

Thematic review series: Lipid Posttranslational Modifications

Structural biology of protein farnesyltransferase and geranylgeranyltransferase type I

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Abstract More than 100 proteins necessary for eukaryotic cell growth, differentiation, and morphology require post-translational modification by the covalent attachment of an isoprenoid lipid (prenylation). Prenylated proteins include members of the Ras, Rab, and Rho families, lamins, CENPE and CENPF, and the γ subunit of many small heterotrimeric G proteins. This modification is catalyzed by the protein prenyltransferases: protein farnesyltransferase (FTase), protein geranylgeranyltransferase type I (GGTase-I), and GGTase-II (or RabGGTase). In this review, we examine the structural biology of FTase and GGTase-I (the CaaX prenyltransferases) to establish a framework for understanding the molecular basis of substrate specificity and mechanism. These enzymes have been identified in a number of species, including mammals, fungi, plants, and protists. Prenyltransferase structures include complexes that represent the major steps along the reaction path, as well as a number of complexes with clinically relevant inhibitors. Such complexes may assist in the design of inhibitors that could lead to treatments for cancer, viral infection, and a number of deadly parasitic diseases.—Lane, K. T., and L. S. Beese. **Structural biology of protein farnesyltransferase and geranylgeranyltransferase type I.** *J. Lipid Res.* 2006. 47: 681–699.

Supplementary key words prenyltransferase • isoprenoid • Ras • G protein • cancer target • drug design • farnesyltransferase inhibitor • crystal structure

More than 100 proteins necessary for eukaryotic cell growth, differentiation, and morphology require post-translational modification by the covalent attachment of an isoprenoid lipid (prenylation) (1). This modification is catalyzed by three protein prenyltransferases: protein farnesyltransferase (FTase) and protein geranylgeranyltransferase type I (GGTase-I), collectively termed the CaaX prenyltransferases, as well as protein GGTase-II (or RabGGTase) [reviewed in this series in (2)], whose substrates are limited to members of the Rab subfamily of G

proteins. FTase and GGTase-I transfer a 15 or 20 carbon isoprenoid [donated by farnesyl diphosphate (FPP) or geranylgeranyl diphosphate (GGPP)], respectively, to the cysteine of a C-terminal CaaX motif, defined by a cysteine (C) residue, followed by two small, generally aliphatic (a) residues, and the X residue, which contributes significantly to specificity (Fig. 1) (3–9). Kinetic assays and analysis of lipidated CaaX proteins purified from the cell demonstrate the general preference of FTase for methionine, serine, glutamine, or alanine and the preference of GGTase-I for leucine or phenylalanine in the X position. Here, we review the structural biology of FTase and GGTase-I; the biochemical properties of these enzymes have been reviewed extensively elsewhere (1, 10–15).

After covalent attachment of the isoprenoid in the cytoplasm, most CaaX proteins undergo two further prenylation-dependent processing steps at the endoplasmic reticulum (1): proteolytic removal of the aaX tripeptide by the CaaX protease Ras and a-factor-converting enzyme (Rce1), and carboxymethylation of the prenylcysteine residue by the enzyme Isoprenylcysteine carboxyl methyltransferase (Icmt). The fully processed proteins exhibit high affinity for cellular membranes and present a unique structure at their C termini that can serve as a specific recognition motif in certain protein-protein interactions (Fig. 2).

FTase and GGTase-I were first identified in 1990 and 1991, respectively (3, 16). Both enzymes have been identified in a number of species, including mammals (17–21), fungi (22–25), plants (26, 27), and protists (28, 29); the genes encoding FTase and GGTase-I have been cloned from several of these species (20, 30–35). These enzymes have been shown to be essential for the function of these organisms: elimination of these enzymes results in severe defects or lethality in many instances (22, 26, 36–38).

Abbreviations: FPP, farnesyl diphosphate; FTase, protein farnesyltransferase; FTI, farnesyltransferase inhibitor; GGPP, geranylgeranyl diphosphate; GGTase-I, geranylgeranyltransferase type I; GTI, geranylgeranyltransferase inhibitor; PDB ID, Protein Data Bank identifier.

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Manuscript received 17 January 2006 and in revised form 9 February 2006.

Published, JLR Papers in Press, February 13, 2006.

DOI 10.1194/jlr.R600002JLR200

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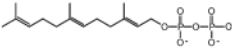
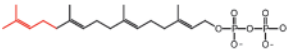
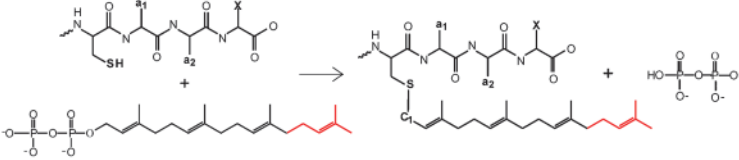
	Farnesyltransferase (FTase)	Geranylgeranyltransferase-I (GGTase-I)
Lipid donor substrate	Farnesyl diphosphate (FPP) – 15 carbon 	Geranylgeranyl diphosphate (GGPP) – 20 carbon 
Protein Recognition motif	CaaX X = Ala, Gln, Ser, Met Phe	CaaX X = Leu Phe, sometimes Met
Select protein substrates	Ras, nuclear lamins, Transducin γ subunit, Rhodopsin kinase, centromeric proteins	Rho, Rac, Rap, heterotrimeric G protein γ subunits
Subunit composition (mammalian)	48kDa (α) 46kDa (β)	<i>identical</i> <i>25% identity</i> 48kDa (α) 43kDa (β)
Metal requirements	Zn ²⁺ , Mg ²⁺ Zn ²⁺ required for catalysis and peptide binding	Zn ²⁺
Reaction		

Fig. 1. Biochemistry of the CaaX prenyltransferases. Ala, alanine; Gln, glutamine; Leu, leucine; Met, methionine; Phe, phenylalanine; Ser, serine.

Since the initial discovery of farnesylated fungal proteins, many more proteins have been demonstrated to contain a prenyl modification, including >100 human proteins. These include members of the Ras (39–42), Rho (43–47), Rac (48), Rap (49, 50), and Rab (51, 52) families, the γ subunit of heterotrimeric G proteins (53, 54), centromeric proteins (55), and many other proteins involved in a variety of cell signaling pathways controlling the cell cycle and apoptosis (56, 57) as well as glycogen metabolism (58), cellular framework (59–61), and visual signal transduction (53, 62–64). Defects in these proteins are associated with a number of human diseases, including Batten disease (65), autosomal recessive retinitis pigmentosa and autosomal dominant retinitis pigmentosa (66–68), Canavan disease (69), Emery-Dreifuss muscular dystrophy type 2 (70–72), and a variety of human cancers. Some of the most studied CaaX proteins are members of the Ras family, mutations in which occur in ~20–30% of all human tumors, making the CaaX prenyltransferases targets for inhibition in cancer therapeutics (73, 74) (see below).

STRUCTURAL CHARACTERIZATION OF THE CaaX PRENYLTRANSFERASES

Over the past decade, high-resolution crystal structures of FTase and GGTase-I complexed with various substrates, products, and inhibitors have advanced our understanding of these enzymes. Structures representing each of the major steps along the reaction pathway of CaaX prenyl-

transferases have been determined (**Fig. 3**) (75, 76). Kinetic studies suggest an ordered binding mechanism in FTase, in which FPP binds to the apo enzyme first, followed by the CaaX substrate (77–80). The release of farnesylated product is the rate-limiting step of the reaction and occurs only in the presence of excess of either substrate, particularly isoprenoid diphosphate (3, 8, 78, 81, 82). This reaction cycle effectively maintains FTase in a substrate- or product-bound complex at all times. Although the GGTase-I reaction has not been studied in such detail, available evidence indicates that it proceeds through a similar pathway (83, 84).

Overall enzyme structure

FTase and GGTase-I exist as $\alpha\beta$ heterodimers and share a common α subunit (48 kDa molecular mass) (16, 30) and a homologous β subunit (46 kDa in FTase, 42 kDa in GGTase-I) (3, 19, 21, 31). Both α and β subunits are composed primarily of α helices, which are arranged in a crescent-shaped superhelix (α subunit) wrapped around an α - α barrel (β subunit) (**Fig. 4A, B**) (76, 85).

The structure of the common α subunit is almost identical in both FTase and GGTase-I, with only slight shifts attributable to differences in the interactions with the divergent β subunit. The helices of the α subunit are folded into seven successive pairs, in a series of right-handed antiparallel coiled coils. These “helical hairpins” are arranged in a crescent-shaped superhelix that wraps around the β subunit. The N-terminal domain (~50

