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Review

Isoprenylated proteins

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Abstract. Isoprenoids are synthesized in all living organisms and are incorporated into diverse classes of end-products that participate in a multitude of cellular processes relating to cell growth, differentiation, cytoskeletal function and vesicle trafficking. In humans, the non-sterol isoprenoids, farnesyl pyrophosphate and geranylgeranyl-pyrophosphate, are synthesized via the mevalonate pathway and are covalently added to members of the small G protein superfamily. Isoprenylated proteins have key roles in membrane attachment and protein functionality, have been shown to have a central role in some cancers and are likely also to be involved in the pathogenesis and progression of atherosclerosis and Alzheimer disease. This review details current knowledge on the biosynthesis of isoprenoids, their incorporation into proteins by the process known as prenylation and the complex regulatory network that controls these proteins. An improved understanding of these processe is likely to lead to the development of novel therapies that will have important implications for human health and disease.

Key words. Isoprenylation; mevalonate pathway; statin.

Introduction

The post-translational modification of proteins by the addition of isoprenoids has been recognized as a key physiological process for facilitating cellular protein-protein interactions and membrane-associated protein trafficking. Protein prenylation occurs by the covalent addition of two types of isoprenoids, farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP), to cysteine residues at or near the carboxy terminus [1]. Although numerous prenylated proteins have been identified, these are likely to these represent only a small proportion of the 0.5–2% of mammalian proteins that are estimated to contain farnesyl or geranylgeranyl groups [2, 3]. A variety of critical intracellular proteins including heterotrimeric G protein subunits and nuclear lamins are prenylated [1] but the largest group, which has been of interest to cell biologists for many years, is the small G proteins. The small G proteins are monomeric G proteins with molecular masses of 20–40-kDa [4] and are often referred to as 'small GTPases'. While the intrinsic GTPase activity is necessary for the termination of function of small G proteins, it is not essential for them to perform their functions.

Although the structural and functional diversity of the prenylated proteins is immense, they carry out their functions via similar biochemical mechanisms. Several comprehensive review articles on mechanistic aspects and enzyme kinetics of the protein prenylation reactions have been published [5, 6] and these aspects will be discussed only briefly. The purpose of this review is to detail recent advances in our understanding of the processes that control the biological functions of the small G protein subset of prenylated proteins and the implications for human health and disease.

Synthesis of isoprenoids

Although isoprenoids have a diversity of structures and functions, they are all derived from the common five-carbon (C_5) building unit isopentenyl diphosphate (IPP) and

Table 1. Isoprenoids.

Basic structure	Name	Selected compounds
C_5	isoprenes	IPP, DMAPP
C_{10}	monoterpenes	essences of flowers, herbs and spices
C_{15}	sesquiterpenes	FPP, essential oils
C_{20}	diterpenes	GGPP, chlorophyll side chain, phylloquinones, tocopherol, giberellins, phytoalexins, taxol
C_{30}	tripertenes	phytosterols, brassinosteroids, toxins, waxes
C_{40}	tetraterpenes	carotenoids
>8 C_5 units	polyterpenes	ubiquinone, plastoquinone
'Partial' isoprenoids	meroterpenes	cytokinins, prenylated proteins

Data from Rodriguez-Concepcion and Boronat [7].

its isomer dimethylallyl diphosphate (DMAPP), also called isoprene units. The simplest isoprenoids contain a single C_5 unit, while 'head-to-tail' or 'head-to-head' addition of isoprene units forms the building blocks of the tens of thousands of more complex isoprenoids (table 1) [7]. Isoprenoids are synthesized in all living organisms, but animals, fungi and archaebacteria synthesize their isoprenoids exclusively through the operation of the mevalonate (MVA) pathway. Details of this biosynthetic pathway and the role of key enzymes in human health have recently been reviewed [8]. Briefly, the MVA pathway converts acetyl-CoA to MVA and thence to IPP (the basic isoprene unit) via a number of enzyme steps (fig. 1). The farnesyl isoprenoid FPP is a 15-carbon lipid that is derived from IPP, while GGPP contains an additional isoprenoid unit and is derived directly from farnesyl pyrophosphate [1, 5]. Several if not most of the enzymes involved in the conversion of acetyl-CoA to IPP were initially identified in peroxisomes, suggesting that these subcellular organelles may have a central role in isoprenoid biosynthesis [9]. However, Hogenboom and colleagues [10] have shown that a number of key MVA pathway enzymes are located within the cytosol, suggesting that functional peroxisomes may not be required for isoprenoid synthesis.

