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Chromosomal Localization of the Genes Encoding Two Forms of the G Protein β Polypeptide, β1 and β3, in Man[1,2]

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The signal-transducing G proteins are heterotrimers composed of three subunits, α, β, and γ. Multiple distinctive forms of the α, β, and γ subunits, each encoded by a distinct gene, have been described. To investigate further the structural diversity of the β subunits, we recently cloned and characterized a novel cDNA encoding a third form of the G protein β subunit, which we have termed β3. The protein corresponding to β3 has not yet been identified. The three forms of the β subunit show 81–90% amino acid sequence identity. Previous studies had localized the human genes for the β1 and β2 subunits to chromosomes 1 and 7, respectively. The present studies were designed to determine whether the gene encoding β3 is linked to either the β1 or the β2 gene. Genomic DNA was isolated from a panel of rodent–human hybrid cell lines and analyzed by hybridization to cDNAs for β1 and β3. Discordancy analysis allowed assignment of the β3 gene to chromosome 12 and confirmed the previous assignment of the β1 gene to chromosome 1. These results were confirmed and extended by using *in situ* chromosome hybridization, which permitted the regional localization of the β1 gene to 1pter → p31.2 and the β3 gene to 12pter → p12.3. Digestion of human genomic DNA with 10 restriction enzymes failed to disclose a restriction fragment length polymorphism for the β3 gene. These data indicate that there is considerable diversity in the genomic organization of the β subunit family. © 1990 Academic Press, Inc.

INTRODUCTION

The guanine nucleotide-binding regulatory proteins, referred to as G proteins,[4] are a family of signal-coupling proteins that mediate numerous transmembrane hormonal and sensory transduction processes. G proteins are essential for carrying extracellular signals generated by activated membrane receptors to intracellular effector enzymes and ion channels (for review see Gilman, 1987). The G proteins share a heterotrimeric structure composed of α, β, and γ subunits. The α subunit contains the guanine nucleotide-binding site, has intrinsic GTPase activity, and is unique to each G protein, conferring specificity for receptor–effector interactions. In response to receptor activation, the α subunit exchanges bound GDP for GTP and dissociates from the βγ dimer. The free α subunit then interacts with a specific signal effector enzyme or ion channel to regulate its activity. Interaction of the α subunit with its effector is terminated when the α subunit hydrolyzes GTP to GDP and subsequently reassociates with the βγ dimer.

Molecular cloning of cDNAs encoding G proteins has disclosed an unexpected diversity among the α subunits and has identified multiple genes that encode a complex superfamily of homologous α subunit proteins. The retinal G protein transducin, which reg-

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[4] Abbreviations used: G proteins, guanine nucleotide-binding regulatory proteins; G_s and G_i, G proteins that mediate stimulation and inhibition, respectively, of adenylyl cyclase; G_t, transducin, the major G proteins of retinal rods and cones; G_{olf}, the major G protein of olfactory neuroepithelium; G_o, a G protein of unknown function purified from the brain; SDS, sodium dodecyl sulfate; cDNA, complementary DNA; Tris HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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ulates activity of cyclic GMP phosphodiesterase in response to light activation of the photoreceptor rhodopsin (Stryer, 1986), is encoded by at least two genes, $G_t\alpha(1)$ in rods and $G_t\alpha(2)$ in cones (Lerea *et al.*, 1986). Activity of the hormone-sensitive adenylyl cyclase system is controlled by at least two G proteins: $G_s\alpha$ is responsible for stimulation of catalytic activity, whereas another group of α subunits, represented by at least three forms of $G_i\alpha$ (Jones and Reed, 1987), mediates inhibition of the enzyme (Gilman, 1987; Gilman, 1984). In olfactory tissue the G protein $G_{olf}\alpha$ mediates sensory signal transduction (Jones and Reed, 1989). The target functions of other G protein α subunits, including $G_o\alpha$, a G protein originally found in bovine brain (Itoh *et al.*, 1986), and $G_z\alpha$ (or $G_x\alpha$) (Fong *et al.*, 1988; Matsuoka *et al.*, 1988), remain largely unknown.

