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Chemical Biology of Lipidated Proteins

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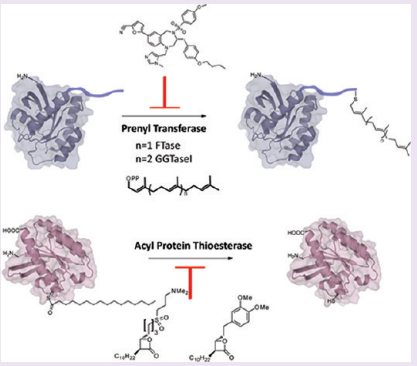
ABSTRACT: Many signaling proteins such as the members of the Ras superfamily of GTPases are posttranslationally modified by covalent attachment of lipid groups, which is crucial for the correct localization and function of these proteins. Numerous lipidated proteins are oncogens often found mutated in several human cancers. Therefore, several therapeutic strategies have been developed based on the inhibition of the enzymes involved in these lipidation steps. Here, we will summarize the results on protein lipidation inhibition, mainly focusing on the small molecules targeting the isoprenylation and acylation of proteins.



Numerous peripheral membrane proteins require a temporary membrane association to fulfill their biological functions. This can be achieved *via* interaction with membrane lipids such as phospholipids or sphingolipids,[1] using different conserved lipid binding domains present in proteins[2] or by covalent attachment of lipid groups, which mediate the specific localization of these proteins in membrane domains. The best-characterized lipidation processes are the isoprenylation of cysteines and acylation (N-terminal myristoylation of glycines or S-palmitoylation of cysteines) (Figure 1).

Myristoylation is the attachment of a 14-carbon myristic acid to a N-terminal glycine and is catalyzed by a N-Myristoyl Transferase (NMT).[3]

Isoprenylation is the posttranslational attachment of an isoprenoid group (either the 15-carbon farnesyl or the 20-carbon geranylgeranyl) to one or two cysteines located at the C-terminus of proteins and it is catalyzed by protein prenyl transferases (PPTases). Around 2% of all cellular proteins are isoprenylated.[4]

The posttranslational attachment of a 16-carbon saturated palmitic acid to cysteines forming a labile thioester is another significant lipid modification. The lability of this thioester bond confers this modification a crucial role in the regulation of protein localization and function due to the reversibility of S-acylation, in contrast to the farnesyl and myristoyl modifications, permanently installed into the proteins. The reversibility has also as a result two potential regulation points, *i.e.*, acylation and deacylation.

As a result of the different lipid modifications and the dynamic nature of palmitoylation, protein lipidation not only plays a key role in controlling membrane association and localization by increasing the protein affinity to membranes but is also essential for correct function of the protein. Hence, there is compelling evidence that lipidation has a crucial role in regulation of signaling and trafficking processes, and may be involved in mediating protein−protein and protein−lipid interactions. The detailed study of the role of lipidation in localization and function has been hampered by the lack of appropriate tools. However, the field of protein lipidation has experienced great advances in recent years with the application of chemical biology approaches. Chemical biology may be defined as the employment of chemical approaches to study biological phenomena. Thus, the design and synthesis of both appropriate small molecules and macromolecules was instrumental to gain new insights into unsolved biological questions in protein lipidation. In this context, chemical biology strategies have given access to fully posttranslationally modified semisynthetic proteins bearing both natural and non-natural lipid modifications or additional tags and fluorophores. These macromolecules, mainly not accessible by other techniques, have been used in biochemical, biophysical, and cell-biological studies and have been crucial in the elucidation of the roles of lipid modifications in protein localization and function.[5−8] Additionally, small molecules such as lipid analogues or inhibitors of the lipidating enzymes have been designed and successfully applied as invaluable tools for detailed studies of lipidated proteins. Recently, reviews have covered the advances in the synthesis and applications of lipidated proteins[9] as well as the use of lipid analogues,[10] mostly in combination with bioorthogonal reactions, to characterize these posttranslational modifications. In this review, we will summarize the chemical biology of lipidated proteins primarily focusing on the small molecules developed as inhibitors of the lipidation processes.

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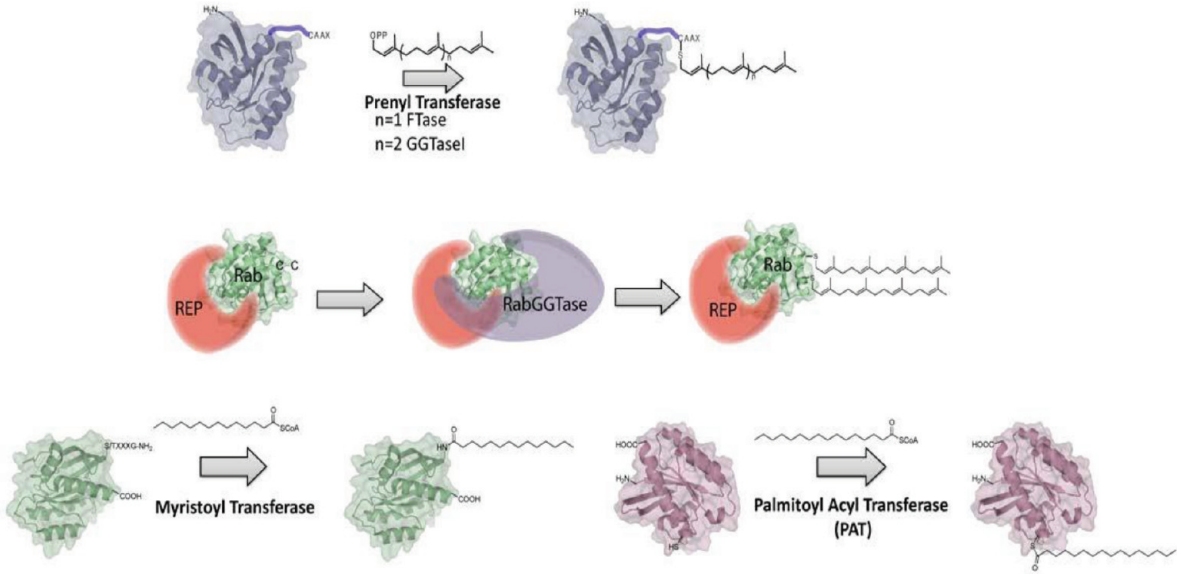

Figure 1. CAAX-box-containing proteins are C-terminally isoprenylated by Farnesyl Transferase (FTase) or Geranylgeranyl Transferase I (GGTase I). Conversely, Rab proteins are prenylated by RabGGTase, which requires previous association of Rab proteins with a Rab escorting protein (REP). Myristoylation is the attachment of a 14-carbon myristic acid to a N-terminal glycine and is catalyzed by a N-Myristoyl Transferase (NMT). Protein Acyl Transferase (PAT) catalyzes the transfer of a palmitic acid to cysteine residues.

PRENYLATION

The majority of the prenylated proteins are solely monoprenylated, and the recognition motif for this modification is a C-terminal sequence known as CAAX-box, where C is a cysteine, A are aliphatic amino acids, and X can be any amino acid and usually determines the nature of the anchored isoprenoid. This step is catalyzed by Farnesyl Transferase (FTase) or by Geranygeranyl Transferase I (GGTase I), which use farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) as lipid donors, respectively. If X is Leu or Ile the protein gets geranylgeranylated, if X is Phe proteins can be both farnesylated or geranylgeranylated, and in almost all other cases proteins are farnesylated.[11] In these CAAX-box containing proteins, two additional posttranslational modifications occur after isoprenylation. First, the Ras converting enzyme 1 (RCE1) cleaves the 3 terminal amino acids, and then the exposed cysteine gets methylated by Icmt (isoprenyl cysteine carboxymethyltransferase) by transfer of a methyl group from *S*-adenosyl methionine. Other proteins can be mono- or doubly isoprenylated, as is the case for the Rab proteins, members of the Ras superfamily of GTPases that possess a crucial role in membrane trafficking.[12] Important differences are encountered in this case. These proteins do not contain the mentioned CAAX-box but a CC or a CXC C-terminal motif. In this latter case, the C-terminal cysteine gets also methylated, which does not occur for a CC-terminus. Another striking difference is the enzyme involved in the lipidation process, Geranylgeranyl Transferase II (GGTase II), also known as Rab Geranylgeranyl Transferase (RabGGTase), which requires association of the Rab proteins to a Rab escorting protein (REP) to exert its function (Figure 1).

FARNESYL TRANSFERASE INHIBITION

Posttranslational isoprenylation is essential for correct localization and function of several proteins. One of the most relevant examples of prenylated proteins are the members of the Ras family, important protooncogens found mutated in ca. 30% of all cancers, especially in pancreatic, colorectal, lung, and bladder cancers.[13] Interference with Ras signaling by inhibition of this isoprenylation step has been widely investigated for the treatment of cancer.

Acting on different points of regulation may block prenylation. Accordingly, several strategies have been considered to achieve this inhibition (for recent reviews extensively covering the different classes of Farnesyl Transferase Inhibitors (FTI), see refs 14 and 15). One option is the depletion of substrates for the prenylating enzymes (either FPP or GPP), generated *via* the mevalonate pathway. Statins, which target the HMGCoA reductase and bisphosphonates, blocking the FPP synthase, are the most relevant examples of these inhibitor classes. Although relative success has been obtained with these strategies, the blockage of the mevalonate pathways may affect many other important cellular processes, such as cholesterol synthesis or hormone production, and therefore more specific strategies are desirable.

