Chromosomal Localization of Genes Encoding Guanine Nucleotide-Binding Protein Subunits in Mouse and Human


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Chromosomal localization of genes encoding guanine nucleotide-binding protein subunits in mouse and human
(recombinant inbred strains/restriction fragment length polymorphisms/gene mapping/synteny/somatic cell hybrids)

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ABSTRACT A variety of genes have been identified that specify the synthesis of the components of guanine nucleotide-binding proteins (G proteins). Eight different guanine nucleotide-binding subunit proteins, two different β subunits, and one γ subunit have been described. Hybridization of cDNA clones with DNA from human–mouse somatic cell hybrids was used to assign many of these genes to human chromosomes. The retinal-specific transducin subunit genes GNAT1 and GNAT2 were on chromosomes 3 and 1; GNA11, GNA12, and GNA13 were assigned to chromosomes 7, 3, and 1, respectively; GNAZ and GNAS were found on chromosomes 22 and 20. The β subunits were also assigned—GNB1 to chromosome 1 and GNB2 to chromosome 7. Restriction fragment length polymorphisms were used to map the homologues of some of these genes in the mouse. GNAT1 and GNA12 were found to map adjacent to each other on mouse chromosome 9 and GNAT2 was mapped on chromosome 17. The mouse GNB1 gene was assigned to chromosome 19. These mapping assignments will be useful in defining the extent of the Gα gene family and may help in attempts to correlate specific genetic diseases with genes corresponding to G proteins.

Guanine nucleotide-binding proteins (G proteins) mediate intracellular responses to a wide variety of extracellular stimuli. They are generally found as heterotrimers composed of an α, β, and γ subunit and are associated with transmembrane receptors. G proteins respond to the activation of specific receptors by ligand (e.g., hormone, neurotransmitter) or by physical stimuli (e.g., light absorption) and participate in the process of transducing these signals into changes in the intracellular level of second messengers. The activated receptor stimulates the exchange of guanosine diphosphate (GDP) bound to the α subunit of the G protein for guanosine triphosphate (GTP), resulting in the disassociation of Gα-GTP from the βγ complex. The Gα nucleotide complex and perhaps the Gαβγ complex as well are able to interact with effector molecules such as adenyl cyclase, phosphodiesterases, phospholipases, and transmembrane channels to evoke a cellular response (1–5).

On the basis of both molecular cloning and biochemical characterization, it has become clear that there are multiple genes that encode a variety of homologous α-subunit proteins. These can be divided into a number of subgroups based on their function and on their similarities in amino acid sequence and cellular localization. Some of the α-subunit proteins, for example, are found only in specific terminally differentiated cells. Thus, the transducin α1 (Tα1) protein is found only in retinal rod photoreceptor cells, while the transducin α2 (Tα2) subunit is present only in cone cells (6). Other Gαi subunit proteins are found to be expressed ubiquitously. For example, Gα1 is found in a large variety of cells; it functions to couple the activation of certain receptors to adenyl cyclase stimulation (1). Another group of Gαi subunit proteins is represented by the Gαi subgroups. They include a small subfamily of polypeptides Gαia1, Gαia2, and Gαia3 (2, 7, 8). These proteins differ from each other by only 10–20% in their amino acid sequences and from the other Gαi proteins by 30–60% in amino acid sequence. Transcripts corresponding to all three Gαi cDNAs have been found in cloned cell lines in tissue culture. However, it is clear that each member of the Gαia subfamily represents a distinct gene product since the amino acid sequence of a particular Gαia protein is almost identical in different mammals. Thus, for example, Gαia1 in rats and humans shows 98% amino acid sequence identity. On the other hand, while the Gαia protein, which is found ubiquitously in brain tissue, has sequence similarity to the Gαia subgroup, it can be clearly distinguished as representing a different family (for review, see ref. 9). Finally, another G protein encoding gene was identified by cDNA cloning and called Gαia. The corresponding protein differs strikingly from other Gαi subunits in amino acid sequences in a number of regions and it appears to be highly enriched in neural tissue (10, 41). Considering the great diversity of interactions that appear to be mediated by G proteins, it is not surprising to find a variety of subunits, and we may anticipate that other homologous genes encoding other Gαi subunit families will be found.

