Evolution of the mammalian G protein α subunit multigene family

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Heterotrimeric guanine nucleotide binding proteins (G proteins) transduce extracellular signals received by transmembrane receptors to effector proteins. The multigene family of G protein α subunits, which interact with receptors and effectors, exhibit a high level of sequence diversity. In mammals, 15 Gα subunit genes can be grouped by sequence and functional similarities into four classes. We have determined the murine chromosomal locations of all 15 Gα subunit genes using an interspecific backcross derived from crosses of C57BL/6J and *Mus spretus* mice. These data, in combination with mapping studies in humans, have provided insight into the events responsible for generating the genetic diversity found in the mammalian α subunit genes and a framework for elucidating the role of the Gα subunits in disease.

G proteins mediate signal transduction in a diverse group of eukaryotic organisms, including yeast[1–3], plants[4], *Dictyostelium*[5–6] and animals[7]. This broad distribution implies that genes encoding G proteins evolved with ancestral eukaryotes, some one and a half billion years ago. The function of heterotrimeric G proteins is to couple extracellular signals to various intracellular effectors, such as adenylyl cyclase, phospholipases and ion channels[8,9]. Multiple genes encoding the α, β and γ subunits of G proteins have been found in several organisms with greatest sequence diversity discovered among the α subunits[7]. At least 15 distinct Gα subunit genes have been identified in mouse and humans. These Gα proteins can be subdivided in four classes, Gs, Gi, Gq and G12 based upon sequence comparisons (Fig. 1). Members of each of the four classes are expressed in invertebrates as well as mammals[10–18].

Functional similarities of the a subunits within each of the four classes are implicit in their evolutionary conservation and have been demonstrated empirically with respect to effector specificity. For example, a subunits of the Gs class (Gαs and Gαolf) activate adenylate cyclase[14,19]; signal transduction through the a subunits of the Gi class is generally inhibited by pertussis toxin ADP ribosylation[8,9]; while α subunits of the Gq class (Gαq, Gα11, 14, 15, and 16) mediate pertussis toxin resistant activation of phospholipase-Cβ[20–22]. The signalling properties of the G12 class α subunits are not well understood. To elucidate the mechanisms responsible for generating the genetic diversity found in the G protein α subunit multigene family we performed a series of genetic and physical mapping studies of Gα subunits in human and mouse.

Murine location of Gα subunit genes

The murine chromosomal location of all 15 guanine nucleotide–binding protein α subunit (*Gna*) genes was determined using an interspecific backcross mapping panel derived from crosses of [(C57BL/6J x *M. spretus*) F1 x C57BL/6J] mice. This mapping panel has been typed for over 850 loci that are well distributed among all 19 mouse autosomes and the X chromosome (ref. 23; N.G.C. and N.A.J., unpublished results). C57BL/6J and *M. spretus* DNAs were digested with several different restriction enzymes and analysed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using probes specific to each *Gna* locus (Table 1). The strain distribution pattern (SDP) of each RFLP was then determined for an average of 150 backcross mice. All backcrosses were to C57BL/6J and, as expected, backcross progeny were either homozygous for the C57BL/6J allele or heterozygous for the C57BL/6J and *M. spretus* alleles. The simple presence or absence of RFLPs specific for *M. spretus* was followed in backcross mice. The chromosomal location of each *Gna* locus was then determined by comparing its SDP with the SDPs for all other loci already mapped in the backcross. The mapping results (Fig. 2) assigned the 15 *Gna* loci to nine different mouse autosomes. The locations of these genes and their flanking markers in mouse were consistent with their locations in humans determined both in this as well as previous studies and with known linkage homologies that have been established between human and mouse (Fig. 2).

The mapping results (summarized in Fig. 1) showed that members of the Gs and G12 classes segregated as unlinked genes (that is, they were not closely linked to any