Alternatively, inhibition has been achieved by direct targeting of the prenylating enzymes (FTase, GGTase I, and RabGGTase), either by substrate competition (isoprenoid-PP or the CAAX peptide) or by coordination to the zinc ion essential for the catalysis. Relevant examples are, among others, some farnesyl-PP analogues[16] or CAAX mimics (peptidic inhibitors based on the CAAX sequence,[17] peptidomimetics,[18] and nonpetidic CAAX-competitive inhibitors), as well as some bisubstrate inhibitors that target both binding sites.[19]

Much expectation was raised in the development of FTIs for cancer treatment. As such, several pharmaceutical companies developed FTIs with variable selectivity, and some of them reached clinical trials (Table 1). The achievement of selectivity for one PPTase *versus* the others is strongly hampered due to the close similarity between their binding sites. Consequently most of the inhibitors target more than one PPTase. This is the case for the Merck inhibitor L778,123 (Table 1, entry 3), which blocks both FTase and GGTase I, albeit with two different binding modes,[20] or BMS-214662, one of the tetrahydrobenzodiazepine (THB) class inhibitors developed by Bristol-Myers

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Table 1. Prenyl Transferase Inhibitors; IC_{50} Values for the Different Prenyltransferase Inhibitors and Their Selectivity Profile

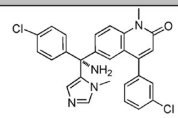
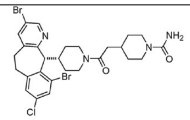
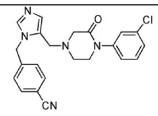
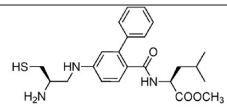
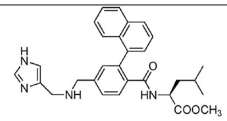
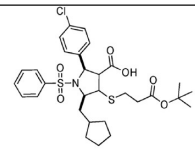
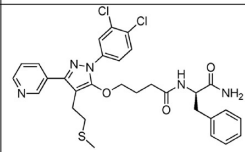
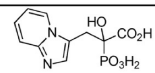
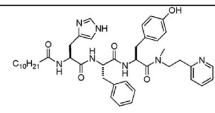
Entry	Inhibitor	Structure	FTase	GGTase I	Rab GGTase
1	Tipifarnib(*15*) (R115777)		✓ 7.9 nM		
2	Lonafarnib(*15*) (SCH66336)		✓ 1.9 nM	>50000	
3	L778,123(*15*)		✓ 2 nM	✓ 98 nM	
4	GGTI-286(*46*)		✓ 240 nM	✓ 183 nM	
5	GGTI-2151(*46*)		10000 nM	✓ 44 nM	
6	P8-H7(*50*)		>100	✓ 2.4 μM	✓ 7 μM
7	GGTI-DU40(*47*)			✓ 0.8 nM (Ki)	
8	3-IPEHPC(*52, 55*)				✓ 1.3 μM
9	Pepticinnamin E derivative		>100 μM	>100 μM	✓ 2.8 μM

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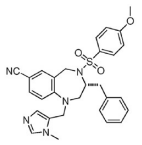
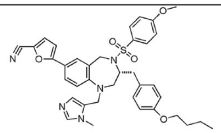
Table 1. continued

Entry	Inhibitor	Structure	FTase	GGTase I	Rab GGTase
10	BMS3		✓ 6 nM	>99.500 nM	✓ 724 nM
11	THB derivative(*60*)		>9700 nM	>99500 nM	✓ 42 nM

Squibb, with inhibitory activity for both FTase and RabGGtase. Alternatively, SCH66336 (Lonafarnib Table 1, entry 2) and R115777 (Tipifarnib, Table 1, entry 1) developed by Schering-Plough and Janssen-Cilag, respectively, selectively inhibit FTase. The basis of their selectivity *versus* other PPTases was revealed by crystallographic studies, showing a selective aromatic stacking interaction of the inhibitors with the CAAX binding site that was not possible in the other enzymes.[21] However, despite the promising results obtained *in vitro* with these inhibitors, this data could not be completely correlated with clinical results in Ras mutated cancers. One of the explanations of this fact may be the alternative prenylation of Ras by GGTase I;[22−24] however, the reasons underlying the poor activity of FTIs are not completely clear. However, FTIs have shown encouraging activity in Ras-independent cancers, thus suggesting that other unknown isoprenylated proteins could be the real target. In agreement with this finding, relevant clinical activity has been detected with Tipifarnib in acute myeloid leukemia and myelodysplatic syndrome,[25] and FTIs in combination with Akt inhibitors have shown a synergic effect on breast cancer.[26] Enhanced activity of FTIs has also been observed in combination with other cancer chemotherapeutics such as taxane.[27] The Ras homologue enriched in brain (Rheb) has been suggested as one of the potential alternative targets. This is supported by the abnormal levels of Rheb found in different cancers[28] and by the fact that Rheb prenylation and thereby Rheb downstream signaling is completely abolished after treatment with an FTI.[29,30] However, some other reports suggest that unprenylated Rheb is still capable of activating S6K, although less efectively.[31] Other possible targets for the action of FTI could be the centromere associated proteins CENP-E and CENP-F.[32,33] The inhibition of farnesylation of CENP-E and CENP-F results in alteration of the microtubule−centromere interaction during mitosis, which correlates with the fact that FTase inhibitors cause accumulation of cells in G2/M phase.[34] However, more detailed studies to detect the prenylated proteins responsible for the antitumorogenic activity in cells sensitive to inhibitors are required to establish their real target.

As an alternative strategy, the selectivity issues that have resulted in dual inhibitors for FTase and GGTase I have also been exploited to minimize the cross-prenylation of Ras protein with GGTase I when FTase is inhibited by simultaneously blocking both PPTases.[35,36] This approach has recently been studied in K-Ras induced lung cancer, resulting in a significant increased reduction of tumor growth and survival.[37] Apart from cancer, FTIs have also been considered for the treatment of parasitic diseases because of the high susceptibility to FTIs shown by parasites such as *Plasmodium falciparum* or *Trypanosoma brucei*, probably due to the fact that they lack GGTase I.[38] More recently, FTase has also emerged as an important target for the treatment of the Hutchinson−Gilford progeria syndrome (HPGS), a premature-aging disease commonly caused by a mutant version of lamin A that results in the accumulation of a farnesylated and carboxymethylated fragment of the protein that can not be removed.[39] As a consequence of the promising results obtained in cells and in mouse models,[40,41] clinical trials are currently underway to study the use of FTIs in children affected by progeria.[42]

GGTASE I AND RABGGTASE INHIBITION

The fact that the administration of GGTase I inhibitors alone results in cell cycle arrest and apoptosis has also promoted the investigation of GGTase I as a target for anticancer drugs.[43,44] In this regard, different inhibitor classes have been reported ranging from peptide-based structures to small molecules to lipid analogues. For instance, a mimic of the prenylation substrate GGPP (GGTI-2Z) has recently been reported by Mattingly and co-workers.[45] As a result of this substrate similarity, this compound targets both GGTase I and RabGGTase. Promising antiproliferative effects have been seen with this inhibitor when used in combination with lovastatin. The synergistic effect of this combination is based on a decrease of substrate availability by treatment with statins and an increased effectiveness of prenylation inhibition at lower concentrations of substrate.

GGTase I acts preferently on proteins containing a leucine residue in the CAAX-box. Sebti and co-workers made use of this particular feature to develop peptide inhibitors based on the CAAL sequence, which showed remarkable selectivity for GGTase I *versus* FTase (Table 1, entries 4 and 5).[46] Despite these advances, it was not until 2006 that Casey and co-workers reported the first non-peptide-based selective inhibitor of GGTase I, named GGTi-DU40 (Table 1, entry 7).[47] This pyrazole-based compound inhibits GGTase I with a K_i of <1 nM by competing with the CAAX binding site. Cellular activity could also be confirmed by inhibition of Rho-dependent rounding of MBA-231 cells.[48] Additional selective inhibitors for

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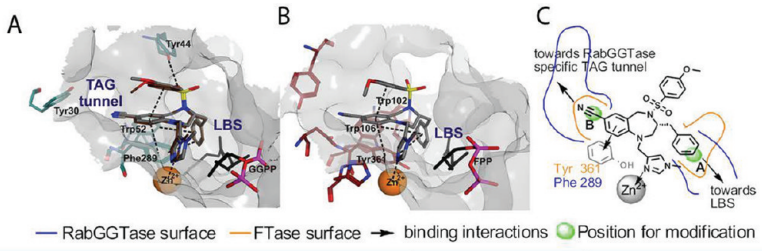

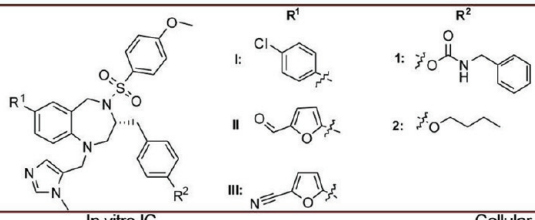

			In vitro IC_{50}				Cellular viability			
cmpd	R^1	R^2	RabGGTase	FTase	GGTase I	Reprenylation[#]	HCT116	A2780	HeLa	PBMC
BMS3	CN	H	724±321	6±3	>99,500	74±34	63±8	43±0	101±2	>10,000
1	CN	1	72±2	<5*	>99,500	81±32	230±59	130±9	151±21	>10,000
2	CN	2	162±10	10±7	>99,500	307±40	75±10	43±9	745±303	>10,000
3	I	OH	>9,500	194±78	>99,500	>30,000	nd	nd	nd	Nd
4	II	OH	206±13	7±3	>99,500	93±42	nd	nd	nd	Nd
5	III	OH	63±2	13±3	>99,500	11±5	2±1	18±1	21±4	>10,000
6	II	1	141±26	>9,700	>99,500	311±193	443±173	589±199	797±330	>10,000
7	III	2	42±13	>9,700	>99,500	49±32	35±1	115±3	101±11	>10,000

Figure 2. Co-crystal structures of BMS3. (A) BMS3:RabGGTase:GGPP. The imidazole coordinates to the zinc ion, whereas the sulfonamide forms hydrogen bonds with Tyr44. The 3-benzyl moiety interacts with Trp52 and Phe289 by T-stacking, and the tetrahydrobenzodiazepine (THB) moiety π-stacks with Phe289. The conformation is further stabilized by internal π-stacking of the THB with the anisylsulfonyl group. The nitrile points toward the TAG tunnel. (B) BMS3:FTase:FPP. The imidazole coordinates to the zinc ion. The 3-benzyl moiety interacts with Trp102 and Trp106 by T-stacking. The THB interacts with Tyr361 and is further involved in internal π-stacking with the anisylsulfonyl group. (C) Schematic representation of the common binding modes of BMS3 in panels A and B. Table: *In vitro* inhibition, cellular prenylation inhibition, and cellular viability data of selected members of the THB library (IC_{50} [nM]). *lowest detection limit, [#]inhibition of cellular prenylation in HeLa cell lines compared to saturated prenylation, measured by reprenylation signal using biotin-GPP. nd = no data. Adapted from Bon *et al.*[60] Copyright 2011 Wiley-VCH Verlag GmbH & Co.

