Protein family review

Protein prenyltransferases

Sebastian Maurer-Stroh, Stefan Washietl and Frank Eisenhaber

Address: Research Institute of Molecular Pathology, Dr Bohr-Gasse 7, A-1030 Vienna, Austria.

Correspondence: Sebastian Maurer-Stroh. E-mail: stroh@imp.univie.ac.at

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Summary

Three different protein prenyltransferases (farnesyltransferase and geranylgeranyltransferases I and II) catalyze the attachment of prenyl lipid anchors 15 or 20 carbons long to the carboxyl termini of a variety of eukaryotic proteins. Farnesyltransferase and geranylgeranyltransferase I both recognize a 'Ca_1a_2X' motif on their protein substrates; geranylgeranyltransferase II recognizes a different, non-CaaX motif. Each enzyme has two subunits. The genes encoding CaaX protein prenyltransferases are considerably longer than those encoding non-CaaX subunits, as a result of longer introns. Alternative splice forms are predicted to occur, but the extent to which each splice form is translated and the functions of the different resulting isoforms remain to be established. Farnesyltransferase-inhibitor drugs have been developed as anti-cancer agents and may also be able to treat several other diseases. The effects of these inhibitors are complicated, however, by the overlapping substrate specificities of geranylgeranyltransferase I and farnesyltransferase.

Gene organization and evolutionary history

Gene organization

Protein prenylation is the posttranslational attachment of either a farnesyl group or a geranylgeranyl group via a thioether linkage (-C-S-C-) to a cysteine at or near the carboxyl terminus of the protein. Farnesyl and geranylgeranyl groups are polyisoprenes, unsaturated hydrocarbons with a multiple of five carbons; the chain is 15 carbons long in the farnesyl moiety and 20 carbons long in the geranylgeranyl moiety (see Table 1). There are three different protein prenyltransferases in humans: farnesyltransferase (FT) and geranylgeranyltransferase 1 (GGT1) share the same motif (the CaaX box) around the cysteine in their substrates, and are thus called CaaX prenyltransferases, whereas geranylgeranyltransferase 2 (GGT2, also called Rab geranylgeranyltransferase) recognizes a different motif and is thus called a non-CaaX prenyltransferase [1]. Each protein consists of two subunits, α and β; the α subunit of FT and GGT1 is encoded by the same gene, *FNTA* (see Table 1).

The genomic organization of the human genes that encode protein prenyltransferases is shown in Figure 1. It is interesting to note that the genes encoding both subunits of non-CaaX prenyltransferases are much smaller (about 6-9 kilobases, kb) than those of the CaaX prenyltransferases (about 30-76 kb). The number of exons in the two types of genes are similar; the differences in gene size therefore result from differing intron lengths.

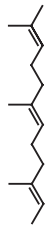
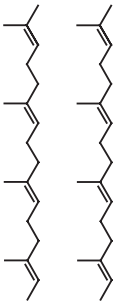
Automatic comparisons of data from expressed sequence tags (ESTs) with genes (for example using the program Acembly, for which the results are available from the NCBI AceView server [2]) shows that all the human protein prenyltransferase genes have multiple alternative splice variants. The extent of translation of the various predicted transcripts and the structures and functions of the resulting proteins remain to be established experimentally; some of the predicted transcripts may be derived from missplicing rather than being real splice variants.

Evolutionary history

Protein prenyltransferases are currently known only in eukaryotes, but they are widespread, being found in vertebrates, insects, nematodes, plants, fungi and protozoa,

Table 1

Features of human protein prenyltransferases

	Farnesyltransferase (FT)	Geranylgeranyltransferase I (GGT1)	Geranylgeranyltransferase II (GGT2 or RabGGT)
Gene name (α subunit)	*FNTA*	*FNTA*	*RABGGTA*
Gene name (β subunit)	*FNTB*	*PGGT1B*	*RABGGTB*
Substrate motif	Carboxy-terminal -Ca_1a_2X box*	Carboxy-terminal -Ca_1a_2X box*	Carboxy-terminal motif such as -CC, -CXC, -CCX, -CCXX, -CCXXX, or -CXXX†
Lipid anchor	 Farnesyl	 Geranylgeranyl	 Geranylgeranyl (one or two moieties per substrate)