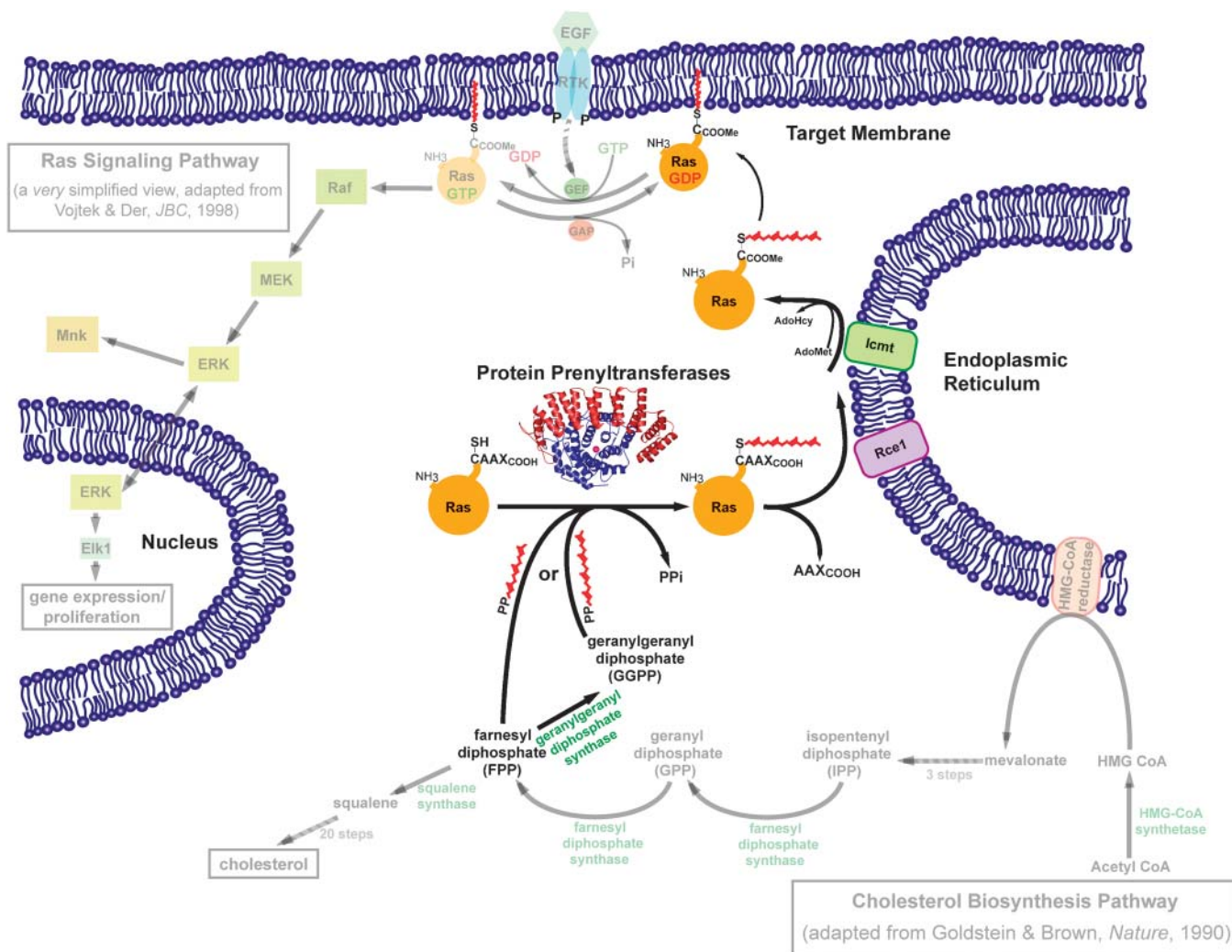


Fig. 2. CaaX prenylation and the eukaryotic cell cycle. The prenylation of CaaX proteins (shown here is Ras, in yellow, as an example) by protein prenyltransferases (red and blue ribbon diagram) and further processing by Rce1 (purple) and Icmt (green) are depicted. Also shown is the cholesterol biosynthesis pathway [adapted from (178)], from which the isoprenoid diphosphate substrates farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) are derived. A simplified depiction of the Ras signaling pathway, after its insertion into the membrane via its lipid tail, is also illustrated [adapted from (179)]. EGF, epidermal growth factor; GEF, guanine exchange factor; HMG CoA, 3-hydroxyl-3-methylglutaryl Coenzyme A; Icmt, isoprenylcysteine carboxyl methyltransferase; MEK, mitogen-activated protein (MAP) or extracellular signal-regulated kinase (ERK) kinase; Mnk, MAP kinase-interacting kinase; Rce1, Ras- and a-factor-converting enzyme; RTK, receptor tyrosine kinase.

amino acids) is disordered in all complexes of FTase and GGTase-I. It has been suggested that this proline-rich domain may interact with other cellular factors, perhaps functioning in enzyme localization, as deletion of this domain does not affect the catalytic activity (86) or the structure of the rest of the protein (87). In the absence of such partners, this domain may be unfolded.

Although the β subunits of FTase and GGTase-I share only 25% sequence identity, they have very similar structures, consisting of 14 α helices in FTase and 13 in GGTase-I. Twelve of the α helices are folded into an α - α barrel (Fig. 4C). Six parallel helices (3β , 5β , 7β , 9β , 11β , and 13β) create the core of the barrel. The other six helices (2β , 4β , 6β , 8β , 10β , and 12β) are parallel with one another and antiparallel with respect to the core helices and form the outside of the barrel. In both enzymes,

one end of this barrel is blocked by a loop containing the C-terminal residues of the β subunit, whereas the opposite end is open to the solvent. This arrangement creates a deep, funnel-shaped cavity in the center of the barrel, with an approximate inner diameter of 15 Å and a depth of 14 Å. This cavity is hydrophobic in nature and lined with a number of conserved aromatic residues. The active sites of both FTase and GGTase-I are located within this cavity.

The α and β subunits form an extensive interface, burying $>3,000 \text{ \AA}^2$, or $\sim 20\%$, of accessible surface area of each subunit (76, 85). The α/β interfaces of both FTase and GGTase-I have lower hydrophobic character than is observed in most subunit interfaces (88). The unusually high polar/charged residue content results in nearly double the typical number of hydrogen bonds.

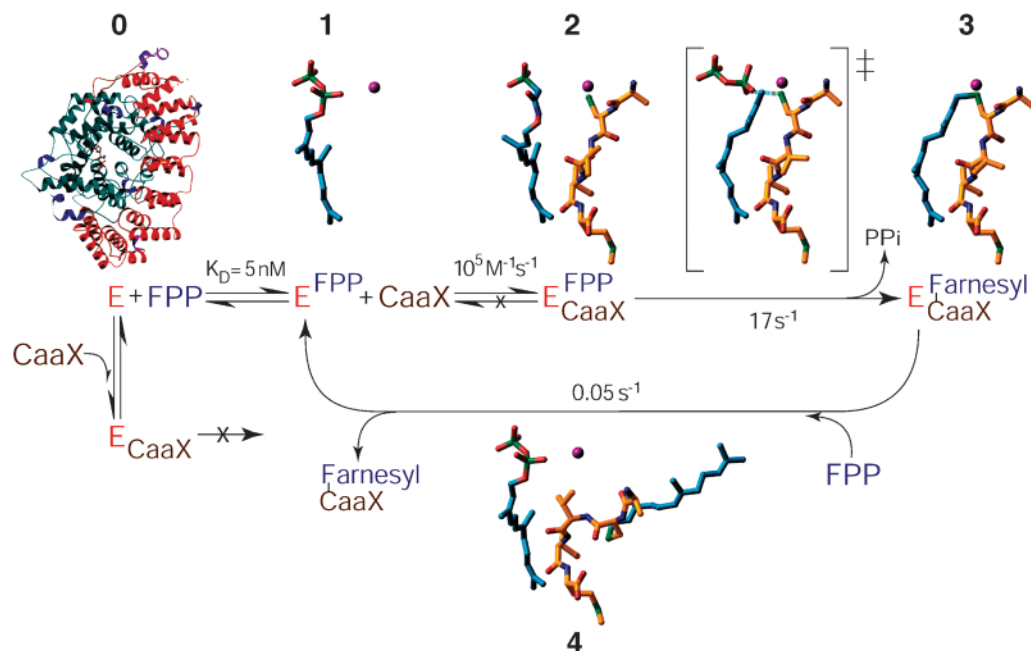


Fig. 3. Structures along the protein farnesyltransferase (FTase) reaction path. Crystallographic studies (75, 85, 95, 99) have produced structures representing the major steps along this path. In each of these complexes, the enzyme acts as a rigid scaffold; therefore, for clarity, only the substrates and products are shown as they bind in the active site. The path begins with the unliganded (apo) enzyme (0) [Protein Data Bank identifier (PDB ID) 1FT1], with the α subunit shown in red and the β subunit in blue. The FPP molecule binds to form a binary substrate complex (1) (PDB ID 1FT2), followed by binding of the CaaX substrate to form a ternary substrate complex (2) (PDB ID 1D8D). The resulting farnesylated product remains bound in the active site (3) (1KZP). Excess substrate, particularly FPP, facilitates product displacement, demonstrated by a complex in which the new FPP molecule and the partially displaced product are bound simultaneously (4) (1KZO). The double dagger symbol indicates a modeled transition state along the reaction coordinate between 2 and 3 (see Fig. 12 for a more detailed view of the transition state). Throughout this figure, the isoprenoid is shown in blue, the CaaX in yellow, and the catalytic zinc ion in magenta. Also shown are the kinetic parameters determined for this reaction (3, 8, 77–81). Reproduced with permission from (75).

Zinc binding site

Both FTase and GGTase-I are zinc metalloenzymes, binding one Zn^{2+} ion per protein dimer (89, 90). Crystal structures of FTase and GGTase-I clearly indicate the presence of a single Zn^{2+} ion bound to the β subunit near the subunit interface (76, 85). In both enzyme structures, the Zn^{2+} ion is coordinated by three strictly conserved residues: D297 β , C299 β , and H362 β in FTase and D269 β , C271 β , and H321 β in GGTase-I. Mutagenesis studies performed on FTase are consistent with Zn^{2+} coordination by residues D297 β , C299 β , and H362 β (91–93) and additionally implicate D359 β in the binding of zinc (92). Crystal structures of FTase (85, 87) reveal a stabilizing hydrogen bond between D359 β and the zinc ligand H362 β , consistent with a secondary coordination shell effect exerted by the former. A similar interaction is observed in GGTase-I (76).

In crystal structures of FTase (94–97) and GGTase-I (76, 97), the CaaX cysteine thiol(ate/ether) is observed coordinating the zinc, consistent with biochemical studies that indicate that the Zn^{2+} ion is required for catalytic activity and that it coordinates the cysteine thiol of the CaaX substrate (12, 19, 32, 81, 89). Comparison of zinc coordina-

tion from high-resolution substrate [1.8 Å (97)] and product [1.65 Å (K. L. Terry et al., unpublished observations)] complexes indicates that the CaaX cysteine thiol(ate/ether) forms a short (2.3 Å) interaction with the zinc ion in substrate complexes and a slighter longer (2.6 Å) interaction in the product complex (**Fig. 5**). The complete zinc coordination sphere for the substrate complex has four close interactions (D297 β , 2.0 Å; C299 β , 2.3 Å; H362 β , 2.1 Å; CaaX cysteine, 2.3 Å) and one longer interaction (D297 β , 2.6 Å; making this residue a bidentate ligand). The prenylated peptide product complex has a similar coordination sphere (D297 β , 2.0 and 2.4 Å; C299 β , 2.3 Å; H362 β , 2.2 Å; CaaX prenyl cysteine, 2.6 Å). Extended X-ray absorption fine structure (EXAFS) data (98) confirm the short interactions with the zinc ion seen in these crystal structures. Longer interaction distances, such as the bidentate interaction of D297 β , in substrate complexes appear to be sufficiently weak to not be detected by this technique.