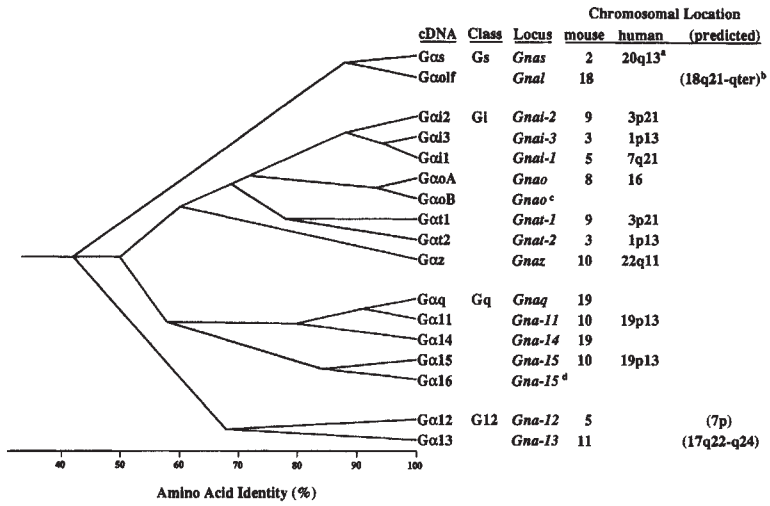


Fig. 1 The mammalian multigene family of G protein α subunits. Amino acid identity comparisons have defined four classes of mammalian α subunits, Gs, Gi, Gq and G12. Branch junctions approximate the values calculated by Bestfit (University of Wisconsin GCG program) for each pair of sequences with an exception; Gαz is about 67% identical to the three Gai sequences , not 57% as implied. Aside from mouse Gα15 and human Gα16, all α subunits that have been sequenced in at least two mammalian species exhibit greater than 97% amino acid identity (only the mouse sequence of Gαq, Gα12 and Ga13 is known).*a*, The human subchromosomal location of *Gnas* was reported previously[66]; human subchromosomal locations of the other Gα subunits were determined in this study. *b* *Gnal* maps in a region of human chromosome 18q21–qter linkage conservation (N.A.J. and N.G.C., unpublished results). The predicted location of other *Gna* loci in human chromosomes is estimated from the regions of linkage homology between mouse and human chromosomes shown in Fig.2. c The comparison of GαoA and GαoB was calculated specifically for the amino acids encoded by the alternatively spliced exons. *d* Although the amino acid sequence of Gα15[37] and Gα16[58] are only 84% identical, in contrast to all other mammalian G protein α subunit homologues, these cDNAs appear to be encoded by mouse and human homologues, hereafter termed *Gna–15* . The evidence that Gα15 and Gα16 are homologues includes Southern blots of mouse and human genomic DNA that were digested with several different enzymes and individually hybridized with radiolabelled probes spanning identical regions of the Gα15 and Gα16 cDNA clones. These probes hybridized to the same simple set of bands under moderate stringency (data not shown). Furthermore, Southern blots of mouse lambda clones and human cosmid clones that contained portions of genomic Gα15 and Gα16, respectively, revealed the same pattern of hybridization as the genomic blots (data not shown). In addition, oligonucleotide and PCR generated probes of Gα11 and Gα15/Gα16 identify overlapping genomic clones from mouse and human DNA that contain both *Gna–11* and *Gna–15* within a single contig (see Fig. 3a). Based on the percent amino acid identity between Gα15 and Gα16 (see Fig. 1), we conclude that the mouse and human homologues of *Gna–15* have diverged at a rate unprecedented among the G protein α subunit genes.

other Gα subunit gene) whereas many Gi and Gq class members segregated as pairs of closely linked genes.

Tandem gene duplication of *Gna–11* and *Gna–15*

To test the possibility that the closely linked pairs of Gα genes arose by tandem gene duplication, the physical linkage of one of the closely linked pairs in the Gq class, *Gna–11* and *Gna–15*, was characterized on cosmid clones isolated from a human chromosome 19 library[24]. Eleven cosmid clones were identified using PCR generated human Gα11 or Gα16 cDNA probes (Gα16 is the human homologue of mouse Gα15, see Fig. 1 legend). All cosmids shown in Fig. 3*a* were previously found to comprise a single contig by restriction enzyme fingerprinting. Three cosmid clones (F18649, F20903 and F23990) containing either *Gna–11* or *Gna–15* were also mapped by flourescence *in situ* hybridization (FISH) to human 19p13.3 (data not shown), as was a Gα16 cDNA probe (Fig. 3*b*). The relative positions of human *Gna-11* and *Gna-15* within this contig are also shown in Fig. 3*a*. Although there was some cross–hybridization between the cDNA probes, three of the cosmid clones on the left side of the contig were strongly positive only with the Gα11 cDNA probe, while five of the clones on the right side were strongly positive only with the Gα16 cDNA probe. Two clones in the middle of the contig gave strong signals with both probes. The transcriptional orientation of these two genes was determined using radiolabelled oligonucleotides and short PCR amplified DNA fragments from the 5' or 3' end of either the mouse Gα11 or Gα15 cDNAs. The hybridization patterns indicated that *Gna-11* and *Gna-15* reside in a head–to–tail array and the distance between the two loci is at most 10–20 kb. Mouse genomic clones of *Gna–11* and *Gna–15* also assemble into a single contig (data not shown). The cumulative data suggest that *Gna–11* and *Gna–15* are tandemly duplicated genes in both humans and mouse and indicate that the duplication arose prior to the divergence of rodents and primates.