*C, cysteine; a_1, and a_2 are amino acids that are preferably aliphatic; the identity of a_1 is more flexible than a_2. X can be C, S, Q, A, M, T, H, V, N, F, G, or I for FT, or L, F, I, V, or M for GGT1 (in decreasing order of affinity). †Requires an escort protein such as REP1.

including several parasites. The chromosomal locations and number of exons from protein prenyltransferase genes in the major eukaryotic model organisms are shown in Table 2. The α and β subunits have different folds, so are unlikely to have arisen from a common ancestor. Molecular phylogenetic analysis shows that orthologous proteins in different species are more closely related to each other than to their paralogs in the same species; the relationships between the β subunits are not fully clear. As well as the known genes, processed pseudogenes resulting from retrotransposition events have been found in the human and mouse genomes [3,4].

The α subunits of protein prenyltransferases consist of tetratricopeptide repeats and are part of the tetratricopeptide repeat superfamily [5], which also includes functionally diverse proteins involved in transcription, co-chaperoning, protein transport, cell-cycle control and phosphorylation. Although evolution of repeat proteins is difficult to analyze and interpret, Zhang and Grishin [6] have deduced convincingly that the *FNTA* and *RABGGTA* genes originated from a common ancestor that already contained multiple tetratricopeptide repeats rather than having independently amplified the number of motifs as the families diverged over time.

The evolutionary history of the β subunits of protein prenyltransferases has received much less attention so far than that of the α subunits [7]. They can, however, be shown using standard sequence analysis tools such as PSI-Blast [8] or HMMer [9] to be part of a superfamily of prenyltransferases. Whereas the β subunits of protein prenyltransferases attach lipid anchors to proteins, other members of the superfamily catalyze different reactions involving polyisoprenes, such as the cyclization of polyisoprene derivatives during the synthesis of hopanoids in bacteria [10], cycloartenol in plants [11], ergosterol in fungi [12] and lanosterol in vertebrates [13,14]; these products are then generally processed further to produce essential substances such as cholesterol, steroid hormones or vitamin D and their equivalents. A merged PFAM [15] domain (PF00432) has been created containing the β subunits of protein prenyltransferases, as well as squalene-hopene cyclases and lanosterol and cycloartenol synthases. Intriguingly, structural superposition of the conserved $(\alpha\text{-}\alpha)_6$ barrel forming one half of bacterial squalene-hopene cyclase (the enzyme that synthesizes hopanoids) [16] with the β subunit of rat FT [17] reveals correspondence not only of their secondary structural elements but also of the parts of their active or binding sites (Figure 2). This would make sense if the different enzymes have retained common reaction mechanisms - or at least common substrate-binding characteristics - during evolution. Indeed, all of the members of the prenyltransferase superfamily bind substrates containing isoprenyl units, although they bind different numbers and variations of such units and the underlying processing and binding mechanisms seem to have diverged widely over time.

