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Invited Review

Protein Prenylation: An (Almost) Comprehensive Overview on Discovery History, Enzymology, and Significance in Physiology and Disease

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Summary. Since 1979, when prenylation has been first discovered as chemical oddity of a yeast mating factor, the two forms of this posttranslational modification of proteins (farnesylation and geranylgeranylation) have been found as wide spread among proteins from Eukarya and their viruses. This review attempts to summarize as comprehensively as possible the enzymological processes of prenylation and the various aspects of their biological significance.

The substrate proteins of prenyltransferases are known to carry a sequence signal composed of a cysteine-containing 4–5 residue stretch at the utmost C-terminal end that is N-terminally preceded by a flexible and polar linker region of *ca.* 10 residues. Postprenylation processing of substrate proteins can involve C-terminal proteolysis, C-terminal carboxyl methylation, and other steps of maturation. The prenyl anchor functions as module for membrane attachment or for protein–protein interaction.

Prenyl anchor carrying proteins fulfill a large array of functions in signaling and regulation of cellular processes. Therefore, they are involved in the pathogenesis of a variety of human diseases, the most prominent one being cancer. Farnesyltransferase inhibitors show surprisingly high efficiency in controlling tumor growth in model systems but, so far, clinical trials with human patients have remained without the desired success. Interference into prenylation pathways appears also a promising treatment principle in a variety of parasitic diseases.

Keywords. Farnesyl; Geranylgeranyl; Prenyl; Farnesyltransferase inhibitor; Cancer; Parasitic disease.

Introduction

The key to the ability of proteins to perform all the functions necessary in cell life is provided by their amino acid sequence. Linear combinations of the 20 amino

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acids create an apparently endless range of functionalities. But at least as important as providing all these features is the task to regulate them properly. Cells of multicellular organisms have to react to extracellular signals to assure coordinated action of different tissues. Protein activity has to be regulated either up or down, and it has to be restricted to the appropriate locations and points in time. Regulation on the level of gene expression and of protein degradation permits only slow responses. For a faster way of control, it is necessary to switch enzymes on and off, often accomplished by reversible posttranslational modifications such as phosphorylation. For both types of regulation, the extracellular signal has to reach its destination first. How is this managed?

The Impact of Posttranslational Modifications on Protein Function

Receptors at the plasma membrane bind signaling molecules like, *e.g.*, hormones or growth factors. They transmit the signals to intracellular proteins through conformational changes as well as posttranslational modifications. The signals are relayed through multiple stations, following cascades of proteins activating or inhibiting the next one, until they reach the nucleus, where gene expression is altered, or they end up regulating the activity of target proteins directly. These signaling pathways are often connected to each other, forming a network of signaling cascades. Some proteins are part of multiple pathways and operate as key regulators of important cellular functions such as cell proliferation and differentiation, cell shape and mobility, and many more. Defects in cell signaling exclude a cell from communication and can lead to uncontrolled cell growth. Cancer is the most prominent disease with malfunction of cell signaling.

Another important topic is protein trafficking and localization. Of course, there are localization signals in the amino acid sequence of proteins determining the compartment they are transported to, but this is by far not the only way. Glycosylation provides an additional level of control in protein sorting *via* the endoplasmatic reticulum (ER) and the *Golgi*, as the polysaccharides become modified in certain trafficking steps. But glycosyl-modifications also act as specific signals for recognition by receptors, and they can stabilize the structure of a protein. Yet another problem arises with proteins which change their localization multiple times in their lives. Some proteins have to associate reversibly with membranes, especially those participating in the signaling pathways mentioned above, making protein localization an important feature in the search for new anti-cancer therapies. Membrane association is often accomplished by lipid modifications like palmitoylation [1, 2] or myristoylation [3–8]. Attachment of glycosylphosphatidylinositol (GPI) lipid anchors binds proteins to membranes, too [9–14]. The lipid modification which has been discovered last is prenylation, the attachment of an isoprenoid group to a cysteine residue at or near the carboxyl-terminus [15]. Prenylation is believed to occur in 0.5% of all cellular proteins in eukaryotes [16]. But since the first discovery of a prenylated protein, it has been a long way to understand the function of prenylation and make use of it to fight cancer, and today there is still a long way to go.

Prenylation: How It All Began

The first evidence for prenylation of a carboxyterminal cysteine residue was discovered in 1979 in Japan for Rhodotorucine A, a mating factor peptide of the yeast *Rhodosporidium toruloides* [17]. In the mature form, an *S*-farnesylcysteine was found at the carboxy-terminus. In the following five years, further fungal peptidyl sex hormones covalently modified by a farnesyl group on a C-terminal cysteine were revealed. The function of this modification was unclear, but it was stoichiometric and stable, indicating that it is an important component for the function of the mating factors. The independent discovery of prenylation in mammalian cells in 1984 resulted from investigations concerning the effect of compactin, an inhibitor of cholesterol biosynthesis. Compactin blocks the enzyme 3-hydroxy-3-methylglutaryl-CoA-reductase (*HMG*-CoA reductase), responsible for the synthesis of mevalonic acid, a crucial intermediate of the isoprenoid pathway. Cultured cells treated with compactin showed cell cycle arrest [18] and altered cell morphology [19]. The fact that these effects could be reversed by supplying the cells with mevalonate, but not with cholesterol, dolichol, ubiquinone, or isopentenyladenine, the major products of isoprenoid biosynthesis, revealed a critical function in cell cycling and shape for one or more non-sterol isoprenoids [19]. Following the fate of radiolabeled mevalonate in cultured 3T3 fibroplasts with their mevalonate synthesis blocked by lovastatin, covalent incorporation of an isoprenoid derivative into cellular proteins was observed [20]. Further investigations showed isoprenylation to be a phenomenon widespread in mammalian cells [21], with proteins in different compartments like the nuclear envelope, the plasma membrane, and the cytosol being involved [22–24]. In 1986, a new discovery connected the previous data: a gene of the yeast *Saccharomyces cerevisiae* required for posttranslational modification of both the RAS proteins and a-mating factor called RAM was found [25]. Comparison of the amino acid sequences of the yeast RAS proteins and the precursor polypeptide for a-factor displayed no obvious sequence similarity except for the C-terminus, consisting of a cysteine followed by two residues with aliphatic sidechains and an utmost C-terminal amino acid residue. This so called C*aaX*-motif was found in a variety of other proteins as well, but was believed to signal modification by a palmitoyl group at that time. In 1985, new eukaryotic protein carboxyl methylation reactions were discovered including modification of the α-subunit of *cGMP* phosphodiesterase [26] and nuclear lamin B [27]. These reactions did not fit in any known type of such an activity. The combined findings of the aforementioned proteins containing a C*aaX*-motif and some fungal mating factors being carboxymethylated at the C-terminus led to the hypothesis that the C*aaX* sequence signal linked prenylation, proteolytic cleavage, and methylation [28, 29]. This assumption was supported in 1988 by the discovery that the yeast a-factor contains a C-terminal farnesylcysteine methyl ester [30, 31] and, in 1989, by the ascertainment that all mammalian Ras proteins are isoprenylated on the conserved cysteine residue [32]. The palmitoylation, which was formerly believed to modify this amino acid [33], could be assigned to upstream cysteine residues in a subset of Ras proteins. At the same time, the first isoprenylated protein identified turned out to be lamin B [34–36]. The major isoprenoids bound to mammalian protein were identified as *trans*, *trans*-farnesyl and all-*trans*-geranylgeranyl in 1990 [37–39],

followed by the finding that nuclear lamin B, mammalian Ras, and yeast RAS are farnesylated [40, 41], while a γ-subunit of a mammalian heterotrimeric G-protein was shown to be geranylgeranylated [42–44]. The discovery, that Ras farnesylation is required for the ability of oncogenic forms to transform cells [45–47], boosted the efforts in this field of research [48, 49], leading to the characterization of the farnesyltransferase enzyme and development of specific inhibitors of protein prenylation for the use in cancer therapy [50].

Definition

After recapitulating the first steps in the research on prenylation, let us take a closer look on the subject of interest. What exactly is meant when talking about prenylation? Prenylation is the covalent attachment of one or more isoprenoids

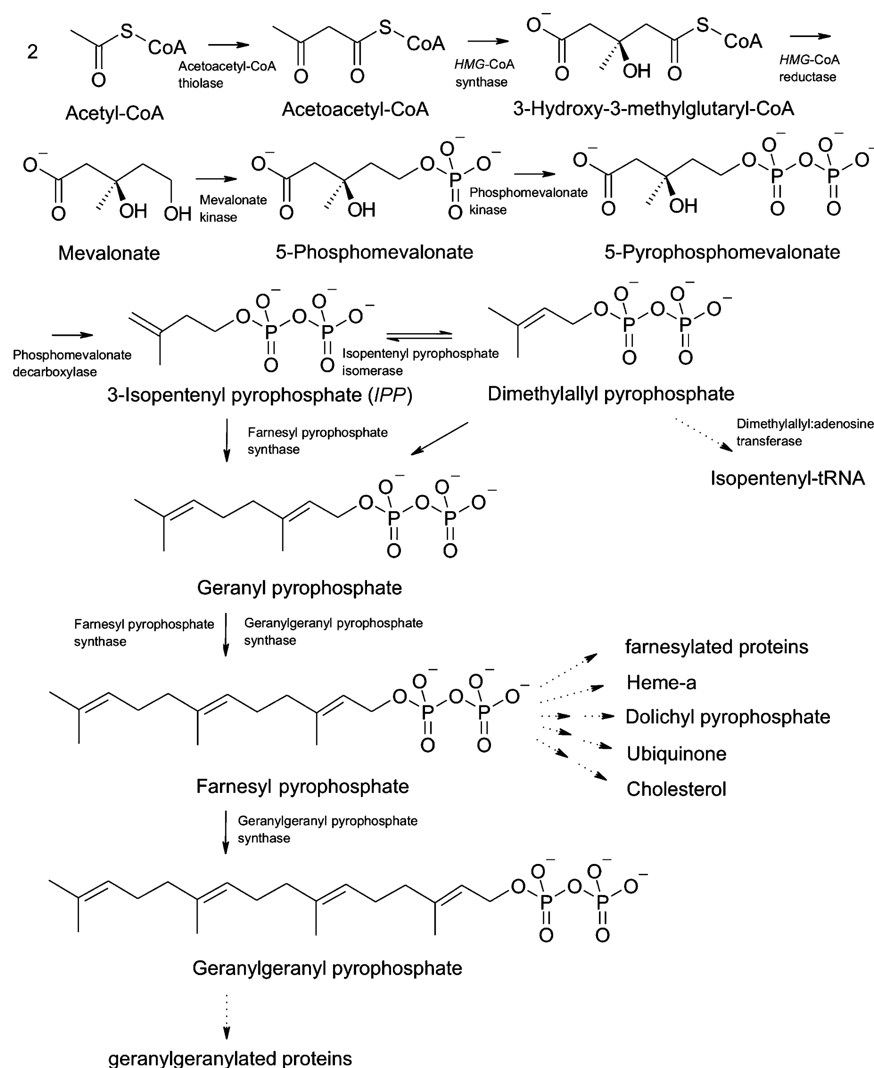

Fig. 1. Pathway for isoprenoid biosynthesis in mammalian cells; the pathway presentation is inspired by the respective figures in Ref. [22]

to cysteine residues at or near the carboxy-terminus of a protein. It occurs in all eukaryotes and possibly also in archaebacteria [16]. The isoprenoid can be a farnesyl-group built of three isoprene entities or a geranylgeranyl-group built of four units. Although the first prenylated proteins discovered were farnesylated, geranylgeranylation appears to be the predominant modification [16]. The isoprenoids are synthesized as farnesyl (*FPP*) or geranylgeranyl pyrophosphate (*GGPP*), an activated form which is then cleaved to link them to the sulfur atom of the cysteine *via* a thioether bond. But how are these isoprenoids built in the cell?