In addition to its role in production of FPP and GGPP, MVA is also a precursor of other isoprenoid groups that are incorporated into diverse classes of end-products, including cholesterol. Thus, MVA synthesis is tightly regulated with the goal of maintaining MVA to non-sterol isoprenoid synthesis while avoiding overproduction of cholesterol. Conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to MVA by HMG-CoA reductase is the rate-limiting step within this pathway, but the activity of HMG-

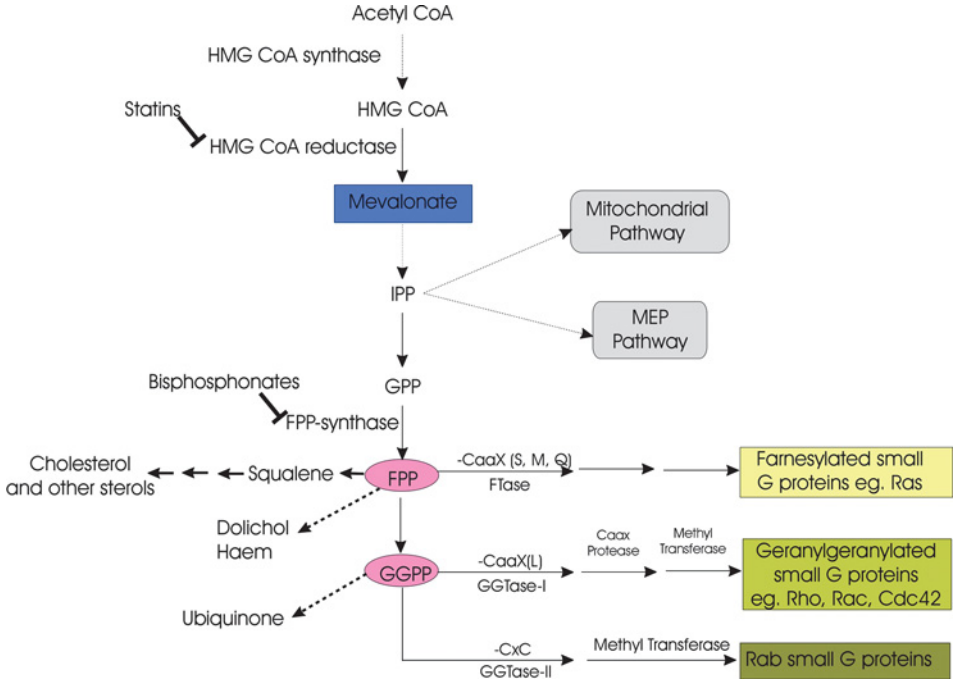

Figure 1. The MVA pathway. MVA is the precursor of cholesterol and the prenylation enzyme substrates, FPP and GGPP. In addition to prenylation, a number of small G protein groups undergo further post-prenylation processing. The production of cholesterol and non-sterol products is controlled by feedback regulation of HMG-CoA synthase and reductase, and low-density lipoprotein receptors. Adapted from Lobell [1].

CoA synthase and the low-density lipoprotein (LDL) receptor are also involved in feedback regulation [11]. In the absence of LDL, the activity of the HMG-CoA synthase and reductase enzymes is high to synthesize MVA for production of cholesterol and non-sterol isoprenoids. This is mediated by transcriptional regulation of a sterol regulatory element (SRE-1) in the LDL receptor gene, which enhances transcription in the absence of sterols but not when they are present [12]. In contrast, when LDL is present, HMG-CoA synthase and reductase activities decline by more than 90%, and the cells produce only small amounts of MVA needed for non-sterol end-products [11, 13]. In addition to regulating MVA synthesis, individual cells are able to regulate MVA disposition. The enzymes of the non-sterol pathways generally have higher affinities than those of the sterol pathway for MVA-derived substrates [13], so when MVA is limiting, it is preferentially shunted into the high-affinity non-sterol pathways [11]. Importantly, while depletion of MVA by the HMG-CoA reductase inhibitors generally results in accumulation of unprenylated proteins [14], unprenylated forms of Ras and Ras-related proteins may maintain partial function [15, 16] and interfere with the activity of other prenylated proteins [17, 18]. It also appears that in uninhibited cells, intermediates in the isoprenoid biosynthetic pathway may play a role in regulating the expression of Ras and Ras-related proteins [19]. Further studies are needed to confirm these observations and elucidate the pathways that result in the observed *in vitro* effects.