GGTase I were later developed by Tamanoi and co-workers. A library of approximately 4000 dihydropyrroles and tetrahydropyridines was generated from resin-bound allenoates by means of phosphine catalysis and a split-and-pool combinatorial synthesis strategy. Several members of this library showed selective submicromolar inhibition of GGTase I by competing with the protein substrate (Table 1, entry 6).[49,50] These compounds showed also cellular activity by inhibiting proliferation of different cell lines such as the breast cell line MCF-7 and the leukemic Jurkat cell line, causing cell cycle arrest at G1 phase.

A major advance in this field was reached when it was observed that dual FTI and RabGGTase inhibitors were effective in tumor cell lines with no mutations in Ras proteins and that the apoptotic activity of these compounds was mainly related to their inhibition of RabGGTase and not to the inhibition of FTase, thus promoting RabGGTase as an emerging target for anticancer therapy.[51] After this finding, interest in the development of specific inhibitors of Rab GGTases has strongly increased.

One of the first selective inhibitor classes described for RabGGTase was the phosphonocarboxylates. Most representative examples are 3-PEHPC and 3-IPEHPC (Table 1, entry 8) derived from the bisphosphonates risedronate and minodronic acid, respectively. While risedronate inhibits osteoclast-mediated bone resorption by blocking FPP synthase, these phosphonocarboxylate analogues act differently and inhibit RabGGTase by blocking selectively the second prenylation reaction.[52] Hence, Rab proteins containing doubly geranylgeranylated cysteines are affected by these compounds, while the synthesis of monoprenylated Rabs such as Rab13, Rab18, or Rab 23 are not inhibited. Despite the presence of the carboxylic acid, phosphonocarboxylates display a certain affinity for mineral ions. They inhibit the prenylation of Rab GTPases required for osteoclast function and have therefore been considered for the treatment of diseases characterized by an excess of osteoclast-mediated bone resorption.[53,54] The main drawback of this compound class is their weak potency. This may be increased by the change of the pyridine ring in 3-PEHPC by a imidazo[1,2-a]pyridine core, resulting in a 25-fold more potent compound (3-IPEHPC) both *in vitro* and in cells with an IC_{50} of 1.3 μM (Table 1).[55] However, selectivity of phosphonocarboxylates also needs to be considered in the light of the recent finding that these compounds cause substrate depletion by blocking the GGPP synthase responsible for substrate production.[52,55]

Members of the of dihydropyrrole and tetrahydropyridine libraries generated by Tamanoi and co-workers showed also dual inhibition of both GGTase I and RabGGTase. Moreover, selective inhibition of RabGGTase in the micromolar range was also detected for compounds bearing an additional hydrophobic tail attached to the pyrrolidine core, thus resulting in

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one of the first potent and selective inhibitors of RabGGTase. Cellular activity could be confirmed by proving a decrease in the levels of prenylated Rab5 after treatment with inhibitor.[50]

The structure of FTase inhibitor Pepticinnamin E was used by Waldmann and co-workers for the design of a library containing 468 peptides (Table 1, entry 9).[56,57] From this library, 33 RabGGTase inhibitors could be identified by means of an *in vitro* fluorometric Rab prenylation assay with a NBD labeled FPP analogue.[58] Cellular activity of these peptide-based inhibitors was investigated by means of a reprenylation assay. Briefly, cells were incubated with the peptides, and then a recombinant RabGGTase and a Biotin-GPP analogue were added in order to prenylate an overexpressed EYFP-Rab7 with the biotinylated lipid PP. Reprenylation could then be quantified by detection with a streptavidin-coupled horseradish peroxidase. An inhibition of RabGGTase in cells resulted in increased levels of reprenylation of EYFP-Rab7 after addition of the recombinant RabGGTase, thus confirming cellular activity of the inhibitors. Some of the peptides showed similar activity for all PPTases, but also selective RabGGTase inhibitors could be identified. Important information was collected from co-crystal structures of some of these peptide-based inhibitors with RabGGTase in order to gain a better insight into their binding mode. Although a clear trend could not be established due to the high flexibility of the peptide backbone, relevant information could be extracted from these X-ray structures for the future synthesis of specific inhibitors of Rab GGTases.

One of the most potent RabGGTase inhibitors described, with an IC_{50} value in the nanomolar range, belongs to the tetrahydrobenzodiazepines (THB) developed by Bristol-Myers Squibb (Table 1, entry 10). These highly apoptotic compounds inhibit both FTase and RabGGTase. However, the apoptotic activity correlates better with inhibition of RabGGtase than with FTase.[59] This was confirmed by the induction of apoptosis observed using RNAi directed against the RabGGTase α- and β-subunits, while this effect could not be seen in the case of RNAi directed against FTase-β or GGTase-β.[51] This compound class was used as starting point by Waldmann, Goody, and co-workers for the design of potent and selective RabGGTase inhibitors.[60] After co-crystallization of BMS3 with both FTase and RabGGTase, striking structural differences were observed in both enzymes that could be exploited for achieving selectivity. The main differences are a bigger lipid binding site (LBS) for RabGGTase and a tunnel adjacent to the GGPP binding site (TAG tunnel), not present in FTase (Figure 2). Thus, it was envisaged that inhibitors designed to target both pockets would display important selectivity for RabGGTase *versus* FTase. With this aim, a library of THB derivatives was synthesized bearing additional substitutions to target the LBS and the TAG tunnel. Extension to one site or the other led to more potent albeit nonselective inhibitors. However, as expected, simultaneous extension to both sites with a combination of 2-cyanofuran or furanaldehyde directed toward the TAG tunnel and a benzylcarbamate to target the LBS led to the first nanomolar and selective inhibitors of RabGGTase (Table 1, entry 11). The binding mode was confirmed by co-crystallization confirming the binding to both the TAG tunnel and the LBS. Importantly, cellular activity was proven by using the above-mentioned reprenylation assay reporting IC_{50} values similar to those from the *in vitro* assays. Furthermore, the most potent compound inhibited proliferation of three different cancer cell lines (both Ras-transformed and non-Ras transformed), while no general toxicity could be seen in blood cells, thus confirming that RabGGTase is an interesting target for anticancer treatment.

In conclusion, much work has been carried out in the development of PPTase inhibitors in recent years. After the discouraging results obtained with FTase inhibitors in cancer treatment, GGTase I and mainly RabGGTase have evolved into promising targets for cancer therapy, and selective and potent inhibitors for each PPTase are now available. Future studies with these inhibitors may provide significant information regarding the suitability of PPTases as therapeutical targets.

MYRISTOYLATION

Myristoylation is found exclusively in eukaryotes and viral proteins and involves the covalent addition of myristoyl groups (14-carbon saturated fatty acids) to the N-terminal glycine residue of a protein. Rarely, the myristoyl group can be attached to a different residue and the linkage can be made *via* a thioesther bond, in analogy to S-palmitoylation. Myristoyl groups are transferred from myristoyl-CoA by myristoyl-CoA:protein N-myristoyltransferase (NMT). Although NMT is considered to be a cytosolic protein, some studies suggest that up to 50% of the total protein amount may be associated with a noncytosolic fraction.[61] NMT is essential to most eukaryotes, including mammals.[62] Among the possible lipidations, N-terminal myristoylation is one of the most investigated experimentally, and progress in its understanding has been assisted by the availability of reliable bioinformatics prediction methods and databases such as MYRbase (http://mendel.imp.ac.at/myristate/myrbase/). However, it remains difficult to reliably *in silico* predict the N-myristoylation patterns of full genomes as the myristoylation motif (MGxxxSxxx) carries a large inherent sequence variation.[63] Inhibitors of protein N-terminal myristoylation has recently been review elsewhere.[64,65]

PALMITOYLATION

Palmitoylation may occur at the N- or C-terminal region of proteins or at the membrane-interface domains of transmembrane proteins. Modification of a N-terminal cysteine residue *via* an amide linkage is named N-palmitoylation; O-palmitoylation corresponds to modification of serine or threonine residues by an ester attachment; and S-palmitoylation denominates the attachment of a palmitate group to the cysteine residue of a protein through a thioester linkage. O-Palmitoylation is catalyzed by members of the MBOAT (membrane-bound O-acyl transferase) protein family. Substrates for O-palmitoylation and to some extent amide linkage formation do not seem to contain specific enzyme recognition sequences and are even considered subject to nonenzymatic spontaneous events.[66,67]

The two-signal hypothesis of palmitoylation states that a protein requires two modifications in order to get stably membrane anchored. Most palmitoylated proteins are, consequently, first lipid modified by N-myristoylation or S-farnesylation, which increases weakly their affinity to membranes, and only subsequently palmitoylated after specific recognition.[68] The kinetic membrane trapping model, which is a revised version of the two-signal hypothesis, suggests that the first lipid modification, or another mechanism that provides a weak affinity to membranes (polycationic stretches, transmembrane region, *etc.*), functions more as membrane targeting rather than as a specific recognition motif for the palmitoylating

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enzyme.[69] According to the kinetic trapping model, a protein carrying a single lipid modification (or other affinity element) cycles on and off membranes until it encounters a membrane with an appropriate palmitoylating catalytic enzyme that, once it recognizes the protein substrate, acylates it and thereby associates it permanently to the membrane. Proteins that do not follow this model and are exclusively palmitoylated have been reported.[68] In these cases, there is no apparent motif for S-palmitoylation and the modified cysteine residues can be found within the 25 most N-terminal amino acids of the protein.