The Isoprenoid Pathway

The isoprenoid pathway (also called mevalonate pathway, see Fig. 1) starts from acetyl-CoA, which is condensed in two steps to *HMG*-CoA. The next step involves the reduction to mevalonic acid by the *HMG*-CoA reductase, which can be inhibited by statins as mentioned above. Mevalonic acid is then phosphorylated twice by mevalonate kinase and decarboxylated forming isopentenyl pyrophosphate, the building block of all isoprenoids. Then, this is condensed stepwise to build geranyl pyrophosphate (2 units), farnesyl pyrophosphate (3 units), and geranylgeranyl pyrophosphate (4 units). The last two are the substrates for protein prenylation, but there are lots of other important products of this pathway [51]. Condensation of two *FPP* molecules yields squalene, the basic structure for the sterols, with cholesterol being the main product. Other derivatives of *FPP* participate in the synthesis of heme-a, dolichyl pyrophosphate, or ubiquinone.

Amino Acid Sequence Motifs for Prenylation

With the synthesis of the isoprenoids finished, the cell has to know where to attach the lipid anchors and which anchor type to select. Obviously, this information is somehow encoded in the C-terminal amino acid sequence. Historically, major attention was focused on the identity of a handful of C-terminal residues of the few known substrate proteins and on the results of enzymological tests with short peptides. The results have been summarized in form of motifs (for example, the C*aaX* motif has been mentioned above already).

However, these simplified recognition models started to blur (i) when it became clear that model peptide results cannot be extrapolated to the situation of whole proteins and (ii) when the number of known prenylated proteins and the knowledge about their C-terminal sequence variability diversified. Major improvement in the understanding of the prenylation signal was gained with crystallographic studies of prenyltransferases [52–55].

If the binding complex consisting of a substrate protein C-terminus and the prenyltransferase is considered, two regions of the sequence signal can be distinguished [56]: Region 1, a stretch of the utmost C-terminal (typically, the last 4) amino acid residues, is buried in the catalytic cleft of the prenyltransferase and experiences the most intense interaction with the enzyme. The amino acid type variability of these residues is most restricted. Region 2, a segment of *ca.* 10 residues, serves as linker connecting the C-terminal stretch bound by the enzyme with the rest of the substrate protein. These residues are, as a trend, small with a

flexible backbone and also polar (to allow interaction with the aqueous surrounding) but not every residue needs to comply with these requirements. It is sufficient if the linker as a whole provides the necessary properties. The more C-terminal residues of the linker interact with the mouth of the catalytic cavity and are, therefore, more restricted in their amino acid type variability [56].

Clearly, the presence of the linker region is necessary for the possibility of prenylation in principle since it provides accessibility to the region 1 residues for the prenyltransferase. But the identity of the handful of utmost C-terminal residues (of region 1 and the residues from region 2 at the mouth of the catalytic cavity) is critical for the recognition by the specific type of prenyltransferase and, consequently, for the type of prenyl anchors that can be attached to the substrate protein.

For example, the Ca_1a_2X motif consists of the modified cysteine and three more amino acids. When this motif has been identified, it was believed to contain two aliphatic residues carboxy-terminal of the cysteine, hence it was named C*aaX*. Today, it is known that there is quite a lot of flexibility for these residues, especially for a_1. Therefore, it should better be called C*XXX*-motif. Targets for farnesylation as well as geranylgeranylation contain this motif, and the type of isoprenoid added is largely determined by the last residue [57, 58] with some minor influence of *X*2. Farnesylation tolerates many C-terminal residues, and takes place with highest efficiency for *X* being methionine (M, Met), serine (S, Ser), alanine (A, Ala), glutamine (Q, Gln), or cysteine (C, Cys), but there are also examples for farnesylated C*XXX* with threonine (T, Thr), histidine (H, His), valine (V, Val), asparagine (N, Asn), phenylalanine (F, Phe), glycine (G, Gly), and isoleucine (I, Ile) in the ultimate position. Geranylgeranylation is largely restricted to proteins with leucine (L, Leu) in the terminal position, but also occurs with F, I, V, and M [59, 42]. Mutation of the C-terminal residue of Ras proteins to leucine changes the type of isoprenoid added [60, 42].

These data, combined with the fact that the prenyltransferases which modify C*XXX*-proteins are able to bind and prenylate short peptides with the adequate sequence (in fact, a C*XXX* tetrapeptide is sufficient) [61], might imply that all necessary information for prenyl anchor selection is included in those four residues. However, there are some exceptions to these rules. K-Ras4B, with a sequence ending in CVIM is farnesylated normally, but becomes geranylgeranylated under conditions where farnesylation is blocked. This alternative prenylation seems to be enabled by a stretch of basic residues upstream of the C*XXX*-motif [62]. N-Ras also seems to be capable of alternative geranylgeranylation [63]. RhoB ends with CKVL, but is found both farnesylated and geranylgeranylated *in vivo*, depending on presence or absence of certain upstream cysteines. These examples demonstrated the influence of the context for the prenylation signal in region 1. This is also supported by the fact that the C*XXX*-motif of $G_{i\alpha}$ becomes prenylated when fused to a Ras protein, while it is unmodified in the original context; apparently, the C-terminal tetrapeptide is not sufficiently accessible to the prenyltransferase in $G_{i\alpha}$.

Another motif for prenylation is less well defined. C-terminal sequences like CC, C*X*C, CC*X*, CC*XX*, CC*XXX*, or C*XXX* can be subjected to geranylgeranylation [64, 65]. If there are two cysteines available, usually both become prenylated [66, 67]. This type of motif is almost exclusively found in the Rab family of small

Table 1. Selected prenylated proteins [430]

Protein	Function	Sequence[a]	Prenyl group	Transferase[b]
H-Ras	Growth, differentiation	CVLS	15C	FT
K-RasA	Growth, differentiation	CIIM	15C	FT
K-RasB	Growth, differentiation	CVIM	15C	FT
N-Ras	Growth, differentiation	CVVM	15C	FT
2′,5′ oligoadenylate synthetase 1	Growth, differentiation, and apoptosis	CTIL	20C	GGT I
Rap1A	Regulation of cell adhesion	CLLL	20C	GGT I
Rap1B	Activation of the MEK-ERK cascade	CQLL	20C	GGT I
Rac1	Secretion at plasma membrane	CLLL	20C	GGT I
RalA	Regulation of actin cytoskeleton	CCIL	20C	GGT I
Cdc42/G25K (brain)	Filopodia formation	CCIF	20C	GGT I
Cdc42/G25K (placenta)	Filopodia formation	CVLL	20C	GGT I
RhoA	Assembly of actin stress fibers and focal adhesion sites	CLVL	20C	GGT I
RhoB	Assembly of actin stress fibers and focal adhesion sites; gene transcription	CKVL	15C and 20C	GGT I
RhoC	Assembly of actin stress fibers and focal adhesion sites	CPIL	20C	GGT I
Rab1A, 1B, 2	Vesicular trafficking	GGCC	20C	GGT II
Rab3a	Vesicular trafficking	DCAC	20C	GGT II
Rab6	Vesicular trafficking	GCSC	20C	GGT II
HDJ2	Protein import into mitochondria, co-chaperone of Hsp70	CQTS	15C	FT
Inositol-1,4,5-triphosphate-5-phosphatase II	Inactivates inositol triphosphate	CPNL	20C	GGT I
Ptp4a1	Protein-tyrosine phosphatase	CCIQ	15C	FT
S. cerevisiae RAS2	Adenylyl cyclase activation	CIIS	15C	FT
Heterotrimeric G-protein (γ-subunit)	Serpentine receptor linked	CAIL	20C	GGT I
S. cerevisiae a-factor	Mating pheromone	CVIA	15C	FT
R. toruloides Rhodotorucine A	Mating pheromone	CTVA	15C	FT
Lamin A	Nuclear membrane component	CSIM	15C	FT
Lamin B	Nuclear membrane component	CYVM	15C	FT
Cenp-F	Centromere (kinetochore) protein for G_2/M transition	CKVQ	15C	FT
Phosphorylase kinase, α-subunit	Muscle glycogen metabolism	CAMQ	15C	FT
Phosphorylase kinase, β-subunit	Liver glycogen metabolism	CQMQ	15C	FT
Phosphorylase kinase, γ-subunit	Muscle glycogen metabolism	CLIS	15C	FT
Transducin (γ-subunit)	Vision	CVIS	15C	FT

(*continued*)

Table 1 (*continued*)

Protein	Function	Sequence[a]	Prenyl group	Transferase[b]
Retinal *cGMP* phosphodiesterase α-subunit	Vision	CCIQ	15C	FT
Retinal *cGMP* phosphodiesterase β-subunit	Vision	CCIL	20C	GGT I
Rhodopsin kinase	Vision	CVLS	15C	FT
RhoE	Regulation of the actin cytoskeleton	CTVM	15C	FT
Rap2a	unknown	CNIQ	15C	FT
Rap2b	unknown	CVIL	20C	GGT I
Rheb	unknown	CSVM	15C	FT
PxF	Peroxisome assembly	CLIM	15C	FT
Interferon induced guanylate binding protein-1	Binds *GMP*, *GDP*, and *GTP* in macrophages	CTIS	15C	FT
Interferon induced guanylate binding protein-2	Binds *GMP*, *GDP*, and *GTP* in macrophages	CNIL	20C	GGT I
Hepatitis delta antigen	Viral particle assembly	CRPQ	15C	FT

[a] Unless noted otherwise, the C-terminal tetrapeptides sequences are from human proteins; [b] FT farnesyltransferase, GGT geranylgeranyltransferase

*GTP*ases. In contrast to the C*XXX*-motif, short peptides are no substrate for prenylation [68, 69]. In addition, the C-terminal regions do not become prenylated when fused to proteins which normally do not exhibit this type of prenylation signal. The correct polypeptide sequence as well as the right conformation is required for prenylation [70]. Taken together, these results suggest that Rab-type prenylation motifs are only the site of possible geranylgeranylation. But other factors do also influence the recognition by the corresponding prenyltransferase (including protein recognition factors such as the Rab escort protein 1, see Table 1).