Finally, for many years, the biosynthesis of the isoprenoids was thought to occur solely via the MVA pathway. However, experimental data on the synthesis of specific isoprenoids in plants and micro-organisms could not be explained exclusively by the MVA pathway and led to the identification of a non-MVA pathway [20, 21]. In keeping with the convention set by the MVA pathway, this pathway is named after its first committed precursor, methylerythritol 4-phosphate (MEP). Whilst isoprenoids are synthesized in all living organisms, experimental evidence has shown that most organisms use only one of the two pathways for the biosynthesis of their precursors. The MEP pathway is the only one present in most eubacteria and the malaria parasite, *Plasmodium falciparum*, but it is absent from archaebacteria, fungi and animals, which synthesize their isoprenoids exclusively through the operation of the MVA pathway. By contrast, plants use both the MEP and MVA pathway for isoprenoid synthesis, although they are localized in different compartments [7, 22]. As the MEP pathway is used exclusively for isoprenoid synthesis by the majority of pathogenic bacteria, enzymes of this pathway have been proposed as highly attractive targets for the design of novel antiinfective drugs [23]. These drugs have a theoretical advantage of being relatively specific for microbes, given that the MEP pathway enzymes are not present in mammalian cells [22].

Protein prenylation

Studies in the early 1980s identified that membrane localization of the small G proteins was critical to their function, and that this localization was dependent on a post-translational modification that resulted in attachment of hydrophobic prenyl groups that anchor the small G proteins to intracellular membranes [24]. As discussed above, these prenyl groups are donated to the carboxy-terminal region of small G proteins by the two isoprenoids FPP and GGPP that are derived from MVA. The importance of the protein prenylation is underscored by the nature of the estimated 300 prenylated proteins in the human proteome [25], many of which participate in a multitude of signal transduction pathways related to cell growth, differentiation, cytoskeletal function and vesicle trafficking.

Farnesylation of proteins is catalysed by farnesyl transferase (FTase), while geranylgeranylation is catalysed by two geranyltranferases (GGTases I and II) [5, 25]. These enzymes catalyse the addition of farnesyl diphosphate and geranylgeranyl diphosphate, respectively, to the cysteine residue in the CaaX sequence where 'a' is aliphatic and 'X' (usually serine, methionine, glutamine, alanine or threonine) determines which prenyl group is added [26–28]. FTase and GGTase I are heterodimers that share an α subunit and have homologous but distinct β subunits [29]. GGTase II (also known as Rab GGTase), together with Rab escort protein, modifies proteins that end in CC or CaC and are found exclusively in the Rab family of small G proteins [1, 6, 29]. In addition to prenylation, proteins with carboxy-terminal CaaX sequences typically undergo two further post-translational modifications [30–32]. Following prenylation, the aaX is cleaved from the s-prenyl protein by the endoprotease Rce-1 [33], resulting in a C-terminal prenylated cysteine that is carboxymethylated. The final modification is methylation of the new carboxy terminus by an s-adenosylmethione-dependent protein carboxymethylase [31, 32]. The catabolism of prenylated proteins remains an area of active investigation. Zhang and colleagues [34] have purified a prenylcysteine lyase that converts prenyl cysteine substrates with a free amino terminus to cysteine and an unidentified isoprenoid product. Thus, proteolytic degradation appears to occur prior to breakdown of the prenyl cysteine.

The substrate specificity rules for FTase and GGTase I noted above are not absolutely stringent, with many examples of cross-prenylation [1, 29]. For example, when K-RasB which has a classical FTase CaaX box is inhibited by FTase inhibitors, it becomes a substrate for geranylgeranylation by GGTase I [35, 36]. The latter reaction is made possible by an upstream polybasic sequence that alters GGTase I substrate specificity [37]. Furthermore, RhoB contains a GGTase I CaaX box but is found in both

farnesylated and geranylgeranylated forms in cells, due to the ability of GGTase I to both geranylgeranylate and farnesylate this substrate [38]. A recent study suggests that at least in the case of RhoB, the prenylation type is able to specify the cellular localization, with the geranylgeranylated form localized to multivesicular late endosomes and farnesylated RhoB localized to the plasma membrane [39]. Therefore, effector functions of RhoB may not only depend on the level of activated protein in the cell, but also on the relative abundance of the two prenylated forms.

Despite the immense literature on protein prenylation, the functional significance of prenylation remains an area of active investigation. While previous studies have clearly confirmed prenylation as a key mechanism in post-translational attachment of proteins to membranes, the diverse cellular compartments in which farnesylated proteins occur suggests that other factors that determine the specificity of subcellular localization have yet to be identified. Several prenylated proteins, such as some Ras proteins, also undergo palmitoylation or myristolyation. Palmitoylation of Ras proteins has been shown to stabilize the association of prenylated proteins with the plasma membrane [6] and may play an important role in membrane localization and protein function. Wang and Sebti [40] have shown that a Rho B mutant that lacks a palmitoylated cysteine is unable to localize and function properly. Other studies have also suggested that palmitoylation is an important post-translational modification with functional consequences that are independent of prenylation [41, 42]. Furthermore, *in vivo* experiments in knockout mice that lack Rce-1, the enzyme that catalyses the step following prenylation, died of dilated cardiomyopathy [43]. Finally, protein S-acylation has recently been proposed to be involved in cooperation between prenylation and carboxymethylation, regulating cycling between intracellular membrane compartments to control signalling activity [44]. Thus, post-translational modifications that follow prenylation may be potential candidates as factors that confer membrane-binding or functional specificity to the prenylated proteins.