The β and γ polypeptides are tightly associated with each other as a $\beta\gamma$ complex. In contrast to the highly specific role exhibited by each distinctive α subunit, the $\beta\gamma$ complexes of the various G proteins appear to have similar or equivalent biological activities (Katada *et al.*, 1984; Northrup *et al.*, 1983; Neer *et al.*, 1984). Nevertheless, several important functional (Cerione *et al.*, 1987; Fukada *et al.*, 1989) and structural (Roof *et al.*, 1985; Mumby *et al.*, 1986) differences have been observed among the β and γ (Fukada *et al.*, 1989; Evans *et al.*, 1987; Hildebrandt *et al.*, 1985; Hurley *et al.*, 1984; Robishaw *et al.*, 1989) subunits of the G proteins, which demonstrate the existence of multiple forms of the β and γ polypeptides.

At least three genes encoding a small family of G protein β polypeptides have been identified (Fong *et al.*, 1986, 1987; Gao *et al.*, 1987; Sugimoto *et al.*, 1985; Codina *et al.*, 1986; Levine *et al.*, 1990b; Amatruda *et al.*, 1988). The three forms of the β subunit differ from each other by only 10–19% in their amino acid sequences. The β polypeptide encoded by the β1 gene is 36 kDa, whereas the β polypeptide encoded by the β2 gene is 35 kDa. The protein corresponding to the β3 gene has not yet been identified (Levine *et al.*, 1990b). Previous studies had localized the human genes for the β1 and β2 subunits to chromosomes 1 and 7, respectively (Blatt *et al.*, 1988). The present studies were designed to determine whether the gene encoding the β3 polypeptide is linked to either the β1 or β2 gene.

MATERIALS AND METHODS

Preparation and Analysis of DNA

High-molecular-weight genomic DNA was extracted from human peripheral blood leukocytes (Ahn *et al.*, 1986) or human × rodent hybrid cell lines (Modi *et al.*, 1989). Hybrid cell lines were obtained by PEG-mediated fusion of fresh human lymphocytes and mutant (HPRT) rodent cells (mouse RAG or Chinese hamster E36) and were propagated in HAT medium. Karyotypic and allozymic characterization of each cell line was performed on the same passage from which genomic DNA was extracted. The entire hybrid panel, which contains 44 cell lines, was the same as that described previously (Modi *et al.*, 1989). For analysis of genomic DNA, restriction endonucleases were purchased from Bethesda Research Laboratories or New England Bio Labs and used according to the manufacturer's directions. Each DNA sample (5–10 μg) was digested with an excess of the various restriction enzymes, size fractionated by electrophoresis on 1% agarose gels, and transferred to nitrocellulose membranes (Southern, 1975). DNA blots were hybridized with radiolabeled DNA probes as previously described (Levine *et al.*, 1988). After hybridization the filters were washed four times at 25°C in 2× SSC (1× SSC is 0.15 *M* NaCl, 15 m*M* sodium citrate) with 0.5% SDS for 5 min and up to either 60°C in 1× SSC with 0.5% SDS two times for 15 min (moderate stringency) or 65°C in 0.1× SSC with 0.5% SDS two times for 30 min (high stringency).