No consensus amino acid sequence for palmitoylation has been reported, but specific cysteines are modified, indicating that a specificity of sequence recognition may exist. This is further accentuated by the fact that the palmitoylation machinery recognizes artificial constructs expressing known palmitoylation motifs.[70,71] N-Myristoylated proteins are usually palmitoylated at cysteine residues adjacent to the N-myristoylated glycine, but palmitoylation occurs at cysteine residues up to 20 amino acids away. Palmitoyl groups, donated by acyl-CoA, are catalytically added to proteins by enzymes named protein S-acyl transferases (PATs). These enzymes are present in all eukaryotic genomes examined so far and are classified into class 1 or 2 depending on whether they modify previously S-farnesylated or N-myristoylated proteins.[72] PATs can be divided into three categories depending on their structure: ankyrin-repeat containing, heterodimeric, or monomeric.[73,74] All known PATs are integral membrane proteins and thus substrate recognition and catalysis can only occur if the protein substrates have previously been associated to the membrane *via* another affinity tag (lipidation, transmembrane domain, *etc.*).[74] The DHHC motif (Asp-His-His-Cys) belongs to the catalytic PAT domain, and mutation of the cysteine residue abolishes palmitoylation.[75,76] DHHC motifs are usually located within a cysteine-rich domain (CRD), between two transmembrane regions. This motif is pointing toward the cytosol, which may aid in bringing closer the weakly membrane adherent substrates to the catalytic site.[75,77,78]

Most genomes encode for more than one PAT, and these enzymes are differently localized. The mechanisms that dictate their specific subcellular distribution, substrate specificit, and mode of regulation are until today unknown. Extracellular factors, as well as different posttranslational modifications have been suggested to influence PAT activity.[74] Recently, a large-scale study proposed that DHHC proteins are themselves palmitoylated, and this may contribute to their substrate specificity, trafficking to specific membrane microdomains, protection from ubiquitination, and/or recruitment of different regulators.[79] Deletion studies with the yeast DHHC protein family showed that although deletion of all of the DHHC-containing proteins is lethal, there is redundancy in PAT function since for some of the proteins assayed, substantial palmitoylation persists in cells multiply deficient for DHHCs.[80] S-Palmitoylation has two unique aspects when compared to other lipidations. It affects strongly the membrane affinity of a protein—a palmitoyl moiety alone provides a 100 times stronger membrane association than a single N-myristoyl moiety—and it is reversible. Reversibility offers a flexible, rapid, and precise mode of regulation of protein activity at a relative time scale comparable to O-phosphorylation. Depalmitoylation returns the protein to a state where it is rapidly cycling on and off membranes and trafficking through a vesicle-independent mechanism. The S-acylation status of a protein is likely to change several times during its lifetime and can occur as result of the activation or deactivation of signaling pathways.[68,81]

Four soluble human thioesterases have been characterized as depalmitoylating enzymes to date. The palmitoyl protein thioesterases PPT1 and PPT2 function in the lysosomes during protein degradation and do not contribute to signaling dynamics. In contrast, the acyl protein thioesterases APT1 and APT2 are small proteins, largely cytosolic and widely conserved from yeast to humans.[66,68,82] The crystal structures of PPT1 (bovine) alone or in complex with palmitate or an inhibitor have been resolved.[66] No dramatic structural changes could be identified among the different situations, and consequently the mechanisms of substrate recognition and catalysis are still not clear. The APT1 apo-structure has also been resolved,[83] and the free status is characterized by dimerization and blocking of the active site, suggesting that substrate binding may lead to dimer dissociation and catalysis. APT1 does not recognize a specific protein sequence, and it has been suggested that a conformational change or dissociation of a binding partner in the vicinity of the palmitoyl group has to occur, in order for the substrate to become more accessible to cleavage. APT1 does depalmitoylate not only the C-terminus of small GTPases, in both D- and L-amino acid forms,[84] but also other G-proteins,[85] ghrelin (deoctanoylation),[86] viral glycoproteins,[87] and lysophospholipids.[88] In terms of substrate recognition, APT2 is less well explored.

Many questions remain regarding PAT and APT/PPT activity: The mechanism of palmitate transfer and the role of the DHHC domain in the reaction are unclear. Does the DHHC domain work as a zinc binding motif? What is the mechanism of substrate and palmitoyl-CoA recognition? What are the domains or factors that regulate PAT and APT specificity? How do the APTs keep control over steady state protein localization? Myristoylation and palmitoylation both work in concert with other lipid modifications, as well as additional posttranslational modifications, such as phosphorylation, to facilitate targeting to membranes and appropriate cellular destination. These mechanisms are just now beginning to be defined.

The human palmitoylme includes Ras isoforms, many members of the Src family of protein tyrosine kinases, subunits of G proteins and G protein-coupled receptors, rhodopsin, and several neuron-specific proteins.[89] In neurons, protein palmitoylation is assumed to play a key role in targeting proteins for transport to nerve terminals and for regulating trafficking at the synapse.[81] As S-palmitoylation is reversible, it induces cycles of modification on its substrates. The addition of a palmitate by a specific PAT at the ER or Golgi results in increased affinity of the protein to a specific membrane. Modified proteins can then leave the organelles and be further trafficked to other compartments, *via* vesicles, on the basis of their sorting information. Depalmitoylation by the action of a APT enzyme forces the return of a protein to a state where it samples different membranes, finally ending up finding a PAT (highly likely at the Golgi), which closes the cycle by repalmitoylating the protein. The small GTPases N- and H-Ras that are key regulators of the mitogen-activated protein kinase (MAPK) cascade are examples of proteins that cycle between different intracellular membranes due to dynamic S-palmitoylation. Similar endomembrane cycling dynamics were recently reported for Giα, a protein that couples to GPCRs, as well as for GAP43.[90] Recently, Schratt and co-workers showed with a functional screen that microRNA-138 regulates APT1 in

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dendritic spine morohogenesis, suggesting an additional layer of regulation.[91]

PAT INHIBITION

Global inhibition of lipidation at the level of fatty acid biosynthesis typically leads to lethal phenotypes in eukaryotic cells. A good example is the use of 2-bromo palmitate (Figure 3a), which is a highly efficient palmitoylation inhibitor at the level of palmitic acid biosynthesis but displays high toxicity, thus being of limited use in cell-based experimentation.[92] Given the importance of the palmitate lipid anchor for correct Ras cellular localization and signaling, targeting Ras palmitoylation may define a new class of anticancer agents. In this context, a first approach has consisted in a global inhibition of protein palmitoylation by inhibiting palmitic acid biosynthesis. This strategy was shown to induce Ras mislocalization and loss of activity with severe toxicity due to various off targets.[93] In comparison to 2-Br-palmitate, small molecule PAT-inhibitors should be less toxic but cannot be expected to be very specific. To date only two Ras protein acyltransferase inhibitors have been reported. Cerulenin (Figure 3b) was the first established palmitoylation inhibitor of Ras proteins and since recently of several additional proteins. The cerulenin-induced antiproliferative effect was shown to be exclusively due to its ability to inhibit Ras palmitoylation (IC_{50} = 4.5 μM) and not to its ability to inhibit fatty acid synthase (fas) given that some cerulenin analogues displaying an antiproliferative effect were inactive against fas.[93] The second PAT inhibitor (Figure 3c) inhibits the MAKP signaling pathway, thereby inducing a significant antiproliferative effect in various human cancer cell lines (with IC_{50} values typically in the low micromolar range).[72] It is likely that wide-spectra inhibition of mammalian PATs will lead to various side effects related to failure of the generic sorting mechanism at the level of the Golgi, as well as diminished metabolism.[84]

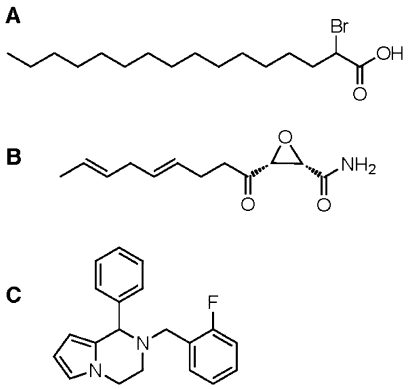

Figure 3. Targeting Ras palmitoylation. Structures of reported PAT inhibitors.

INHIBITION OF DEPALMITOYLATING ENZYMES

In sharp contrast to inhibition of palmitoylation, interference with the depalmitoylating enzymes should allow for viable phenotypes by only down-regulating biological responses (for example, signaling) by disturbing the intracellular localization of the target protein. A good example is the human S-depalmitoylating enzyme APT1, which upon inhibition leads to attenuated MAPK-mediated signaling *via* Ras-delocalization.[94,95] Inhibition of thioesterase activity responsible for Ras depalmitoylation increases the pool of palmitoylated Ras proteins, leading to the expectation that such inhibition will not reduce oncogenic Ras signaling. However, since palmitoylated H- and N-Ras remain membrane-bound, permanent unspecific cellular membrane exchange leads to loss of normal localization by entropy-driven redistribution to all endomembranes. Thus, inhibition of depalmitoylation counterintuitively will lead to down-regulation rather than inhibition of signaling, allowing cell viability. This observation verifies the strategy that disturbing dynamic lipidation cycles at the stage of delipidation leads to an entropy-driven loss of precise localization, thus affecting the signaling network. This concept can be translated and applied in a broader sense to a plethora of acylated proteins, which are depending on precise localization for their biological role, thus allowing for small-molecule regulation of hard-to-affect targets relying on dynamic acylation. When considering the relative number of turnovers during the lifetime of a cell in a eukaryotic organism, S-acylation is most likely to be effected at the deacylation stage by inhibition of the corresponding hydrolases, whereas N- and O-lipidations are best targeted at the transferase stage of the cycle because of the persistence of the modification.

DEVELOPMENT OF APT INHIBITORS

Recently, Waldmann *et al.* reported APT inhibitors designed on the basis of the β-lactone core. The first inhibitor developed, Palmostatin B (Figure 4a), was designed using a knowledge-based strategy, denoted protein structure similarity clustering (PSSC).[94,96] In the PSSC strategy, proteins are assigned to clusters on the basis of similarity in the three-dimensional structure in their ligand sensing cores. Using this approach structural similarity was observed between the enzymes APT1 and gastric lipase. The hydrolase inhibitor tetrahydrolipstatin was employed to design a focused compound collection with a β-lactone core structure (Figure 4b). This compound collection was screened for inhibition of the enzyme APT1 and provided the inhibitor palmostatin B as the most potent compound. Palmostatin B inactivates APT1 by reversible covalent modification of the enzyme active site, thus acting as a slow substrate. Furthermore, a direct interaction between APT1 and palmostatin B in cells was demonstrated. Palmostatin B inhibits APT1 selectively compared to the intracellular phospholipases A1, A2, Cβ, and D. Palmostatin B caused H-Ras specific delocalization from the plasma membrane to endomembranes in MDCKF3 cells, where K-Ras 4B (nonpalmitoylated) was not affected.[94] However, APT1 is an enzyme with very broad substrate scope, and the very unusual and exceptionally wide substrate tolerance provided inspiration to alternatively employ the structural characteristics of the different substrates as guiding arguments for the design of a more potent family of inhibitors. The activity of palmostatin B is mainly determined by the stereochemistry of its electrophilic β-lactone core and the aliphatic substitution at its α-position. Ideation based on substrate similarity indicates that selective recognition will be enhanced by the introduction of functionalities that enable hydrogen bonding and electrostatic interactions similar to those of a selected number of substrates. The design principle resulted in a low nanomolar inhibitor of APT1, denoted Palmostatin M (Figure 4C), possessing superior characteristics for cell-based experimentation.[97] Palmostatin M perturbs the acylation cycle, as well as the H- and N-Ras signaling activity, at the level of depalmitoylation, thereby leading to decreased