The other components of the G protein heterotrimer also show diversity. It has been reported that there are two distinct β subunits (β1 and β2), and both are expressed in a variety of different tissues (11, 12). Furthermore, there is clearly diversity in the γ subunits, since the only cDNA that has been cloned and sequenced thus far is found to be restricted to retinal tissues; yet γ subunits are found associated with β in all tissues that have been examined (13). If Gαi subunits can interact combinatorially with β and γ subunits, a large variety of complexes with different functional properties could be formed. It is necessary, therefore, to determine the limits of diversity in members of the G-protein family. One way to further examine the organization of this multigene family is to classify individual genes by their specific chromosomal locations. In this paper, we report the relative map positions of a number of G-protein-determining genes.

Abbreviations: G protein, guanine nucleotide-binding protein; Tα1 and Tα2, transducin α1 and α2 protein subunits; RI, recombinant inbred; RFLP, restriction fragment length polymorphism; SDP, strain distribution pattern.

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MATERIAL AND METHODS

Nomenclature. Probes of cDNA clones and polypeptides are referred to as they have been in the literature (14). Thus, the transducin α subunits are Tα1 and Tα2. The members of the Gi subfamily are Gαi1, Gαi2, Gαi3, etc. However, the corresponding genes 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. The third letter refers to the nature of the a-subunit subfamily. Thus, the two transducin a genes would be GNAT1 and GNAT2. The members of the Gi subfamily are GNAI1, GNAI2, and GNAI3. The genes that encode the β1 and β2 subunits are referred to as GNB1 and GNB2.

Genomic DNA Isolation and Digestion. Mouse DNA from parental and B × D recombinant inbred (RI) strains were purchased from The Jackson Laboratory; A × B/B × A RI DNA was prepared from liver and kindly donated by M. Nesbitt (University of California, San Diego). Microcell hybrid clones retaining various mouse chromosomes in a rat or hamster background were grown and DNA was extracted as described (15-17). The human × mouse somatic cell hybrid lines were the same as described (18) and DNA was prepared as described (18). High molecular weight DNA was digested with a 4- to 6-fold excess of restriction enzyme (Bethesda Research Laboratories). DNA fragments were separated by agarose gel electrophoresis and transferred to GeneScreen Plus (DuPont) by the method of Southern or by the alkaline method (19, 20).

DNA Probes. cDNA probes were obtained as follows: GNAT1 and GNAT2 were isolated from bovine retina and were provided by M. Lochrie (California Institute of Technology, Pasadena, CA) (21); GNAI1, GNAI2, GNAI3, and GNAS were isolated from rat olfactory epithelium by Jones and Reed (7). GNB1 and GNB2 were isolated from human HL-60 myeloid leukemia cells by T. Amatruda (California Institute of Technology) (11).

Probes were labeled by nick-translation or by the random oligonucleotide priming method (22) and hybridization was carried out at 65°C in 6 × SSC (1 × SSC = 0.15 M sodium chloride/15 mM sodium citrate)/2% bovine serum albumin/2% Ficoll 400/2% polyvinylpyrrolidone/1% sodium dodecyl sulfate (SDS)/10% dextran sulfate for restriction fragment length polymorphism (RFLP) analysis, or in 0.5 M sodium phosphate, pH 7/1 mM EDTA/1% SDS/1% bovine serum albumin at 52°C-68°C for somatic cell hybrid analysis. Membranes were washed at 55°C-65°C with 0.1 × –0.2 × SSC/0.1% SDS and autoradiographed with Kodak XAR-5 x-ray film with an intensifying screen at -70°C. The methods for the use of RFLPs and RI strains of mice for mapping in our laboratory have been described (23).

RESULTS

Mapping with RFLPs in Recombinant Inbred Mice. To demonstrate that we could clearly follow each gene that corresponded to a specific cDNA probe without apparent cross-hybridization with other G protein-determining genes, the restriction fragment patterns for heterologous probes were examined. Each of the probes derived from either bovine, rat, or human was hybridized and washed at high stringency (0.1 × SSC, 65°C) with DNA from A/J mice that was digested with a variety of restriction enzymes. Fig. 1 shows that each Gαn subunit probe gave a unique pattern of restriction fragments that could easily be distinguished. In addition, the hybridization pattern of probes isolated from human cells (HL60 myeloid cells) corresponding to Gαn2 (T. Amatruda, personal communication) was essentially the same as the pattern found with the Gαn2 cDNA probe derived from rat olfactory epithelial cells. Homologous cDNA probes corresponding to a specific isoform of Gα from rat, human, and bovine tissue each hybridized with the same set of restriction fragments in the mouse. This demonstrates that each probe detects a unique set of restriction fragments. Thus, while there is a great deal of similarity in the amino acid sequences of the α subunits, high stringency hybridization with mouse DNA distinguishes between them. These results support the assumption that each probe from the different sources is characteristic for at least one gene in the mouse genome.