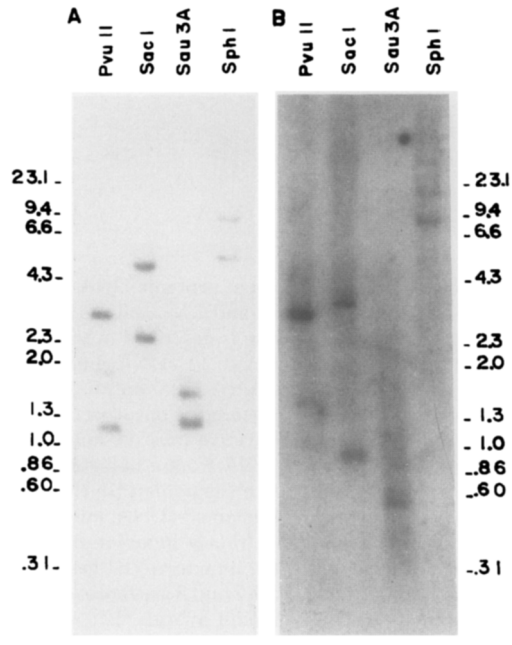


FIG. 1. Blot hybridization analysis of human genomic DNA. Ten micrograms of human genomic DNA was digested with the indicated restriction endonuclease and analyzed by blot hybridization to the human β1 (**B**) or human β3 (**A**) cDNA probe. Molecular weight size markers (λ DNA/*Hin*dIII fragments) are shown on the left and right.

In situ hybridization of tritiated probes to metaphase chromosomes derived from peripheral human lymphocytes was performed as previously described (Modi *et al.*, 1987), with the following modifications.

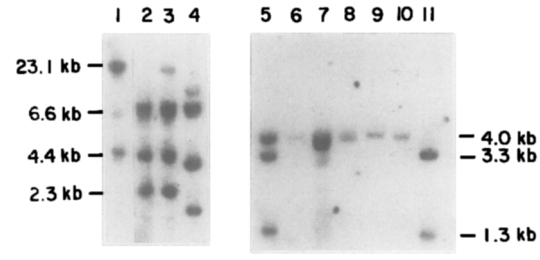


FIG. 2. Southern blot analysis of genomic DNA from rodent–human hybrid cell lines for GNB1 and GNB3 chromosome assignment. The experimental procedure is described in the text. Lanes **1–4** were hybridized with the human β1 cDNA. Lane **1** contains human DNA; lane **2** is a representative lane containing DNA from a mouse–human hybrid that was scored as negative for the human GNB1 gene; lane **3** is a representative positive lane; lane **4** is a representative lane containing DNA from a hamster–human hybrid that was scored as negative for the human GNB1 gene. Lanes **5–11** were hybridized with the human β3 cDNA; lane **5** is a representative lane containing DNA from a hamster–human hybrid that was scored as positive for the human GNB1 gene; lane **6** is a representative negative lane; lanes **7** and **8** are representative lanes containing DNA from mouse–human hybrids that were scored as negative for the human GNB3 gene; lane **9** contains mouse DNA; lane **10** contains hamster DNA; lane **11** contains human DNA.

Before denaturation the slides were treated with 0.5% acetic anhydride in 0.1 *M* triethanolamine buffer (pH 8.0) at room temperature for 10 min (Hayashi *et al.*, 1978). Probe concentrations in the hybridization solution ranged from 0.10 to 0.30 μg/ml. Slides were washed in 50% formamide in 2× SSC and in 2× SSC alone, both at 40°C before an autoradiographic exposure of 10–30 days.

A search was made for restriction fragment length polymorphisms by digesting genomic DNA from 6 to 10 unrelated Caucasian subjects with 10 restriction enzymes (*Ava*II, *Bam*HI, *Bgl*II, *Eco*RI, *Hin*dIII, *Msp*I, *Pst*I, *Pvu*II, *Rsa*I, and *Taq*I). DNA transfer and hybridization were performed following Dean *et al.* (1987).

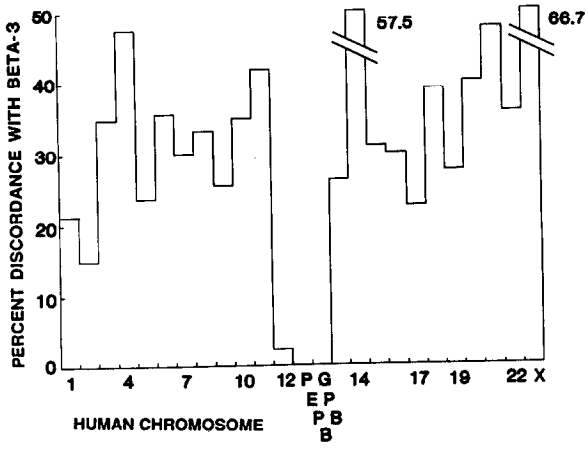


FIG. 3. Discordancy diagram illustrating results of the Southern blot analysis of the somatic cell hybrid panel. Localization of the GNB3 gene to chromosome 12.