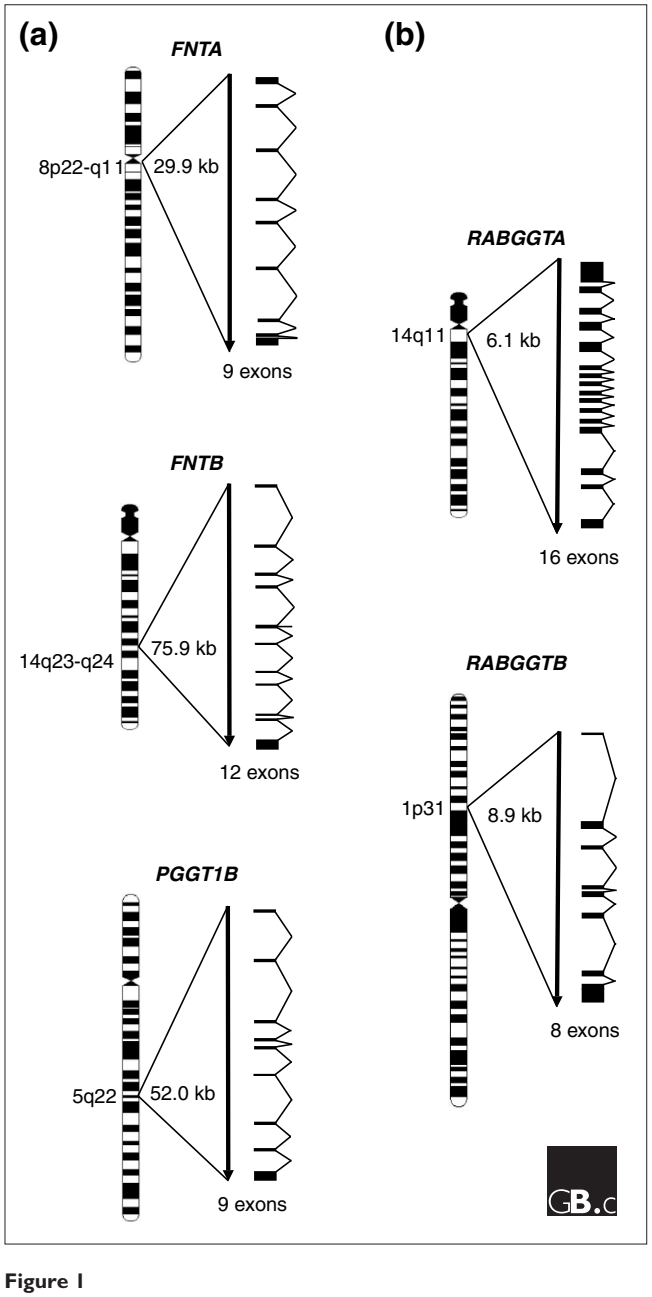


Figure 1
Gene structures and chromosomal locations of human protein prenyltransferase subunit genes. The chromosome diagrams and the locations of the genes on the cytogenetic map are according to the NCBI MapViewer [67]. The sizes of the genes are indicated but are not drawn to scale. **(a)** Genes encoding CaaX protein prenyltransferases are relatively long; **(b)** genes encoding non-CaaX protein prenyltransferases are much shorter.

Table 2

Protein prenyltransferase genes in model organisms

Organism	Subunit	Chromosome	Number of exons
Mus musculus	FTA (GGT1A)	8	9
	FTB	12	6
	GGT1B	18	9
	GGT2A	14	15
	GGT2B	3	8
Drosophila melanogaster	FTA (GGT1A)	2	3
	FTB	3	4
	GGT1B	2	4
	GGT2A	3	1
	GGT2B	2	2
Caenorhabditis elegans	FTA (GGT1A)	4	4
	FTB	5	6
	GGT1B	2	6
	GGT2A	4	9
	GGT2B	3	6
Arabidopsis thaliana	FTA (GGT1A)	3	5
	FTB	5	14
	GGT1B	2	11
	GGT2A	4	8
	GGT2B	3	9
Saccharomyces cerevisiae	FTA (GGT1A)	11	1
	FTB	4	1
	GGT1B	7	1
	GGT2A	10	1
	GGT2B	16	1

Characteristic structural features

The CaaX prenyltransferases FT and GGT1 and the non-CaaX prenyltransferase GGT2 share the same heterodimeric structure [1] (Figure 3). In the α subunits of both types of protein prenyltransferases, seven tetratricopeptide repeats are formed by pairs of helices (helices 2 to 15) that are stabilized by conserved intercalating residues. The α subunits of GGT2 in mammals and plants also have an immunoglobulin-like domain between the fifth and sixth tetratricopeptide repeat, as well as leucine-rich repeats at the carboxyl terminus. The functions of these additional domains in GGT2 are as yet undefined, but they are apparently not directly involved in the interaction with substrates and Rab escort proteins (see below) [18,19]. The tetratricopeptide repeats of the α subunit form a right-handed superhelix, which embraces the $(\alpha\text{-}\alpha)_6$ barrel of the β subunit [20]. The β subunits include most of the substrate- and lipid-binding pockets [20] and their tight association with the respective α subunits is required for proper function [21]. Compared with FT, GGT2 has a larger hydrophobic pocket in the β subunit to accommodate the longer lipid [22].