Isoprenoid diphosphate binding in binary complexes

In the cocrystal structures of FTase and GGTase-I with FPP and GGPP prenyl substrate, respectively [Protein Data Bank identifiers (PDB IDs) 1FT2 and 1N4P], the iso-

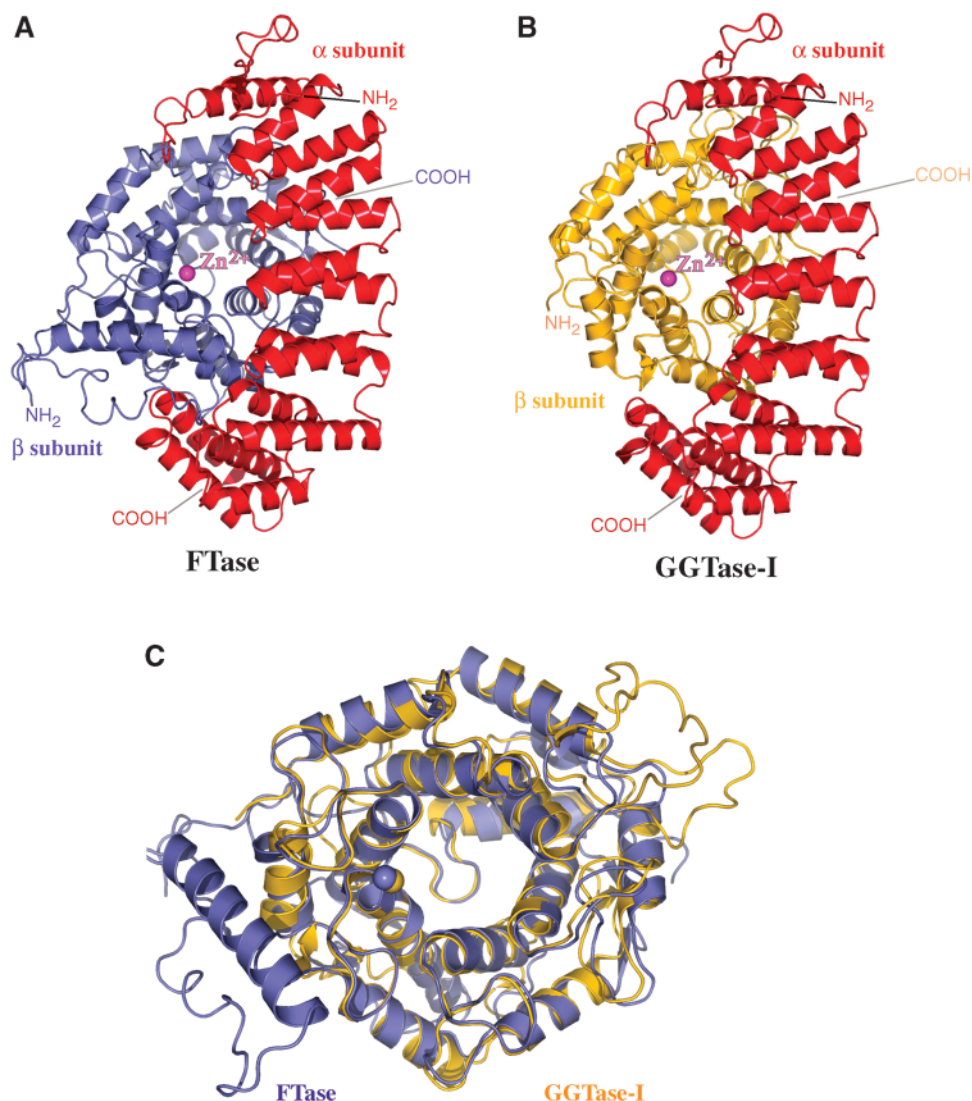


Fig. 4. Structures of the CaaX prenyltransferases. Overall structures of FTase (A) and geranylgeranyltransferase type I (GGase-I) (B), with the α subunit shown in red, the β subunit in blue and yellow, respectively, and the catalytic zinc ion in magenta. C: Superposition of FTase (blue) and GGase-I (yellow) demonstrates the structural homology of the β subunit of these enzymes.

prenoid binds along one side of the hydrophobic cavity of the β subunit α - α barrel (Fig. 6A, B; complex 1 in Fig. 3) (76, 99). The diphosphate moiety of both FPP and GGPP binds in a positively charged cleft at the rim of the α - α barrel near the α / β subunit interface. This group forms several hydrogen bonds in each enzyme (K164 α , H248 β , R291 β , and Y300 β in FTase; K164 α , H219 β , R263 β , K266 β , and Y272 β in GGase-I). The farnesyl portion of FPP binds in an extended conformation along one side of the hydrophobic cavity of the α - α barrel, interacting with a number of conserved aromatic residues. The first three isoprene units of GGPP bind in a similar conformation (with similar hydrophobic interactions with aromatic residues in the GGase-I active site), but the fourth isoprene unit is turned $\sim 90^\circ$ relative to the rest of the molecule. This conformation is significantly different from that of the other known structures with bound isopren-

oids [i.e., FTase (99), RhoGDP dissociation inhibitor (GDI) (47), and the phosphatidylinositol-3-OH kinase-1 (PI3K) complex (100)], in which the isoprenoid is completely extended. Superposition of GGase-I and RabGGase (101) by alignment of all enzyme C_α atoms illustrates the similarity of their respective GGPP binding sites; the 24 residues surrounding the GGPP molecule in GGase-I are identical or structurally conservative substitutions in RabGGase. The high degree of homology in the prenyl binding site indicates a similar mode of binding for the GGPP molecule in RabGGase.

Isoprenoid diphosphate substrate specificity: the ruler hypothesis

CaaX prenyltransferase activities are highly selective for their isoprenoid diphosphate substrates: FTase for FPP and GGase-I for GGPP. Binding studies demonstrate,

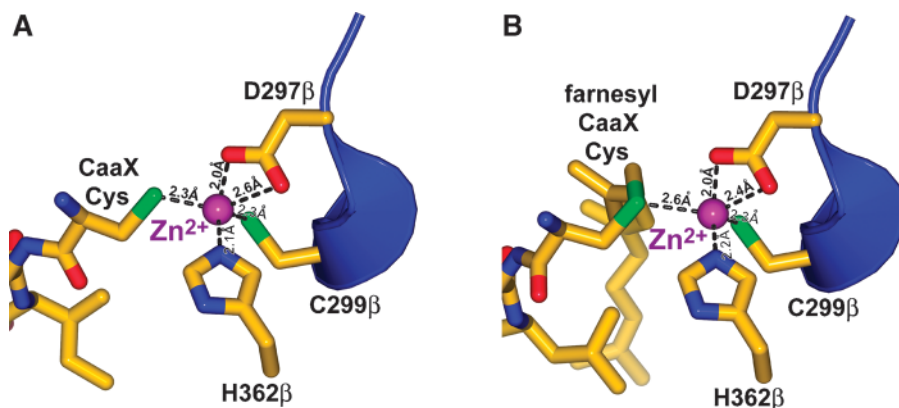


Fig. 5. Zinc coordination in FTase. The catalytic zinc ion (magenta) is shown in a ternary substrate complex (A) (PDB ID 1TN6) and a farnesylated peptide-product complex (B), interacting with three enzyme residues (D297 β , C299 β , and H362 β) as well as the cysteine (Cys) of the CaaX motif. Note the longer interaction distance between the Zn²⁺ ion and the CaaX cysteine in the product complex.

however, that FTase can bind both FPP and GGPP with nanomolar affinity, yet the enzyme is unable to transfer the geranylgeranyl group to substrate proteins (17, 78, 79, 89, 102), indicating that GGPP is an inhibitor of FTase. GGTase-I similarly binds both FPP and GGPP, although it binds FPP with somewhat lower affinity (79, 103). Unlike FTase, GGTase-I can (mis)transfer a farnesyl group to substrate proteins, but with reduced efficiency (83).