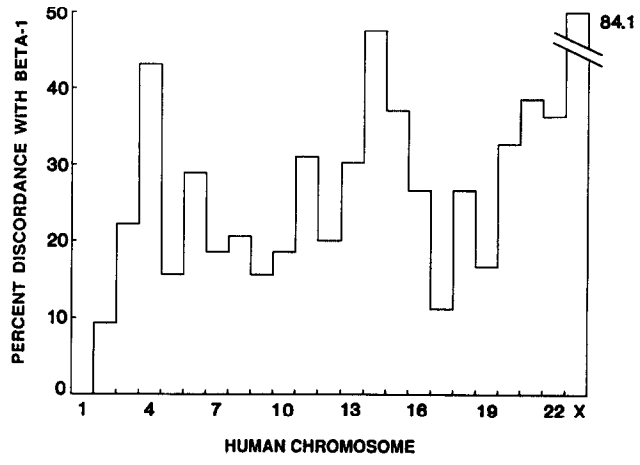


FIG. 4. Discordancy diagram illustrating results of the Southern blot analysis of the somatic cell hybrid panel. Localization of the GNB1 gene to chromosome 1.

DNA Probes

Full-length cDNA probes encoding human retinal β1 and β3 (Levine *et al.*, 1990b) were radiolabeled to specific activities of 0.5 to 2.0 × 10^9 dpm/μg by ran-

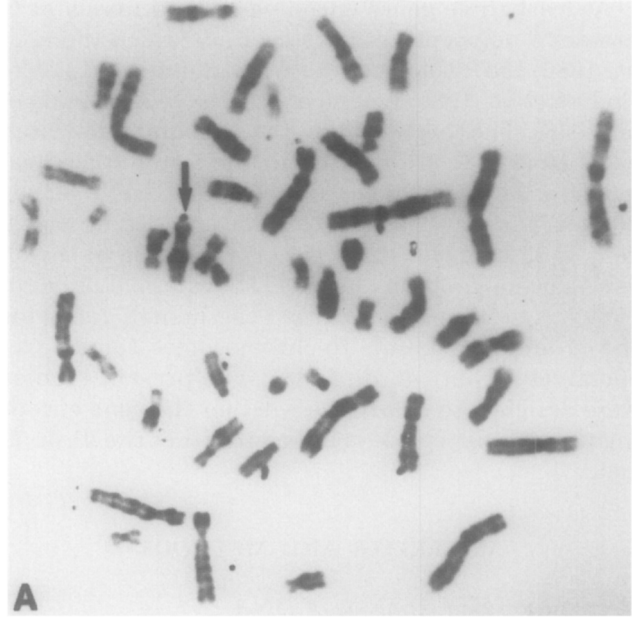


FIG. 5. Chromosome mapping of the human GNB3 gene to locus 12pter → p12.3. Human metaphase chromosomes were hybridized with the ^{3}H-labeled human β3 cDNA probe. The arrow shows the presence of autoradiographic grains representing hybridization of the β3 probe to a site on chromosome 12.

dom primer extension (Feinberg and Vogelstein 1982) using [α-^{32}P]dCTP (New England Nuclear). Specific activities for ^{3}H-labeled probes used for *in situ* hybridization were 2 to 8 × 10^{7} dpm/μg. The genes encoding the α and β subunit mRNAs are referred to by the nomenclature adopted at the Ninth International Workshop on Human Gene Mapping (1987). The letters GN refer to the guanine nucleotide-binding protein polypeptide. The third letter indicates whether the protein is an α (A), β (B), or γ (G) subunit, whereas the fourth letter refers to the nature of the α subunit family (e.g., T refers to transducin, I refers to inhibitory, and S refers to stimulatory).

