe was used to map a homologous human gene to chromosome 7. In the present study a bovine $\alpha_i$ cDNA probe was used to identify and clone two genomic fragments of the human $\alpha_{i1}$ gene. A radiolabeled *Xba*I-*Sma*I restriction fragment of bovine $\alpha_i$ cDNA was hybridized to a Southern blot prepared from *Eco*RI-treated human genomic DNA. The blot was washed under low-stringency conditions prior to autoradiography; bands corresponding to DNA restriction fragments of ~10 kb and ~4.3 kb were present (data not shown). To clone the 4.3-kb restriction fragment, a 4–6-kb size-fractionated genomic library was prepared from *Eco*RI-treated human T-cell DNA. The library was screened with the bovine $\alpha_i$ probe; filters were washed under low-stringency conditions. Instead of a single clone containing a genomic fragment of the $\alpha_{i1}$ gene, two non-cross-hybridizing clones,

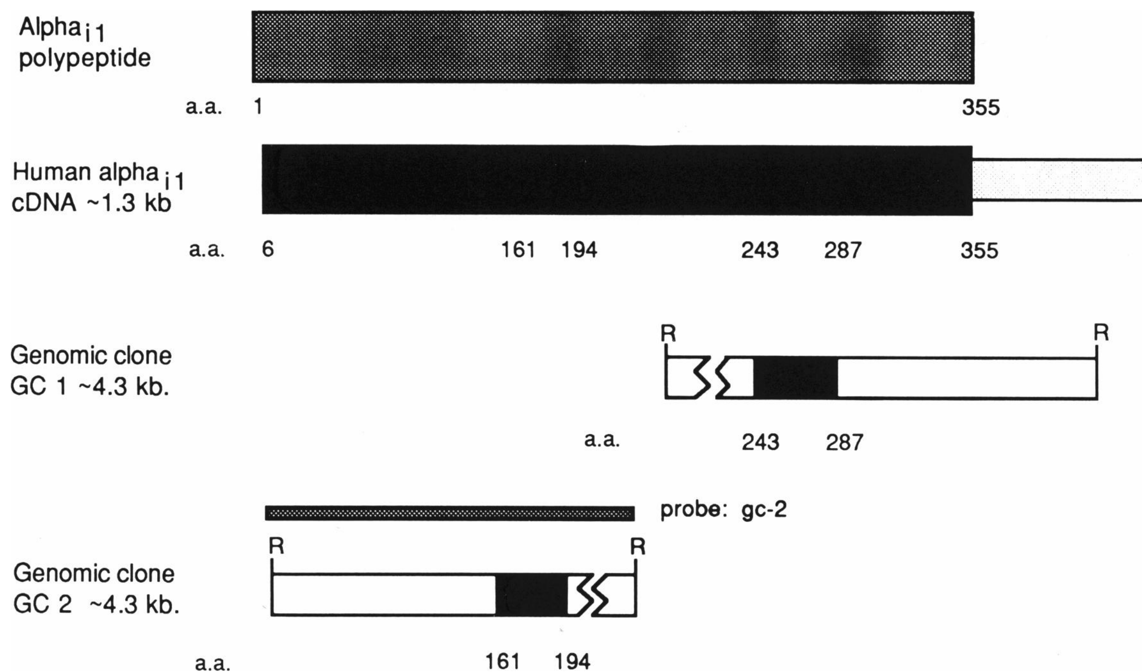


**Figure 1** Clones GC1 and GC2 are fragments of the human $\alpha_{i1}$ gene. The bovine $\alpha_i$ cDNA encoded a polypeptide 355 amino acids in length (Nukada et al. 1986). The amino acid sequence predicted by a truncated human $\alpha_{i1}$ cDNA (Bray et al. 1987) was identical to bovine $\alpha_i$ amino acids 6–355. The human $\alpha_{i1}$ cDNA had a 264-bp 3′-untranslated region. Genomic clone GC1 contained an exon that encoded amino acids 243–287 of the human $\alpha_{i1}$ gene. Genomic clone GC2 encoded amino acids 161–194 of the human $\alpha_{i1}$ gene. The nucleotide sequence of the coding regions was identical to the corresponding region in the human $\alpha_{i1}$ cDNA. The full-length *Eco*RI-*Eco*RI insert of GC2 (gc-2) was radiolabeled and used to probe Southern blots prepared from somatic-cell hybrid lines. R = *Eco*RI.