Superposition of FTase and GGTase-I in binary isoprenoid substrate complexes by aligning all enzyme C α atoms illustrates the similarity of binding of FPP and GGPP in their respective enzymes (**Fig. 7A**) (76). There is a slight divergence in the binding of the first isoprene unit, but the second and third units are essentially indistinguish-

able. The binding sites for GGPP in GGTase-I and FPP in FTase are very similar, constituting a cavity lined with conserved aromatic residues. The primary differences are at the bottom of this cavity, where the fourth isoprene unit of GGPP binds in GGTase-I. Here, FTase has the bulky tryptophan and tyrosine residues (W102 β and Y365 β), whereas the corresponding positions in GGTase-I (and RabGGTase) are occupied by the smaller residues threonine and phenylalanine to accommodate the fourth isoprene unit of the GGPP molecule, which cannot fit in FTase. Therefore, FTase and GGTase-I can function as simple length-discriminating molecular rulers. This hypothesis was tested by introducing mutations into FTase that are predicted to convert it to a geranylgeranyltransferase

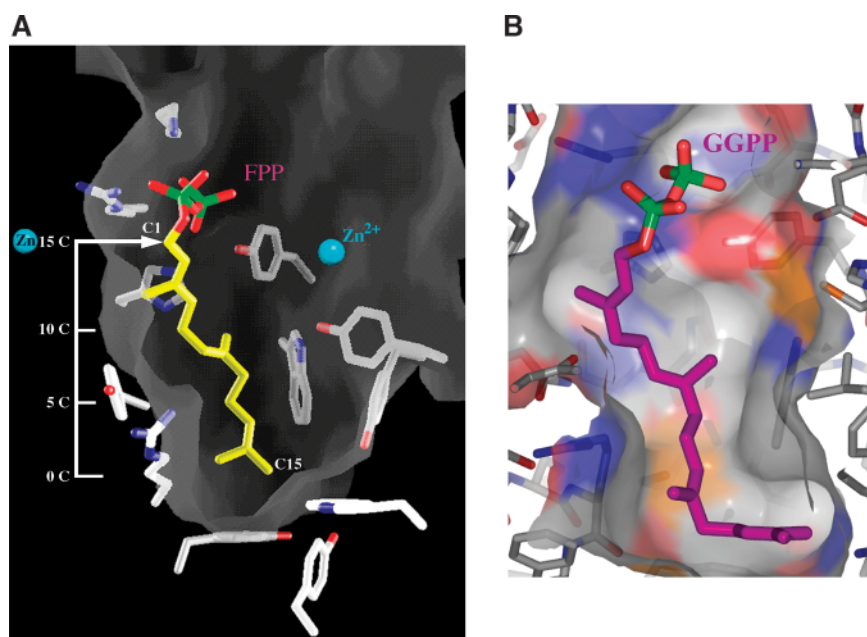


Fig. 6. Isoprenoid diphosphate binding in the CaaX prenyltransferases. FPP (yellow) in FTase (PDB ID 1FT2) (A) and GGPP (purple) in GGTase-I (PDB ID 1N4P) (B) bind along one side of the hydrophobic pocket. Reproduced with permission from (99).

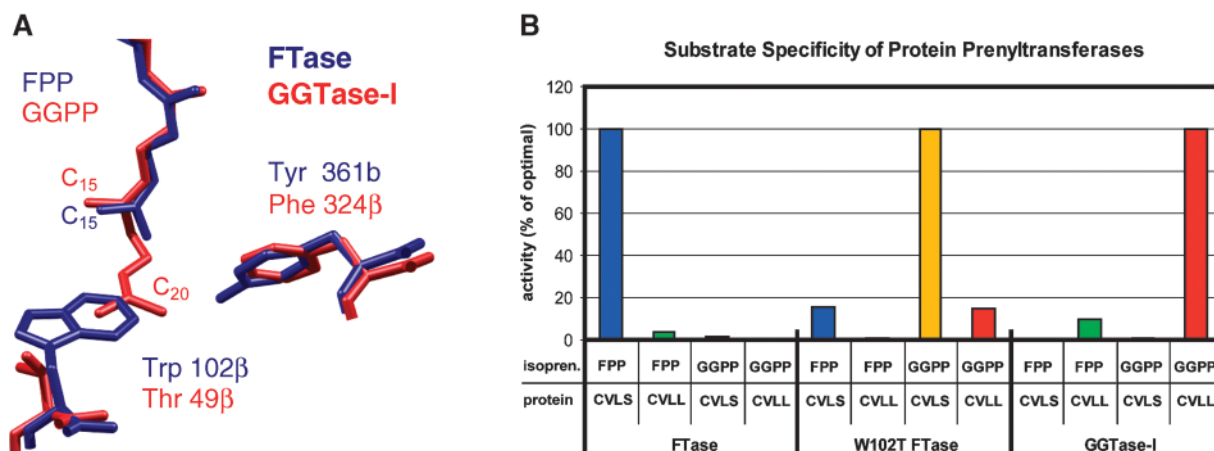


Fig. 7. CaaX prenyltransferase isoprenoid diphosphate substrate binding pocket. A: Comparison of isoprenoid binding in FTase (PDB ID 1KZ0) and GGTase-I (PDB ID 1N4P). In FTase (red), the larger tryptophan (Trp) fills the space where the fourth isoprene binds in GGTase-I (blue) and is one of the primary determinants of isoprenoid specificity. Thr, threonine; Tyr, tyrosine. B: Altered substrate specificity of the W102T mutant. Prenylation reactions assayed the activity of human wild-type FTase, wild-type GGTase-I, and the W102T mutant with four substrate combinations: FPP + Ras-CVLS (blue), FPP + Ras-CVLL (green), GGPP + Ras-CVLS (yellow), and GGPP + Ras-CVLL (red). Reproduced with permission from (76).

(76). A mutation at the W102 β residue of FTase to a threonine (the corresponding residue in GGTase-I) (W102T mutant) was created. Steady-state activity assays indicated that this single mutation results in an FTase enzyme greatly preferring GGPP over FPP as its isoprenoid substrate without significantly altering CaaX sequence specificity (Fig. 7B).

Peptide and isoprenoid binding in ternary complexes

Crystal structures of the CaaX prenyltransferases with bound peptide substrates and nonreactive isoprenoid analogs revealed the conformation of the bound CaaX substrate (complex 2 in Fig. 3) (76, 94, 95). Each of these FTase and GGTase-I complexes includes a peptide derived from the K-Ras4B (CVIM Ca₁a₂X sequence; PDB IDs 1D8D and 1QBQ) or Rap2b (CVIL Ca₁a₂X sequence; PDB ID 1N4Q) protein substrate, respectively, taking advantage of the ability of these enzymes to prenylate peptides as short as the CaaX tetrapeptide (3, 6, 18). The structure of the enzyme in these complexes is essentially identical to the previously determined structures, with the exception of a few minor side chain rearrangements in the vicinity of the diphosphate binding site compared with the unliganded FTase structure (PDB ID 1FT1) (85).

In each of these structures, the isoprenoid forms a large part of the CaaX sequence binding surface, with its second and third isoprene (and fourth, in the GGPP analog) units in direct van der Waals contact with the CaaX motif, particularly the a₂ and X residues (Fig. 8A, B). The peptide substrate sandwiches the isoprenoid against the wall of the hydrophobic cavity, burying 115 and 140 Å² (in FTase and GGTase-I, respectively) of accessible isoprenoid diphosphate analog surface area and completely sequestering the third isoprene unit from the bulk solvent. The location and conformation of the nonreactive isoprenoid analogs used in these studies are similar to those observed in the

binary complex with FPP and GGPP. Furthermore, the enzyme residues that interact with the isoprenoid diphosphate substrate molecule were also observed to interact with the analogs. These structures, therefore, are consistent with the ordered binding pattern observed by kinetics in which isoprenoid diphosphate binding precedes peptide binding (77, 78).

In the FTase and GGTase-I ternary substrate complexes, the peptide Ca₁a₂X motif binds in an extended conformation in the hydrophobic cavity of the β subunit. As predicted from spectroscopic studies (81), the cysteine residue of the peptide Ca₁a₂X sequence directly coordinates the zinc ion (Fig. 5). The side chain of the peptide valine residue (position a₁) points into solvent without making any hydrophobic interactions with the isoprenoid. The isoleucine side chain of the peptide substrate (a₂) is positioned in close proximity to the isoprenoid, making a hydrophobic interaction, whereas the backbone carbonyl oxygen of this residue participates in a hydrogen bond with the enzyme (R202 β in FTase, R173 β in GGTase-I). The C-terminal carboxylate of the X residue (methionine in FTase, leucine in GGTase-I) forms hydrogen bonds with Q167 α and a buried water molecule coordinated by three enzyme residues (H149 β , E198 β , and R202 β in FTase; H121 β , E169 β , and R173 β in GGTase-I). The residue in position X is also in van der Waals contact with a number of enzyme residues (Y131 α , A98 β , S99 β , W102 β , H149 β , A151 β , and P152 β in FTase; T49 β , H121 β , A123 β , and F174 β in GGTase-I). These interactions bury a large portion of the accessible surface area of the residue in position X. As noted, the only directional, bonded interactions between the peptide CaaX sequence and the protein are the Zn²⁺-to-cysteine thiol(ate) interaction and the hydrogen bonds involving the carbonyl oxygen of the a₂ isoleucine residue and the C-terminal carboxylate. Together, these interactions help anchor the CaaX peptide into its observed substrate binding conformation.

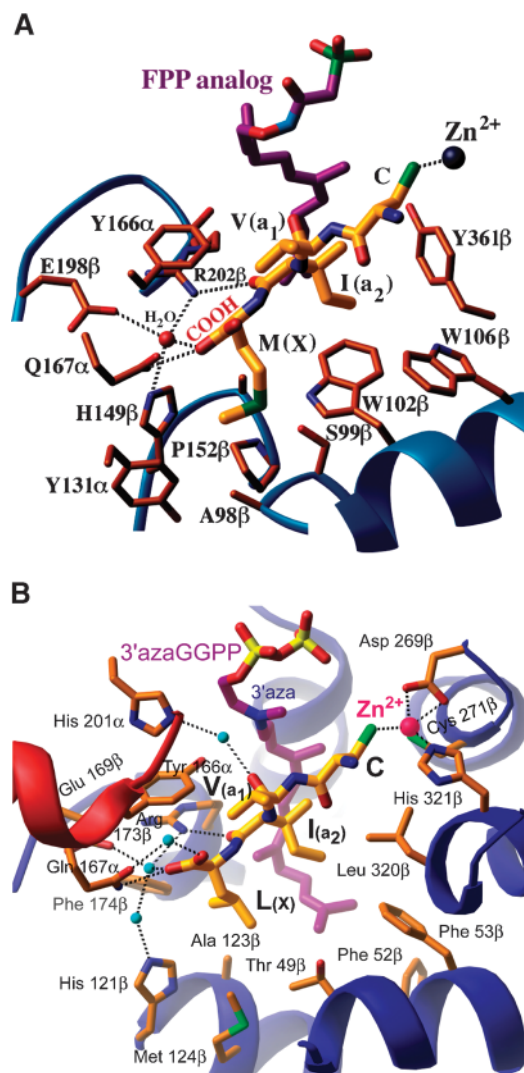


Fig. 8. CaaX substrate binding in the CaaX prenyltransferases. CaaX substrates bind in FTase (A) and GGTase-I (B) opposite the isoprenoid diphosphate substrate (PDB IDs 1D81 and 1N4Q). Also shown are the residues of the enzymes interacting with the CaaX motif, as well as the isoprenoid diphosphate analog (purple) and the catalytic zinc (dark blue in FTase, magenta in GGTase-I). Asp, aspartic acid; Glu, glutamic acid; His, histidine. Reproduced with permission from (76, 95).