Gi α subunit gene pairs are closely linked

Within the Gi class, we determined the human chromosomal location of both pairs of closely linked genes encoding Gαi and transducin isotypes by FISH analysis using human cDNA probes[25,26]. *Gnai-3* and *Gnat-2*, which cosegregated on murine chromosome 3, mapped to human 1p13 while *Gnai-2* and *Gnat-1*, which cosegregated on murine chromosome 9, mapped to human 3p21 (data not shown). These mapping results are also consistent with the notion that some of the Gi α subunit genes were generated by tandem gene duplication that occurred prior to the divergence of rodents and primates.

Evolution of mammalian Gα subunit genes

Multigene families are thought to be generated by a number of different mechanisms including (1) reverse transcription, a process that is likely responsible for pseudogene formation, (2) tandem gene duplication, which is thought to arise from unequal crossing over and (3) genome duplication or tetraploidization. There is considerable evidence that the eukaryotic genome has undergone multiple genome duplication events with the most recent duplication event occurring approximately 300 million years ago, long before the divergence of the lineages leading to mouse and man (for a review see ref. 27). A simple model to explain our mapping results is that the Gα multigene family was created through a series of genome duplications and one tandem duplication that occurred in the progenitor of the Gi and Gq genes after its divergence from the Gs and G12 progenitors (Fig. 4). According to this model all Gi and Gq class genes arose from a single tandem gene pair.

Three Gi class genes, *Gnai-1*, *Gnao* and *Gnaz*, do not readily conform to the simple model that all mammalian Gi and Gq class genes arose from a single tandem gene pair. *Gnai-1* is similar to *Gnai–3* in amino acid sequence and genomic organization suggesting that if it has a partner it is probably most closely related to transducin

Table 1 *Gna* loci mapped in interspecific backcross mice

Locus	Probe[a]	Reference	Enzyme	Restriction fragment sizes (kb)[b] C57BL/6J	*Mus spretus*[c]
Gnas	mouse: nt 142–834	66	*PstI*	3.7, 2.4, 1.2, 1.1	4.7, 2.4, 1.3
Gnal	rat: 3' untranslated region	19	*SphI*	1.8	4.9
Gnai–1	rat: 2.0 kb *Eco*RI fragment	67	*HindIII*	13.0, 8.0, 6.6, 4.5, 4.2, 3.2	14.0, 5.4, 4.3, (3.0), 2.4
Gnai–2	rat: 1.34 kb cDNA	66	*MspI*	3.1, (1.4, 0.7, 0.6, 0.5)	2.5, (1.4, 0.7, 0.6, 0.5)
Gnai–3	rat: 2.4 kb *Eco*RI/*Eco*RV fragment	67	*XbaI*	9.4, 6.1, 4.4, 4.2, 3.5	7.0, 6.1, 4.4, 4.0,3.8, 3.5
Gnao [d]	mouse: *GnaoA*, nt 725–1046	33	*PvuII*	3.5, 0.9	3.9, 2.8
	mouse: *GnaoB*, nt 728–1049	33	*KpnI*	16.0, 6.4	13.0, 6.4, 3.8
Gnat–1	human: nt 717–1075	26	*BglII*	5.2, 3.1, 2.7, 1.9	5.2, 3.7, 2.7
Gnat–2	human: nt 849–1207	26	*BamHI*	7.0, ~1.1	5.0, (~0.9)
Gnaz	human: nt 631–818	29	*XbaI*	12.0, 6.6	8.3, 6.6
Gnaq	mouse: nt 147–701	11	*XbaI*	16.0, 4.4, 3.0	16.0, 3.0, 1.8
Gna–11	mouse: nt 641–828	11	*HindIII*	9.4	18.0
Gna–14 [e]	mouse: nt 631–819	37	*PvuII*	4.2, 3.8, 2.7, 1.8, 0.8, 0.7	6.6, (5.4), 4.1, (3.5, 1.0,), 0.8
Gna–15 [f]	mouse: nt 653–840	37	*PvuII*	2.5, 0.5	2.9, 0.5
	nt 670–1180	37	*PvuII*	6.0, 2.5, 0.5	3.6, 2.9, 0.5
Gna–12	mouse: nt 703–1061	68	*BglII*	14.0	21.0
Gna–13	mouse: nt 694–1056	68	*SphI*	5.1	2.9