It is difficult to estimate the effect of alternative splicing on the structure of protein prenyltransferases. We would expect that the integrity of the structure of the β subunits would be more sensitive to non-terminal truncations than are the α subunits, because the modular structure of the

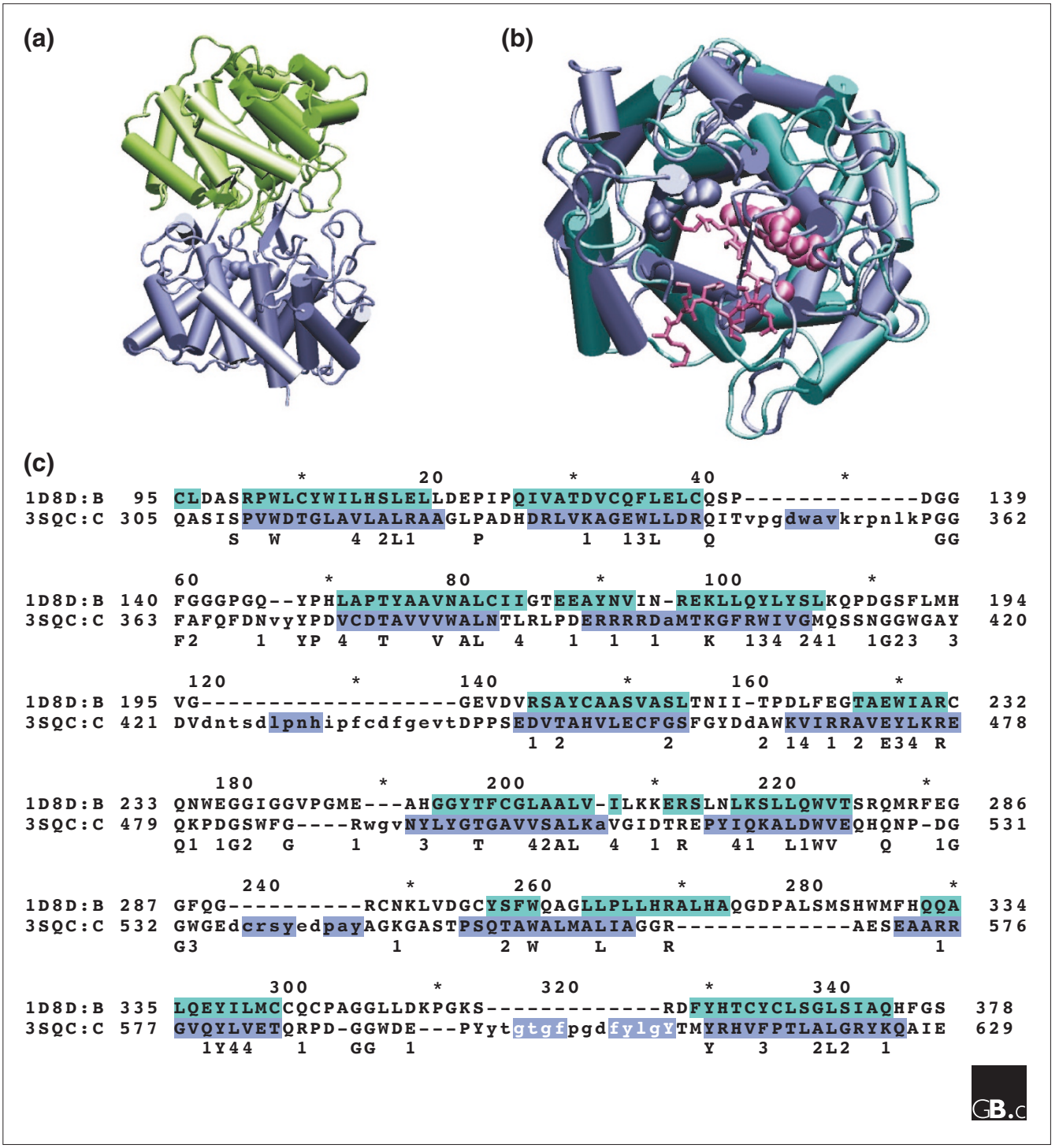


Figure 2

The structural relationship between protein prenyltransferase β subunits and squalene-hopene cyclases. **(a)** The complete structure of squalene-hopene cyclase from the bacterium *Alicyclobacillus acidocaldarius* (Protein Data Bank (PDB) identifier 3SQC [68]). **(b)** Structural superposition of the conserved $(\alpha\text{-}\alpha)_6$ barrels of squalene-hopene cyclase (blue) and the rat FT β subunit (cyan; see Figure 3 for the full structure of the FT). Blue spheres, functionally important residues in the half of the squalene-hopene cyclase that is shown (the sphere indicates the van der Waals radius); pink spheres, farnesyl-pyrophosphate and Zn^{2+} bound to the β subunit of FT; pink residues in stick representation, the Ca_1a_2X peptide of the substrate protein. **(c)** Alignment of the two proteins on the basis of the structural superposition. Secondary structural elements are colored as in (b); black residues with colored background represent α helix and white residues with colored background represent β sheet. Conserved residues and similar residues are shown below the alignment; 1, polar; 2, small; 3, aromatic; 4, hydrophobic. The lower-case letters represent residues within 3SQC that were not easily superimposed on the 1D8D structure (that is, gaps in 1D8D). The molecular representations were created using VMD [69].

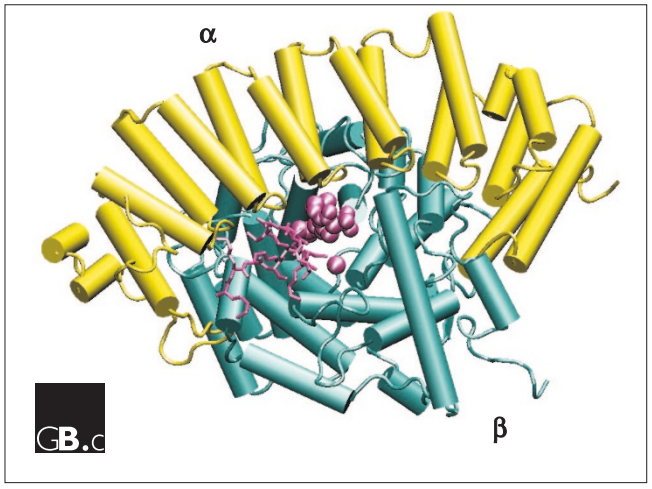


Figure 3