Further insight into the role of zinc in peptide substrate binding is derived from a crystal structure of zinc-depleted rat FTase with bound FPP analog and a K-Ras4B-derived peptide (PDB ID 1D8E) (95). The overall structure of the enzyme is essentially identical to the other FTase structures, proving that zinc is not required to maintain the proper three-dimensional fold of FTase. Furthermore, the conformations of the zinc ligands (D297 β , C299 β , and H362 β) remain largely undisturbed. Both the FPP analog and peptide are observed bound in the active site without zinc. The FPP analog conformation is unchanged from the other ternary complexes, supporting the observation that zinc is not required for FPP binding (89). In this complex, the a_2 and X residues of the CaaX peptide bind in the same manner as in the presence of zinc, whereas the cysteine and valine residues bind much differently, forming a β -turn. The cysteine thiol(ate) has shifted ~ 9 Å from its position when in coordination of the zinc ion. This altered CaaX binding conformation of the peptide in the absence of zinc indicates that it is the zinc-thiol(ate) interaction that anchors the cysteine residue, holding the peptide in an extended conformation. This observation is consistent with the finding that peptide affinity is greatly enhanced in the zinc metalloenzyme (89). This zinc-depleted structure also indicates that, although not strictly required for peptide binding, the zinc ion is required both to orient the cysteine thiol(ate) for catalysis and to stabilize a productive peptide substrate conformation.

CaaX protein substrate specificity

FTase and GGTase-I possess distinct but overlapping protein substrate specificities that are determined by the degenerate Ca_1a_2X sequence motif, recognizing a number of protein substrates with a variety of sequences (3–9). To identify the basis for peptide selectivity by the CaaX prenyltransferases, structures were determined of FTase and GGTase-I complexed with the appropriate isoprenoid diphosphate analogs and a collection of specific and cross-reactive peptide substrates, including sequences derived from the C terminus of the oncoproteins K-Ras4B (CVIM; PDB ID 1TNO in GGTase-I), H-Ras (CVLS; PDB ID 1TN8 in FTase), and TC21 (CVIF; PDB IDs 1TN7 in FTase and 1TNB in GGTase-I), the signal transduction proteins Rap2a (CNIQ; PDB ID 1TN6 in FTase), RhoB (CKVL; PDB ID 1TNU in GGTase-I), and Cdc42 (CVLL; PDB ID 1TNZ in GGTase-I), and the heterotrimeric G protein γ_2 subunit (CAIL; PDB ID 1TNY in GGTase-I) (97).

CaaX protein substrate specificity

A superposition of these ternary complexes by aligning all C_α atoms (**Fig. 9**) reveals that the CaaX portion of all protein substrates adopts a common binding mode in the FTase and GGTase-I active sites and suggests a series of rules that define peptide substrate selectivity for the CaaX prenyltransferases. The presence of two fixed anchor points within the recognition motif (cysteine and C terminus) that make specific interactions with the enzyme discriminates against peptides that are either too long or too short (i.e., only tetrapeptides) or that lack a cysteine at the correct position. The a_1 residue is solvent-exposed and can accommodate any amino acid, as observed in solution studies (6, 76, 94, 95). Both the a_2 and X positions are buried in the active site; consequently, they are the major determinants of peptide selectivity through steric and electrostatic complementarity between the amino acid side chains and enzyme residues.

In these structural studies, it was observed that the binding sites on FTase and GGTase-I that recognize the a_2 peptide residue have unique steric and aromatic properties, suggesting that the a_2 residue may affect Ca_1a_2X recognition. The structures indicate that the a_2 binding site can accommodate a variety of amino acids, including valine, isoleucine, leucine, phenylalanine, tyrosine, proline, threonine, and methionine, consistent with previous studies of FTase peptide selectivity in mammalian and

FTase Ca₁a₂X Peptides

Rap2A (CNIQ)
KRAS (CVIM)
TC21 (CVIF)
HRas (CVLS)

GGTase-I Ca₁a₂X Peptides

RhoB (CKVL)
KRAS (CVIM)
TC21 (CVIF)

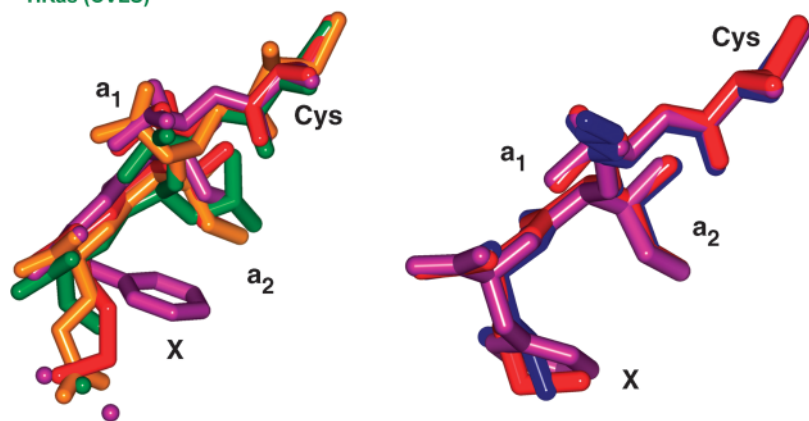


Fig. 9. Comparison of Ca₁a₂X substrate binding in FTase and GGTase-I. Superpositions of four FTase substrate complexes (left panel) and three GGTase-I substrate complexes (right panel) demonstrate that cognate and cross-reactive peptides adopt a common binding mode (PDB IDs 1D81, 1TN6, 1TN7, 1TN8, 1TNO, 1TNU, and 1TNB). In FTase, however, the C-terminal phenylalanine residue of TC21 binds in a different pocket than the C-terminal methionine, glutamine, and serine residues. Reproduced with permission from (97).

yeast systems (6, 104). Comparison of these sites illustrates the increased aromatic character of FTase relative to GGTase-I, which has subtle effects on CaaX specificity but a more marked effect on inhibitor binding (see below) (Fig. 10).

The X position is the primary sequence determinant that specifies whether a peptide is a substrate for FTase,

GGTase-I, both, or neither. The structures reveal that FTase and GGTase-I have different X-residue binding pockets, each with distinct electrostatic properties. The X-residue binding pocket in FTase is more polar and accepts the residues methionine, serine, and glutamine (as well as alanine, threonine, and cysteine to a lesser extent). This pocket in GGTase-I has more hydrophobic character and accepts leucine and phenylalanine (as well as isoleucine and valine to a lesser extent). Both pockets discriminate against bulky amino acids such as tyrosine, tryptophan, and arginine through steric complementarity. Interestingly, this study also revealed a previously unobserved secondary X residue binding site in FTase, in which the C-terminal phenylalanine residue of the TC21-derived peptide binds. This pocket may also accommodate leucine, asparagine, or histidine.

Together, these structures outline a series of rules that govern substrate peptide selectivity. These rules were used to identify potential protein substrates within the human genome, including a number of possible CaaX prenyltransferase substrates not identified previously.

Product formation

In both FTase and GGTase-I, the structures of the product complex (complex 3 in Fig. 3) (PDB IDs 1KZP and 1N4R) and ternary substrate complexes are virtually identical (75). There are no observed changes in the polypeptide backbone; differences are confined to changes in side chain rotamers of those active site residues that interact with the diphosphate moiety of the isoprenoid diphosphate substrate and are a consequence of the pyrophosphate product release. Equally, the peptide exhibits no appreciable differences between its reactant and product structures (Fig. 11A). The isoprenoid, however, moves toward the peptide in the product structure, with the C₁ atom nearly 7 Å closer to the Zn²⁺ ion compared with its position as a substrate. This motion is localized, as the third (and fourth, in the geranylgeranyl moiety) isoprene unit retains its substrate binding position; only the first and

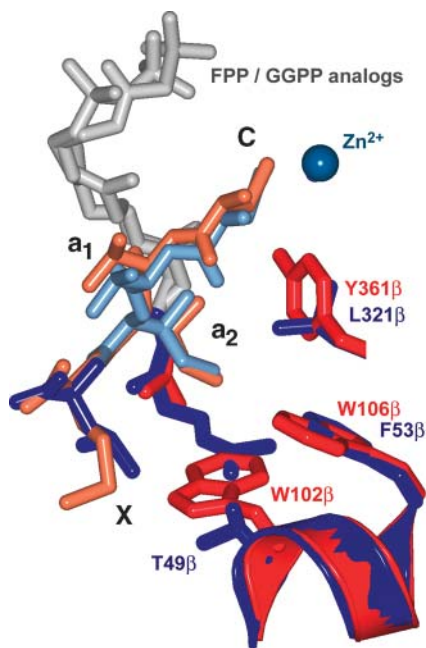


Fig. 10. Comparison of the a₂ binding site in FTase and GGTase-I. Superposition of FTase and GGTase-I shows residues that interact with the a₂ residue of the Ca₁a₂X motif (PDB IDs 1D81 and 1N4Q). Regions of the FTase ternary complex forming the a₂ binding site (enzyme residues W102β, W106β, and Y361β and isoprene 3) are colored red. Corresponding regions of the GGTase-I ternary complex (enzyme residues T49β, F53β, and L321β, isoprenes 3 and 4, and substrate peptide X residue) are colored blue. Portions of the FPP and GGPP analogs (gray) and of the CVIM (pink) and CVIL (light blue) peptides not forming the a₂ site are also shown. Reproduced with permission from (97).