It should be noted that the experimental testing of the prenylation status of a protein is a laborious task. The standard literature method for *in vitro* or *in vivo* analysis of selected candidates involves transcription/translation of a cloned construct and protein prenylation in the presence of ^{3}H-labeled lipid anchor precursors followed by autoradiography/fluorography [71–73]. Necessary controls involve mutations of the C-terminal cysteine expected to be modified, prenyltransferase inhibitor applications, and/or exposition to precursors of alternative prenyl anchors during the prenylation reaction. However, the reportedly long exposure times (weeks/months) contradict the need for several repetitions of the experiment. Optimization of protein expression and incubation conditions is typically not avoidable. In our own experience, many attempts with the standard technology ended up without reportable result; *i.e.*, the signals in initial experiments were often below the detection limit. Scientific literature research showed that rarely a

lab has studied the prenylation status of more than a single target, apparently as a consequence of the tenacious methodology. A more sensitive protocol has recently been described [74].

With so much experimental information on prenylated proteins, it should be possible to recognize the capability for prenylation from the amino acid sequence of query protein sequences. Indeed, a computerized prediction tool called PrePS (Prenylation Prediction Suite) with high prediction accuracy (with a sensitivity of ~95% for true targets and with only ~5% false-positive rate among non-targets with appropriate cysteines) has recently been released and is available at http://mendel.imp.ac.at/sat/PrePS/ [56]. It is clear now how a cell synthesizes the isoprenoids, and where they do become attached to, but which enzymes actually do the job?

Protein Prenyltransferases

In eukaryotes, there are three distinct types of prenyltransferases [68, 75, 76]. Farnesyltransferase (FT) [77] as well as geranylgeranyltransferase type I (GGT I) [57] recognize the C-terminal C*XXX*-motif, while geranylgeranyltransferase type II (GGT II) [78, 66, 64], also called RabGGT, prenylates C-terminal cysteines in a less defined arrangement such as CC, C*X*C, or the like. All of them are heterodimeric metalloenzymes [79], with subunits designated α and β [80]. Yeast genetics revealed that FT and GGT I share the same α-subunit, while their β-subunits are distinct, which is also true for mammalian prenyltransferases [81–83]. The respective β-subunits are homologous to each other as well as the α-subunits.

The 3D structures of eukaryote prenyltransferases are known from crystal X-ray diffraction. The α- and β-subunits are almost exclusively composed of

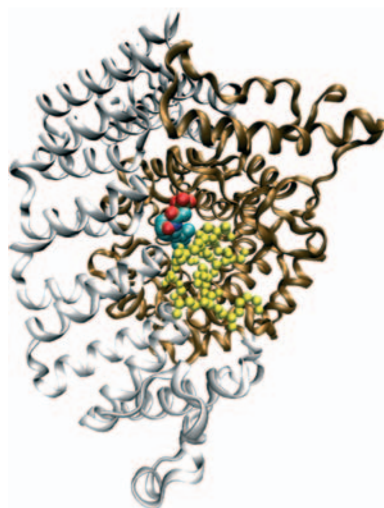

Fig. 2. 3D structure of the eukaryote farnesyltransferase; this figure is based on the co-crystal structure of rat protein farnesyltransferase complexed with a K-ras4B peptide substrate (TKCVFM) and a farnesyl pyrophosphate (*FPP*) analogue at 2.0 Å resolution (PDB accession 1D8D) [53]; the α-subunit is colored off-white; the β-subunit is ochre; the *FPP* analogue is shown as CPK model; the substrate peptide is yellow; the figure was produced with VMD [428]

α-helical and loop segments and jointly form the substrate binding sites (see Fig. 2 for FT and Fig. 3 for GGT I). The enzyme GGT II is very similar to the GGT I structure (see Fig. 4), whereas the escort protein belongs to a completely different fold class.

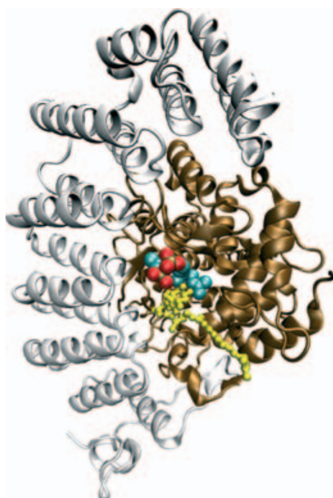

Fig. 3. 3D structure of the eukaryote geranylgeranyltransferase I; this figure is based on the co-crystal structure of rat protein geranylgeranyltransferase I complexed with a peptide substrate (KKKSKTKCVIL) and a geranylgeranyl pyrophosphate (*GGPP*) molecule at 2.60 Å resolution (PDB accession 1N4S) [55]; the α-subunit is colored off-white; the β-subunit is ochre; the *GGPP* molecule is shown as CPK model; the substrate peptide is yellow; the figure was produced with VMD [428]

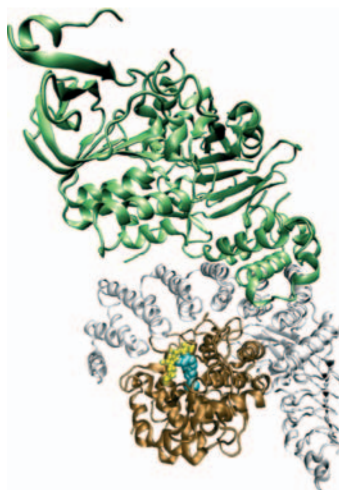

Fig. 4. 3D structure of the eukaryote geranylgeranyltransferase II; this figure is based on the co-crystal structure of rat protein geranylgeranyltransferase II complexed with a peptide model (AAAA), an isoprenoid, and the rab escort protein-1 at 2.70 Å resolution (PDB accession 1LTX) [54]; the α-subunit is colored off-white; the β-subunit is ochre; the rab escort protein-1 is presented in lime; the isoprenoid molecule is shown as CPK model; the substrate peptide is yellow; the figure was produced with VMD [428]

As mentioned above, FT and GGT I recognize short peptides with a C*XXX* motif, which made it easier to purify these enzymes [84–86] and study their structure with X-ray crystallography, as well as the structure of the enzyme in complex with substrate analogs [87]. Therefore, much more information is available on the mechanism and structure of type I prenyltransferases, especially of FT, than of RabGGT.

The catalytic activity of all prenyltransferases depends on a Zn^{2+}-ion bound at the active site [88]. High concentrations of Mg^{2+} are required for maximum activity of FT and GGT II, while conflicting reports are available on the Mg^{2+}-requirements of GGT I [82, 89].

The Zn^{2+}-ion is absolutely required for catalytic activity and enhances peptide substrate binding of FT and GGT I, but not binding of prenyl pyrophosphate, suggesting activation of the cysteine thiol group by the metal ion [88]. Coordination with Zn^{2+} lowers the pK_a of the thiol by approximately two units, resulting in a Zn^{2+}-coordinated thiolate-anion at physiological *pH* [90]. In the absence of zinc, the enzymatic activity of FT and GGT I is restored by Co^{2+} or Cd^{2+} [91], and increased absorption at 340 nm (charge transfer band) of Co^{2+}-substituted FT upon C*XXX*-substrate binding supplied the first evidence of a direct interaction between the peptide substrate and the metal ion [92]. The catalytical role of Zn^{2+} has been confirmed by X-ray crystallography [93]. Substitution of Zn^{2+} with Cd^{2+} increases the affinity for the peptide substrate fivefold, while reducing product formation sixfold. This indicates a direct participation of the metal ion in catalysis. Product formation is also decreased for C3-(fluoromethyl)-farnesyl pyrophosphate proportional to the number of fluor atoms [94], indicating a carbocation-like transition state with a partial negative charge on the cysteine sulfur, partial positive charge at carbon 1 of *FPP*, and another partial negative charge at the oxygen atom connecting C1 to the α-phosphate [95]. Stabilizing these developing charges seems to be an important feature for the catalytic activity of prenyltransferases (see Fig. 5).

Mg^{2+} seems to activate pyrophosphate as a leaving group. The *pH*-dependency of FT reveals two deprotonation steps increasing the rate of product formation: ionization of the zinc-coordinated thiol with a pK_a of 6.0 and dissociation of a

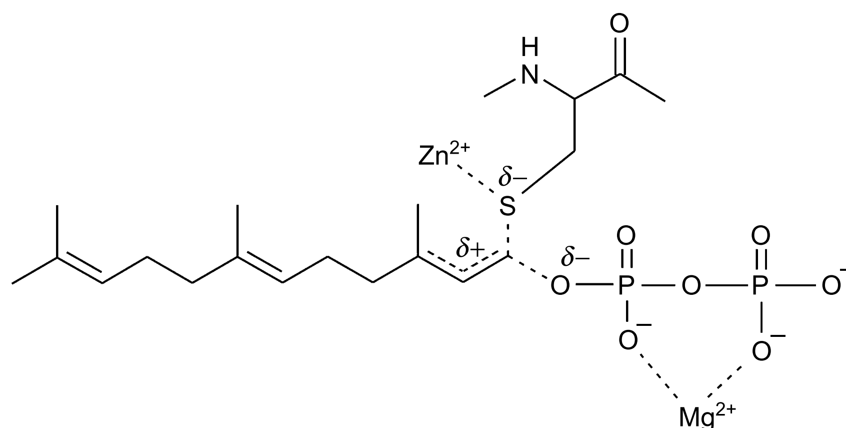

Fig. 5. Proposed transition state of the reactive species in the transfer of a farnesyl group by FT; this figure follows the model of the reaction mechanism described in Ref. [429]

hydroxyl-group of the pyrophosphate of *FPP* with $pK_a = 7.4$. The second step is important for binding of Mg^{2+}. The latter reaction does not take place without Mg^{2+} or with farnesyl monophosphate [96]. Mg^{2+} can be substituted by Mn^{2+}, which has been shown to coordinate the diphosphate of the isoprenoid by X-ray crystallography. In conclusion, maximum catalytic activity demands a Zn^{2+}-coordinated thiolate nucleophil and a Mg^{2+}-coordinated diphosphate leaving group.