GC1 and GC2, were identified. Both clones contained a 4.3-kb insert. Each genomic clone contained at least one exon of the human $\alpha_{i1}$ gene, as determined by nucleotide sequencing and comparison with the published human $\alpha_{i1}$ cDNA sequence (Bray et al. 1987). GC1 encoded amino acids 243–287 of human $\alpha_{i1}$; GC2 encoded amino acids 161–194. The nucleotide sequence of the genomic clones was identical to the sequence of the $\alpha_{i1}$ cDNA in these regions (fig. 1).

The complete pBSGC2 plasmid DNA was radiolabeled by nick-translation and used to map the human $\alpha_{i1}$ gene by chromosomal in situ hybridization. Analysis of 120 metaphases of normal human peripheral blood lymphocyte chromosomes revealed that 14% of all silver grains were located on or beside chromosome 7 between bands q11.2 and q22; 7.4% of all silver grains were on or beside band q21 (fig. 2). No other chromosome was associated with a significant number of silver grains. On the basis of these results, the human $\alpha_{i1}$ gene was assigned to chromosome 7 at band q21.

The regional chromosomal location of the human $\alpha_{i1}$ gene was confirmed using Southern blots prepared from human/mouse somatic-cell hybrid lines that contained all or part of human chromosome 7. A radiolabeled probe (gc-2) derived from GC2 hybridized to a blot prepared from REW-11, a cell line that contained intact human chromosome 7 (Shows et al. 1982). The same probe hybridized to a 4.6-kb band on a blot prepared from 1CF/KO16 (Arfin et al. 1983), a cell line that contained the portion of chromosome 7 from centromere to band q22 (L.-C. Tsui, personal communication). The band was also present on a blot prepared from GM1059, a somatic-cell line that contained an intact human chromosome 7 as well as a chromosome 7 with a deletion between bands q22 and q32 (Zengerling et al. 1987). DNA markers Met, pJ3.11, 7C22, and B79a map within this deleted region (Estivill et al. 1986; Zengerling et al. 1987). The signal intensity of the band produced by gc-2 was consistent with the intensity produced by other probes for DNA markers outside the deleted region. The 4.6-kb band was not present on a Southern blot prepared from Rag22-2, a cell line that contained human chromosome 7 from q22 to qter (K.-H.

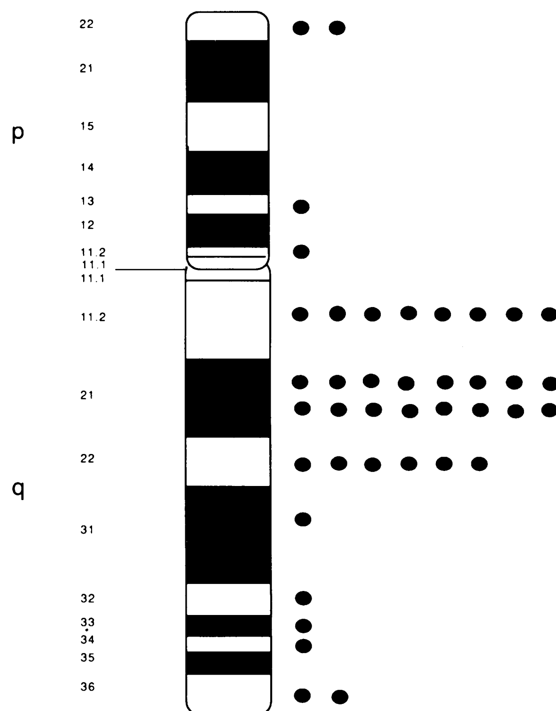


**Figure 2** Ideogram of chromosome 7, depicting the distribution of silver grains when the pBSGC2 probe was used. Analysis of 120 metaphase chromosomes revealed that 14% of all silver grains were on chromosome 7 between bands q11.2 and q22. Some 7.4% of all silver grains were on or beside band q21. No other chromosome was associated with a significant number of silver grains.

