

Protein Prenyltransferases

Key References

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Overview

Protein prenylation involves attachment of either the 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoid to conserved carboxyl-terminal cysteine residues of proteins. Three known enzymes catalyze this type of modification; these are termed protein farnesyltransferase (FTase), protein geranylgeranyltransferase type I (GGTase-I), and protein geranylgeranyltransferase Type II (GGTase-II, also known as Rab GGTase). FTase and GGTase-I are quite closely related and utilize similar mechanisms for substrate recognition and catalysis, while GGTase-II is somewhat distinct in both regards. Proteins prenylated by FTase or GGTase I undergo further processing that includes the removal of three C-terminal amino acids by Rce1 protease and the methylation of C-terminal cysteine by isoprenyl cysteine methyltransferase (Icmt).

FTase was the first protein prenyltransferase to be identified. Purified FTase is a heterodimer consisting of a 48 kDa α subunit and a 46 kDa β subunit. The α subunit of FTase was subsequently found to be the same as the α subunit of GGTase-I. To date, approximately 30 proteins have been identified as substrates of FTase in mammalian cells, and they include all four isoforms of Ras proteins, some Ras-related proteins such as Rap2A, RhoB, and Rheb, nuclear lamin B and prelamin A, several proteins involved in visual signal transduction, some γ -subunits of heterotrimeric G proteins and enzymes such as the PRL tyrosine phosphatases and cytosolic phospholipase A(2) γ . Recently, a proteomics method to identify farnesylated proteins has been developed by applying TAS (tagging via substrate) technology.

Most prenylated proteins are modified by the geranylgeranyl isoprenoid. The major enzyme in this regard is GGTase-I, which

is also composed of two non-identical subunits, the aforementioned 48 kDa α subunit and a 43 kDa β subunit. Both FTase and GGTase-I catalyze prenylation reactions using either protein or peptide substrates containing the carboxyl-terminal CaaX motifs, so they are also termed CaaX prenyltransferases. Target proteins of GGTase-I include most Ras-related small G proteins such as those in the Rac, Rho, and Rap subfamilies, and most γ -subunits of heterotrimeric G proteins. Crystallographic analysis of FTase and GGTase I has revealed a set of rules that govern their substrate peptide selectivity.

The third member of this protein prenyltransferase family is GGTase-II. This enzyme has two subunits, a 60 kDa α subunit and a 38 kDa β subunit, that comprise its catalytic unit. Unlike FTase and GGTase-I, GGTase-II requires a third polypeptide for activity, this being a 95 kDa protein termed Rab escort protein (REP) that binds the Rab protein substrate and presents it to the catalytic dimer. GGTase-II most likely catalyzes the geranylgeranylation of all Rab proteins, the majority of which terminate in paired cysteine motifs. Furthermore, the enzyme is capable of di-geranylgeranylation, i.e., modifying both cysteine residues at the carboxyl-terminus of an individual Rab protein.

Development of inhibitors of protein prenyltransferases is an active area of research following the finding that oncogenic forms of Ras proteins require farnesylation for their ability to transform cells. Several representative inhibitors of FTase that have been synthesized are listed below in the accompanying table and include both substrate analogs and compounds identified from screening programs. Currently, several inhibitors are being evaluated in clinical trials

as anti-cancer agents and clinical activities have been detected particularly with hematopoietic malignancies and breast cancer. However, these effects are unlikely to be due to the inhibition of Ras, as farnesyltransferase inhibitors fail to inhibit K-Ras4B which becomes alternatively modified by a geranylgeranyl group when its farnesylation is inhibited. Thus, the mechanism of action of farnesyltransferase inhibitors needs to be further investigated. Interest in the development of GGTase-I inhibitors as therapeutics has also increased of late.