The complete structure of rat FT (PDB identifier 1D8D [70]). The spheres in the center represent the farnesyl-pyrophosphate and Zn^{2+}, and the amino acids in stick representation are the CaaX motif of the protein substrate. The molecular representation was created using VMD [69].

tetratricopeptide repeat motifs in the α subunits would allow truncations and additions without severe consequences for the hydrophobic packing of the structure.

Enzyme mechanism

CaaX prenyltransferases recognize the carboxy-terminal Ca_1a_2X motif (see Table 1) [23,24] of substrate proteins, usually after binding farnesyl-pyrophosphate [25] or geranylgeranyl-pyrophosphate [26]. The lipid anchors are then transferred by a catalytic mechanism that depends on formation of a complex between a Zn^{2+} cation and the cysteine of the Ca_1a_2X motif [20]. High concentrations of Mg^{2+} are required for optimal enzymatic activity of FT [27], though this is apparently not the case for GGT1 [28]. The Zn^{2+} is suggested to be required for the proper conformation of the substrate peptide [17]. The major conformational change in the transfer step seems to be a rotation of the prenylpyrophosphate in the binding pocket and not of parts of the enzyme itself. A detailed picture of the reaction pathway that involves electrophilic and nucleophilic mechanisms is given by a series of structures representing the different states [28] as well as by kinetic measurements [29].

In contrast to FT and GGT1, GGT2 does not require a very specific carboxy-terminal motif [30] apart from the availability of several cysteines close to the carboxyl terminus that are often arranged -CC, -CXC, -CCX, -CCXX, -CCXXX or, in a few cases, with only a single cysteine as in -CXXX. If the motif consists of two cysteines in close proximity, two geranylgeranyl moieties are usually added.