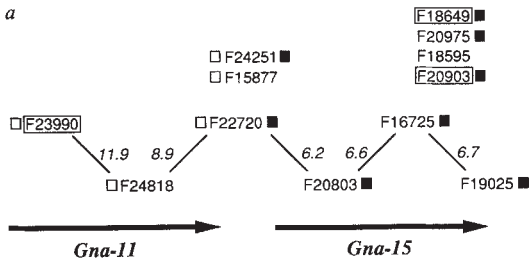
[a]All probes that specify the inclusive nucleotides (nt) were generated by PCR amplification. Probes to 4 loci, *Gnal*, *Gnai–1*, *Gnai–2* and *Gnai–3*, were derived from restriction fragments of cDNA clones. The species of origin of each probe is indicated.
[b]Restriction fragments shown in parentheses were usually lighter in intensity and not visible in all samples.
[c]The underlined restriction fragment sizes indicate the fragments typed in the interspecific backcross analysis. Where more than one fragment was typed, the RFLPs cosegregated.
[d]Probes specific to either the *GnaoA* or *GnaoB* splice variant were mapped; these loci co–segregated. The data shown in Fig. 2 is based on the *GnaoB* typing.
[e]The 3.5 kb *M. spretus*–specific fragment was light in intensity and difficult to follow but it appeared to be unlinked to the chromosome 19 locus.
[f]Two PCR generated probes were used to map *Gna–15*. The data was combined to generate the results shown in Fig. 2.

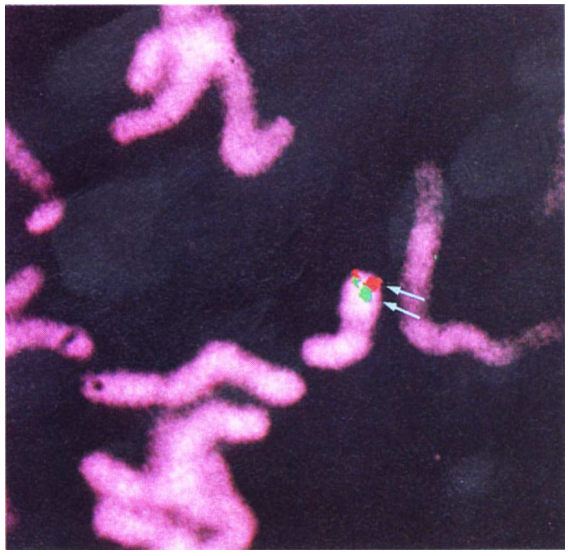
(Fig. 1; ref. 28). *Gnai–1* may have originally had a partner but lost it through deletion or other mutation (a common occurence in the evolution of duplicated genes), or alternatively, it may have a functional partner yet to be identified. Identification and characterization of cosmid clones for *Gnai–1* and flanking regions should help resolve this question. *Gnaz* also maps separately from all other Gα genes (Fig. 1) and is the most divergent member of the Gi class. The intron organization of *Gnaz* is unique, with one intron in the 5' untranslated region and a second intron within the coding region in place of the eight introns that are conserved in all other mammalian Gi class genes[28]. The structure of *Gnaz* resembles an incompletely processed pseudogene suggesting that it may have been derived by reverse transcription. If *Gnaz* was derived by reverse transcription, it was presumably recruited as a functional gene prior to divergence of rodents and primates because its amino acid sequence is highly conserved in rats and humans[29,30].