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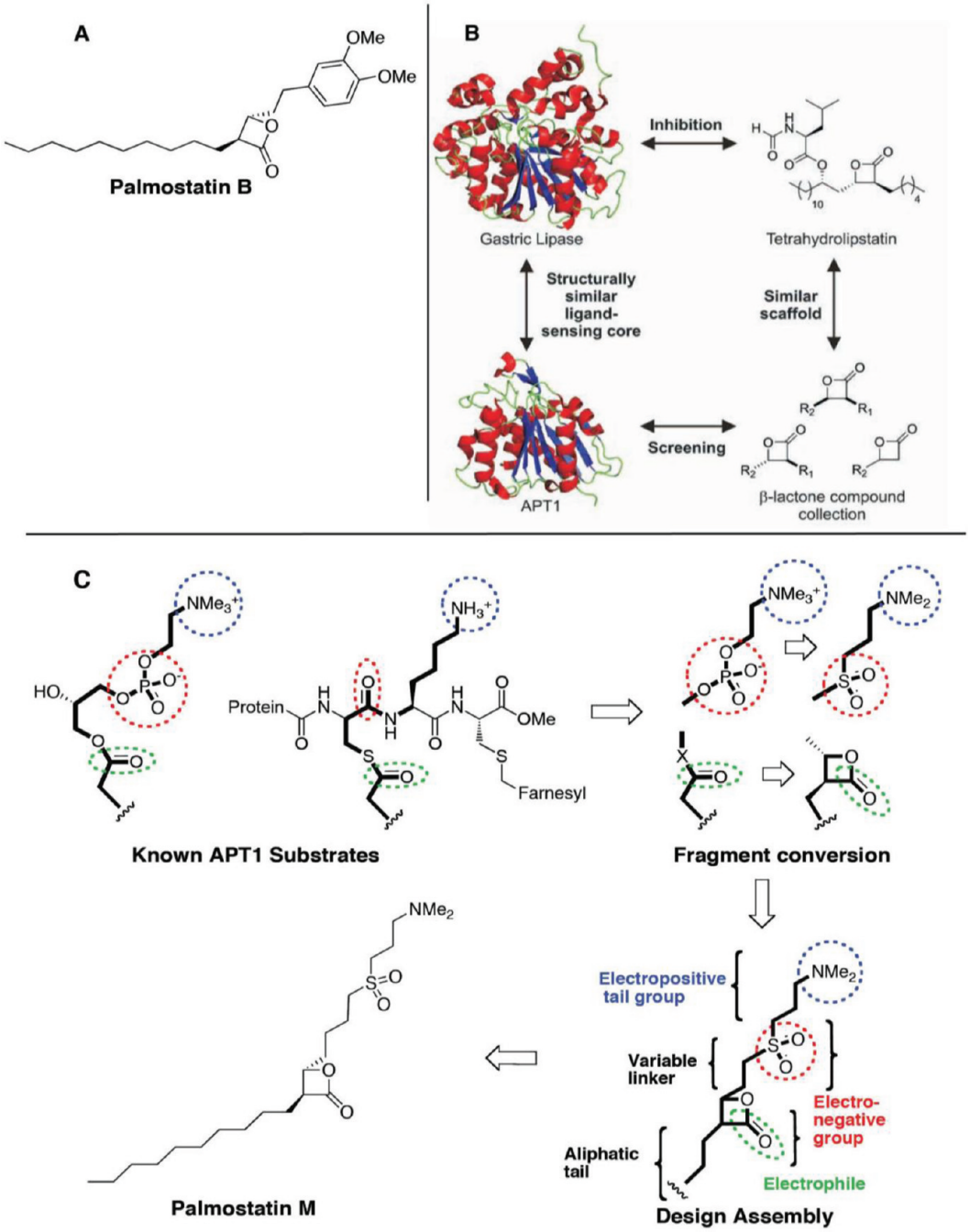

Figure 4. (A) Structure of the cell-permeable small molecule APT1-inhibitor denoted Palmostatin B. (B) Development of Palmostatin B through the knowledge-driven PSSC approach over a β-lactone library. (C) Development of a second-generation APT1 inhibitor. Comparison of two known native APT1 substrates, lysophospholipid and the N-Ras C-terminus resulted in the identification of a common recognition motif consisting of a negatively charged group at a distance of five to six bonds (red) from the (thio)-ester functionality (green) and a positively charged tail group at 10 to 12 bonds distance (blue). To design a inhibitor, fragments were joined through a variable spacer to a trans-β-lactone core, which addresses the stereochemical preference of APT1 and serves as covalent modifier of the nucleophilic residue in the active site of the enzyme. Variation in spacer length enables the identification of the optimal distance between fragments. A lipophilic tail mimicking the palmitate moiety was introduced on the opposite side of the β-lactone core to create affinity to the lipid-binding pocket of the enzyme. Based on these criteria, a small focused library of three series of inhibitors was designed and synthesized, which resulted in the discovery of Palmostatin M.

MAP-kinase signaling and partial endothelial to mesenchymal phenotypic reversion of H-Ras-transformed MDCK-F3 cells. Further evaluation of Palmostatin B and substrate analogue based inhibitor Palmostatin M by activity-based proteome profiling (ABPP) in Hela cells revealed that the isoenzyme APT2 also is targeted by the Palmostatins, in addition to PPT1 and APT1 (Figure 5).[98] Notably, no other intracellular esterases (such as phospholipases A1, A2, C, and D) relevant to Ras signaling were identified as targets for the palmostatins, which proves the selectivity of the probes. Taken together, these findings suggest that no further hydrolases employing a similar mechanism of catalysis and possibly no further hydrolases in general are involved in Ras depalmitoyation in cells.

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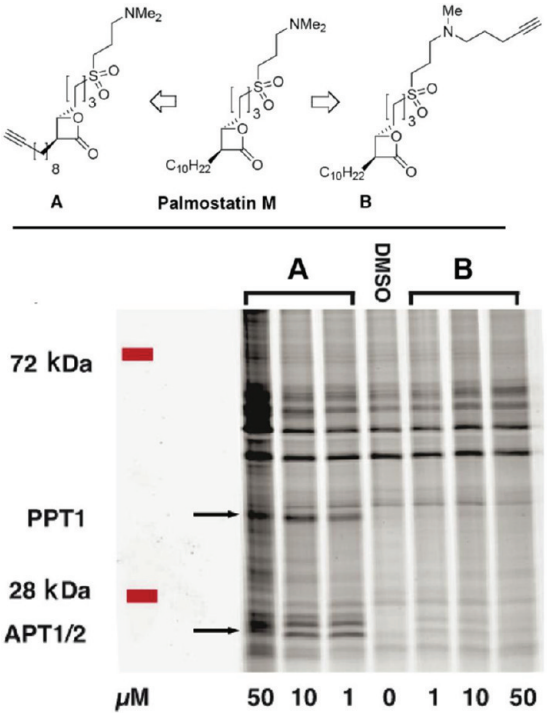

Figure 5. Cellular proteome labeling profile for Palmostatin M derived probes A and B after 20 min incubation at various probe concentrations (50/10/1 μM). Fluorescent gel highlighting three major bands corresponding to PPT1 (MW = 34.193 kDa), APT1 (MW = 24.67 kDa), and APT1/2 (MW = 24.73 kDa) Conditions: Biotin-Rhodamine-N3 = 20 μM, TCEP:HCl = 0.5 mM, ligand = 50 μM, $CuSO_4$ = 0.5 mM, HeLa cells = 2×10^6.

CONCLUDING REMARKS

Numerous signaling proteins are posttranslationally modified by lipidation. Since this lipidation is essential for a correct localization and function of these proteins, the enzymes responsible for the covalent attachment of lipids (FTase, GGTase, Rab GGTase, NMTs, PATs) or lipid removal (APTs) have been considered interesting targets for blocking aberrant signaling processes. Despite the initial promising results obtained *in vitro* with FTIs, similar success could not be translated to the clinic. Alternatively, dual inhibitors of FTase and GGTase or the use of FTase in combination with cytotoxic agents have overcome some of these limitations. Furthermore, FTIs have also shown good results in cancer cells not dependent on Ras. Alternatively, good correlation between apoptotic activity and selective inhibition of RabGGTase *versus* the other PPTases has been recently observed in different studies. This, together with the lack of toxicity in noncancer cell lines, suggests that RabGGTase may be indeed a promising target for cancer therapy. A general consideration of PPTIs is that they may target a broad range of lipidated proteins. In this context, the identification of their target proteins is a major goal to understand the mechanisms underlying their cytotoxic activity. Recent advances in this field, such the methods reported by Maurer-Stroh and co-workers[99] or Alexandrov and co-workers, for the identification of the Rab proteins subset affected by RabGGTase inhibitors,[100] may contribute enormously to this understanding .

The development of APT-inhibitors has gained little attention in the pharmaceutical industry, as it has intuitively been expected that inhibition of depalmitoylation would increase the portion of Ras in active palmitoylated form, which would facilitate oncogenic signaling. However, the discovery that dynamic palmitoylation/depalmitoylation events are required to maintain proper Ras localization indicates that inhibition of Ras depalmitoylation will attenuate Ras-mediated signaling, thus making the depalmitoylation event more attractive to target, compared to the palmitoylation step. Since the palmitoylation reaction of proteins at the Golgi is a rather unspecific generic sorting machinery for palmitoylated proteins, PATs are unwanted targets for small-molecule inhibitors. In contrast, targeting APTs opens a new and unexpected entry toward development of conceptually new therapeutic approaches in oncology that target depalmitoylation specifically. However, the selectivity of both Palmostatin B and the APT-enzymes remains to be investigated further. It can be expected that many biological functions are regulated by reversible palmitoylation events controlling protein localization and that additional thioesterases will be identified in the near future. Such a development will further increase the importance of thioesterase inhibitors such as palmostatin B, as well as of semisynthetic lipidated proteins to probe biological function *in vivo*.