GGT2 recognizes the structural features of a complex of the substrate with an escort protein (Rab escort protein (REP), previously known as component A) and then scans the carboxyl terminus for prenylatable cysteines [30]. The catalytic mechanism of lipid transfer from geranylgeranyl-pyrophosphate to the protein substrate also requires Zn^{2+}, and the following model has been presented for how the double geranylgeranylation could take place on the basis of insights from the reaction pathway of FT [28]. After attachment of the first prenyl group, the lipid chain is translocated over the enzyme surface into another hydrophobic groove upon binding of the second geranylgeranyl-pyrophosphate. Finally, binding of a third geranylgeranyl-pyrophosphate releases the whole complex of the now doubly geranylgeranylated substrate with its escort protein; the escort protein is also involved in the transport of the substrate to the target membrane [31,32].

Localization and function

The results of systematic oligonucleotide microarray experiments catalogued in the human gene-expression index database [33,34] show that protein prenyltransferases are expressed in a variety of tissues. Both CaaX prenyltransferases seem to be active (that is, their α and β subunits are coexpressed) in a range of tissues, and the non-CaaX prenyltransferase subunits are also expressed in several more tissues. It should be noted, however, that many of the expression levels listed [33,34] are close to the detection threshold and could therefore result from cross-hybridization between close homologs rather than true expression. Interestingly, the α subunits also appear to be expressed in tissues that lack expression of the corresponding β subunits; this suggests that single subunits, or isoforms of them, might have additional, prenylation-independent functions in the cell.

As the α subunit of CaaX prenyltransferases is shared between FT and GGT1, its expression must be higher than that of each β subunit if it is to form 1:1 complexes with the β subunits of both enzymes. These higher expression levels seem to be transcriptionally regulated by different promoters; in order to produce recombinant CaaX prenyltransferases in the laboratory, it is thus necessary to downregulate expression of the α subunits when coexpressing with β subunits [35].

Lipid anchors are common posttranslational modifications that can direct the subcellular localization of proteins. Other lipid modifications, such as myristoylation [36-38], palmitoylation [39,40] and glycosylphosphatidylinositol (GPI) anchors [41,42], are mainly important for attachment of the protein to membranes, but lipid modification by protein prenyltransferases seems to have a more complex role: the farnesyl and geranylgeranyl moieties attached to the substrates are directly involved in protein-protein interactions as well as in protein-membrane interactions [43]. The importance of protein prenyltransferases is illustrated by the

involvement of their substrates in critical cellular pathways and diseases [44].

Substrates and functions of CaaX prenyltransferases

Typical substrates that are farnesylated by FT include many members of the Ras superfamily of small GTPases (H-Ras, K-Ras, N-Ras, Ras2, Rap2, RhoB (which is also geranylgeranylated), RhoE, Rheb, TC10, and TC21), as well as the nuclear lamina proteins lamin A and B, the kinetochore proteins CENP-E and CENP-F, fungal mating factors, cGMP phosphodiesterase α, γ subunit variants of G proteins, DnaJ heat-shock protein homologs, rhodopsin kinase, the peroxisomal membrane proteins Pex19 and PxF and paralemmin (a neural protein suggested to be involved in membrane dynamics). GGT1 preferentially geranylgeranylates some of the other small GTPases (such as Rac1, Rac2, RalA, Rap1A, Rap1B, RhoA, and RhoB (which is also farnesylated, as noted above), RhoC, Cdc42, Rab8 (which is also geranylgeranylated by GGT2), Rab11, and Rab13, as well as some γ-subunit variants of G proteins, cGMP phosphodiesterase β and the plant calmodulin CaM53. Typically, prenylation by CaaX protein prenyltransferases is accompanied by further posttranslational processing, most often involving cleavage of the carboxy-terminal tripeptide ($-a_1a_2X$) followed by carboxymethylation of the carboxyl terminus [45-47]. Palmitoylation is another modification that sometimes takes place after prenylation [48].