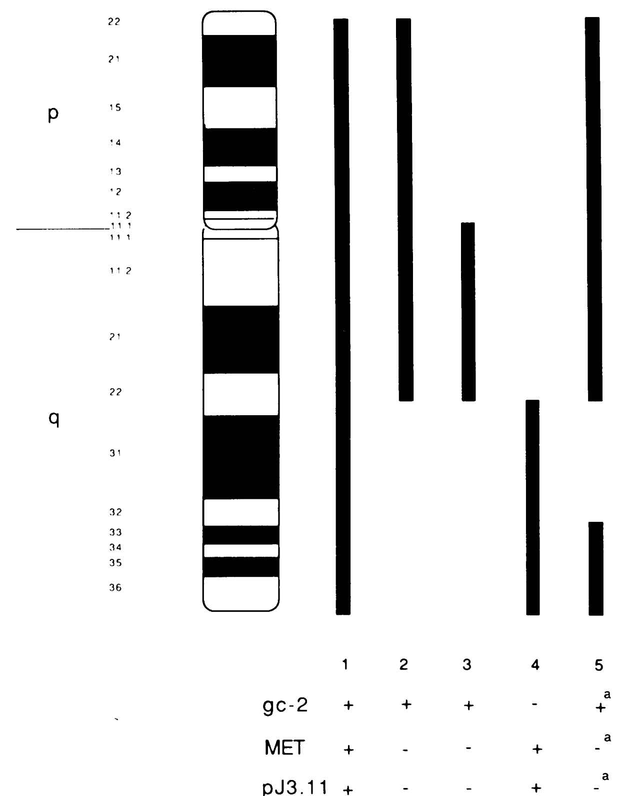


**Figure 3** Localization of human $\alpha_{i1}$ gene by using somatic-cell lines. Southern blots prepared from human/mouse somatic-cell hybrid lines containing portions of human chromosome 7 were used to confirm the regional chromosomal location of the human $\alpha_{i1}$ gene. The somatic-cell lines used were as follows: 1, REW-11 (intact human chromosome 7); 2, JSR-17S (7pter→q22); 3, 1CF/KO16 (7cen→q22); 4, Rag22-2 (7q22→qter); 5, GM1059 (intact chromosome 7 and 7pter→q22::q32→qter). Radiolabeled gc-2 hybridized to Southern blots prepared from REW-11 and JSR-17S; the probe did not hybridize to Southern blots prepared from cell lines that lacked human chromosome 7 (data not shown). Radiolabeled gc-2 hybridized to a 4.6-kb band on a Southern blot prepared from 1CF/KO16 and GM1059; the 4.6-band was not detected on a Southern blot prepared from Rag22-2. These results confirmed the localization of the human $\alpha_{i1}$ gene between the centromere of chromosome 7 and band q22. Although gc-2 hybridized to a Southern blot prepared from JSR-17S, probes for Met and pJ3.11 did not hybridize to the same filter. The human $\alpha_{i1}$ gene was therefore centromeric with respect to Met and pJ3.11. A superscript "a" denotes that somatic-cell line GM1059 contained an intact human chromosome 7 in addition to chromosome 7 with a deletion between bands q31 and q32. The signal intensity of the band produced by gc-2 was consistent with the intensity produced by other probes for markers outside the deleted region. In contrast, the signal intensity produced by radiolabeled probes for Met and pJ3.11 was reduced by ~50% (as determined by scanning densitometry) compared with that produced by probes for markers outside bands q31 and q32. These results for Met and pJ3.11 had been noted elsewhere (Zengerling et al. 1987).

Grzeshik, unpublished data; L.-C. Tsui, personal communication) (fig. 3).

In contrast to the results for gc-2, probes for pJ3.11 and Met, two DNA markers that flank the cystic fibrosis locus (Beaudet et al. 1986), hybridized to a Southern blot prepared from Rag22-2. These probes did not hybridize to blots prepared from cell lines that lacked the region of chromosome 7 distal to q22 (JSR-17S and 1CF/KO16) (fig. 3). On the basis of these results, the human $\alpha_{i1}$ gene is located closer to the centromere of chromosome 7 than are Met, pJ3.11, and the cystic fibrosis locus. In addition, the human $\alpha_{i1}$ gene maps closer to the centromere than do DNA markers 7C22 and B79a.

In summary, the gene for the $\alpha_{i1}$ subunit of human G protein was mapped to chromosome 7 at band q21. Although the $\alpha_{i1}$ gene mapped near the cystic fibrosis locus, detailed chromosomal mapping by means of somatic-cell hybrid lines demonstrated that the $\alpha_{i1}$ gene was centromeric with respect to the cystic fibrosis locus. An abnormal $\alpha_{i1}$ gene is therefore unlikely to contribute to the pathophysiology of this

disease. The detection of restriction-fragment polymorphisms within the $\alpha_{i1}$ locus will permit genetic mapping of the $\alpha_{i1}$ gene with respect to both other genes and DNA markers in this region of chromosome 7.

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