RESULTS

Southern Blot Analysis of Genomic DNA

Although the three β proteins share extended regions of identical amino acid sequence, the extensive divergence in nucleotide sequence among the three cDNAs (Levine *et al.*, 1990b) is consistent with the notion that the three proteins are the products of distinct genes. Hybridization of human genomic DNA with cDNA probes indicated that the GNB1 and GNB3 genes are encoded by separate genomic regions. Each probe hybridized to a complex but distinct pattern of restriction fragments (Fig. 1). No restriction fragment length polymorphisms were found with any of the 10 enzymes used to analyze the GNB3 gene.

To map the GNB1 and GNB3 genes we used rodent × human hybrid cell lines. Because the hybrids used in this study were derived from mouse and hamster cells, many of which contain a complete rodent genome, it was important to establish differential hybridization patterns between the human and rodent β genes. The β subunit cDNAs encoding β1 and β3 were used to probe Southern blots of mouse, hamster, and human DNA that had been digested with a battery of restriction endonucleases. Digestion of human DNA with *Pvu*II yields at least four hybridizing GNB3 gene fragments, 12.0, 6.4, 6.0, and 3.4 kb in length (Fig. 2). The human β3 cDNA also hybridized with several fragments from the hamster and mouse GNB3 gene (Fig. 2), but the patterns can be easily distinguished from that of the human GNB3 gene. DNAs isolated from human–mouse and human–hamster hybrids were digested with *Pvu*II and analyzed for the presence or absence of the human GNB3 gene. A discordancy analysis of the entire panel of 44 hybrid cell lines indicates that the lowest discordancy values are obtained with respect to chromosome 12 (1 discordant hybrid of 42, or 2.4% discordancy) and its associated isozyme markers lactate dehydrogenase B, *LDHB* (2 discordant hybrids of 42, or 4.8% discordancy), and glyceraldehyde-3-phosphate dehydrogenase, *GAPDH* (no discordant hybrids out of 43). All other chromosomes and isozyme markers have substantially higher discordancy values (15–67%) (Fig. 3).