To use the probes to map the corresponding genes, parental mouse DNAs (A/J, C57BL/6J, and DBA/2J) were digested with a battery of restriction endonucleases and inspected for polymorphism after hybridization with specific probes. When a polymorphism was found by using a particular restriction enzyme, then DNA from the appropriate RI strains digested with the same enzyme was hybridized to the probe and the polymorphic pattern was scored for resemblance to one parental strain or the other. This procedure was followed with all of the available probes and the resulting strain distribution pattern (SDP) of polymorphisms is shown in Table 1. Linkage was determined by comparing these newly determined SDPs to the SDPs determined for previously mapped genes. Linkage results are summarized in Table 2 and Fig. 2. Linkage and statistical analysis were calculated as described (24).

Several RFLPs were associated with the Tα1 probe. SDPs were determined in the B × D RI strains for polymorphisms found by using Kpn I and Xba I; the SDPs were identical. Comparison of this SDP with other markers indicates linkage on chromosome 9 to the LTW-3 (a liver protein variant) gene and the FV-2 (Friend virus insertion) locus. The SDP was also obtained for an EcoRI polymorphism found in the A × B and B × A RI strains. Although conclusive linkage to a known marker was not established in this system, weak linkage [≥8 centimorgans (cM)] to the β-galactosidase gene on chromosome 9 was indicated. Interestingly, the SDP for a PsI I polymorphism identified with GNAI2 was identical to that of GNAT1 in 42 of 42 A × B and B × A strains. Thus,
Table 1. Strain distribution patterns in RI strains

<table>
<thead>
<tr>
<th>Probes (positions)</th>
<th>B × D RI strain</th>
<th>A × B RI strain</th>
<th>B × A RI strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Map position in RI strains

<table>
<thead>
<tr>
<th>Gene</th>
<th>Probe</th>
<th>RFLP</th>
<th>RI strain</th>
<th>Linkage</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNA1T</td>
<td>Gsa1</td>
<td>*</td>
<td>A × B</td>
<td>Tsa1 0.0 (0–2.4) cM</td>
<td>9</td>
</tr>
<tr>
<td>GNA2T</td>
<td>Gso2</td>
<td>*</td>
<td>A × B</td>
<td>No significant linkage to known markers</td>
<td>2</td>
</tr>
<tr>
<td>GNA1S</td>
<td>Gsa3</td>
<td>*</td>
<td>EcoRI, Pst I</td>
<td>A × B</td>
<td>Gsa1 0.0 (0–2.4) cM</td>
</tr>
<tr>
<td>GNA2S</td>
<td>Gsa4</td>
<td>*</td>
<td>EcoRI</td>
<td>Xba I</td>
<td>B × D</td>
</tr>
<tr>
<td>GNA1T</td>
<td>Gsa1</td>
<td>*</td>
<td>B × D</td>
<td>Tsa1 0.0 (0–2.4) cM</td>
<td>19</td>
</tr>
<tr>
<td>GNA2T</td>
<td>*</td>
<td>Pvu II</td>
<td>B × D</td>
<td>No significant linkage to known markers</td>
<td>19</td>
</tr>
<tr>
<td>GNA1S</td>
<td>Gsa2</td>
<td>*</td>
<td>BamHI</td>
<td>B × D</td>
<td>No significant linkage to known markers</td>
</tr>
<tr>
<td>GNA2S</td>
<td>Gsa3</td>
<td>*</td>
<td>B × D</td>
<td>No significant linkage to known markers</td>
<td>19</td>
</tr>
</tbody>
</table>

*No RFLP detected using 24 restriction enzymes.*
Table 3. Comparative map positions in mouse and human