For FT, both X-ray structures of different states of the enzyme as well as kinetic studies are available, giving the opportunity to deduce the presumable reaction mechanism [97, 52, 98, 93]. Both α- and β-subunit are mostly α-helical (see also Fig. 2). Helices 2 to 15 of the α-subunit fold into seven tetratricopeptide repeats forming a right-handed superhelix of the shape of a crescent [99], enveloping part of the β-subunit. In contrary, 12 helices of the β-subunit form an α–α barrel. The *FPP* binding pocket is proposed to be a cleft at the center of the barrel, surrounded by hydrophobic amino acids. A single zinc ion is located at the junction of a hydrophilic groove of the α-subunit near the interface of the subunits and the *FPP*-binding site. It is penta-coordinated to Cys299β, Asp297β as a bidentate ligand and His362β as well as one water molecule in the apoenzyme. The importance of these residues for enzymatic activity has been confirmed by site directed mutagenesis [100, 101].

The structure of FT in complex with *FPP* revealed binding of the hydrocarbon tail of *FPP* by the conserved residues Trp102β, Tyr200β, Tyr205β, Tyr251β, and Trp303β, accounting for the increased intrinsic fluorescence of the enzyme upon isoprenoid binding [102]. Arg202β adopts a different conformation for further interactions, stabilized by Asp200β and Met193β. Cys254β and Gly250β also participate in substrate binding. The diphosphate group binds to a positively charged groove, hydrogen-bonded with His248β, Arg291β, and Tyr300β, as well as possibly Lys164α and Lys294β. Replacement of Lys164α with Asn results in decreased catalytical activity, underscoring the importance of this residue [103]. The depth of the hydrophobic binding pocket, delimited by the large amino acids Trp102β and Tyr205β, corresponds to the length of *FPP*, keeping the charged diphosphate group in close proximity to the zinc ion. According to these findings, the pyrophosphate of a *GGPP* bound at the active site would be out of reach for the Zn^{2+}, illustrating the isoprenoid substrate selectivity of FT. This explanation is called the molecular ruler hypothesis and is supported by the discovery that *GGPP* is a competitive inhibitor of FT, but not a substrate [88]. On the other hand, even though *FPP* binds to GGT with 330-fold less affinity than *GGPP* [104], it still serves as a substrate.

The C*XXX*-peptide substrate is bound in an extended conformation [105] with the *X*3 residue near the bottom of a cleft at the subunit interface, and *X*2 next to the isoprenoid, both of them interacting with Tyr166α. Molecular modeling yielded Met, Ser, Gln, and Ala as possible *X*3 residues, but shows sterical restrictions for Leu. Experiments with peptides of the sequence KKSSCVL*X* identified Met, Ser, Gln, Ala, and Cys as best substrates [106]. Arg202β forms a hydrogen bond with the C-terminal carboxylate of the peptide. The sidechain of the *X*1 residue of the C*XXX*-motif is directed to the solvent, giving an explanation for the farnesylation of at least one protein with a polar amino acid at this position [107]. The cysteine sulfur atom replaces the water molecule coordinating the Zn^{2+}. *FPP* contributes significantly to the formation of the binding pocket of the peptide. *Van der Waals*

contacts between the C*XXX* peptide and *FPP* exclude the majority of the second and the whole third isoprene unit from the solvent. These results suggest an ordered sequential mechanism with binding of the isoprenoid at first, followed by binding of the peptide substrate [108].

For the actual reaction, a movement of the first two isoprene units of *FPP* is necessary to get carbon 1 next to the cysteine sulfur. While the chemical step of the reaction is relatively fast, release of the product is the rate limiting step of the reaction [102, 109]. It requires binding of a new *FPP* displacing the farnesylpeptide to another hydrophobic pocket [93, 110] and is accompanied by a conformation change of the C*XXX* peptide from extended to β-turn [111, 112]. The nature of the *XXX* residues also affects dissociation of the complex through interaction with the new isoprenoid substrate. Not enough, the FT-farnesylpeptide-*FPP*-complex is stable and requires binding of a peptide [93], but it remains uncertain, whether a new substrate peptide is needed for product release or the interaction with a C*XXX*-endoprotease, which cleaves prenylated C*XXX*-sequences. From these results, it can be concluded that product release from FT may take place at a subcellular localization rich in *FPP* and/or newly synthesized protein substrates. A reasonable region would be the ER, where further modifications will occur.

In contrast to FT and GGT I (both are able to prenylate short C*XXX* peptides), GGT II recognizes a complex of the substrate with a cofactor called Rab escort protein (REP) [54, 113–116] and, then, scans the carboxy terminus for cysteines [117]. This model explains the fact that motifs like CC or C*X*C do not signal prenylation themselves, as noted above.

The reaction mechanism seems to be similar to FT. *GGPP* is bound in a cavity of an α-α barrel, with the diphosphate next to a positively charged cluster and the Zn^{2+}-ion, coordinated in analogy to FT. GGT II also possesses a similar exit groove. Following the putative reaction pathway, binding of the second isoprenoid would displace the monoprenylated peptide to the second pocket, and after covalent addition of a second lipid anchor, a third *GGPP* would trigger the release of the product [93]. Different from FT, the active site cavity of GGT II is terminated by the smaller Ser48β and Leu99β (instead of the respective Trp102β and Tyr205β residues in FT), adjusting the size for *GGPP* binding [118].

Post-Prenylation Modifications

CXXX-Proteolysis

For many C*XXX*-proteins, posttranslational modification is not finished with prenylation. The lipidated proteins become cleaved by an endoprotease between the cysteine and *X*1, releasing a tripeptide and leaving the prenylcysteine in the ultimate position. In analogy to type I prenyltransferases, the protease recognizes short prenylated peptides, suggesting that all relevant signals are included in the immediate sequence environment of the prenylated C*XXX*-peptide. In fact, a prenylated cystein-dipeptide is the minimum requirement sufficient for cleavage. Additional conditions are (i) that the carboxyl-terminus is not esterified and (ii) that the amino acids are in *L*-configuration [119]. The highest activity has been measured for big hydrophobic sidechains in positions *X*1 and *X*2 [120].

Standard inhibitors of serine-, cysteine-, aspartyl-, and metalloproteases showed no effect, but the protease was inhibited by substrate analogues, especially the aldehyde derivative of benzyloxycarbonyl (*BOC*)-farnesylcysteine [121]. Further inhibition analysis suggests that the endopeptidase is a thiol protease [122], but sequence analysis argues for a metalloprotease [123]. These results indicate the possibility of multiple C*XXX*-proteases. It should be noted that, according to the current knowledge, there is only one C*XXX* peptidase with function shown *in vitro* and *in vivo* and this one is called Rce1 (Ras converting enzyme 1), which cleaves farnesylated as well as geranylgeranylated proteins [124]. The human Rce1 has been shown to hydrolyze substrates with both isoprenoids [125]. Rce1 is a polytopic integral membrane protein localized in the membrane of the ER [126]. Knockout mice deficient for Rce1 showed gel mobility for Ras corresponding to the uncleaved protein, and further processing of Ras was blocked [127]. 50% of Ras were cytosolic, and the partial membrane association was due to farnesylated, uncleaved Ras. There was no detectable C*XXX*-endopeptidase activity with either farnesylated or geranylgeranylated substrates [128]. Deletion of Rce1 was lethal, the mice died between embryonic day 15 and the first week after birth.

In *Saccharomyces cerevisiae*, deletion of Rce1 caused only a partial loss of endoproteolytic activity. During processing of the farnesylated a-mating factor from yeast, two C*XXX*-endopeptidases are involved [129], which are not homologous to each other. There is Rce1p, the yeast homolog to mammalian Rce1, also responsible for Ras2-processing in yeast, and there is Afc1p [130–132]. The latter is a polytopic integral membrane protein like Rce1p and a Zn^{2+}-metalloprotease, according to sequence analysis. It acts as a C*XXX*-endopeptidase for model compounds as well as an N-terminal a-factor-endopeptidase [133, 134]. The C-terminal processing of prenylated proteins by Afc1p *in vivo* has not been shown. The human homolog of Afc1p is a zinc-metalloprotease, too, and it is localized at the ER like Rce1, but its function in mammals is still unknown [130, 135, 126].

Carboxyl Methylation

After proteolytic cleavage, the now C-terminal prenylcysteine of C*XXX*-proteins can be carboxymethylated [136]. The isoprenylcysteine carboxyl methyltransferase (ICMT) utilizes *S*-adenosylmethionine as a substrate [137] and modifies both farnesylated and geranylgeranylated proteins [138]. It is an integral membrane protein localized at the membrane of the ER in mammals [139] and yeast [140], but it may also be found at the *Golgi* and the nuclear membrane. The smallest unit recognized as substrate is *S*-prenylcysteine [141].

Like proteolysis by Rce1, methylation of C*XXX*-proteins is essential. Mice deficient for ICMT die by embryonic day 11, which is earlier than Rce1-deficient mice [142, 143]. There are multiple possible explanations for this result. ICMT may be involved in methylation of some prenylated Rab proteins. Experiments with the competitive inhibitor *S*-farnesylthioacetic acid (*FTA*) [141] showed influence of ICMT on various cellular processes like the capacitive Ca^{2+}-influx [144], tumor-necrosis-factor α induced necrosis [145], and induction of apoptosis [146], but these results are not only indicative, since *FTA* is not specific for ICMT [147].

Another possible reason is that the *XXX*-tripeptide is not as troublesome for the function of prenylated proteins as is a C-terminal carboxylate-anion, which seems quite plausible for the case of membrane association.

Rab proteins do not undergo proteolytic cleavage, but those ending in C*X*C become methylated [66], while those ending in CC do not [148]. This methylation activity is also dependent on *S*-adenosylmethionine, but seems to be provided by a different enzyme [149].

Other Post-Prenylation Modifications

Additional to C*XXX*-proteolysis and methylation, some prenylated proteins require extra proteolytic cleavage steps. At first, there are farnesylated fungal peptide pheromones like a-mating factor from yeast [150]. The a-factor is synthesized as a 36 amino acid propeptide, and after standard processing for C*XXX*-proteins, there are two more cleavages at the N-terminus [151]. The first one removes 7 residues and is catalyzed by Afc1p, which has been mentioned above [133], and in the second step 14 amino acids are removed by Axl1p, another Zn^{2+}-metalloprotease [152]. Both steps require farnesylated and membrane associated substrates, but it is unknown whether the farnesyl group is recognized specifically by the endoproteases or just provides the required membrane attachment [151].

The other example is lamin A. The mature protein is not prenylated at all, but is synthesized as a prelamin A precursor, which includes a C*XXX*-motif at the C-terminus and undergoes the complete processing followed by an endoproteolytic cleavage 15 amino acids upstream of the site of farnesylation, releasing mature lamin A and a farnesylated peptide [153–156]. The proteolysis takes place at the nuclear membrane [157] and is performed by a serine protease, which specifically cleaves the conserved hexapeptide RSY↓LLG in farnesylated prelamin A. The current hypothesis proposes sterical hindrance of the cleavage site in the unprenylated molecule, which is removed by binding of the isoprenoid in an extra pocket of the endoprotease [158, 159].