Because several prenylated substrates are involved in diseases, inhibition of protein prenyltransferases has great potential for medical applications. A boom in the field was triggered by the finding that inhibition of FT in mice that have tumors derived from H-Ras-transformed cells leads to tumor regression, while the inhibitor has no adverse effect on the organism [49]. This led to successful completion of clinical phase I trials of farnesyl transferase inhibitors (FTIs), but in phase II trials the efficacy of the inhibitors towards a broad spectrum of different cancer cells (such as K-Ras-transformed cells) was far below the high expectations that arose from the phase I trials. Surprisingly, however, beneficial effects were found for other, non-neoplastic diseases; for example, diabetic retinopathy and macular degeneration [50]. The unexpected physiological effects of FT inhibition are partly due to a striking cross-specificity between the two CaaX prenyltransferases: both FT and GGT1 can use either farnesyl-pyrophosphate or GGPP to a certain extent to transfer lipids to several of each others' substrates as well as their own [51,52], and several substrates can be either farnesylated or geranylgeranylated. The substrates probably compete *in vivo* for the enzymes loaded with the preferred polyprenyl-pyrophosphate, and the type of modification that is added depends on the relative affinity of the substrates for the enzymes.

In cancer cells, FTIs are known to affect growth in soft agar (anchorage-independent growth), cell-cycle progression at the G1-S phase and the G2-M phase checkpoints, the morphology of the cytoskeleton (formation of actin stress fibers) and induction of apoptosis. The substrate proteins that have been suggested to be involved in these effects include H-Ras [49], Rheb [53], CENP-E [54] and RhoB [55]; FTI effects are reviewed in more detail elsewhere [56]. The effects of FT inhibition in cells transformed with H-Ras differ from those on cells transformed with K-Ras. This difference has been attributed to the ability of GGT1 to alternatively prenylate some proteins, through the cross-specificity mentioned above, including K-Ras but not H-Ras (see Figure 4). RhoB can be both farnesylated and geranylgeranylated; Prendergast and colleagues [57] have suggested that the different levels of farnesylated and geranylgeranylated RhoB in FTI-treated cells compared with untreated cells could contribute to the observed effects of FTIs.

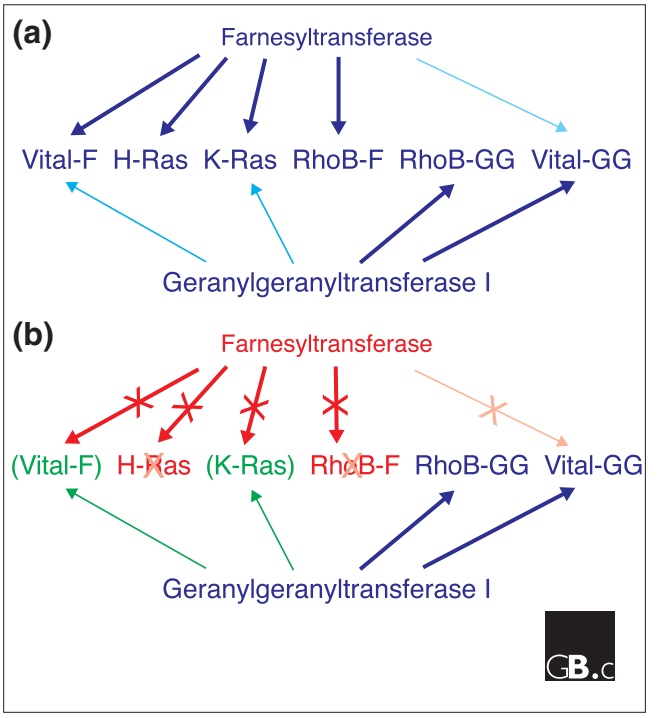


Figure 4
Differential prenylation of substrates of CaaX protein prenyltransferases **(a)** under normal conditions and **(b)** in the presence of a FT inhibitor. Substrates are shown in the middle of each panel, with the two enzymes above and below. 'Vital-F' and 'Vital-GG' represent all the proteins for which farnesylation (Vital-F) or geranylgeranylation (Vital-GG) are essential; RhoB-GG represents geranylgeranylated RhoB and RhoB-F represents farnesylated RhoB. Thick arrow, normal prenylation activity; thin arrow, reduced prenylation activity through cross-specificity of the non-preferred enzyme; arrows with crosses, blocked prenylation activity during inhibition of FT; brackets, substituting prenylation activity by GGT1 during inhibition of FT.