<table>
<thead>
<tr>
<th>Probe</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNα1</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>GNα2</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>GNα3</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>GNβ2</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>GNβ3</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>GNβ4</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>GNβ2</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The exact number of genes encoding α subunits of G proteins is not known (1–8, 10, 21). There are at least eight α genes, two β genes, and more than one γ gene based on molecular cloning studies and hybridization experiments in mammals. It is likely that additional genes exist. Recently, a number of groups have reported the independent isolation of GNA1-like genes. Jones and Reed (7) isolated three distinct forms of GNA1 from rat corresponding to GNA11, GNA12, and GNA13, while Beals et al. (25) obtained the corresponding cDNA clones from human B-cell libraries. Based on nucleotide sequence homology, GNA11 appears to correspond to the cDNAs isolated by Sullivan et al. (26) and Itoh et al. (27), while GNA12 corresponds to those of Nukada et al. (28) and Michel et al. (29). GNA13 is distinct from the other genes and appears to correspond to the human cDNA isolated by Didsbury and Snyderman (30). Southern blot comparisons show that these three cDNAs are clearly different (Fig. 1). Neer et al. (31) have cloned two cDNAs, which they call α1 and α2. Based on the Southern blot banding patterns and chromosome assignments, α1 appears to be GNA12, and α2 is probably GNA11. Neer and coworkers have mapped the GNA11 gene to human chromosome 7. However, they found that α2 hybridized to two human-specific bands in EcoRI-digested DNA, which segregates to different human chromosomes. Neer et al. (31) scored one of these bands, assigning it to chromosome 12. In HindIII-digested DNA we observe these bands with GNA12, two of them are concordant with chromosome 3 and one segregates with chromosome 12. Repeating the experiment using two other restriction enzymes we confirmed the chromosome 3 assignment. Thus, in humans there may be two genes with significant cross-reactivity to GNA12; alternatively, the chromosome 12 assignment may correspond to a pseudogene with a sequence that is similar to GNA12.

We have shown that the GNA12 gene in mouse maps adjacent to the GNA11 gene on chromosome 9. Recently, Ashley et al. (32) have mapped a GNA11 gene to chromosome 9 in the mouse. In the human, GNA11 maps to chromosome 3 in a region that shows syntenic relationships with the distribution of markers on mouse chromosome 9. Thus, it is reasonable to suggest that GNA11 and GNA12 may be closely linked on chromosome 3 in humans as they are on chromosome 9 in mice.

Although several members of the G protein multigene family are closely related (e.g., GNA11 and GNA12; GNA11, GNA12, and GNA13; GNB1 and GNB2), these relationships are not observed at the level of chromosomal organization. Some clustering of related genes is found on chromosome 9 in mouse; however, the genes that are potentially clustered are not those that are most closely related in DNA sequence. It is difficult at this point to assign any significance to this arrangement. Recently, Nakafuku et al. (33) found a G protein in yeast with a great deal of conserved sequence that was homologous to Gαs. This suggests that the G protein family may be very ancient. Some genes may have remained tightly linked through evolution (34). The syntenic group in which GNA11 and GNA12 are found also contains Aty-1 (aminoacylase-1) and Bgl (β-galactosidase) and extends over a region >10 cM (35). GNAS may also lie in a human–mouse synteny group, as several human chromosome 20 markers are also found on mouse chromosome 2. Ashley et al. (32) also found that GNAS mapped to mouse chromosome 2. The genes mapped thus far do not necessarily account for all the members of the GNA gene family, and we expect that more members of this group will be found.

The GNB1 and GNB2 proteins share 90% protein sequence homology within a species, while between species each of these proteins is identical. This suggests that divergence of each gene has been constrained and implies that these two genes may interact with different effector or receptor molecules to elicit potentially different responses in signal transduction. The two β genes map to different human chromosomes. While no position has yet been determined for GNB2 in mouse, on the basis of studies with somatic cell hybrids, it clearly does not lie on chromosome 19 adjacent to GNB1 (data not shown).

G proteins have been implicated in the etiology of at least one human disease, pseudohyoparathyroidism type Ia (36). Decreased levels of Gαs, observed in erythrocytes, result in a partial uncoupling of receptor-bound parathyroid hormone and intracellular adenylate cyclase levels. Other hormones also appear to be affected. It is likely that G proteins are involved in other diseases (2, 37, 38). An examination of the genes mapped in this study reveals at least one G protein gene in the vicinity of a known retinal marker. GNA12 maps on mouse chromosome 17 near the locus corresponding to retinal degeneration slow (rds) (39, 40). The extent of diversity among the Gα-subunit genes is not known; eventually
some of them may be found to be associated with diseases that result from defects in sensory transducing systems.

Note Added in Proof. Kaziro and coworkers (41) isolated a human genomic clone and a rat cDNA clone designated Gsa that corresponds in sequence to the Gsa clone (10).