Functional regulation of prenylated proteins

The largest family of prenylated proteins are the intracellular GTP-binding proteins that transduce extracellular signals into intracellular changes via downstream effectors. These proteins include some of the heterotrimeric G protein subunits (the Giα subunit contains a CaaX motif but is not prenylated [45]) and the small G protein superfamily. The small G proteins are also commonly referred to as GTPases, despite the fact that both heterotrimeric and small G proteins have GDP/GTP-binding and GTPase activity. While both groups are technically GTPases, the small G proteins are monomeric proteins that are structurally and functionally distinct from the heterotrimeric G proteins. In contrast to the heterotrimeric G proteins that are activated by agonist-bound G protein-coupled receptors (GPCRs), GTP exchange on small G proteins is controlled by guanine nucleotide exchange factors (GEFs) that catalyse the exchange of GDP for GTP.

The small G proteins comprise a superfamily, with more than 100 proteins that have only been identified in eukaryotes, from yeast to humans [4]. Of the small G proteins, the Ras superfamily has been most closely studied and is structurally classified into five major subfamilies: Ras, Rho/Rac, Rab, Sar1/ARF and Ran [4, 46]. The functions of many of the small G proteins can be classified broadly as follows: Ras subfamily members mainly regulate gene expression; the Rho/Rac/Cdc42 proteins of the Rho family regulate both cytoskeletal reorganization and gene expression; the Rab and Sar1/Arf proteins regulate intracellular vesicle trafficking, and the Ran family members regulate nucleocytoplasmic transport during the G_1, S and G_2 phases of the cell cycle [4].

These small G proteins function as 'molecular traffic lights' in a multitude of signal transduction avenues by cycling between GDP-bound (inactive) and GTP-bound (active) conformations [47–49]. In resting cells, most Ras-related proteins are maintained in the GDP-bound inactive state, with activation regulated by three main classes of proteins that control guanine-nucleotide hydrolysis (fig. 2). In the resting state, activation is prevented by guanine nucleotide dissociation inhibitors (GDIs). Following dissociation of GDIs, the small G proteins are activated by guanine nucleotide exchange factors (GEFs) that catalyse the exchange of GDP for GTP that results in interaction with downstream effectors. Inactivation occurs via hydrolysis of GTP, a slow process that is catalysed by GTPase-activating proteins (GAPs). The key roles of each of these proteins in the regulation of prenylated protein cycling are discussed in detail in the following sections.

Guanine dissociation inhibitors

Although the GDIs are considered to be one of the pivotal regulators of small G protein function, only recently have the mechanisms controlling the GDI-G protein interaction begun to be elucidated [50, 51]. Only four GDIs have been identified to date; three human Rho GDIs that bind to the carboxyl terminus of the G protein in a 1:1 ratio and sequester G proteins to prevent activation, and a Rab GDI that has a different structure but performs the same function for proteins in the Rab family [50]. RhoGDI-1 has been shown to form complexes with RhoA, Rac1, Rac2 and Cdc42 and binding and inhibitory activity towards all