Substrates and functions of non-CaaX prenyltransferases

The main substrates for prenylation by GGT2 are the Rab family of proteins, the largest group of small GTPases in the

Ras superfamily. There are at least 60 different Rabs in humans [58]. They interact with the Rab escort protein REP, which is required for the prenylation of Rabs by GGT2 [30], and are involved in the docking of transport vesicles to their specific target membranes [59].

As with CaaX protein prenyltransferases, deficiencies in prenylation by non-CaaX protein prenyltransferases are relevant to diseases [59,60]. A mutation inactivating a start codon of the major transcript of the α subunit of GGT2 is one of the many mutations involved in the recessively inherited Hermansky-Pudlak syndrome and related disorders [61,62], in which platelet synthesis, platelet organelle function and pigmentation are affected. X-linked choroideremia (CHM) results in retinal degeneration, with symptoms starting from night blindness in young people and progressing over decades until vision is completely lost [63]. It is caused by loss-of-function mutations in the *CHM* gene, which encodes Rab escort protein 1 (REP1) [64]. Loss-of function mutations in the *Rab27a* gene cause Griscelli syndrome, whose symptoms are similar to Hermansky-Pudlak syndrome and other diseases associated with insufficient Rab prenylation [60].

Frontiers

There are several issues that merit further study in the regulation of protein prenyltransferases. Firstly, it is not clear how the concomitant transcription of the two subunits from two different chromosomes is regulated or where and how the subunits meet to build up functional prenyltransferases. Secondly, given that there are multiple splice variants, it is likely that additional variants of subunits will be found to have distinct functions or regulatory roles; an example is a variant of the FT/GGT1 α subunit that has been reported to be directly involved in signaling by transforming growth factor β and activin [65]. Interpretation of results in areas ranging from molecular biology to clinical trials must take into account possible isoforms with varying functions or altered interactions to avoid erroneous conclusions.

A third issue is the striking differences in gene size and intron length between the two types of protein prenyltransferases. One of several possible factors that could have caused this is a difference in evolutionary selection pressures. Whereas FT and GGT1 partly compensate each other functionally, there is no counterpart for GGT2. Furthermore, formation of a complex between the substrate and an escort protein is necessary for recognition by GGT2 and the conservation of additional binding sites at the surface is therefore required. Also, the severity of the effect when the prenylation of different substrates is abolished may vary. Finally, the size of the genomic region containing the gene might alter its accessibility to the transcription machinery and the time needed to complete transcription, so gene size may affect or be affected by expression levels. The implications of these factors for the exact evolutionary history of the protein prenyltransferase genes (such as the relative ages of the subunits and the order of duplication events) remain to be established.

Finally, more research is also needed on the effects of FTIs. After the rush to develop inhibitors, basic research is now needed as well as clinical trials in order to improve the understanding of the basic processes involved [66]. For example, it cannot be ruled out that some effects of FTIs are not a direct consequence of inhibiting prenylation but are instead due to cross-reactivity with proteins from completely different pathways. It is tempting to speculate that one of the proteins that are evolutionarily related to the protein prenyltransferases (such as other prenyltransferases) could be affected by FTIs; the selectivity of existing FTIs, which do not inhibit even the much more closely related GGTs, makes this scenario most unlikely, however. The next task is to identify clearly the proteins whose altered prenylation causes the observed effects of FT inhibition. Given the multiplicity and heterogeneity of these effects, it is clear that they cannot be attributed to one single farnesylated protein that lacks a lipid modification because of FT inhibition; rather, alterations in the function of several proteins probably cause the observed effects, with variations depending on the cell type, disease and organism. Further research may eventually lead to FTIs being used successfully to treat cancers and other diseases.

Acknowledgements

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