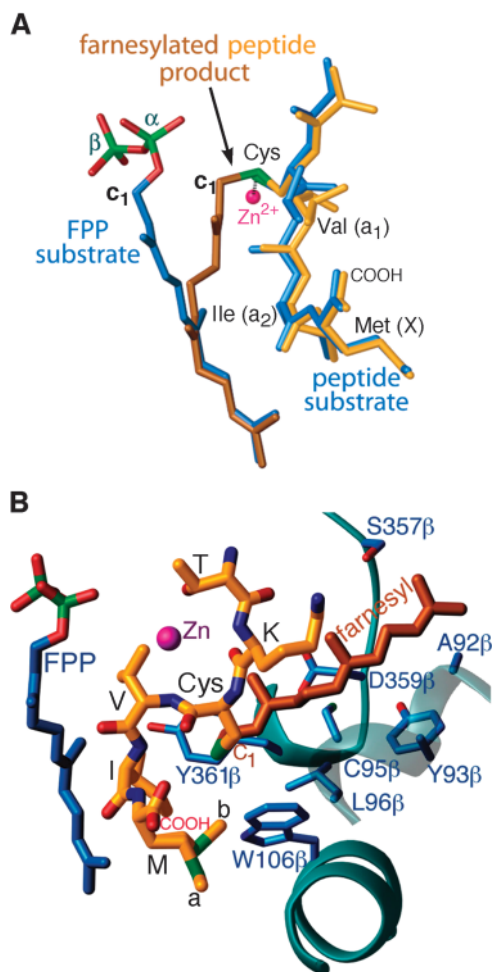


Fig. 11. Comparison of the binding of substrates and products in the CaaX prenyltransferases. **A:** In FTase, FPP (dark blue) rotates through the second isoprene unit to close the distance between the C1 atom and the CaaX substrate (light blue) (PDB ID 1D8D) to form a farnesylated peptide product (brown and yellow) (PDB ID 1KZP). Ile, isoleucine; Val, valine. **B:** Simultaneous binding of FPP and translocated product in FTase (PDB ID 1KZO). FPP (blue) binds along one side of the active site. The CaaX portion of the translocated product (yellow) no longer coordinates the zinc ion (magenta), making a type I- β turn, placing the farnesyl portion (brown) of the product in a conserved “exit groove” (residues shown in cyan). Reproduced with permission from (75).

second isoprene units shift, and they do so by rotating through the second isoprene unit. These results suggest that only a portion of the isoprenoid moves substantially during catalysis.

Product release

We note that the product complex described above (complex 3 in Fig. 3) does not represent the final step in this reaction. As described above, the rate-limiting step of the prenylation reaction is product release (78, 105), requiring binding of the next substrate(s) (82). This final step is represented by structures of FTase and GGTase-I complexed with both isoprenoid substrate and prenylated peptide product (complex 4 in Fig. 3) (PDB IDs 1KZO and 1N4S), which reveal that binding of the fresh isoprenoid

diphosphate substrate molecule moves the prenyl group of the prenylated peptide to a new binding site, the “exit groove” (Fig. 11B) (75, 76). This movement is accompanied by a conformational change in the CaaX moiety. Therefore, there are two CaaX peptide conformations in the product: extended (complex 3 in Fig. 3) and type I β -turn (complex 4 in Fig. 3). NMR studies of peptides bound to FTase revealed a similar β -turn, which was postulated to correspond to a reactant rather than a product complex (106, 107). In the displaced product, the X and a_2 residues remain in their original positions, indicating that the peptide substrate is competitive with respect to the displaced product. The three C-terminal amino acids of the prenylated peptide product make extensive van der Waals contacts with the new isoprenoid substrate, suggesting that the amino acid sequence of the CaaX motif may modulate product release. Multiple binding sites for product molecules have been observed in processive enzyme systems, in which substrate binding is involved in product release or translocation [e.g., the P and E sites of the ribosome (108)]. We note that this complex represents a direct observation of both substrate and product bound simultaneously to an enzyme that catalyzes an apparently nonprocessive reaction.

Implications of substrate-mediated product release

Unlike FTase and GGTase-I, RabGGTase is a processive enzyme, adding two prenyl groups to its Rab substrates (52) via a process that appears to proceed without dissociation of the monoprenylated intermediate (109) [reviewed in this series in (2)]. Biochemical and structural studies indicate that, like FTase and GGTase-I, RabGGTase has only one prenyl diphosphate binding site (101, 110). The structure of RabGGTase (PDB ID 1DCE) revealed a striking 7.5 Å diameter hydrophobic tunnel, originating from a shallow groove analogous to the exit groove in FTase and GGTase-I and extending through the β subunit (101, 110). The first geranylgeranyl isoprenoid attached to Rab may be sequestered in this tunnel after translocation of the monoprenylated product from the site of catalysis, which may be associated with the binding of fresh GGPP substrate, as observed for FTase and GGTase-I (109, 111). This translocation would allow the unmodified cysteine residue to interact with the zinc ion in preparation for the second catalytic reaction. This mechanism would explain why RabGGTase preferentially modifies Rab substrates that have one geranylgeranyl group already attached (52, 109). In addition, the potential to insert different lengths of the geranylgeranyl moiety through the tunnel may explain how RabGGTase is able to modify cysteine residues at a variety of positions within a few residues of the C termini of Rab substrates.

Additionally, this unusual substrate-mediated product release may have a significant biological role. We propose that it provides a mechanism for the regulated handover of the prenylated protein product to the next step in this processing pathway (76). In the CaaX prenyltransferases, the prenylated product remains tightly bound; therefore,

it is shielded from the cytoplasm, preventing aggregation or association with an incorrect membrane compartment. Only upon binding of an additional isoprenoid diphosphate molecule is the product isoprenoid displaced into the exit groove, presenting the product for delivery to the next processing step. The mechanism for this handover is analogous to one of the roles of Rab escort protein (REP), which escorts RabGGTase products to their final destination (112), and is similar to that of the GDI proteins, which extract prenylated G proteins such as Rho from membranes by binding the isoprenoid moiety in a hydrophobic pocket and transporting them throughout the cell (47).

Transition state model

The chemical mechanism and nature of the transition state is still an area of active investigation. We have developed a transition state model for catalysis by CaaX prenyltransferases consistent with the observed structural and kinetic information (Fig. 12) (75, 76). The proposed model retains elements of both the postulated electrophilic and nucleophilic components of the reaction mechanism (80, 113, 114). In this model, the CaaX motif binds as observed in the ternary substrate complex, coordinating the Zn^{2+} ion, whereas the isoprenoid diphosphate rotates through its second isoprene unit to bring the C_1 atom into

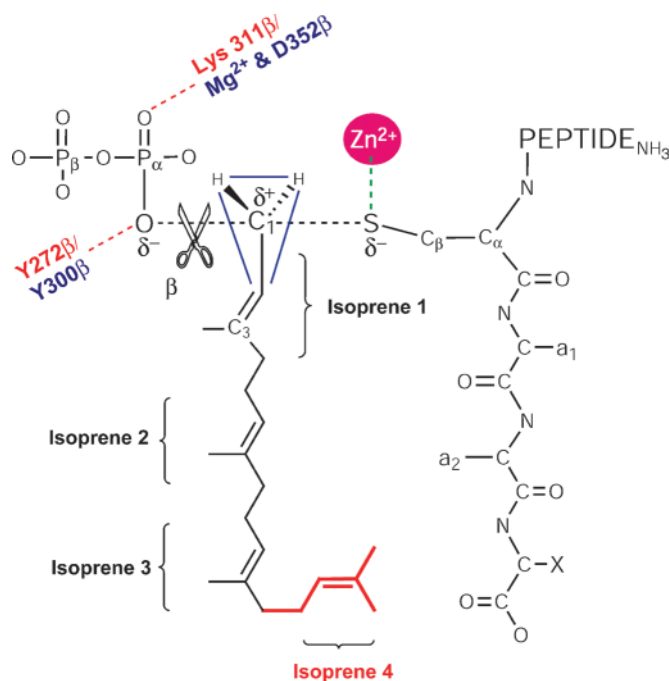


Fig. 12. Proposed transition state model for the CaaX prenyltransferases. The scissile phosphoether bond between the diphosphate and prenyl groups and the nascent thioether bond between the CaaX cysteine and the prenyl group are shown as black dotted lines. Hydrogen bonds predicted to stabilize the phosphate in the transition state are shown as red dotted lines (residues of FTase are labeled in blue, GGTase-I in red). In this model, the magnesium ion, in turn coordinated by D352 β , in FTase is replaced by K311 β in GGTase-I. Lys, lysine.

alignment for thioether bond formation, maintaining the binding conformation of the β -phosphate in its substrate conformation. In this reaction, the isoprenoid C_1 atom undergoes inversion of configuration during the reaction (115–117). The developing negative charge on the diphosphate moiety (localized mainly on the α -phosphate) is stabilized by several interactions with the enzyme, particularly residues K164 α and a β subunit tyrosine (Y300 β in FTase, Y272 β in GGTase-I). Kinetic studies demonstrated that FTase, but not GGTase-I, requires millimolar levels of Mg^{2+} for full catalytic efficiency (19, 89). It has been hypothesized that the Mg^{2+} ion required by FTase stabilizes the developing negative charge on the diphosphate as the bond breaks between the α -phosphate and the C_1 atom of the farnesyl group. Structural studies suggest that Mg^{2+} is coordinated by residue D352 β of the enzyme and the FPP diphosphate moiety (75). Sequence alignments reveal that in GGTase-I, a lysine occupies the position corresponding to D352 β in FTase. Superposition of the structures of FTase (75, 85, 95, 96, 99) and GGTase-I (76) shows that this lysine residue adopts a conformation that positions the positively charged side chain N_ϵ at the site of Mg^{2+} in FTase, allowing it to stabilize the diphosphate group. Mutagenesis studies (118, 119) indicate that Mg^{2+} sensitivity is dependent on these two residues; a lysine at 352 β of FTase abolishes Mg^{2+} dependence, whereas an aspartate at 311 β in GGTase-I introduces Mg^{2+} dependence.