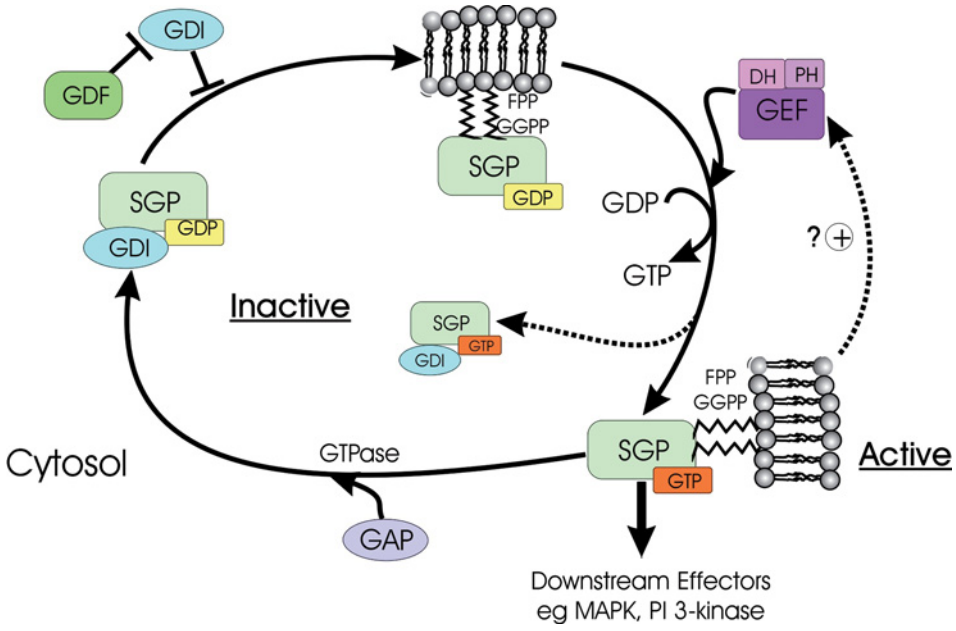

Figure 2. Schematic representation of the GTPase cycle. Under unstimulated conditions, the major cellular fraction of small G proteins are found in the cytosol bound to guanine nucleotide dissociation inhibitors (GDIs), which are negatively regulated by GDI displacement factors (GDFs) . Cellular signals stimulate release of GDI that is followed by dissociation of GDP and binding by GTP, a process catalysed by GEFs. The active form of GTPase is bound to the cell membrane via farnesylated and geranylgeranylated residues. Intrinsic GTPase activity stimulated by GTP-ase activativating proteins (GAPs) leads to inactivation. See text for further details.

these proteins seems to be similar, at least *in vitro* [50]. The Rho GDIs prevent activation of the G proteins via three distinct mechanisms [reviewed in ref. 51]. Briefly, GDIs are able maintain Rho GTPases as soluble cytosolic proteins by forming high-affinity complexes in which the isoprenoid moiety of the GTPase is shielded from the solvent by its insertion into the hydrophobic pocket formed by the immunoglobulin-like β sandwich of the GDI. Although the complex of a Rho protein with a GDI is generally considered to be biologically inert, this complex itself may have some effector activity. Secondly, GDIs inhibit the dissociation of GDP from Rho proteins, preventing GTPase activation by GEFs. Interaction of GDIs with the side chain of the essential Thr35 residue in switch I of the GTPase stabilizes Mg^{2+} coordination, thereby stabilizing nucleotide binding and preventing the GEF-catalysed nucleotide exchange reaction [52, 53]. Finally, the GDIs are able to interact with the GTP-bound form of the GTPase to prevent interactions with effector targets [50, 51]. Through these multiple actions, GDIs act as a major regulator of Rho GTPase activity and function, but are themselves also regulated by other proteins termed GDI displacement factors, or GDFs. The mammalian Ypt-interacting protein 3 (Yip3, also called prenylated Rab acceptor 1) has been shown to be a GDF for Rab9 [54] and a number of other proteins and lipids have been shown to induce release of the Rho proteins from Rho GDI [55–58]. DerMardirossian and Bokoch [51] have proposed that the activity of kinases that modulate the phosphorylation state of Rho GDIs might also act in regulating activation of the GDIs, and further studies on these pathways is currently in progress.

Guanine nucleotide exchange factors

After dissociation from the GDIs, the small G proteins associate with plasma membranes and GDP is exchanged for GTP, a process that is normally extremely slow and is therefore catalysed by GEFs. The first mammalian Rho GEF was identified as an oncoprotein from diffuse *B* cell lymphoma cells and was therefore designated Dbl [59]. Since then, many other GEFs have been identified in humans [60] that in most cases contain an ~200-residue Dbl homology (DH) domain. The DH domains are responsible for catalysing the exchange of GDP for GTP within Rho GTPases by interacting with switch regions of the Rho GTPases to facilitate nucleotide exchange activity [59, 60]. Non-conserved residues that occur within the interface between DH domains and GTPases form a 'specificity patch' [59] that provides the core variability in primary sequence, allowing the selective pairing of individual Rho proteins with different GEF partners [61]. The structural changes that result from DH binding to GTPases and lead to nucleotide dissociation appear to be conserved in the mechanisms of guanine nucleotide exchange used by both Ras and Rho proteins [62]. That is, the general mechanistic features of GEF-catalysed GDP

dissociation from Rho family proteins appear to be conserved for Ras family proteins and their GEFs.

In addition to the DH domain, the GEFs have an adjacent C-terminal pleckstrin homology (PH) domain. The PH domain sequence of ~100 amino acids is present in many signalling molecules and binds to the lipid products of phosphatidylinositol 3-kinase. PH domains are thought to localize Dbl proteins to plasma membranes, and cooperation between DH and PH domains appears to facilitate exchange activity more than the DH domains alone [63, 64]. Various other roles for PH domains, such as involvement in membrane orientation and allosteric regulation of GEF activity, have been proposed [60] but are not yet fully elucidated.