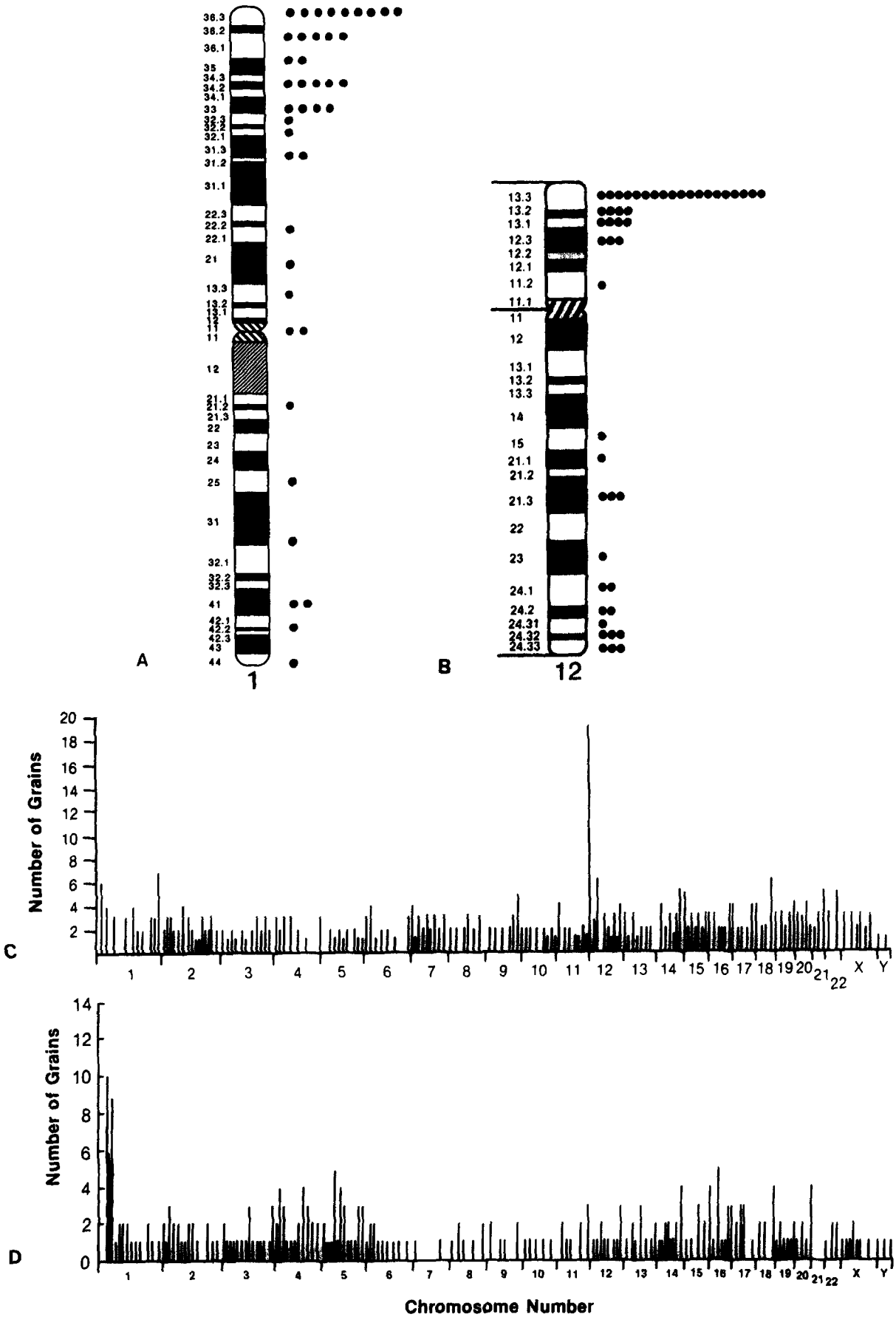
A similar analysis was performed using the human β1 cDNA. Digestion of human DNA with *Bgl*II generated several diagnostic hybridizing fragments that could be readily distinguished from restriction fragments generated from the hamster and mouse β1 genes (Fig. 2). A discordancy analysis of the hybrid cell line panel indicated that the lowest discordancy values are obtained with respect to chromosome 1 (no discordant hybrids out of a total of 44) and its associated isozyme markers phosphoglucomutase-1, *PGM1* (4 discordant hybrids of 43, or 9.3%). All other chromosomes and isozyme markers showed higher discordancy values (9.3–84%) (Fig. 4).

In Situ Hybridization

To define further the chromosomal location of the human GNB1 and GNB3 genes, we performed *in situ* hybridization using metaphase chromosomal preparations. A representative chromosome spread hybridized with the ^{3}H-labeled β3 probe is shown in Fig. 5. A discrete accumulation of silver grains is detected on one homolog of chromosome 12. The distribution of silver grains observed over metaphase chromosome spreads was plotted on a histogram in which a standardized idiogram of the haploid human genome was divided into units scaled to the average diameter of a silver grain (0.35 μm). For the β1 cDNA clone a total of 304 grains were observed in 73 metaphase cells examined. Of this total, 29 grains (9.5%) were located at 1pter → p31.2 (Fig. 6). With the β3 cDNA clone 436 grains were recorded in 120 cells. Thirty grains (6.9%) were localized at 12pter → p12.3 (Fig. 6). These results permit the regional localization of these two genes to these locations.