INHIBITION OF CaaX PRENYLTRANSFERASE ACTIVITY

Three major classes of farnesyltransferase inhibitors (FTIs) and geranylgeranyltransferase inhibitors (GTIs) have been identified: isoprenoid diphosphate-derived, peptide-competitive (peptidomimetic and nonpeptidomimetic), and bisubstrate-mimicking [reviewed in this series in (120)]. Hundreds of compounds have been discovered or developed as inhibitors of either FTase or GGTase-I (or both); many of these have clinical potential as anticancer (121–125), antiparasitic (29, 126–128), antifungal (129–131), and antiviral (132–134) therapeutic agents. Some of these have been characterized by X-ray crystallographic studies, revealing a molecular basis for their inhibitory activities.

Isoprenoid diphosphate-derived inhibitors

A number of FPP and GGPP analogs have been developed that are competitive inhibitors of FTase and GGTase-I, respectively (135–141). Systematic structure-activity analysis of a series of FPP-based inhibitor molecules indicated that the most potent inhibitors retain a hydrophobic farnesyl group and a negatively charged moiety mimicking the diphosphate (138), consistent with the interactions observed previously between the enzyme and the terminal phosphate in the FTase and GGTase-I complexes (75, 76, 94–97, 99, 142, 143). That study also revealed the dramatic effect of hydrophobic chain length on FTase

activity, because elongation of the farnesyl group by a single carbon resulted in a decrease in IC_{50} of >200-fold, consistent with the structure-derived ruler hypothesis.

Several isoprenoid analog inhibitors have been characterized in crystal structures of various complexes (76, 94–97, 144, 145). Some of these inhibitors are described in this review (see above), including the FPP analogs farnesyl protein transferase inhibitor II (95–97) and α -hydroxyfarnesylphosphonic acid (94) and the GGPP analog 3'-aza GGPP (76, 97). In each of these structures, the isoprenoid analogs bind in a manner similar to their substrate counterparts, interacting extensively with both the enzyme and the CaaX substrate. These inhibitors continue to be invaluable in the structural characterization of FTase and GGTase-I; they bind much like the isoprenoid diphosphate

substrates but will not react with the peptide, allowing the crystallization of ternary substrate complexes.

Peptide-competitive inhibitors

Peptidomimetics. Initial characterization of FTase demonstrated that this enzyme is inhibited by short Ca_1a_2X peptides (3, 4, 6). The most effective inhibitory peptides contain a nonpolar aliphatic or aromatic amino acid in the a_1 or a_2 position, particularly the latter, although some of these peptides can also serve as FTase substrates. Inclusion of an aromatic residue such as phenylalanine in the a_2 position eliminates prenylation, however, resulting in a purely competitive inhibitor with respect to the peptide. Interestingly, the removal or substitution of the amino group on the cysteine of a tetrapeptide inhibitor restores

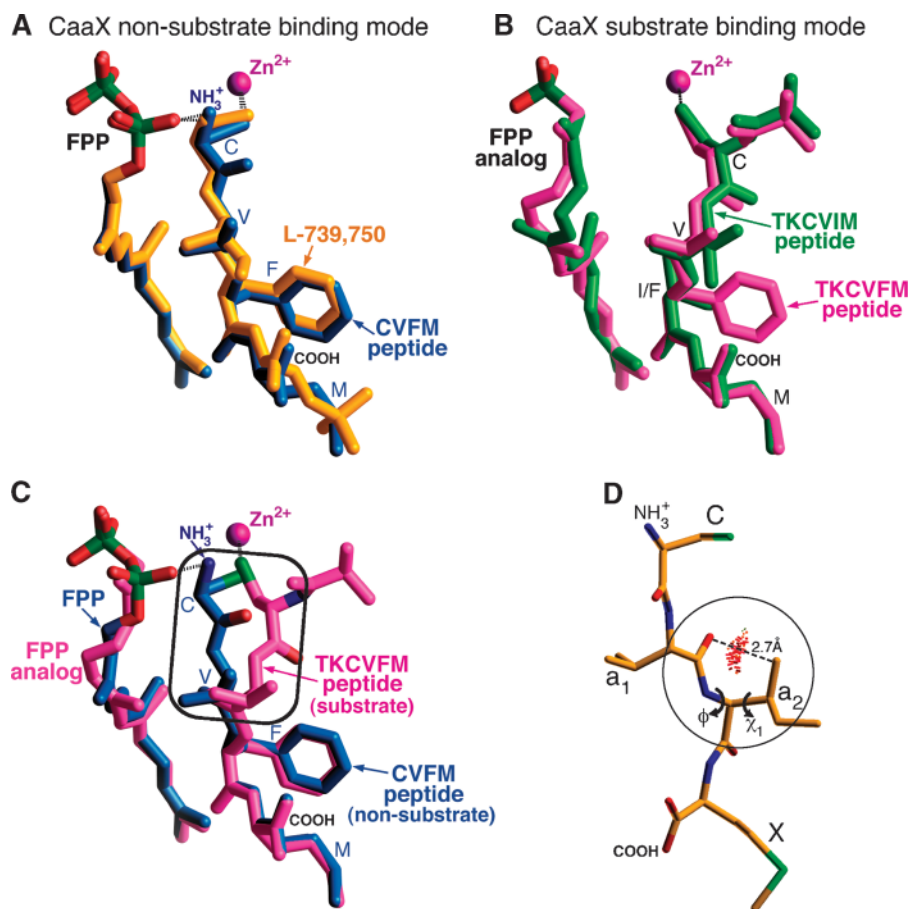


Fig. 13. Comparison of CaaX nonsubstrate and substrate conformations. A: CaaX tetrapeptide nonsubstrate inhibitor binding conformation. L-739,750 peptidomimetic (PDB ID 1JQC) and CVFM tetrapeptide (PDB ID 1JCR) are shown with the FPP molecules observed in these two complexes colored yellow (with peptidomimetic) and blue (with CVFM). B: Peptide substrate binding conformation. TKCVFM (PDB ID 1JCS) and the K-Ras4B-derived peptide TKCVIM (PDB ID 1D8D) are both substrates and adopt the same backbone conformation. The N-terminal residue and the lysine side chain of each of these peptides are omitted here for clarity. Also shown are the FPP analogs, which are colored according to their observed conformations with these peptides. C: Comparison of the hexapeptide substrate TKCVFM and the non-substrate tetrapeptide inhibitor CVFM. A boxed region highlights the differences between the substrate and nonsubstrate conformations. D: Energetically unfavorable steric contact (red spikes) between the C_{γ2} methyl group of the isoleucine residue in the a₂ position and the carbonyl oxygen of the a₁ residue when the phenylalanine of the CVFM tetrapeptide is replaced with isoleucine. Consequently, a tetrapeptide with a β -branched residue (isoleucine or valine) in the a₂ position cannot adopt the nonsubstrate binding mode observed for CVFM. Reproduced with permission from (96).

catalytic activity (4, 7). Because these molecules are peptides, they have three significant disadvantages for potential *in vivo* activity: poor cellular uptake, rapid degradation, and FTase farnesylation (in some cases), which results in depletion of the inhibitor. To overcome these disadvantages, a number of peptidomimetic inhibitors have been developed and characterized (96, 121, 146–150).

The mechanism for tetrapeptide inhibition was studied using the crystal structures of FTase complexed with the nonsubstrate tetrapeptide CVFM (PDB ID 1JCR), the substrate hexapeptide TKCVFM (PDB ID 1JCS), and the peptidomimetic inhibitor L-739,750 (PDB ID 1JCQ), developed by Merck Research Laboratories (96). This inhibitor compound, mimicking the tetrapeptide CIFM, is the metabolic product of the prodrug L-744,832; administration of this prodrug in rats resulted in tumor regression without any observed systemic toxicity (121). Each of these peptides/peptidomimetics bind in the peptide binding site, anchored by cysteine coordination of the catalytic zinc ion and hydrogen bonds (both direct and water-mediated) between the C terminus and the enzyme. The two inhibitors (the nonsubstrate CVFM peptide and L-739,750) adopt a similar conformation, in which there is an ~ 3 Å distance shift of the inhibitor cysteine C_α atom relative to the substrate, although the cysteine still coordinates the zinc ion (Fig. 13A, C). This alternative conformation is stabilized by a hydrogen bond between the N terminus of the inhibitor and an oxygen on the α -phosphate of the FPP molecule, as well as by hydrophobic interactions between the phenylalanine-derived side chain of the inhibitor and several aromatic residues of the enzyme. In both complexes, the inhibitor binds in the space between the isoprenoid and the zinc ion and interferes with the movement of the isoprenoid during the reaction, preventing bond formation. The substrate TKCVFM hexapeptide, however, adopts the same conformation as the K-Ras4B-derived TKCVIM peptide in an analogous complex (Fig. 13B). In each of these complexes, the side chain in the a_2 position adopts essentially the same conformation; this conformation places steric constraints on the ϕ backbone angle between the a_1 and a_2 residues. β -Branched amino acids (isoleucine and valine) in the a_2 position form an unfavorable steric interaction between their $C_{\gamma 2}$ methyl group and the carbonyl oxygen of the a_1 residue in the nonsubstrate backbone conformation adopted by the CVFM peptide (Fig. 13D). Therefore, it was predicted that, of the CaaX cognate tetrapeptides, only peptides with branched residues (i.e., isoleucine or valine) in the a_2 position are substrates, whereas peptides with other non-branched aliphatic amino acids are poor substrates or non-substrates (provided that the N terminus is not blocked).