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KEYWORDS

Farnesyl Transferase (FTase) and Geranylgeranyl Transferase (GGTase): formed by an α subunit and a β subunit; the α subunit is almost identical in both enzymes; the β subunit contains a hydrophobic pocket whose substrate specificity for FPP or GPP is determined by the residue present at the bottom of this site; Rab geranylgeranyl transferase (GGTase): also formed by an α and a β subunit and (di)geranylgeranylates members of the Rab family; this is done in a ternary complex of Rab GTPase, RabGGTase and REP, in which REP recognizes unprenylated Rab GTPases and presents them to RabGGTase; TAG tunnel (Tunnel adjacent to the GGPP): present in RabGGTase and absent in the FTase and GGTase that can be used to design selective inhibitors for RabGGTase; Lipid binding site (LBS): the binding site for the different PPP present in all of the PPTases; N-myristyl transferase (NMT): transfers myristate from myristyl-CoA to the N-terminal glycine of many important eukaryotic and viral proteins; Palmitoyl acyl transferases (PAT): a family of around 20 members, each possessing diverse and overlapping substrate specificity; PATs transfer palmitoyl residues from Palmitoyl-CoA to cysteine residues in proteins; Palmitoyl protein thioesterase (PPT): cleaves palmitoyl residues from defolded and proteolytically digested proteins in lysosomes as a part of the protein degradation machinery; Acyl protein thioesterase (APT): removes palmitoyl groups from palmitoylated proteins at membranes, thereby avoiding unspecific entropic redistribution in the cell by allowing for retrapping at the Golgi

REFERENCES

(1) Snook, C. F., Jones, J. A., and Hannun, Y. A. (2006) Sphingolipid-binding proteins. *Biochim. Biophys. Acta 1761*, 927−946.

(2) Stahelin, R. V. (2009) Lipid binding domains: more than simple lipid effectors. *J. Lipid Res. 50*, S299−S304.

(3) Towler, D. A., Eubanks, S. R., Towery, D. S., Adams, S. P., and Glaser, L. (1987) Amino-terminal processing of proteins by N-myristoylation - substrate-specificity of N-myristoyl transferase. *J. Biol. Chem. 262*, 1030−1036.
(4) Gelb, M. H. (1997) Protein biochemistry - protein prenylation, et cetera: Signal transduction in two dimensions. *Science 275*, 1750−1751.
(5) Weise, K., Triola, G., Brunsveld, L., Waldmann, H., and Winter, R. (2009) Influence of the lipidation motif on the partitioning and association of N-Ras in model membrane subdomains. *J. Am. Chem. Soc. 131*, 1557−1564.
(6) Weise, K., Kapoor, S., Denter, C., Nikolaus, J., Opitz, N., Koch, S., Triola, G., Herrmann, A., Waldmann, H., and Winter, R. (2011) Membrane-mediated induction and sorting of K-Ras microdomain signaling platforms. *J. Am. Chem. Soc. 133*, 880−887.
(7) Chen, Y. X., Koch, S., Uhlenbrock, K., Weise, K., Das, D., Gremer, L., Brunsveld, L., Wittinghofer, A., Winter, R., Triola, G., and Waldmann, H. (2010) Synthesis of the Rheb and K-Ras4B GTPases. *Angew. Chem., Int. Ed. 49*, 6090−6095.
(8) Ismail, S. A., Chen, Y. X., Rusinova, A., Chandra, A., Bierbaum, M., Gremer, L., Triola, G., Waldmann, H., Bastiaens, P. I., and Wittinghofer, A. (2011) Arl2-GTP and Arl3-GTP regulate a GDI-like transport system for farnesylated cargo. *Nat. Chem. Biol. 7*, 942−949.
(9) Brunsveld, L., Kuhlmann, K., Alexandrov, K., Wittinghofer, A., Goody, R. G., and Waldmann, H. (2006) Lipidated Ras and Rab peptides and proteins - synthesis, structure, and function. *Angew. Chem., Int. Ed.. 45*, 6622−6646.
(10) Hang, H. C., Wilson, J., and Charron, G. (2011) Bioorthogonal chemical reporters for analyzing protein lipidation and lipid trafficking. *Acc. Chem. Res. 44*, 699−708.
(11) Zhang, F. L., and Casey, P. J. (1996) Protein prenylation: Molecular mechanisms and functional consequences. *Annu. Rev. Biochem. 65*, 241−269.
(12) Itzen, A., and Goody, R. S. (2011) GTPases involved in vesicular trafficking: Structures and mechanisms. *Semin. Cell Dev. Biol. 22*, 48−56.
(13) Bos, J. L. (1989) Ras oncogenes in human cancer - a review. *Cancer Res. 49*, 4682−4689.
(14) Ramos, M. J., Sousa, S. F., and Fernandes, P. A. (2008) Farnesyltransferase inhibitors: A detailed chemical view on an elusive biological problem. *Curr. Med. Chem. 15*, 1478−1492.
(15) Basso, A. D., Kirschmeier, P., and Bishop, W. R. (2006) Farnesyl transferase inhibitors. *J. Lipid Res. 47*, 15−31.
(16) Patel, D. V., Schmidt, R. J., Biller, S. A., Gordon, E. M., Robinson, S. S., and Manne, V. (1995) Farnesyl diphosphate-based inhibitors of Ras farnesyl-protein transferase. *J. Med. Chem. 38*, 2906−2921.
(17) Reiss, Y., Goldstein, J. L., Seabra, M. C., Casey, P. J., and Brown, M. S. (1990) Inhibition of purified P21ras farnesyl-protein transferase by Cys-Aax tetrapeptides. *Cell 62*, 81−88.
(18) James, G. L., Goldstein, J. L., Brown, M. S., Rawson, T. E., Somers, T. C., Mcdowell, R. S., Crowley, C. W., Lucas, B. K., Levinson, A. D., and Marsters, J. C. (1993) Benzodiazepine peptidomimetics - potent inhibitors of Ras farnesylation in animal cells. *Science 260*, 1937−1942.
(19) Patel, D. V., Gordon, E. M., Schmidt, R. J., Weller, H. N., Young, M. G., Zahler, R., Barbacid, M., Carboni, J. M., Gullobrown, J. L., Hunihan, L., Ricca, C., Robinson, S., Seizinger, B. R., Tuomari, A. V., and Manne, V. (1995) Phosphinyl acid-based bisubstrate analog inhibitors of Ras farnesyl-protein transferase. *J. Med. Chem. 38*, 435−442.
(20) Beese, L. S., Reid, T. S., and Long, S. B. (2004) Crystallographic analysis reveals that anticancer clinical candidate L-778,123 inhibits protein famesyltransferase and geranylgeranyltransferase-I by different binding modes. *Biochemistry (Moscow) 43*, 9000−9008.
(21) Beese, L. S., and Reid, T. S. (2004) Crystal structures of the anticancer clinical candidates R1 15777 (Tipifarnib) and BMS-214662 complexed with protein farnesyltransferase suggest a mechanism of FTI selectivity. *Biochemistry (Moscow) 43*, 6877−6884.
(22) Zhang, F. L., Kirschmeier, P., Carr, D., James, L., Bond, R. W., Wang, L., Patton, R., Windsor, W. T., Syto, R., Zhang, R. M., and Bishop, W. R. (1997) Characterization of Ha-Ras, N-Ras, Ki-Ras4A, and Ki-Ras4B as in vitro substrates for farnesyl protein transferase and geranylgeranyl protein transferase type I. *J. Biol. Chem. 272*, 10232−10239.
(23) Whyte, D. B., Kirschmeier, P., Hockenberry, T. N., NunezOliva, I., James, L., Catino, J. J., Bishop, W. R., and Pai, J. K. (1997) K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J. Biol. Chem. 272*, 14459−14464.
(24) James, G. L., Goldstein, J. L., and Brown, M. S. (1995) Polylysine and Cvim sequences of K-Rasb dictate specificity of prenylation and confer resistance to benzodiazepine peptidomimetic in vitro. *J. Biol. Chem. 270*, 6221−6226.
(25) Epling-Burnette, P. K., and Loughran, T. P. (2010) Suppression of farnesyltransferase activity in acute myeloid leukemia and myelodysplastic syndrome: current understanding and recommended use of tipifarnib. *Expert Opin. Invest. Drugs 19*, 689−698.
(26) Balasis, M. E., Forinash, K. D., Chen, Y. A., Fulp, W. J., Coppola, D., Hamilton, A. D., Cheng, J. Q., and Sebti, S. M. (2011) Combination of farnesyltransferase and Akt inhibitors is synergistic in breast cancer cells and causes significant breast tumor regression in ErbB2 transgenic mice. *Clin. Cancer Res. 17*, 2852−2862.
(27) Moasser, M. M., Sepp-Lorenzino, L., Kohl, N. E., Oliff, A., Balog, A., Su, D. S., Danishefsky, S. J., and Rosen, N. (1998) Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones. *Proc. Natl. Acad. Sci. U.S.A. 95*, 1369−1374.
(28) Gromov, P. S., Madsen, P., Tomerup, N., and Celis, J. E. (1995) A novel approach for expression cloning of small GTPases: Identification, tissue distribution and chromosome mapping of the human homolog of rheb. *FEBS Lett. 377*, 221−226.
(29) Basso, A. D., Mirza, A., Liu, G. J., Long, B. J., Bishop, W. R., and Kirschmeier, P. (2005) The farnesyl transferase inhibitor (FTI) SCH66336 (lonafarnib) inhibits Rheb farnesylation and mTOR signaling - Role in FTI enhancement of taxane and tamoxifen anti-tumor activity. *J. Biol. Chem. 280*, 31101−31108.
(30) Zheng, H., Liu, A. L., Liu, B., Li, M. H., Yu, H. L., and Luo, X. J. (2010) Ras homologue enriched in brain is a critical target of farnesyltransferase inhibitors in non-small cell lung cancer cells. *Cancer Lett. 297*, 117−125.
(31) Li, Y., Inoki, K., and Guan, K. L. (2004) Biochemical and functional characterizations of small GTPase Rheb and TSC2 GAP activity. *Mol. Cell. Biol. 24*, 7965−7975.
(32) Ashar, H. R., James, L., Gray, K., Carr, D., Black, S., Armstrong, L., Bishop, W. R., and Kirschmeier, P. (2000) Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. *J. Biol. Chem. 275*, 30451−30457.
(33) Hussein, D., and Taylor, S. S. (2002) Farnesylation of Cenp-F is required for G2/M progression and degradation after mitosis. *J. Cell Sci. 115*, 3403−3414.
(34) Crespo, N. C., Ohkanda, J., Yen, T. J., Hamilton, A. D., and Sebti, S. M. (2001) The farnesyltransferase inhibitor, FTI-2153, blocks bipolar spindle formation and chromosome alignment and causes prometaphase accumulation during mitosis of human lung cancer cells. *J. Biol. Chem. 276*, 16161−16167.
(35) Graham, S. L., DeSolms, S. J., Ciccarone, T. M., MacTough, S. C., Shaw, A. W., Buser, C. A., Ellis-Hutchings, M., Fernandes, C., Hamilton, K. A., Huber, H. E., Kohl, N. E., Lobell, R. B., Robinson, R. G., Tsou, N. N., Walsh, E. S., Beese, L. S., and Taylor, J. S. (2003) Dual protein farnesyltransferase-geranylgeranyltransferase-I inhibitors as potential cancer chemotherapeutic agents. *J. Med. Chem. 46*, 2973−2984.
(36) Li, D., Qiao, Y. Q., Gao, J. B., Qiu, Y. G., Wu, L., Guo, F., and Lo, K. K. W. (2011) Design, synthesis, and characterization of piperazinedione-based dual protein inhibitors for both farnesyltransferase and geranylgeranyltransferase-I. *Eur. J. Med. Chem. 46*, 2264−2273.
(37) Liu, M., Sjogren, A. K. M., Karlsson, C., Ibrahim, M. X., Andersson, K. M. E., Olofsson, F. J., Wahlstrom, A. M., Dalin, M., Yu,