There does not appear to be an invariant need for DH and PH domains within small G proteins for them to act as nucleotide exchange factors. Members of the DOCK180 family of proteins act as GEFs for the Rho GTPases and have critical roles in cell migration, phagocytosis and cytoskeletal remodelling [59, 65]. All members of this family possess a 'Docker' [66] or dock homology region 2 (DHR-2) domain which appears to be involved in catalysing the GDP-GTP exchange reaction. The DHR-2 domain has been shown in some studies to be sufficient to promote GDP-GTP exchange in a manner similar to the tandem DH-PH domains found in Dbl proteins [65, 67]. However, other studies have shown that at least in some cases, an ELMO (for engulfment and motility) accessory protein is necessary to stimulate GDP-GTP exchange [59, 68].

Finally, positive cooperativity has recently been demonstrated whereby Ras, when activated by the Ras GEF Son of sevenless (Sos) and bound to GTP, enhances the GEF activity of Sos [69]. This reaction is mediated by an interaction between GTP-bound Ras and Sos that is structurally similar to the binding of Ras to the phosphoinositide 3-kinase effector [69]. This positive feedback has been postulated to greatly enhance localized GEF activity to concentrate the active pool of Ras at the inner membrane site of receptor clustering [59].

In summary, the GDP-GTP exchange reaction is thought to be the rate-limiting step in the GTP-binding/GTP hydrolytic cycle of GTPases and the GEFs have therefore been proposed as key regulators of the GTPases. However, Dbl proteins may also function as effectors of Rho GTPases [70] and some Rho GTPases may not be regulated by GEF activity. Clearly much has still to be understood regarding the complex relationships between GTPases and GEFs.

GTPase-activating proteins

GTP-bound forms of the GTPases are converted to the inactive GDP-bound forms by the action of intrinsic GTPases. Under normal circumstances, hydrolysis of GTP by intrinsic GTPases occurs slowly, but can be accelerated by a further group of regulatory proteins known as GAPs. At least 160 human genes are predicted to encode proteins that resemble GAPs [71, 72] (see also http://www.massgeneral.org/cancer/GAP_review.htm), suggesting that these proteins have important and widespread roles in the regulation of the small GTPases.

GAPs are classified according to their GTPase subfamily (Ras-GAP, Rap-GAP, etc) with sequence homology within subfamilies but not between families [73]. As with the GEFs, GAPs contain a phosphoinositide-binding PH domain but also commonly contain a Src homology domain (SH3) that binds proline-containing peptides [72].

The typical model of GAP catalysis has been well established from studies of the Ras/Ras-GAP (p120 GAP) system. Ras-GAP works by stabilizing the existing catalytic machinery of Ras via supplementing an external arginine residue, termed the arginine finger [74]. In contrast, Rap1-GAP uses an asparagine, termed the asparagine thumb, as the active site to stimulate GTP hydrolysis rather than an arginine finger [75]. Li and colleagues [76] have demonstrated that the mechanisms of Rheb GTP hydrolysis and stimulation by its GAP (TSC2) also use an asparagine thumb, similar to the Rap1-GAP/Rap catalytic mechanism.

Most of the predicted GAPs also contain potential or confirmed enzymatic domains indicating that the regulatory control of GTPase activity is highly complex. Studies have shown that protein-protein interactions, phosphorylation, lipid interactions, subcellular translocation and targeted degradation all have roles in the modulation of GAP activity [reviewed in ref. 72] and further mechanisms may exist but have yet to be discovered. This extreme diversity suggests that these proteins might not just act as GAPs but also have effector roles downstream of activated GTPases.

In addition, another distinct group of proteins that functionally acts as GAPs are the regulators of G protein signalling (RGS) proteins. This large family has a conserved RGS domain of 120–130 amino acids that is flanked by N and C termini of varying lengths, and is capable of interacting with heterotrimeric G protein α subunits to accelerate the GTP hydrolysis rate of the Gα subunit, thereby promoting termination of the G protein signal [77]. Recent studies have suggested that RGS proteins may facilitate scaffolding of G protein with G protein coupled receptors and membrane channels to form a macromolecular signalling complex that promotes entry into the G protein cycle by physically aiding the dissociation of Gα-GTP and G$\beta\gamma$ subunits [77]. In addition to their negative regulatory effects, the additional C- and N-terminal modular protein-binding domains and motifs within the RGS proteins are able to facilitate multiple novel regulatory interactions in other signalling pathways [78].