Many proteins undergo palmitoylation in addition to prenylation. In fact, all of the Ras proteins in humans except K-Ras4B possess cysteines upstream of the prenylation site which are subject to palmitoylation. This modification is known to occur at membranes and mediates membrane interactions, suggesting that prenylation guides proteins to the membrane, but a second anchor is necessary for stable binding to the lipid bilayer. In fact, K-Ras4B, which is not palmitoylated, exhibits a polybasic region (PBR) consisting of a stretch of six consecutive lysine residues upstream of the C*XXX*-motif. This feature is known to promote membrane association by electrostatic interaction with the negatively charged phospholipid head groups [160, 168].

Functions of Prenylation

Membrane Association

Although there is a large pool of cytosolic prenylated proteins, almost every protein known to be prenylated interacts with membranes in some way [161, 162]. This is

not surprising facing the similarity to other posttranslational lipid modifications like palmitoylation or myristoylation, which mediate membrane association [163]. The isoprenoids are hydrophobic groups, and hydrophobicity is increased further by proteolysis and methylation [164]. Proteolytic removal of the *XXX*-tripeptide may allow better interaction of the prenyl group with the lipid bilayer and methylation may contribute through enhanced hydrophobicity [165] as well as altered conformation and charge [28]. This makes membrane localization an easy choice when searching for the function of prenylation. Support comes from the results that site directed mutagenesis of the prenylated cysteine residue abolishes membrane association of Ras and Rab proteins. Without a doubt, prenylation, proteolysis, and methylation trigger membrane association in some way. But how exactly does this interaction look like? Is it really only a lipid–lipid interaction, based solely on hydrophobicity?

Prenylated proteins are specifically localized to a wide variety of subcellular membrane compartments. With only two different isoprenoid anchors available, it is impossible to provide the needed selectivity. Experiments with cells deficient in C*XXX*-proteolysis showed that Ras is mislocalized to intracellular membranes instead of the plasma membrane if only farnesylated, indicating that prenylation alone offers only very unspecific membrane attachment. Additionally, in most cases prenylation alone is not sufficient for stable membrane binding [166, 160]. A second anchor is needed like a palmitoyl group [167] or a polybasic region [168]. Since palmitoylation occurs at the membrane [169], this led to the "kinetic bilayer trapping"-hypothesis [163], proposing that palmitoylation stabilizes the membrane association initially provided by isoprenylation [32].

But how are proteins brought to the plasma membrane? Since C*XXX*-processing occurs at the ER, it was proposed that H-Ras and N-Ras could travel with the vesicles of the secretory pathway to the plasma membrane [170] where they are palmitoylated. In contrast, K-RasB exhibits a polylysine domain and is believed to utilize a different route to its destination [171]. There is evidence for interaction of K-RasB with tubulin, as disruption of the microtubule by taxol results in mislocalization of K-RasB. Lamin A is another example where the isoprenoid merely guides the modified protein to the membrane for further processing. But it is not involved in the function of the mature protein, since the prenylated peptide is finally removed.

In conclusion, isoprenylation seems to bring proteins in proximity to membranes, where other factors determine the precise localization. But even with added palmitoylation or polybasic region, there is still not enough selectivity to explain the diversity observed in the targeting of prenylated proteins. Supporting experimental data come from oncogenic H-Ras mutants with changes in amino acid sequence upstream of the prenylation and palmitoylation sites, which were mislocalized and lacked transforming activity [167].

The Ras proteins display their greatest divergence in the carboxy-terminal region of about 20 amino acids directly upstream of the CXXX-motif. This so-called hypervariable region contributes to the avidity and specificity of plasma membrane interactions [172], which may be possible through binding to receptors in the membrane [173]. Experiments with *GFP*-fusion-proteins support this theory [174]. *GFP* with the C*XXX*-motif of Ras proteins was found localized to endo-

membranes, while fusion-proteins including the hypervariable domain are localized correctly to the plasma membrane.

Another subfamily of small *GTP*ases, called the Rab proteins, consists of more than 60 members [175] regulating the vesicular traffic and apparently, require the most precise targeting of all prenylated proteins [176]. They also exhibit a hypervariable domain, which has been demonstrated to influence their subcellular localization [177]. Switching of the hypervariable domains of Rab5 and 7 reversed their membrane localization.

Rab proteins interact with two types of auxiliary proteins showing high structural and functional homology. The first one is the Rab escort protein (REP), which has been mentioned above. It binds unprenylated Rab proteins, presents them to GGT II and may also escort the processed Rab proteins to their proper membrane localization [178]. The other one called Rab guanine nucleotide dissociation inhibitor (RabGDI) is able to extract Rab proteins in the inactive, *GDP*-bound state from membranes, thus facilitating the recycling of Rab proteins to their original membrane [179, 180]. In contrast to REP, RabGDI does not bind to unprenylated Rab proteins [181]. Both of these proteins possess the characteristics needed for escorting Rab proteins to their target membrane, where the complex may be recognized and dissociated by a specific receptor. Mutational analysis showed that a Rab1-mutant unable to bind RabGDI, but able to become geranylgeranylated and thus to bind to REP, was localized correctly [182], indicating that REP initially delivers Rab proteins to their site of action [183], while RabGDI provides recycling through the cytosol, but how is the task of target membrane recognition by the hypervariable region accomplished?

Studies focusing on Ypt proteins, the yeast homologs of Rab, showed that hypervariable domain of Ypt1 bound to GDI drapes over the GDI surface in an extended conformation, making it highly accessible for recognition by target membrane components. The same seems to be true for mammalian Rab:GDI-complexes. The hypervariable domains, which do not only differ in sequence but also in length, create a unique surface region for each Rab:GDI-complex facilitating specific delivery to target membranes. Given the high similarity of REP to GDI, REP may utilize the same mechanism for escorting Rab proteins to membranes after prenylation.

A putative GDI displacement factor (GDF) for the GDI:Rab9-complex has already been discovered [184] and been named prenylated Rab acceptor 1 (PRA1) [185] and Ypt-interacting protein 3 (Yip3) [186] in yeast, respectively. It displays all features necessary for the function as a receptor, including membrane association and high affinity for GDI:Rab9. It stimulates dissociation of the complex and membrane association of Rab9 [186]. But the presumable receptor is not specific for Rab9 and functions for a whole subset of Rab proteins instead [185]. In yeast, a total of six Yip proteins have been found and there is some evidence indicating that others than Yip3 play a role in Ypt targeting [187]. Although database searches showed many more members for the corresponding mammalian protein family (in human there are 16), there are still far fewer than Rab proteins. This leads to the assumption that Yip/PRA proteins mediate membrane targeting of multiple Ypt/Rab proteins. Further specification of localization, that means transport to certain membrane microdomains, may be accomplished by interaction

of Rab proteins with their specific effectors. This could occur either by stabilizing membrane association of Rab through binding to effectors already localized in a microdomain, or by recruitment of effectors after activation of the Rab protein, creating its own microenvironment [188].

The Prenyl Anchor as Specific Recognition Unit

So far, we viewed prenylation only as unspecific guide to membranes with following precise targeting by protein–protein interactions between parts of the prenylated protein and membrane constituents. Should this be all there is? If the function of prenylation was just to provide general hydrophobic interactions, nature would have picked a poor choice. Isoprenoids have a branched and quite rigid structure, making them a far worse hydrophobic ‘‘glue’’ than, *e.g.*, palmitate [189]. And after all, there are various experimental results indicating that the isoprenoid groups participate in specific recognition through lipid-protein interactions [190].

First of all, there is evidence for differential prenylation. While the α-subunit of retinal *cGMP*-phosphodiesterase is farnesylated, the β-subunit is geranylgeranylated, suggesting that there must be some important difference in the functionality of the isoprenoids to legitimate the effort of different prenylations in one enzyme [191]. RhoB exists in isoforms with both isoprenoids [192, 193]. It was proposed that farnesylated RhoB may be growth promoting, while geranylgeranylated RhoB inhibits growth. Anyway, it is quite sure that prenyl anchor size does matter: G-protein β-γ complexes are directed to specific receptors depending on isoprenoid length [194]. Rhodopsin kinase looses its ability of translocation to membranes upon light induction with the wrong isoprenoid attached, which is caused by a defect in the interaction with photon activated rhodopsin [195]. Geranylgeranylated normal Ras possesses growth inhibitory activity, while oncogenic Ras with a geranylgeranyl moiety has transforming activity, but not normal biological function, suggesting that membrane association requirements of normal and oncogenic Ras might be different [60]. Myristoylation can replace prenylation for membrane association required for oncogenic activities [48, 196] but leads to activation of transforming activity in normal Ras. Thus, it seems that myristoylation does not facilitate normal Ras functions [197]. The geranylgeranylated forms of K-RasB and H-Ras have been shown to exhibit cellular localizations different from the normal variants. In addition, in yeast only farnesylated RAS is capable of stimulating adenylate cyclase activation and the same is true for interaction of mammalian Ras with its effector Raf [198, 199]. Taken together, these results indicate that oncogenic Ras only requires a generalized membrane association, whereas normal Ras function requires farnesylation [167].

Furthermore, there are multiple examples of direct protein-protein interactions dependent on prenylation. For K-Ras, interaction with tubulin has been shown to require prenylation [200]. Processing of lamin A [153, 154], as well as cleavage and methylation of all C*XXX*-proteins depend on prenylation.

Of course, it is possible to argue that these interactions may simply depend on membrane association, but there is evidence that this is not the case. For example, detergent-solubilized adenylate cyclase of *S. cerevisiae* is only activated by farnesylated Ras, suggesting direct recognition of the isoprenoid [201, 202]. The same is

true for prelamin A endoprotease, which requires prenylation even when solubilized with detergents. Furthermore, farnesylated prelamin A is processed faster than the geranylgeranylated one. *N*-Acetyl-farnesylmethylcysteine (*AFMC*) is a noncompetitive inhibitor of prelamin A endoprotease, indicating that the isoprenoid is bound in a location distinct from the active site.

Other data indicate that membrane association may be mediated by lipid-protein interactions, too. An unidentified binding activity for H-Ras has been found in the plasma membrane. It showed high affinity, but low capacity and, thus, was saturable [203]. There was no competition for binding by unfarnesylated H-Ras but by different farnesylated proteins and peptides as well as by *AFMC*. Another small analog, *S*-farnesylthiosalicylic acid (*FTS*), releases H-Ras and K-Ras from the membrane to the cytosol at high concentrations, where they are exposed to increased degradation [204, 205]. At lower concentrations, *FTS* increases the lateral diffusion of a green fluorescent protein (GFP)-K-Ras-fusion-protein, indicating that the protein is released from some restraints to its mobility [206]. Similar studies revealed that *N*-acetyl-*S*-farnesylcysteine (*AFC*) dislodges farnesylated Ras proteins from the membrane [207], while *N*-acetyl-*S*-geranylgeranylcysteine (*AGGC*) extracts geranylgeranylated Rho proteins [208]. All these results support the assumption, that prenyl modifications are at least part of a unit that is specifically recognized by certain target proteins.