Gnao presents the most provocative exception to the model of tandemly duplicated Gi and Gq class genes. *Gnao* encodes two functionally distinct proteins[31]; the amino acids encoded in the first six exons are common to both Gαo proteins but the remainder of the two proteins are encoded by two different pairs of exons that are selected through alternative splicing[32–34] (Fig. 4). This unusual gene organization may have derived from a pair of tandemly duplicated genes. Deletion of the transcription termination signal of the upstream gene, along with the amino terminal two–thirds of the downstream gene, could have generated the present *Gnao* gene structure. The *Drosophila Gnao* homologue also undergoes alternative splicing, but in this case two transcripts, containing individual translation start sites in their respective first coding exons, initiate from distinct promoters and then splice to a common acceptor site at the beginning of exon 2 (ref. 15). The structure of *Drosophila Gnao* could also be interpreted to have derived by internal deletion of a tandem gene pair, in this case removing nearly the entire coding region and transcription termination signals of the upstream gene.

Invertebrate Gα subunit genes

Invertebrates express homologues of all four classes of mammalian Gα genes, indicating that these four classes evolved prior to the divergence of vertebrate and invertebrate lineages, approximately 570 million years ago. Indeed, the genomic organization of the Gi and Gq





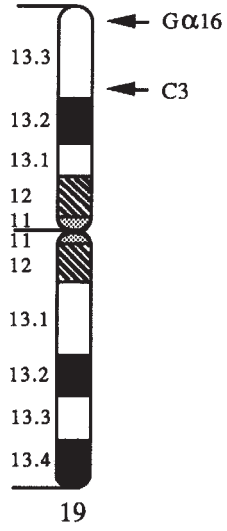


Fig. 3a *Gna–11* and *Gna–15* are encoded by two genes in a tandem head–to–tail array. An overlapping set of human cosmid clones was previously established by restriction fragment fingerprinting and was again identified by hybridization to human Gα11 (open squares) and/or Gα16 (filled squares). L values, shown next to the slanting lines that connect the cosmids, define the minimum spanning set based on restriction fragment fingerprinting. Additional cosmids are positioned above the spanning set members they most overlap. The boxed cosmid clones were mapped to human 19p13.3 by FISH. Transcriptional direction (arrowhead at the 3' end) was defined by hybridization to oligonucleotides and specific cDNA fragments (see methodology). The human chromosome 19 library was also screened with the Gα11 probe at lower stringency (final wash 0.1 × SSC, 1 % SCS, at 55 °C) but no additional clones were identified. *b* Flourescence *in situ* hybridization shows cDNA for Gα16 is located telomeric to Complement Factor 3 (C3) in human metaphase chromosome band 19p13.3. The approximate positions of the 2 genes within the band are noted by the arrows on the ideogram. Human chromosome preparations were hybridized simultaneously with cDNAs for Gα16, labelled with biotin–11–dUTP, and C3, labelled with digoxigenin. Hybridization was detected with streptavidin conjugated fluorescein isothiocyanate (FITC) and antibody–conjugated Texas Red, respectively. A computer enhanced image is shown.

class genes in *Drosophila* and mammals show remarkable conservation of the intron/exon boundaries[12,16] in addition to amino acid sequence conservation. If tandem duplication of the Gi and Gq class genes occurred prior to divergence of vertebrate and invertebrate lineages, it may not have been rigourously conserved. For example, *Drosophila* express *Gnai* homologues but not transducin, the gene that is closely linked to *Gnai* in mammals. It is unlikely that a functional invertebrate transducin awaits discovery as invertebrate vision is thought to be mediated by Gαq coupling to phospholipase C[12,35,36] in contrast to transducin regulation of cGMP phosphodiesterase.

Expression patterns of Gα subunit gene pairs

An interesting feature of each Gα subunit gene pair is that one member is always expressed in a wide range of tissues, whereas expression of the second member is more restricted. In the case of *Gna-11* and *Gna-15*, the upstream gene (*Gna-11*) is ubiquitously expressed while the downstream gene (*Gna-15*) is predominantely expressed in haematopoietic cell–types[37]. Regarding the other gene pairs, *Gnaq*, *Gnai–2* and *Gnai–3* are widely expressed while their respective partners, *Gna–14*, *Gnat–1* and *Gnat–2* exhibit more restricted expression[7,37]. For example, *Gnat–1* and *Gnat–2* are specifically expressed in rod and cone photoreceptor cells, respectively. In our model (Fig. 4) we assume that it is the upstream gene that is always widely expressed although this remains to be confirmed. Comparison of the promoters in each of these evolutionarily related gene pairs may reveal similar mechanisms for spacial and temporal regulation of gene expression.