Table 2. Selected isoprenylation defects and human disease

	Gene/ inheritance	Clinical disorder/comments
Isoprenoid biosynthesis		
MVA	AR	mevalonic aciduria (MA) and hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS) two conditions form a spectrum [reviewed in ref. 8]
REP1	X-linked	Rab escort protein – assists GGTII mutations associated with choroidaenia (retinal degeneration)
GTPases		
Mutations in Ras family of small G proteins identified in 10–15% of all human cancers.		
GDI		
GDI1	*Gdil*	non-specific X-linked mental retardation
GEF		
Bcr	*Bcr*	acute and chronic leukaemia
LARG GEF	*LARG/MLL*	acute myeloid leukaemia
Tiam1	*Tiam1*	association with renal cell carcinoma
ARHGEF6 (Cool-2/αPix)	*Arhgef6*	non-specific X-linked mental retardation
GAP		
Neurofibromin (domain with homology to RasGAP)	*nf1* AD	neurofibromatosis
p120 Ras GAP	*RASA1*	capillary malformation-arteriovenous malformation syndrome (CM-AVM)
TSC2 GAP	*tsc1/tsc2* AD	tuberous sclerosis complex
Phosphatidylinositol (4, 5)-bisphosphate 5-phosphatase	*ocrl1* AR	oculocerebrorenal syndrome of Lowe protein contains a functional RhoGAP domain
Oligonephrin-1	*ophn1*	non-specific X-linked mental retardation

* AD, autosomal dominant; AR, autosomal recessive [data derived from refs. 60, 72, 127, 128].

Isoprenylation and human disease

Cancer

Given the ubiquitous nature of the prenylated proteins and their diverse roles in cellular biology, to date surprisingly few human diseases have been identified that result from defects in isoprenoid biosynthesis or regulation (table 2). The notable exception to this is human malignancy, where detailed research on the oncogenic potential of prenylated proteins has been stimulated by the fact that mutations within the Ras family could be identified in as many as 10–15% of all human cancers [see refs. 79, 80 for reviews]. Recent research by Mijimolle and colleagues [81] utilizing a farnesyl transferase knockout mouse has provided new insights into the role of farnesylated proteins in neoplasia by showing that farnesylated proteins apparently are not required for the initial malignant cellular transformation but are critical for tumour progression and maintenance.

Of the GTPase regulatory proteins, a number of GEFs have also been associated with human malignancy. The rearrangement of *BCR* that is caused by the reciprocal chromosome translocation and formation of the Philadelphia chromosome (Ph) is most well known for its involvement in human leukaemias. Different lengths of the 5′ region of *BCR* become fused with the *ABL* tyrosine kinase, and while the molecular pathways by which Bcr-Abl proteins induce transformation have yet to be clarified, all *BCR-ABL* chimaeras possess constitutively activated Abl kinase activity, which appears to be essential for oncogenesis [reviewed in ref. 82]. A gene rearrangement of another GEF gene has also been identified in a patient with acute myelogenous leukaemia, with fusion of the leukaemia-associated Rho GEF (LARG) and the mixed lineage leukaemia (MLL) gene postulated to result in alteration of the LARG GEF function [83]. Finally, tumours and cell lines of renal cell carcinoma have been shown to have a missense mutation in the GEF Tiam-1 (T lymphoma invasion and metastasis 1), which results in an increase in Tiam1 transforming activity by an unknown mechanism [84].

The central role of prenylated proteins in malignancy has directed drug discovery programs over the last decade towards development of farnesyl transferase inhibitors

(FTIs) as novel anticancer drugs [85, 86]. Many CaaX peptidomimetics, as well as FTIs and geranylgeranyl transferase inhibitors (GGTIs) have been developed that are highly potent and with low toxicity, and two such compounds are currently in phase III trials [87, 88]. FTIs inhibit anchorage-independent growth of a wide variety of cancer cells, cause changes in cell cycle progression, induce apoptosis of cancer cells and inhibit their attachment to the extracellular substratum [reviewed in ref. 89]. Their antiproliferative and proapoptotic activity appears to be related to inhibition of multiple cellular pathways, including PI3K/Akt, mammalian target of Rapamycin (mTOR) and S6 kinase [summarized in ref. 25]. In addition, Lackner and colleagues [90] have shown that FTIs not only inhibit FTase, but can also inhibit GGTase II (Rab GGTase) resulting in blocking of post-translational modification of Rab proteins, leading to cell death. Thus, interfering with endosomal trafficking pathways may be a novel action of the FTIs.