Protein Prenyltransferases

CURRENTLY KNOWN TYPE	FTase (F0175)	GGTase-I (G7761)	GGTase-II
SUBUNIT COMPOSITION (MAMMALIAN)	48 kDa (α), 46 kDa (β)	48 kDa (α), 43 kDa (β)	95 kDa (REP), 60 kDa (α), 38 kDa (β)
SACCHAROMYCES CEREVISIAE GENE PRODUCT	RAM2 (α), RAM1/DPR1 (β)	RAM2 (α), CDC43 (β)	BET4 (α), BET2 (β)
METAL REQUIREMENTS FOR ENZYME ACTIVITY	Zn ²⁺ , Mg ²⁺	Zn ²⁺	Unclear
ISOPRENOID SUBSTRATES	FPP (F6892)	GGPP (G6025)	GGPP (G6025)
PROTEIN SUBSTRATES	Ras (R9894), nuclear lamins, transducin γ subunit, others	Rho, Rac (R3012), trimeric G protein γ subunit	Rab proteins (R6022)
PROTEIN SUBSTRATE MOTIF A	-CaaX, X = Met, Ser, Gln, Ala	-CaaX, X = Leu	-CC, -CXC, X = Any amino acid
ISOPRENOID ANALOG INHIBITORS	(α -Hydroxyfarnesyl) phosphonic acid (H9279), O-Farnesyl phosphonoacetylhydroxamate, fluorinated phosphonates, polycarboxylic acids	3-Aza-GGPP	Not known
-CAAX ANALOG AND OTHER INHIBITORS	L-744,832 (L7287), B581 (B2559), B956, FTI-254, FTI-277 (F9803), SCH66336, R115777, BMS-193269, BMS-214662, BMS-225975, L-778,123 ^b , AZD3409 ^b , Chaetomelic acid A, Manumycin (M6418)	GGTI-298 (G5169), GGTI-2147, GGTI-2154 Not known	Not known Not known
RADIOLIGANDS OF CHOICE	[³ H]-FPP	[³ H]-GGPP	[³ H]-GGPP
TISSUE EXPRESSION	Ubiquitous	Ubiquitous	Ubiquitous
PHYSIOLOGICAL FUNCTION	Modification of signaling proteins and others	Modification of Rho-family proteins and others	Modification of Rab-family proteins
DISEASE RELEVANCE	Cancer	Cancer, cardiovascular disease	Choloideraemia

Abbreviations

L-744,832: (2S)-2-[[[(2S)-2-[[[(2S,3S)-2-[[[(2R)-2-Amino-3-mercaptopropyl]amino]-3-methylpentyl]oxy]-1-oxo-3-phenylpropyl]amino]-4-(methylsulfonyl)-butanoic acid-1-methylethyl ester
B581: N-(2S)(2R)-Amino-3-mercaptopropylamino)-3-methylbutyl)-Phe-Met-OH
B956: N-[2S,3Z,5S,6E,8R]-8-Amino-9-mercapto-5-(1-methylethyl)-1-oxo-2-(phenylmethyl)-3,6-nonadienyl]-L-methionine
FTI-254: N-[4-[[[(2R)-2-Amino-3-mercaptopropyl]amino]benzoyl]-L-methionine methyl ester
SCH-66336: (+)-4-[2-[4-(8-Chloro-3,10-dibromo-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]-pyridin-11(R)-yl)-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide
R115777: (B-6-[Amino(4-chlorophenyl)(1-methyl-1H-imidazole-5-yl)-methyl]-4-(3-chlorophenyl)-1-methyl-2(1H)-quinoline
BMS-193269: N-[2-(1H-Imidazol-4-yl)ethyl]-L-valyl-(3S)-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-methionine
BMS-214662: (R)-7-Cyano-2,3,4,5-tetrahydro-1-(1H-imidazole-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H,4-benzodiazepine

BMS-225975: (R)-7-Cyano-2,3,4,5-tetrahydro-1-(1H-1-methyl-imidazol-5-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H,4-benzodiazepine hydrochloride
L-778, 123: {4-[[5-[[4-(3-Chlorophenyl)-3-oxo-1-piperazinyl]methyl]-1H-imidazo-1-yl]methyl]-benzonitrile}
AZD3409: Isopropyl(2S)-2-[(2-(4-fluorophenethyl)-5-[[[(2S,4S)-4-[(3-pyridinylcarbonyl)sulfanyl]tetrahydro-1H-pyrrol-2-yl]methyl]amino]benzoyl]amino)-4-(methylsulfanyl)butanoate
GGTI-298: N-[4-[[[(2R)-2-Amino-3-mercaptopropyl]amino]-2-(1-naphthalenyl)benzoyl]-L-leucine methyl ester
GGTI-2147: 4-[[[N-(imidazol-4-yl)methyleneamino]-2-(1-naphthyl)benzoyl]leucine methyl ester
FPP: Farnesyl pyrophosphate
GGPP: Geranylgeranyl pyrophosphate
REP: Rab-Escort Protein required for activity of this enzyme

FOOTNOTES

- a:** CaaX: Single letter abbreviations for amino acids. "a" residues are undefined, but generally aliphatic.
b: These FTI compounds also inhibit GGTase-I.