Gα subunit genes in human and mouse disease

Mutations in Gα subunit genes are associated with human genetic disorders and malignancies. Albright's hereditary oesteodystrophy and related diseases exhibit Gαs deficincies, in some cases shown to be due to missense mutations in *Gnas*, that presumably express non–functional proteins truncated at either the N– or C–terminus[38,39]. In constrast, McCune–Albright Syndrome[40] and some tumours and thyroid hyperplasias[41,42] have been shown to express constitutively activated Gα subunits. While the function of Gαs in coupling hormonal stimulation to cAMP synthesis is well known, many of the Gα genes mapped here have only recently been identified and little is known about their function. As characterization of these gene products progresses, the genetic maps shown

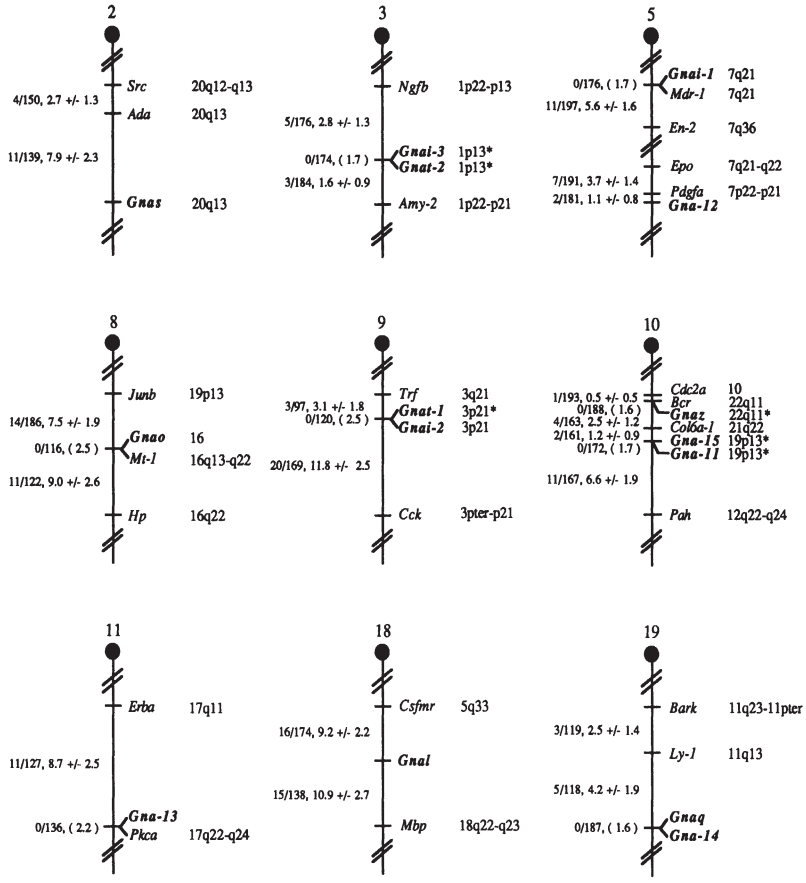


Fig. 2 Chromosomal locations of 15 *Gna* loci in the mouse genome. *Gna* loci were mapped by interspecific backcross analysis. The number of recombinant N_2 animals is presented over the total number of N_2 animals typed (ranging from 101–197 animals) to the left of the chromosome maps between each pair of loci. The recombination frequencies, expressed as genetic distance in centimorgans (± one s.e.), are also shown. The upper 95% conficence limit of the recombination distance is given in parentheses when no recombinants were found between loci. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns. The positions of loci on human chromosomes, where known, are shown to the right of the chromosome maps. The murine chromosomal locations of four Gna loci have been reported previously, *Gnas* (64), and *Gnai–2*, *Gnat–1* and *Gnat–2*[70]. Our results are in good agreement with these assignments, with the exception of *Gnat–2* in mouse, and further refine the position of these genes on the mouse linkage map. The *Gnat–2* locus was originally mapped to mouse chromosome 17 by recombinant inbred strain analysis using a full length bovine cDNA probe[70,71]. In contrast, we placed *Gnat–2* on mouse chromosome 3, tightly linked to *Gnai–3* and in agreement with the mapping positions of flanking markers on human chromosome 1p. References for the map positions of most loci in human chromosomes can be obtained from OMIM (Online mendelian inheritance in man), a computerized database of human linkage information (William H. Welch Medical Library, Johns Hopkins University).