For lamin B, a receptor residing in the nuclear envelope has been identified [209], but only little is known about the actual interaction. *S. cerevisiae* a-factor has a specific receptor, too, which requires a prenylated and methylated peptide pheromone for binding [210]. Studies on the heterotrimeric G_t-protein revealed inhibition of formation of the $G_{t\alpha\beta\gamma}$-complex by *S*-prenylcysteine analogs. Since the inhibition is independent of palmitoylation, it seems likely that $G_{t\alpha}$ possesses a prenyl binding domain for $G_{t\gamma}$ [211, 212].

First direct evidence for the existence of prenyl binding pockets for recognition of prenylation comes from RabGDI, which has already been mentioned before in context with the influence of the hypervariable region. Binding of Rab proteins by RabGDI requires prenylation [181, 213] and is selective for geranylgeranylation over farnesylation [180]. By X-ray crystallography, it was shown that the hypervariable region is held in place by multiple interactions. Binding of the polypeptide chain of the Rab proteins to a Rab binding platform at the top of the GDI leads to a conformational change exposing a cavity at the bottom of the GDI which is rich in hydrophobic residues and binds to a prenyl group of the Rab protein [214]. Other investigators found a second prenyl binding site just below the Rab binding platform [215]. Both sites also have a nearby second groove, which fits the usually digeranylgeranylated Rab proteins. Since RabGDI extracts *GDP*-bound Rab proteins from membranes, it seems feasible that a first interaction with the target leaves the isoprenoid(s) bound to the pocket close to the Rab binding platform, weakening the association with the membrane. Later, the isoprenoid(s) are translocated over the GDI-surface to the second prenyl binding pocket which has been exposed by the first contact, ending in a more stable binding and optimal presentation of the hypervariable region [188]. Since REP shows a high degree of homology to RabGDI [116, 216–218], it may also contain a similar prenyl binding pocket.

There are other GDIs for Rho and Rac proteins with a function similar to that of RabGDI [219–221]. Structure analyses in solution of Rho:GDI- and Rac:GDI-complexes support a model with two different binding domains [222, 223]. One of them binds to the peptide and exhibits the *GTP*ase inhibiting activity, while the other one is responsible for membrane extraction and binding of the isoprenoid. For example, one experiment demonstrated the possibility of sequential binding of a farnesylated peptide analog of the C-terminus and a C-terminal truncated Rac1 [224]. The crystal structures of RhoGDI in complex with Cdc42 [225] and RhoGDI-2 in complex with Rac2 [226] confirmed this model. Recent studies on *cGMP* phosphodiesterase δ subunit (PDEδ) revealed structural similarity to the domain of RhoGDI that contains the prenyl binding pocket [227, 228], indicating a function that depends on prenylation of PDE$\alpha\beta$. PDEδ seems to solubilize the PDE$\alpha\beta$-dimer from the membrane [229–231]. Other experiments showed that PDEδ can interact with a wide range of farnesylated and geranylgeranylated proteins, suggesting that it acts as a cytosolic carrier of prenylated proteins [227, 228]. Another prenyl binding pocket found by comparison to the one in RhoGDI is part of the β-galactoside-binding protein galectin-1 [232]. Galectin-1 preferentially interacts with the active, *GTP*-bound forms of H-Ras and K-Ras [233, 234]. It is recruited to the membrane by active Ras, where it seems to stabilize Ras in its active state and to enhance membrane association with specific microdomains.

In conclusion, there is no doubt that recognition of isoprenoid groups by specific prenyl-binding pockets seems to be an important feature of protein isoprenylation. The structures of known prenyl-binding domains differ from each other, despite their analogy at first sight, enabling high selectivity towards specific prenylated target proteins [235].

Functional Contribution of Post-Prenylation Processing

If prenyl groups are part of regions specifically recognized by other proteins, the same is true for the other processing steps of C*XXX*-proteins. Once again, it is quite easy to propose a function in simply increasing membrane affinity. This might be true anyway, since proteolytic cleavage removes a part of the polypeptide chain which may interfere with optimal interaction of the prenyl group with membrane lipids, although it creates a negative charge in direct proximity of the isoprenoid. Methylation nullifies this effect, enhancing hydrophobicity of the C-terminus once more, especially for farnesylated proteins, while the influence on hydrophobicity of geranylgeranylated proteins is negligible [236, 165, 237].

However, there is evidence that postprenylation modifications also participate in specific interactions. *S. cerevisiae* a-mating factor requires a fully processed C-terminus for recognition and export by a membrane transporter [210, 238]. Transducin with a non-methylated γ-subunit exhibits a reduction by a factor of 10 in activation of its downstream effectors [239–241]. Association of K-Ras with microtubules, which is believed to be important for trafficking to the target membrane, as well as membrane association of K-Ras also depends on methylation [242–244]. At last, interaction of lamin B with an unidentified protein of the inner nuclear membrane demands the fully processed, mature lamin [245].

Other examinations found an influence on protein stability. While RhoA showed increased degradation without methylation [246, 247], the half-life of Ras was longer. Anyway, it remains unclear whether this is due to mislocalization or altered recognition by the degradation apparatus of the cell.

While prenylation has been shown to be a stable modification, methylation in principle is fully reversible [28]. Consequential, a regulatory function has been hypothesized [248, 249]. But until today, no specific methylesterase has been found and there is also not the slightest sign for cycles of methylation/demethylation in any investigated protein [250]. In conclusion, post-prenylation processing mainly seems to be dedicated to supplement the features of prenylation, but even this function is crucial, at least in some proteins.

Function and Clinical Relevance of Isoprenylated Proteins

In mammals, it is estimated that there are approximately 50 farnesylated proteins [251] and 100 proteins geranylgeranylated by GGT I, along with more than 70 Rab proteins, most of them if not all undergoing prenylation by GGT II [75, 56]. Many prenylated proteins exhibit important regulatory functions of the cell, especially in signal transduction but also in proliferation, differentiation, cell survival and death, regulation of the cytoskeleton, cell migration and adhesion, as well as vesicular traffic [252–256]. Thus, it is not surprising that more than one prenylated protein is involved in human diseases, the most prominent of them being cancer.

Ras Proteins

The four Ras proteins H-Ras, N-Ras, and splice variants A and B of K-Ras are master regulators in signal transduction pathways controlling cell proliferation and differentiation [257]. They are members of the large family of small *GTP*ases, which function as molecular switches, cycling between the active, *GTP*-bound and the inactive, *GDP*-bound state. Mutated versions of Ras have been shown to be involved in a wide variety of human cancers [258–262]. Those mutations are usually constitutively activated forms which are chronically *GTP*-bound [263]. All four Ras proteins undergo farnesylation [32], and this modification has been shown to be critical for localization to the inner surface of the plasma membrane and at least some of the normal biological functions. Fortunately, transformation of cells by oncogenic Ras depends on prenylation, too [196, 48, 264]. Since oncogenic mutations of elements lying upstream of Ras in the signal pathways, *e.g.*, some tyrosine kinases [265–267], also exert their transforming activity through hyperactivation of Ras, controlling the farnesylation of those proteins has become a topic of high interest in cancer research.

Closely related are the Rap proteins, more precisely Rap1A and B which are geranylgeranylated and the farnesylated Rap2A and B. Mutational activation of Rap1A signaling is associated with myeloproliferation [268].

Rab Proteins

The Rab proteins are the only known targets of GGT II, although some of them have been shown to be modified by GGT I. They carry out important functions in

the assembly of transport vesicles and their targeting in the exo- and endocytotic pathway of mammals [269, 270]. Prenylation is crucial for their membrane association [271] and is also necessary for interaction with RabGDI [181], which enables recycling of Rab proteins through the cytosol back to their original membranes.

The most popular diseases associated with Rab prenylation and function are not correlated to a certain Rab protein but with general defects in Rab modification [271]. Loss of the GGT II α-subunit causes the *Hermansky-Pudlak* syndrome. This is a disfunction of vesicle traffic between the trans-*Golgi* and lysosomes [272] with phenotypic effects on platelet synthesis, platelet organelle function, and pigmentation, while X-linked choroideremia is associated with loss of REP [116, 114, 273]. The symptom is retinal degeneration, resulting in night blindness in young people and complete loss of vision in the worst case. The specific loss of Rab27a function causes the *Griscelli* syndrome with a disease pattern very similar to the *Hermansky-Pudlak* syndrome [274].

Other Small GTPases

Other proteins of the Ras superfamily also exhibit important functions in the cell. Most of them are geranylgeranylated [193, 275]. The Rho proteins regulate the actin cytoskeleton with involvement of RhoA and B in the formation of actin stress fibers and focal adhesions [276, 277]. Cdc42 is associated with formation of filopodia as well as control of the cell cycle and cell polarity. Rac has functions in the secretion at the plasma membrane, membrane ruffling [278], and the oxidative burst of phagocytic cells [279–281]. Some members of the Rho family also play a role in apoptosis.

From these important functions, it is quite clear that they are also connected with some types of cancer [282]. Rho proteins are especially correlated to tumor angiogenesis and metastasis [283–285], which seems quite believable, considering their field of action.

Non Ras-Related Proteins

The heterotrimeric G-proteins play a key role in transmitting extracellular signals from cell surface receptors to the intracellular signaling cascades. They are *GTP*ases like the Ras superfamily but, in contrast to the monomeric Ras proteins, they are built from three subunits designated α, β, and γ. Some γ-subunits have been shown to require prenylation for their proper function [286], while the α-subunits are not modified by isoprenoids, despite the fact that they possess a C*XXX*-like motif at the C-terminus [287]. Like Ras, the G-proteins are involved in malignant transformation of cells by constitutive activation of their downstream signaling pathways [288–291].

The nuclear lamins are an important component of the nuclear membrane, involved in the mitotic membrane disassembly and assembly [292]. Farnesylation is required for maturation of lamin A [153] and an integral part of mature lamin B, while lamin C is not prenylated.

There are many more proteins with prenylation dependent functions which did not receive as much attention as *GTP*ases or the lamins. The spectrum ranges from

fungal mating peptides [293] like a-factor and rhodotorucine A to the large antigen of the hepatitis delta virus, which requires prenylation for viral particle assembly [294]. Other examples are the kinetochore proteins Cenp-E and F [295] and various proteins involved in vision like transducin [296], retinal *cGMP* phosphodiesterase, and rhodopsin kinase [297].