H. M., Chen, Z. G., Yang, S. H., Young, S. G., and Bergo, M. O. (2010) Targeting the protein prenyltransferases efficiently reduces tumor development in mice with K-RAS-induced lung cancer. *Proc. Natl. Acad. Sci. U.S.A. 107*, 6471−6476.

(38) Bendale, P., Olepu, S., Suryadevara, P. K., Bulbule, V., Rivas, K., Nallan, L., Smart, B., Yokoyama, K., Ankala, S., Pendyala, P. R., Floyd, D., Lombardo, L. J., Williams, D. K., Buckner, F. S., Chakrabarti, D., Verlinde, C., Van Voorhis, W. C., and Gelb, M. H. (2007) Second generation tetrahydroquinoline-based protein farnesyltransferase inhibitors as antimalarials. *J. Med. Chem. 50*, 4585−4605.

(39) Mehta, I. S., Bridger, J. M., and Kill, I. R. (2010) Progeria, the nucleolus and farnesyltransferase inhibitors. *Biochem. Soc. Trans. 38*, 287−291.

(40) Mallampalli, M. P., Huyer, G., Bendale, P., Gelb, M. H., and Michaelis, S. (2005) Inhibiting farnesylation reverses the nuclear morphology defect in a HeLa cell model for Hutchinson-Gilford progeria syndrome. *Proc. Natl. Acad. Sci. U.S.A. 102*, 14416−14421.

(41) Fong, L. G., Frost, D., Meta, M., Qiao, X., Yang, S. H., Coffinier, C., and Young, S. G. (2006) A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria. *Science 311*, 1621−1623.

(42) Kieran, M. W., Gordon, L., and Kleinman, M. (2007) New approaches to progeria. *Pediatrics 120*, 834−841.

(43) Philips, M. R., and Cox, A. D. (2007) Geranylgeranyltransferase I as a target for anti-cancer drugs. *J. Clin. Invest. 117*, 1223−1225.

(44) Bergo, M. O., Sjogren, A. K. M., Andersson, K. M. E., Liu, M., Cutts, B. A., Karlsson, C., Wahlstrom, A. M., Dalin, M., Weinbaum, C., Casey, P. J., Tarkowski, A., Swolin, B., and Young, S. G. (2007) GGTase-I deficiency reduces tumor formation and improves survival in mice with K-RAS-induced lung cancer. *J. Clin. Invest. 117*, 1294−1304.

(45) Sane, K. M., Mynderse, M., LaLonde, D. T., Dean, I. S., Wojtkowiak, J. W., Fouad, F., Borch, R. F., Reiners, J. J., Gibbs, R. A., and Mattingly, R. R. (2010) A Novel Geranylgeranyl transferase inhibitor in combination with lovastatin inhibits proliferation and induces autophagy in STS-26T MPNST cells. *J. Pharmacol. Exp. Ther. 333*, 23−33.

(46) Hamilton, A. D., Vasudevan, A., Qian, Y. M., Vogt, A., Blaskovich, M. A., Ohkanda, J., and Sebti, S. M. (1999) Potent, highly selective, and non-thiol inhibitors of protein geranylgeranyltransferase-I. *J. Med. Chem. 42*, 1333−1340.

(47) Peterson, Y. K., Kelly, P., Weinbaum, C. A., and Casey, P. J. (2006) A novel protein geranylgeranyltransferase-I inhibitor with high potency, selectivity, and cellular activity. *J. Biol. Chem. 281*, 12445−12450.

(48) Casey, P. J., Peterson, Y. K., Kelly, P., and Weinbaum, C. A. (2006) A novel protein geranylgeranyltransferase-I inhibitor with high potency, selectivity, and cellular activity. *J. Biol. Chem. 281*, 12445−12450.

(49) Castellano, S., Fiji, H. D. G., Kinderman, S. S., Watanabe, M., de Leon, P., Tamanoi, F., and Kwon, O. (2007) Small-molecule inhibitors of protein geranylgeranyltransferase type I. *J. Am. Chem. Soc. 129*, 5843−+.

(50) Tamanoi, F., Watanabe, M., Fiji, H. D. G., Guo, L., Chan, L., Kinderman, S. S., Slamon, D. J., and Kwon, O. (2008) Inhibitors of protein geranylgeranyltransferase I and Rab geranylgeranyltransferase identified from a library of allenoate-derived compounds. *J. Biol. Chem. 283*, 9571−9579.

(51) Lackner, M. R., Kindt, R. M., Carroll, P. M., Brown, K., Cancilla, M. R., Chen, C. Y., de Silva, H., Franke, Y., Guan, B., Heuer, T., Hung, T., Keegan, K., Lee, J. M., Manne, V., O'Brien, C., Parry, D., Perez-Villar, J. J., Reddy, R. K., Xiao, H. J., Zhan, H. J., Cockett, M., Plowman, G., Fitzgerald, K., Costa, M., and Ross-Macdonald, P. (2005) Chemical genetics identifies Rab geranylgeranyl transferase as an apoptotic target of farnesyl transferase inhibitors. *Cancer Cell 7*, 325−336.

(52) Seabra, M. C., Baron, R. A., Tavare, R., Figueiredo, A. C., Blazewska, K. M., Kashemirov, B. A., McKenna, C. E., Ebetino, F. H., Taylor, A., Rogers, M. J., and Coxon, F. P. (2009) Phosphonocarboxylates inhibit the second geranylgeranyl addition by Rab geranylgeranyl transferase. *J. Biol. Chem. 284*, 6861−6868.

(53) Coxon, F. P., Helfrich, M. H., Larijani, B., Muzylak, M., Dunford, J. E., Marshall, D., McKinnon, A. D., Nesbitt, S. A., Horton, M. A., Seabra, M. C., Ebetino, F. H., and Rogers, M. J. (2001) Identification of a novel phosphonocarboxylate inhibitor of Rab geranylgeranyl transferase that specifically prevents Rab prenylation in osteoclasts and macrophages. *J. Biol. Chem. 276*, 48213−48222.

(54) Lawson, M. A., Coulton, L., Ebetino, F. H., Vanderkerken, K., and Croucher, P. I. (2008) Geranylgeranyl transferase type II inhibition prevents myeloma bone disease. *Biochem. Biophys. Res. Commun. 377*, 453−457.

(55) McKenna, C. E., Kashemirov, B. A., Blazewska, K. M., Mallard-Favier, I., Stewart, C. A., Rojas, J., Lundy, M. W., Ebetino, F. H., Baron, R. A., Dunford, J. E., Kirsten, M. L., Seabra, M. C., Bala, J. L., Marma, M. S., Rogers, M. J., and Coxon, F. P. (2010) Synthesis, chiral high performance liquid chromatographic resolution and enantiospecific activity of a potent new geranylgeranyl transferase inhibitor, 2-hydroxy-3-imidazo[1,2-*a*]pyridin-3-yl-2-phosphonopropionic acid. *J. Med. Chem. 53*, 3454−3464.

(56) Guo, Z., Wu, Y. W., Tan, K. T., Bon, R. S., Guiu-Rozas, E., Delon, C., Nguyen, U. T., Wetzel, S., Arndt, S., Goody, R. S., Blankenfeldt, W., Alexandrov, K., and Waldmann, H. (2008) Development of selective RabGGTase inhibitors and crystal structure of a RabGGTase-inhibitor complex. *Angew. Chem., Int. Ed.. 47*, 3747−3750.

(57) Tan, K. T., Guiu-Rozas, E., Bon, R. S., Guo, Z., Delon, C., Wetzel, S., Arndt, S., Alexandrov, K., Waldmann, H., Goody, R. S., Wu, Y. W., and Blankenfeldt, W. (2009) Design, synthesis, and characterization of peptide-based Rab geranylgeranyl transferase inhibitors. *J. Med. Chem. 52*, 8025−8037.

(58) Wu, Y. W., Waldmann, H., Reents, R., Ebetino, F. H., Goody, R. S., and Alexandrova, K. (2006) A protein fluorescence amplifier: Continuous fluorometric assay for Rab geranylgeranyltransferase. *ChemBioChem 7*, 1859−1861.

(59) Manne, V., Rose, W. C., Lee, F. Y. F., Fairchild, C. R., Lynch, M., Monticello, T., and Kramer, R. A. (2001) Preclinical antitumor activity of BMS-214662, a highly apoptotic and novel farnesyltransferase inhibitor. *Cancer Res. 61*, 7507−7517.

(60) Bon, R. S., Guo, Z., Stigter, E. A., Wetzel, S., Menninger, S., Wolf, A., Choidas, A., Alexandrov, K., Blankenfeldt, W., Goody, R. S., and Waldmann, H. (2011) Structure-guided development of selective RabGGTase inhibitors. *Angew. Chem., Int. Ed.. 50*, 4957−4961.