An interesting feature regarding the middle of mouse chromosome 10 is that it has nine markers within approximately 11 cM, including *Gnaz, Gna–11* and *Gna–15,* that are dispersed on five different human chromosomes. In contrast, the mean length of all conserved regions of linkage homology between mouse and human is estimated to be 10.4 +/– 2.4 cM[72]. Similarly, human *Gna–11* and *Gna–15* map with a group of genes on 19p13 that are dispersed to four murine chromosomes (N.A.J. and N.G.C., unpublished results). *Determined in this study by FISH with either human genomic cosmid (*Gnaz, Gna–11, Gna–15*) or human cDNA (Gαi2, Gαi3, Gαt1, Gαt2, Gα16) probes.(See also refs 73–76.)

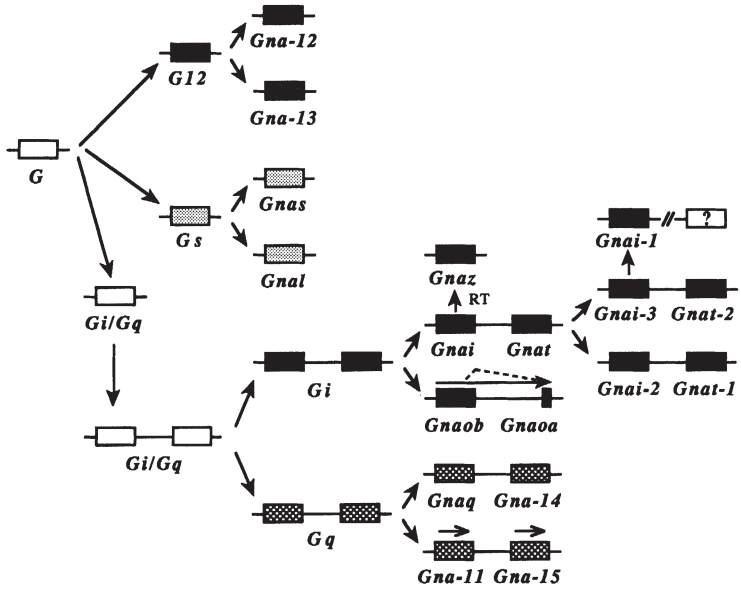


Fig. 4 A model depicting some of the possible evolutionary events giving rise to the genetic diversity found in the Gα subunit mutigene family. The Gq and Gi class α subunits are predicted to exhibit similar genomic organization and are modelled to descend from a common, tandemly duplicated progenitor, but may have independently undergone tandem duplication. Boxes represent the genes. Arrows above the genes point towards the 3' end of the transcription unit where their relative orientation is known. The dotted line above *Gnao* depicts alternatively spliced exons. The open box next to *Gnai–1* suggests the possibility of a tandemly duplicated gene. RT indicates that *Gnaz* may be derived from a *Gnai* progenitor through reverse transcription. G12, Gs and Gi/Gq represent progenitors of the 4 classes of G protein α subunits that are expressed in vertebrates and invertebrates. G symbolizes an ancient progenitor gene.

here will provide an important tool in establishing causal associations between Gα subunit genes and human as well as mouse disease.

Methodology

Interspecific backcross mapping. Interspecific backcross progeny were generated by mating (C57BL/6J × *M. spretus*) F1 females and C57BL/6J males as described[23]. A total of 205 N_2 progeny were obtained; a random subset of these N_2 mice were used to map the α subunits of guanine nucleotide–binding protein (*Gna*) loci (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer and hybridization were performed essentially as described[43]. All blots were prepared with Zetabind nylon membrane (AMF–Cuno). The probes and RFLPs for the loci used to position the *Gna* loci in the interspecific backcross have been reported. These include: *Src* and *Ada*, chromosome 2 (ref. 44); *Ngfb* and *Amy–2*, chromosome 3 (ref. 45); *Mdr–1*, *En–2*, *Epo* and *Pdgfa*, chromosome 5 (ref. 46); *Junb*, *Mt–1* and *Hp*, chromosome 8 (refs 47,48); *Trf* and *Cck*, chromosome 9 (ref. 49); *Cdc2a*, *Bcr*, *Col6a–1* and *Pah*, chromosome 10 (ref. 50); *Erba* and *Pkca*, chromosome 11 (ref/ 51); *Csfmr* (*Fim–2*) and *Mbp*, chromosome 18 (ref. 52); and *Bark* and *Ly–1*, chromosome 19 (ref. 53). Recombination distances were calculated as described (ref. 54) using the computer program SPRETUS MADNESS. Gene order was determined by assuming the least number of recombination events required to explain the allele distribution patterns.