Inhibitors of Prenyltransferases

Ras mutations have been detected in ~30% of all human cancers. The discovery that all Ras proteins are farnesylated and require this modification for membrane targeting, normal biological function and malignant cell transformation as well [45] boosted the efforts invested in the development of farnesyltransferase inhibitors (FTI) as anti-cancer therapeutics [298]. Different strategies of targeting FT divide FTIs into three classes:

1) Farnesyl pyrophosphate analogs compete with *FPP* for the binding to FT
   a) Competitive inhibitors of the C*XXX*-binding site
   b) Peptidomimetic inhibitors
2) Non-peptidomimetic inhibitors derived from high throughput screenings
3) Bisubstrate analogs that mimic the features of both FT substrates [299]

Inhibition at the peptide substrate binding site turned out to be more specific [300–302], while *FPP* analogs also inhibited GGT I and *FPP*-converting enzymes like squalene synthase [303, 304].

Preclinical Studies

In preclinical experiments with cultured cells and mouse models, FTIs exhibited remarkable effects as anti-cancer drugs with IC_{50} values in the low nanomolecular range [303, 305–317]. Various cancer cell lines were affected by FTI treatment [316, 318], with complete inhibition of transformation at best. Other effects included inhibition of anchorage-dependent growth [318], changes in cell cycle progression (G_1/S and G_2/M phase checkpoints depending on cell type), induction of apoptosis [319], and effects on actin stress fibers and cell morphology [320].

The knockouts of the FT β-subunit in mice [321] showed that FT is required for tumor progression, but not for its initiation. Furthermore, it revealed that, while FT is critical during embryonic development, conditional knockouts of age up to 18 months showed no severe defects, indicating that FT is dispensable for postnatal development and adult homeostasis. This is in accordance with *in vivo* studies showing that FTIs are well tolerated and suggests the use of FTIs for tumor prevention (after surgical removal of tumors to prevent recurrence).

However, preclinical studies yielded two surprising outcomes, too. Inhibition of tumor growth was independent of the Ras status [318]. Some cell lines without any Ras mutations were affected by FTI treatment, indicating that the anti-tumor effects of FTIs are at least not alone mediated by inhibition of farnesylation of the Ras proteins [322, 309]. This raises the question, which proteins are the true targets. On the other hand, while cell lines bearing H-Ras mutations generally

Table 2. Ras mutations in solid malignancies [380]

Cancer	Frequency %	Type	Cancer	Frequency %	Type
Pancreas	80	K	Lung	30–50	K
Colon	40–50	K	Liver	10	N
Thyroid	50–80	H,N,K	Kidney	10	H
Head & Neck	25	H,N,K	Ovary	25	K
Bile Duct	>50	N	Endometrial	18–40	K
Breast	<5	H,K	Glioma	<5	wt, overexpression

respond very well to FTIs, increased resistance was observed for mutated K-Ras and N-Ras [63]. Unfortunately, in human cancers K-Ras is mutated most frequently (Table 2).

Different explanations for FTI resistance of cancer cell lines have been proposed. One possibility is that transforming activity of K-Ras and N-Ras can be rescued by alternative geranylgeranylation due to crossreactivity of GGT I [323, 323, 63]. However, the experimental results on the alternative prenylation of K-Ras and N-Ras are partially conflicting [324, 325]. To date, there is no evidence for this phenomenon in any other protein investigated. This is due to problems in the experimental procedures. The ratio of *FPP* to *GGPP* depends on the intracellular concentration of their precursor mevalonate [326], making it difficult to perform radioactive labeling experiments with physiological pools of *FPP* and *GGPP*. Worsening the case even more, *FPP*-synthase is downregulated by *FPP*, while *GGPP*-synthesis is stimulated by *FPP* [327]. Thus, inhibition of FT increases cellular concentration of *FPP* and in consequence raises also the level of available *GGPP*. In addition, oncogenic K-Ras exhibits pleiotropic effects on mevalonate-metabolism [328]. At last, incorporation of a radioactive label does not reveal the fraction of the protein that is prenylated. Thus, it is hard to distinguish between experimental artifacts and results reflecting the true *in vivo* situation concerning alternative prenylation.

Experimental data show that FTIs induced tumor regression in cell lines bearing K-Ras mutations, although prenylation of K-Ras was not blocked. This result supports the thesis of alternative prenylation and provides further evidence that proteins other than Ras mediate the effects of FTIs [329].

While tumor-suppressive effects of FTIs are restricted for K-Ras mutations, association of K-Ras with its effector Raf-1 has been shown to be effectively inhibited by FTI [330]. This result suggests that FTI resistance is due to activation of other effectors downstream of Raf-1 by geranylgeranylated K-Ras or mutational activation [331].

Irrespective of alternative prenylation of K-Ras and N-Ras, interrupted treatment with FTIs sometimes led to development of resistance. A possible explanation is mutation of FT. Indeed, it has been reported that the mutation Y361L increased resistance of FT for inhibition by FTIs. Nevertheless, it is still able to farnesylate some of its normal substrates, namely those terminating in CIIS [332].

In conclusion, different mechanisms could contribute to FTI resistance of cancer cells. While continuous treatment should overcome some of the troubles, alter-

native prenylation of K-Ras and N-Ras (and possibly others) demands for another solution. Combined treatment with geranylgeranyltransferase I inhibitors (GGTI) should prevent alternative prenylation, and GGTIs caused tumor regression in cultured cells and mice [329] (cell cycle arrest in G_0/G_1 phase) [333], but there are conflicting reports on the tolerance of these compounds [334, 335]. A new development are dual prenyltransferase inhibitors (DPTI) which serve the same purpose and yielded promising results in *in vivo* models [336].

Since cell lines bearing mutations in H-Ras or no Ras mutations at all appeared to be most sensitive to treatment with FTIs, other proteins which mediate their tumor suppressing effects are still waiting to be discovered. RhoB has been observed both farnesylated and geranylgeranylated. Thus, it has been suggested as a key target for FTI [337, 338]. A shift in RhoB prenylation towards the geranylgeranylated form was proposed to induce phenotypic reversion and loss of anchorage independence [339–343]. In contrast to this hypothesis, overexpression of farnesylated RhoB also induces apoptosis [344]. Another model assigns RhoB a tumor suppressive function [345], documented by the fact that RhoB levels in head and neck carcinoma decreased with tumor stage [346]. Recapitulating, there are different assumptions concerning the influence of RhoB, but there is no direct evidence for a role in mediating apoptotic effects of FTIs.

Since FTI treatment in most cell types induces cell cycle arrest in G_2/M phase, prenylation of the kinetochore proteins Cenp-E and F was proposed to be a target for FTIs [295]. Again, there is no evidence for this thesis.

Conflicting data are available on the influence of p53 on the action of FTIs. While some experiments suggested that cell lines with wild-type p53 are most sensitive to FTI treatment [347, 348], *in vivo* studies in transgenic mice indicated a p53-independent apoptotic activity of FTIs [349]. A possible explanation could be two different pathways of action of FTIs. Anyway, since defects in p53 lead to accelerated tumorigenesis and resistance to various cytotoxics, a p53 independent way for induction of apoptosis by FTIs indicates that they may be active against these tumors [350].

Indeed, two ways for induction of apoptosis by FTIs have been revealed. The first one is mediated by release of cytochrome c from the mitochondria into the cytosol, resulting in activation of caspases [320]. Interestingly, the common α-subunit of FT and GGT I becomes cleaved by caspase-3, indicating that inactivation of prenylation contributes to progress of apoptosis [351]. The other way of action is inhibition of the phosphoinositide 3-OH kinase/AKT2-mediated cell survival and adhesion pathway, but this effect is not mediated by Ras or RhoB [352]. Therefore, other proteins involved in this pathway could be targets for FTIs.

To date, no specific protein has been identified as the key target for FTI treatment. Thus, their effects are mediated either by a not identified protein or inhibition of farnesylation of several proteins is required for antitumor activity.

Recently, inhibition of RabGGT has been identified as a possible mechanism of certain Bristol-Myers Squibb FTIs known to be strong inducers of apoptosis. Knockdown of RabGGT as well as of its substrates Rab5 and Rab7 resembled the proapoptotic activity of the FTIs [353]. The frequent overexpression of both subunits of RabGGT in human tumors supports the idea of RabGGT as a new target in cancer therapy, providing a novel pathway for induction of apoptosis by inter-

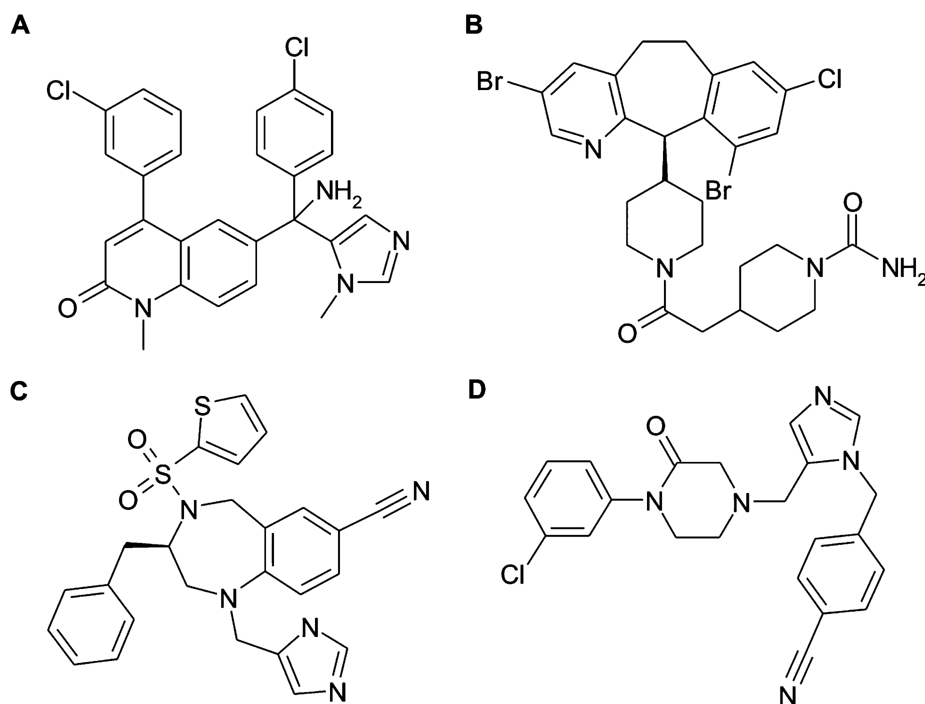

Fig. 6. Chemical structures of farnesyltransferase inhibitors: R115777 (A), SCH66336 (B), BMS-214662 (C), and L778123 (D); the chemical formulas of the drug molecules are based on information from Ref. [336]

fering with vesicular trafficking. However, it remains to be established whether this effect is a feature of other FTIs, too, or is unique to the investigated type of compound.