Nonpeptide inhibitors. Another class of peptide-competitive inhibitors includes molecules that are not derived from peptides but still bind in the CaaX binding site of the enzyme; this class is by far the most prevalent and the most characterized (122–125, 144, 151–165). This class of inhibitors includes compounds produced by the drug discovery and design efforts of Merck Research Laboratories,

Janssen Research Foundation, Bristol-Myers Squibb Co., Schering-Plough, and Abbott Laboratories, some of which have been evaluated in phase I, II, and III clinical trials for the treatment of a variety of human cancers (122, 123). Crystal structures of a number of these inhibitors complexed with the CaaX prenyltransferases have been determined. In each of these structures, the compounds bind in the peptide binding site, typically without inducing large structural changes in the enzyme. Each of these compounds participates in extensive hydrophobic interactions with both the enzyme and the FPP/FPP analog

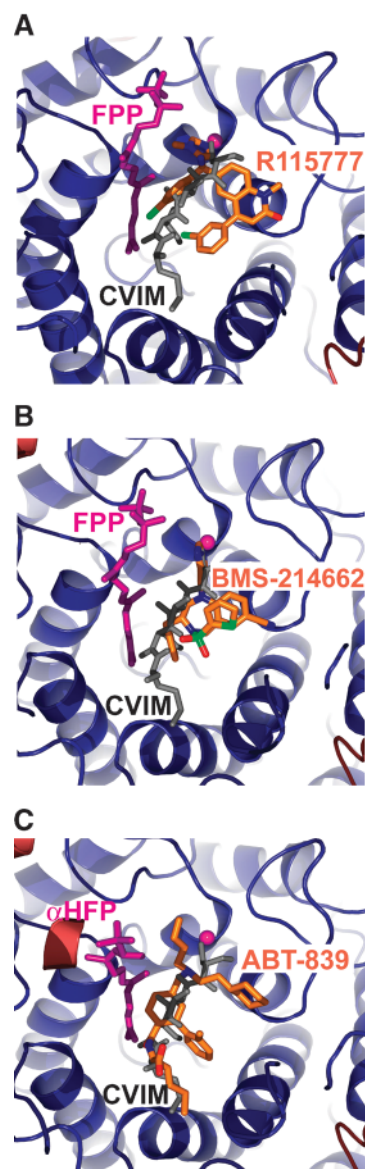


Fig. 14. FTase inhibitors R115777, BMS-214662, and ABT-839. Ribbon cartoons of FTase complexed with FPP or FPP analog α -hydroxyfarnesylphosphonic acid (α -HFP) (purple) and the inhibitor molecules (orange) R115777 (A), BMS-214662 (B), and ABT-839 (C) shown in the same orientation (PDB IDs 1SA4, 1SA5, and 1N94, respectively). Each inhibitor is shown in comparison with the K-Ras4B-derived peptide CVIM (gray) (PDB ID 1D8D). Also shown is the Zn^{2+} ion (magenta), coordinated by the imidazole group of R115777 and BMS-214662.

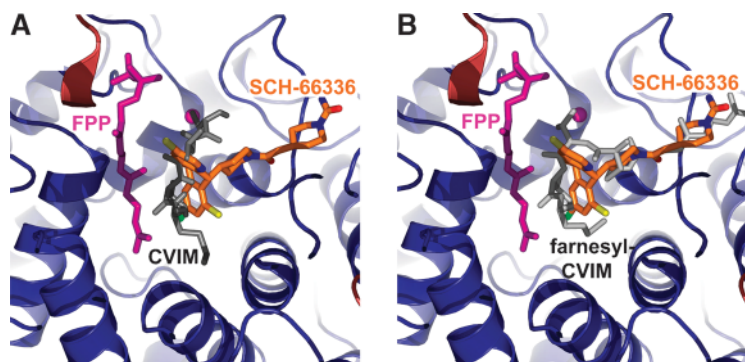


Fig. 15. FTase inhibitor SCH66336. Ribbon cartoons of FTase complexed with FPP (purple) and the inhibitor SCH66336 (orange) (PDB ID 1O5M). The inhibitor is shown in comparison with (A) the K-Ras4B-derived peptide CVIM (dark gray) (PDB ID 1D8D) and (B) the partially displaced peptide product (light gray) (PDB ID 1KZO). Also shown is the Zn^{2+} ion (magenta).

molecule, as well as in a combination of direct and water-mediated hydrogen bonds with the enzyme.

The structures of FTase complexed with FPP (or an FPP analog) and each of the inhibitors R115777 (Janssen; PDB ID 1SA4) (142), BMS-214662 (Bristol-Myers Squibb; PDB ID 1SA5) (142), and ABT-839 (Abbott Laboratories; PDB ID 1N94; one of several Abbott compounds with determined structures) (161) have been determined. All three inhibitors bind in the FTase peptide binding site, overlapping with the binding of the Ca_1a_2 portion of the substrate Ca_1a_2X peptide (Fig. 14). R115777 and BMS-214662 coordinate the catalytic Zn^{2+} ion via an imidazole group; however, ABT-839 does not. ABT-839 is also unique in that it possesses a methionine mimic at one end of the molecule that binds as in the substrate complex (95), making it the only nonpeptidomimetic inhibitor described here that extends into the X residue binding site.

Design efforts from Schering-Plough have resulted in a number of FTase inhibitors, of which a series of tricyclic compounds have been structurally characterized (144). These compounds are composed of three connected rings, with a tail containing two or more rings extending from the central seven carbon ring, representing a series of mono-, di-, and trihalogenated species. SCH66336, an inhibitor in this series, is observed in an unusual conformation in the FTase active site (PDB ID 1O5M) (Fig. 15). This compound does not coordinate the Zn^{2+} ion and only overlaps the a_1 and a_2 portion of the substrate Ca_1a_2X peptide. Additionally, a large portion of the inhibitor is observed binding in the exit groove described above, where the displaced prenyl group of the farnesylated peptide product binds in the translocated product complex. The latter observation may explain the binding affinity toward FTase that this inhibitor exhibits, despite its lack of Zn^{2+} coordination.

Although most of these inhibitors are highly selective toward FTase in preference to GGTase-I, L-778,123 (Merck Research Laboratories) is a nonpeptide inhibitor of both FTase and GGTase-I (154, 155). No steric blocks prevent compounds such as R115777 and BMS-214662 from binding in GGTase-I, although binding is not favored because the GGTase-I a_2 binding site has a reduced aromatic character that does not permit the stabilizing aromatic stacking interactions (166, 167) observed in FTase (see above). These differences in the a_2 binding site may cause the alternative binding mode adopted by L-778,123 in GGTase-I (Fig. 16) (143). In FTase-I (PDB ID 1S63), the peptide-competitive

binding mode is stabilized by aromatic stacking interactions with the aromatic residues that constitute the a_2 binding site. In GGTase-I (PDB ID 1S64), these stacking interactions are not possible, and L-778,123 adopts a GGPP-competitive binding mode that permits the formation of a hydrogen bond in lieu of stacking interactions. In this structure, the inhibitor also interacts with a bound sulfate anion; this synergistic interaction may additionally induce a small change in the GGPP binding site that enhances L-778,123 binding (168, 169).

Bisubstrate mimics

The final class of prenyltransferase inhibitors includes a variety of molecules that mimic a bisubstrate complex and are competitive for both the isoprenoid diphosphate and Ca_1a_2X binding sites. Several of these inhibitors have been synthesized and characterized (170–172), although none has been studied by structural methods.

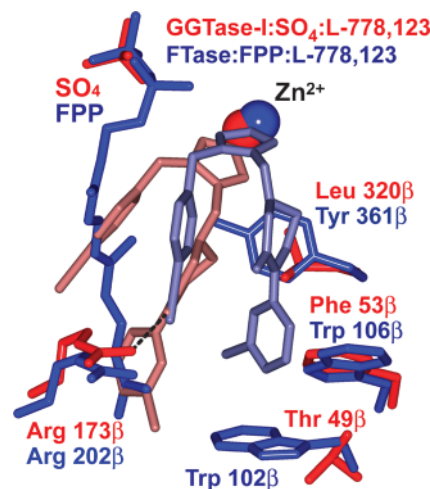


Fig. 16. L-778,123 binding in FTase and GGTase-I suggests a molecular mechanism for inhibitor selectivity. Superposition of FTase (blue) and GGTase-I (red) complexes illustrates the binding modes of L-778,123 in each enzyme (PDB IDs 1S63 and 1S64, respectively). Only the residues that contact the a_2 residue of the Ca_1a_2X peptide, the “ a_2 binding site,” and a conserved arginine (Arg) residue are shown. Aromatic stacking interactions are not possible between L-778,123 and the GGTase-I a_2 binding site, encouraging the inhibitor to adopt a lipid-competitive binding mode that permits the formation of a hydrogen bond. Reproduced with permission from (143).

STRUCTURAL ANALYSIS OF CaaX PRENYLTRANSFERASE AND HUMAN THERAPEUTICS

Since their discovery, FTase and GGTase-I have been targets for cancer therapeutics (73, 74). To this end, a number of FTIs and GTIs have been studied kinetically, structurally, and clinically (reviewed in this series in 120). More recent studies have begun to illustrate the potential of FTIs in the treatment of infection by pathogenic microorganisms, including *Plasmodium falciparum* (the causative agent of malaria) (29, 128), *Trypanosoma brucei* (African sleeping sickness) (126, 173), *Trypanosoma cruzi* (Chagas disease) (35), and *Leishmania mexicana* and *Leishmania major* (leishmaniasis) (35, 127) (reviewed in this series in 174). Another potential application of FTIs and GTIs is as a new class of antifungal and antiviral agents. *Candida albicans* and *Cryptococcus neoformans* (opportunistic fungal pathogens causing life-threatening infections in many immunocompromised patients with AIDS) (24, 25, 175, 176) and viruses (through the use of host prenyltransferase enzymes), including hepatitis C virus and hepatitis delta virus, appear to require prenylation for viability (25, 129, 132, 133).

Although structural characterization of mammalian FTase and GGTase-I has advanced our understanding of these enzymes, there is no structural information available for any of the protein prenyltransferases from human pathogens. FTIs are attractive drug candidates to develop for the treatment of parasitic, fungal, and viral infections because the chemistry, pharmacokinetics, and toxicity of a number of these compounds have been established in humans. Using this “piggy-back” approach to drug development bypasses many of the hurdles involved in designing drugs de novo (177). Structural studies of the protein prenyltransferases from human pathogens would facilitate the design of potent, highly selective inhibitors. ■■

This work was supported National Institutes of Health Grant GM-52382 (L.S.B.).

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