Chromosome 19 cosmid library. The chromosome 19 cosmid library was constructed from a flow sorted preparation of chromosome 19 isolated from a Chinese hamster–human hybrid cell line (UV5HL9–5B) that contains a single chromosome 19 as the only detectable human material[55]. DNA was cloned in the Lawrist 5 vector[56] and clones were propagated in the bacterial host DH5αMCR. Clones were arrayed in microtitre dishes from which high density colony arrays (1536 clones per 8 × 12 cm filter) were prepared on nylon filters (Hybond N, Amersham) for screening. Hybridization probes of PCR amplified DNA fragments of human Gα11 (nt 149–837; ref. 57) and Gα16 (nt 226–1067; ref. 58) were radiolabelled by the random primer method[59] and used to screen a portion of the library representing approximately a 4–fold coverage of chromosome 19. Filters were hybridized at 65 °C for 16 hr in 4×SSC, 1%SDS, 0.1 mg ml^{-1} denatured sheared herring sperm DNA and washed at a high stringency (0.1 × SSC, 0.1% SDS at 70 °C) or moderate stringency (55 °C). Transcriptional orientation was determined on Southern blots and dot blots of cosmid DNA hybridized with the probes indicated above or a series of radiolabelled oligonucleotides to distin guish *Gna–11*, CT161 [TIGTITA(TC)CA(AG)AA(TC)AT (TCA)TT], CT179 [CTGTGCCACCGACACGGAGAAC], CT180 [TCGAGCCATAAGTCAAGGCCAG] and *Gna–15*, CT184 [CCA(AG)ATCAG(GC))GA(GC)CGGGCCAT], CT58 [GAGAA-CGTGATTGCCCTCATC], CT141 [GCCGTCCACGCACCC-GGTGTA], CT186 [GATGGGGCTCCTGAGAGATGC]. Additional probes were PCR amplified from a mouse cDNA clone of Gα11 (ref. 11) (nt 1–118, 642–831 and 810–1076) or mouse Gα15 (ref. 37) (nt 67–602).

Fingerprinting and assembly of cosmid contigs. Cosmids were analysed by a high resolution flourescence–based restriction fragment fingerprinting technique as described previously[60]. The potential overlap between pairs of cosmids was estimated by calculating a log likelihood of overlap (a LOD score, referred to as an L value) based on the occurrence of shared restriction fragments[61]; an L value of less than 6 was not considered significant. Sets of overlapping cosmids were then assembled using a computerized contig assembly program.

FISH. Prometaphase chromosomes were prepared from peripheral blood lymphocytes by using the bromodeoxyuridine synchronization method[62]. cDNAs were either labelled with biotin–11–dUTP by using nick translation and were detected with streptavidin–conjugated Texas Red (Vector Labs) or were labelled with digoxygenin, and were detected with sheep anti–digoxigenin (Boehringer–Mannheim Biochemical) followed by rabbit anit–sheep–conjugated FITC (Vector Labs). Preannealing the probe and *in situ* hybridization were performed as described previously[63] with some modification. Reverse bands were produced by counterstaining with chromomycin A3 followed by distamycin[64]. For precise band assignment, the FITC signal is shown simultaneously with the banding signal by using Zeiss filter set 05 and the resulting image was photographed with Kodak technical pan film. The FITC and Texas red images were recorded and superimposed using the Hamamatsu photon counting system to display the two colour FISH. *Gna–11* and *Gna–15* human cosmid DNA was labelled with biotin and hybridized to metaphase spreads from a normal donor, as described[65]. Sites of hybridization were labelled with avidin–fluorescein and mapped relative to chromosomal bands produced by DAPI and actinomycin staining.

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