DISCUSSION

In this study, we report the chromosomal assignment of the GNB3 gene to human chromosome 12 using somatic cell hybrids. Moreover, these results were confirmed and extended using *in situ* hybridization, which permitted the regional localization of the β3 gene to 12pter → p12.3. In addition, we have confirmed the prior (Blatt *et al.*, 1988) localization of the GNB1 gene to human chromosome 1, and have used *in situ* hybridization to map this gene to 1pter → p31.2. The GNB2 gene had been previously mapped to human chromosome 7 (Blatt *et al.*, 1988). The striking sequence similarities among the G protein β


A
1
B
12
C
Number of Grains
D
Number of Grains
Chromosome Number

subunits suggest that they may have arisen from successive duplications of a common ancestor gene, and might therefore be clustered on a single chromosome. Our results show, however, that the three forms of β are located on different human chromosomes. Thus, similar to the genes for the G protein α subunits, the genes for the β polypeptides are dispersed in the human genome.

The three β proteins show 81–90% structural homology with one another, but contain differences that suggest that these proteins may interact with different α or γ subunits to elicit potentially different responses in signal transduction. Moreover, the distribution of the G protein β subunit genes over three chromosomes suggests that coordinate expression may be regulated by homologous *trans*-acting factors rather that by a common *cis* regulatory mechanism. Indeed, the parallel increase in expression of the genes encoding the β1 and β2 [and $G_i\alpha(2)$ and $G_i\alpha(3)$] proteins in response to hypothyroidism suggests that there is coordinate regulation of these genes by thyroid hormone (Levine *et al.*, 1990a).

In addition to the genes encoding the β subunits, several other genes related to signal transduction biochemistry have been mapped to chromosomes 1, 7, and 12 (Blatt *et al.*, 1988). The signal transduction G proteins are found as heterotrimers composed of α, β, and γ subunits. The genes encoding the α (GNAT2) and β (GNB1) polypeptides of $G_t\alpha(2)$, the major G protein in retinal cones (Lerea *et al.*, 1986), are both located on chromosome 1 (Blatt *et al.*, 1988). In addition, Blatt *et al.* (1988) have mapped the genes encoding the α subunit (GNAI1) of $G_i(1)$ and a β subunit (GNB2) to chromosome 7. Although the genes encoding the γ subunits for $G_t(2)$ and $G_i(1)$ have not yet been mapped, it is tempting to speculate that they may be linked to the genes encoding their respective α and β subunits. Lastly, Neer *et al.* (1987) have previously mapped the gene encoding $G_i\alpha(2)$ (GNAI2) to chromosome 12, where we have mapped the GNB3 gene. Blatt *et al.* (1988) were unable to confirm this assignment of GNAI2 to chromosome 12, and have assigned the GNAI2 gene to human chromosome 3. Although the reason for the difference in assignment for GNAI2 reported by these two groups is unresolved, this discrepancy is consistent with the notion that there are additional homologous α subunit genes that contain sequences with cross-reactivity to the $G_i\alpha(2)$ probe (Strathmann *et al.*, 1989). Further analysis and characterization of these cross-hybridizing loci may provide additional insights into the evolutionary origins of the GNA genes.

In summary, our results show that genes encoding three distinct β subunits of G proteins are located on different human chromosomes. The exact number of β polypeptides remains unknown. Three β genes have been identified; it is likely that additional genes exist (M. I. Simon, California Institute of Technology, personal communication). Analysis of these genes in conjunction with molecular mapping may allow us to address the question of whether the β subunit genes share a common ancestor.

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FIG. 6. Chromosomal mapping of human GNB1 and GNB3 genes. **(A)** Idiogram of chromosome 1 illustrating grain distributions following hybridization with the β1 probe. **(B)** Idiogram of chromosome 12 illustrating grain distributions following hybridization with the β3 probe. **(C)** Histogram depicting the grain distributions over the entire karyotype after hybridization with the β3 cDNA probe. **(D)** Histogram depicting the grain distributions over the entire karyotype after hybridization with the β1 cDNA probe.

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