(61) Shrivastav, A., Selvakumar, P., Bajaj, G., Lu, Y., Dimmock, J. R., and Sharma, R. K. (2005) Regulation of N-myristoyltransferase by novel inhibitor proteins. *Cell Biochem. Biophys. 43*, 189−202.

(62) Yang, S. H., Shrivastav, A., Kosinski, C., Sharma, R. K., Chen, M.-H., Berthiaume, L. G., Peters, L. L., Chuang, P.-T., Young, S. G., and Bergo, M. O. (2005) N-Myristoyltransferase 1 is essential in early mouse development. *J. Biol. Chem. 280*, 18990−18995.

(63) Maurer-Stroh, S., Eisenhaber, B., and Eisenhaber, F. (2002) N-Terminal N-myristoylation of proteins: Prediction of substrate proteins from amino acid sequence. *J. Mol. Biol. 317*, 541−557.

(64) Selvakumar, P., Lakshmikuttyamma, A., Shrivastav, A., Das, S. B., Dimmock, J. R., and Sharma, R. K. (2007) Potential role of N-myristoyltransferase in cancer. *Prog. Lipid Res. 46*, 1−36.

(65) Prasad, K. K., Toraskar, M. P., and Kadam, V. J. (2008) N-Myristoyltransferase: A novel target. *Mini Rev Med Chem 8*, 142−149.

(66) Linder, M. E., and Deschenes, R. J. (2003) New insights into the mechanisms of protein palmitoylation. *Biochemistry (Moscow) 42*, 4311−4320.

(67) Nadolski, M. J., and Linder, M. E. (2007) Protein lipidation. *FEBS J. 274*, 5202−5210.

(68) Smotrys, J. E., and Linder, M. E. (2004) Palmitoylation of intracellular signaling proteins: Regulation and function. *Annu. Rev. Biochem. 73*, 559−587.

(69) Shahinian, S., and Silvius, J. R. (1995) Doubly-lipid-modified protein-sequence motifs exhibit long-lived anchorage to lipid bilayer-membranes. *Biochemistry (Moscow) 34*, 3813−3822.

(70) Huang, K., Sanders, S., Singaraja, R., Orban, P., Cijsouw, T., Arstikaitis, P., Yanai, A., Hayden, M. R., and El-Husseini, A. (2009) Neuronal palmitoyl acyl transferases exhibit distinct substrate specificity. *FASEB J. 23*, 2605−2615.
(71) Navarro-Lerida, I., Alvarez-Barrientos, A., Gavilanes, F., and Rodriguez-Crespo, I. (2002) Distance-dependent cellular palmitoylation of de-novo-designed sequences and their translocation to plasma membrane subdomains. *J. Cell Sci. 115*, 3119−3130.
(72) Ducker, C. E., Griffel, L. K., Smith, R. A., Keller, S. N., Zhuang, Y., Xia, Z., Diller, J. D., and Smith, C. D. (2006) Discovery and characterization of inhibitors of human palmitoyl acyltransferases. *Mol. Cancer Ther. 5*, 1647−1659.
(73) Hemsley, P. A., and Grierson, C. S. (2008) Multiple roles for protein palmitoylation in plants. *Trends Plant Sci. 13*, 295−302.
(74) Tsutsumi, R., Fukata, Y., and Fukata, M. (2008) Discovery of protein-palmitoylating enzymes. *Pfluegers Arch. 456*, 1199−1206.
(75) Baekkeskov, S., and Kanaani, J. (2009) Palmitoylation cycles and regulation of protein function. *Mol. Membr. Biol. 26*, 42−54.
(76) Planey, S. L., and Zacharias, D. A. (2009) Palmitoyl acyltransferases, their substrates, and novel assays to connect them. *Mol. Membr. Biol. 26*, 14−31.
(77) Lobo, S., Greentree, W. K., Linder, M. E., and Deschenes, R. J. (2002) Identification of a Ras palmitoyltransferase in Saccharomyces cerevisiae. *J. Biol. Chem. 277*, 41268−41273.
(78) Roth, A. F., Feng, Y., Chen, L. Y., and Davis, N. G. (2002) The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. *J. Cell Biol. 159*, 23−28.
(79) Yang, W., Di Vizio, D., Kirchner, M., Steen, H., and Freeman, M. R. (2010) Proteome scale characterization of human S-acylated proteins in lipid raft-enriched and non-raft membranes. *Mol. Cell. Proteomics 9*, 54−70.
(80) Roth, A. F., Wan, J. M., Bailey, A. O., Sun, B. M., Kuchar, J. A., Green, W. N., Phinney, B. S., Yates, J. R., and Davis, N. G. (2006) Global analysis of protein palmitoylation in yeast. *Cell 125*, 1003−1013.
(81) Linder, M. E., and Deschenes, R. J. (2007) Palmitoylation: policing protein stability and traffic. *Nat. Rev. Mol. Cell Biol. 8*, 74−84.
(82) Resh, M. D. (2006) Trafficking and signaling by fatty-acylated and prenylated proteins. *Nat. Chem. Biol. 2*, 584−590.
(83) Devedjiev, Y., Dauter, Z., Kuznetsov, S. R., Jones, T. L. Z., and Derewenda, Z. S. (2000) Crystal structure of the human acyl protein thioesterase I from a single X-ray data set to 1.5 angstrom. *Structure 8*, 1137−1146.
(84) Rocks, O., Gerauer, M., Vartak, N., Koch, S., Huang, Z. P., Pechlivanis, M., Kuhlmann, J., Brunsveld, L., Chandra, A., Ellinger, B., Waldmann, H., and Bastiaens, P. I. H. (2010) The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. *Cell 141*, 458−471.
(85) Duncan, J. A., and Gilman, A. G. (1998) A cytoplasmic acyl-protein thioesterase that removes palmitate from G protein alpha subunits and p21(RAS). *J. Biol. Chem. 273*, 15830−15837.
(86) Shanado, Y., Kometani, M., Uchiyama, H., Koizumi, S., and Teno, N. (2004) Lysophospholipase I identified as a ghrelin deacylation enzyme in rat stomach. *Biochem. Biophys. Res. Commun. 325*, 1487−1494.
(87) Veit, M., and Schmidt, M. F. G. (2001) Enzymatic depalmitoylation of viral glycoproteins with acyl-protein thioesterase 1 in vitro. *Virology 288*, 89−95.
(88) Sunaga, H., Sugimoto, H., Nagamachi, Y., and Yamashita, S. (1995) Purification and properties of lysophospholipase isoenzymes from pig gastric-mucosa. *Biochem. J. 308*, 551−557.
(89) Mitchell, D. A., Vasudevan, A., Linder, M. E., and Deschenes, R. J. (2006) Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J. Lipid Res. 47*, 1118−1127.
(90) Tomatis, V. M., Trenchi, A., Gomez, G. A., and Daniotti, J. L. (2010) Acyl-Protein Thioesterase 2 Catalizes the Deacylation of Peripheral Membrane-Associated GAP-43. *Plos One 5*, No. e15045.
(91) Siegel, G., Obernosterer, G., Fiore, R., Oehmen, M., Bicker, S., Christensen, M., Khudayberdiev, S., Leuschner, P. F., Busch, C. J. L., Kane, C., Hubel, K., Dekker, F., Hedberg, C., Rengarajan, B., Drepper, C., Waldmann, H., Kauppinen, S., Greenberg, M. E., Draguhn, A., Rehmsmeier, M., Martinez, J., and Schratt, G. M. (2009) A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis. *Nat. Cell Biol. 11*, 705−U736.
(92) Webb, Y., Hermida-Matsumoto, L., and Resh, M. D. (2000) Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. *J. Biol. Chem. 275*, 261−270.
(93) Lawrence, D. S., Zilfou, J. T., and Smith, C. D. (1999) Structure-activity studies of cerulenin analogues as protein palmitoylation inhibitors. *J. Med. Chem. 42*, 4932−4941.
(94) Dekker, F. J., Rocks, O., Vartak, N., Menninger, S., Hedberg, C., Balamurugan, R., Wetzel, S., Renner, S., Gerauer, M., Schölermann, B., Rusch, M., Kramer, J. W., Rauh, D., Coates, G. J., Brunsveld, L., Bastiaens, P. I. H., and Waldmann, H. (2010) Small-molecule inhibition of APT 1 affects Ras localization and signaling. *Nat. Chem. Biol. 6*, 449−456.
(95) Dekker, F. J., and Hedberg, C. (2011) Small molecule inhibition of protein depalmitoylation as a new approach towards down-regulation of oncogenic Ras signalling. *Bioorg. Med. Chem. 19*, 1376−1380.
(96) Koch, M. A., Wittenberg, L.-O., Basu, S., Jeyaraj, D. A., Gourzoulidou, E., Reinecke, K., Odermatt, A., and Waldmann, H. (2004) Compound library development guided by protein structure similarity clustering and natural product structure. *Proc. Natl. Acad. Sci. U.S.A. 101*, 16721−16726.
(97) Hedberg, C., Dekker, F. J., Rusch, M., Renner, S., Wetzel, S., Vartak, N., Gerding-Reimers, C., Bon, R. S., Bastiaens, P. I. H., and Waldmann, H. (2011) Development of highly potent inhibitors of the Ras-targeting human acyl protein thioesterases based on substrate similarity design. *Angew. Chem., Int. Ed. 50*, 9832−9837.
(98) Rusch, M., Zimmermann, T. J., Buerger, M., Dekker, F. J., Goermer, K., Triola, G., Brockmeyer, A., Janning, P., Boettcher, T., Sieber, S. A., Vetter, I. R., Hedberg, C., and Waldmann, H. (2011) Identification of acyl protein thioesterases 1 and 2 as the cellular targets of the Ras-signaling modulators palmostatin B and M. *Angew. Chem., Int. Ed. 50*, 9838−9842.
(99) Maurer-Stroh, S., Koranda, M., Benetka, W., Schneider, G., Sirota, F. L., and Eisenhaber, F. (2007) Towards complete sets of farnesylated and geranylgeranylated proteins. *PLoS Comp. Biol. 3*, 634−648.
(100) Nguyen, U. T. T., Guo, Z., Delon, C., Wu, Y. W., Deraeve, C., Fraenzel, B., Bon, R. S., Blankenfeldt, W., Goody, R. S., Waldmann, H., Wolters, D., and Alexandrov, K. (2009) Analysis of the eukaryotic prenylome by isoprenoid affinity tagging. *Nat. Chem. Biol. 5*, 227−235.