Clinical Trials

Preclinical studies revealed at least in some cases high antitumor effectivity along with low toxicity of FTIs, leading to quick approval for clinical tests [354, 355]. To date, six compounds have been or are being tested [356], but results for only four compounds are publicly available (see Fig. 6):

1. R115777, Tipifarnib, ZARNESTRA™ (Ortho Biotech. Oncology/Janssen Pharma PV)
2. SCH66336, SARASAR™ (Schering-Plough Res. Inst.) [315]
3. BMS-214662 (Bristol-Myers Squibb Pharma Res. Inst.)
4. L-778123 (Merck Res. Lab.)

R115777: Phase I studies on this compound revealed myelosuppression and neurotoxicity as dose limiting toxicities, while nausea, vomiting, headache, fatigue, anemia, and hypotension were also observed [357–360]. Several phase II trials have also been performed with R115777. In patients with advanced breast cancer, nine partial responses and nine cases of stable disease were observed in 76 patients [361], while for patients with relapsed small-cell lung cancer no response and only

one case of stable disease are reported [362]. Good results were also obtained in the treatment of patients with untreated poor-risk acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS), with up to 33% response rate (8 complete, 2 partial) [336]. A second trial with MDS patients yielded only 2 complete and one partial remission in 27 patients [363]. For patients with multiple myeloma, treatment with R11577 unfortunately yielded no response, but stabilization of the disease was observed in 64% of the patients [364]. Two phase III trials brought no response in patients with advanced colorectal cancer as well as pancreatic carcinoma [336]. In the latter case, R115777 has been tested in combination with the conventional drug gemcitabine but there was no enhancement in response compared to treatment with gemcitabine alone.

SCH66336: Phase I trials utilizing an intermittent dosing schedule showed diarrhea to be dose limiting, while other observed toxicities included nausea, vomiting and fatigue [365]. A continuous dosing schedule also caused neutropenia, thrombocytopenia, confusion, and disorientation [366]. Phase II studies with urothelial cancer, metastatic colorectal cancer, and chemotherapy-refractive advanced head and neck squamous cell carcinoma all resulted in no response, but differing percentages of patients with stable disease [367, 336]. The best results were gained in trials with myeloid malignancies with up to 19% response rate [368].

BMS-214662: Initial tests with oral formulation were abandoned due to severe gastrointestinal toxicity [336]. Therefore, further investigations were performed with intravenous administration. Dose limiting toxicities in phase I trials were nausea, vomiting, diarrhea [369] but, in other administration schemes, also creatinine elevation, acute pancreatitis, and renal failure as well as hypokalemia and cardiovascular problems, somnolence, anorexia, leucopenia, and neutropenia were observed [370, 371]. While phase I studies reported activity in lung, colorectal, prostate, laryngeal, and breast cancer and also antileukemic activity in five patients [363], no literature is available on phase II studies.

L778123: In contrast to the other compounds, L778123 also exhibits considerable inhibition of GGT I [372, 373]. It is able to completely inhibit K-Ras prenylation in cell lines [374], but fails to do so in humans [373]. Phase I trials were performed with intravenous administration [375, 376]. Dose limiting toxicities were revealed to be thrombocytopenia and neutropenia, but no responses were obtained. Since QT_C prolongation in ECG was observed in at least one patient in all trials (indicating a delay in cardiac repolarization, which favors development of cardiac arrhythmias) the development of compound was discontinued.

In conclusion, FTIs yielded promising results in preclinical studies, which could not be fully confirmed in clinical tests. This may be due to unexpected resistance of tumors bearing mutations in K-Ras and N-Ras, which can be alternatively geranylgeranylated. Nonetheless, FTIs showed good results against hematological malignancies [377] and breast cancer. In addition, synergistic effects were observed with conventional drugs as taxanes and platinum-containing compounds [378]. For example, SCH66336 has been shown to exhibit synergy with cisplatin [379]. Preclinical tests on synergistic antitumor activity of R115777 with trastuzumab in certain types of breast cancer yielded positive results, followed by phase I trials demonstrating that administration of the full dose of both agents is safe [380]. Combination with SCH66336 is also under investigation. Synergistic effects

observed for combination of FTIs with taxanes [381] were proposed to be mediated by inhibition of farnesylation of the centromeric proteins Cenp-E and -F, thus enhancing the microtubule-stabilizing effect of the taxanes [382]. Combination with cytotoxics revealed some effects in phase I studies [383], although combination of R11577 with gemcitabine was ineffective in a phase III trial [336].

Applications for the Use of FTIs Besides Cancer Treatment

Viruses utilize the machinery of their host cells for various functions critical for their life cycle. Consequently, some viruses could also take advantage of protein prenylation. Indeed, prenylation of viral proteins has been demonstrated. The hepatitis delta virus (HDV) has an RNA genome encoding only two proteins, the small and the large antigen. These differ only in 19 additional amino acids at C-terminus of the large antigen [384]. This protein possesses a C*XXX*-motif, which is conserved between different HDV isolates [294]. It has been shown to be subject to farnesylation *in vitro* and in cell culture [294, 385, 386]. The HDV large antigen is required for assembly and release of viral particles [387]. The critical role for prenylation in this process has been demonstrated by mutation of the modified cysteine, which impaired viral particle formation [385]. Treatment with FTIs turned out to be highly effective in cell culture as well as in mouse models, reducing virion production below the detection limit [388–390].

This offers a novel antiviral strategy. By targeting a host cell function, which is not under viral control, it can be anticipated to be harder for the virus to develop resistance. Furthermore, the farnesyl moiety seems to be a specific ligand, indicating that substitution by geranylgeranyl may be not sufficient to restore large antigen function in the case of HDV. At last, although also interfering with normal functions of the host cell, FTIs are tolerated surprisingly well by higher eukaryote organisms such as mice or humans.

C*XXX*-motifs are also found in other viral proteins, but prenylation has not been proven for all of them. UL32 of the herpes simplex virus (HSV), which is also believed to be involved in particle formation, is one of these examples [391]. HSV-1 is thought to exploit Ras signaling pathways. Therefore, it has been subjected to FTI treatment. Antiviral activity was observed, indicating a possible dual function of FTI against particle formation as well as the Ras pathway utilized by the virus [392]. Other C*XXX*-boxes were found in the polymerase of hepatitis A virus and the virus causing foot and mouth disease, where prenylation may mediate membrane associated RNA replication [393].

Another alternative application for FTIs is the use against fungal and protozoan pathogens. Although prenylation is also present in some of these organisms, not all of them show the full spectrum of prenylation activities observed in eukaryotes. While *Trypanosoma cruzi*, which causes the *Chagas* disease, completely lacks GGT I activity [394, 395], others are at least less able to compensate for FT inhibition than mammals. Consequently, *Plasmodium falciparium* (malaria) [396, 397], *Trypanosoma brucei* (African sleeping sickness) [395, 398], and *Candida albicans* (candidiasis) [399] showed increased sensitivity against FTI treatment. Positive effects were also achieved for *Leishmania* parasites (leishmaniasis) [395], *Toxoplasma gondi* (toxoplasmosis) [400], and *Schistosoma mansoni* (schistosomiasis) [401].

In search for more targets, database searches revealed additional 11 animal and 8 plant pathogens, suggesting possible new applications for treatment of lymphatic filiariasis (elephantiasis), onchocerciasis (river blindness), aspergillosis, pneumocystosis, amoebiasis, strongyloidiasis, trichinosis, and cryptosporidiosis [76].

Alternative Ways of Targeting Protein Prenylation

With FTI treatment facing the problem of alternative geranylgeranylation, other approaches allowing complete inhibition of protein prenylation gained interest. Apart from dual prenylation inhibitors blocking both FT and GGT I, the other C*XXX*-tail processing enzymes became new targets in anti-cancer therapy.

Deletion of the gene encoding Rce1 reduced Ras-induced malignant transformation of cells, albeit with lower efficiency than FTI treatment. However, increased sensitivity to FTIs has been reported [402], indicating influence on proteins still prenylated under inhibition of FT.

Deletion of the gene for isoprenylcysteine carboxyl methyltransferase (ICMT) showed more effectivity, permitting complete blocking of transformation by oncogenic K-Ras [403].

For inhibition of Rce1, mainly prenylated C*XXX*-peptides are in use. Since chloromethylketone-inhibitors showed effects against Rce1 [402, 404], prenylcysteine-chloromethylketone derivatives have been designed.

Inhibition of ICMT can be reached both by substrate and product analogues. *S*-Adenosylhomocysteine is the product and, therefore, a competitive inhibitor of various methyltransferases [405–407]. Its effects, therefore, exceed inhibition of methylation of prenylcysteines [408]. Reported functions are growth inhibition of tumor cells and antiangiogenetic effects. By reducing proliferation of endothelial cells and increasing their apoptosis [409], the supply of new blood vessels for the tumor is reduced. The second class of ICMT-inhibitors are prenylcysteine derivatives [410]. *N*-Acetyl-*S*-farnesyl-*L*-cysteine (*AFC*) [411] and *N*-acetyl-*S*-geranylgeranyl-*L*-cysteine (*AGGC*) are competitive inhibitors for methylation of C*XXX*-proteins. Again, they also exhibit other effects independent of ICMT [147, 412–415] like dominant inhibition of prenylcysteine-dependent protein interactions. Treatment with these compounds resulted in reduced methylation and mislocalization of Ras. Surprisingly, the same effect was not observed in cell lines with a deletion mutant in the gene corresponding to ICMT, indicating adaptation of cells to sustained loss of ICMT.

Since the structure of prenyl binding pockets is very specific, it may be possible to design *S*-prenyl analogues blocking the action of certain prenylated proteins [416]. The attempt to fight cancer by dislodging Ras proteins from membranes with FTS is a very similar idea [417].

Contrary to the approaches mentioned above, other strategies try to intervene with isoprenoid synthesis. The lowest specificity is exhibited by the statins, which are inhibitors of the enzyme *HMG*-CoA reductase, thus operating by depletion of the cellular mevalonate pool [418]. They were initially designed to lower cholesterol synthesis, but unavoidably also block protein prenylation. Treatment with statins resulted in cell cycle arrest at the G_1/S phase checkpoint [419, 420] and induction of apoptosis in sensitive tumor cells by cytochrome c release and sub-

sequent caspase-3 activation [349, 421]. Reversal of the effect was only achieved by addition of *GGPP*, indicating that the anti-cancer activity of statins is due to critical geranylgeranylated proteins [285, 422, 423], but the definite targets are unknown. Influence of cellular cholesterol on cell survival has also been proposed, giving another explanation for the action of statins [424]. Enhancement of the antiproliferative activity of standard therapeutics is a promising side effect [425, 426].

Similar effects were found for a class of compounds actually designed as anti-osteoporosis drugs called nitrogen-containing bisphosphonates (NCBPs). They turned out to be specific inhibitors of the enzyme *FPP* synthase [427].

In conclusion, much effort is invested in the search for alternative ways to target protein prenylation in cancer therapy. Most of these approaches are still in the preliminary, preclinical phase but they promise innovative applications in the future.

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