However, results of clinical trials with these drugs have not been as positive as was anticipated, and while good results have been achieved in haematological malignancies and breast cancer, other cancers have not responded as well. The phenomenon of 'cross-prenylation' discussed above has frequently been cited as a possibility for this apparent discrepancy: alternative protein prenylation has been postulated to rescue protein function in cells treated with a specific prenylation inhibitor [86, 91]. In addition to the Ras proteins themselves, a number of other prenylated proteins have been shown to be involved in the initiation, invasion and progression of cancer [summarized in ref. 92], and some of these proteins that are not farnesylated may also contribute to the apparent *in vivo* resistance to FTIs. The shortcomings of current FTIs have resulted in a search for other important enzymes within the prenylation pathway as potential anticancer therapeutics. As detailed above, post-prenylation processing involving either palmitoylation or methylation also appears to be important for cellular function, and there is currently considerable interest in targeting these enzymes to control cancer cell proliferation [93].

Prenylated proteins also appear to play a role in both benign and malignant bone disease. The bisphosphonates are drugs that inhibit farnesyl diphosphate synthase resulting in effects on osteoclasts and tumour cells. Inhibition of protein prenylation and Ras signalling within osteoclasts leads to defects in intracellular vesicle transport that result in imperfect formation of the tight-sealing zones or ruffled borders required for bone reabsorption [94]. Aside from their antiproliferative effects via inhibition of Ras, bisphosphonates also appear to have important proapoptotic effects that may be mediated by accumulation of isopentanyl diphosphonate. Isopentanyl diphosphonate can be metabolized to Apppi (triphosphoric acid 1-adenosin-5′-ylester 3-[3-methylbut-3-enyl] ester), an intracellular ATP analogue that can directly induce apoptosis [95].

Phakomatoses

Tuberous sclerosis complex (TSC) and neurofibromatosis type 1 (NF1) are part of a group of conditions known as phakomatoses that have similar clinical features and have recently been linked in a common biochemical pathway that involves mammalian target of rapamycin (mTOR) signalling [96]. TSC1 (also called hamartin) and TSC2 (also called tuberin) are encoded by the tuberous sclerosis complex 1 (*TSC1*) and tuberous sclerosis complex 2 (*TSC2*) genes, respectively. Mutations in either *TSC1* or *TSC2* are associated with the genetic disorder TSC that results in the development of hamartomas in a variety of tissues and is characterised clinically by epilepsy, mental retardation, cardiac myxomas and renal angiomyolipomas. The TSC1 and TSC2 proteins form a heterodimer that is an upstream negative regulator of mTOR, a protein kinase that is a central controller of cell growth. TSC2 contains a region of homology to the catalytic domain of the Rap1 GAP and displays GAP activity specifically towards the small G protein Rheb, inhibiting its ability to stimulate the mTOR signalling pathway [97]. TSC2 has recently been demonstrated to use a novel catalytic mechanism, an 'asparagine thumb', instead of the arginine finger found in Ras GAP [97]. While inactivation of TSC1 or TSC2 causes a similar phenotype, TSC1 is not required for Rheb GAP activity per se, as TSC2 alone appears sufficient to promote GTP hydrolysis of Rheb [97, 98]. Rather, TSC1 appears to play an important role in regulating the physiological function of TSC2 by modulating the protein stability and localization.

Neurofibromatosis results from loss-of-function mutations in the *NF1* gene that lead to deregulation of Ras [99]. A recent study has shown that the mTOR pathway is tightly regulated by neurofibromin (encoded by the *NF1* gene), and mTOR is constitutively activated in NF1-deficient primary cells [96]. This aberrant activation depends on Ras and PI3 kinase, and is mediated by the phosphorylation and inactivation of the TSC-2-encoded protein tuberin by the serine threonine kinase, Akt [96]. Thus the NF1 tumour suppressor appears to be an important regulator of TSC2 and mTOR signalling.

Mental retardation

Four of the 11 genes that have been identified in cases of non-specific X-linked mental retardation (MRX) encode regulators or effectors of the Rho GTPases, suggesting an important role for Rho signalling in cognitive function. Mutations of *OPHN1* are associated with loss of, or dramatic reduction in oligonephrin-1, a Rho GAP

[100]. Oligonephrin-1 is present in neuronal and astroglial cells and colocalizes with actin at the tip of the growing neurites [101]. Govek et al. [102] have recently shown that reduced oligonephrin-1 levels affect dendritic spine morphogenesis, and as dendritic spines are believed to be important for synaptic function, such defects may compromise neuronal function. A similar mechanism may also account for the abnormalities seen in patients with a mutation of *ARHGEF6* (also known as *αPIX* or *Cool-2*), another MRX gene that encodes a protein with homology to the Rho GEFs [103, 104]. Mutations in the *GDI1* gene (leading to reduced binding and recycling of RAB3A) and p21-activated kinase 3 (*PAK3*, a serine/threonine kinase that acts downstream of Rac and Cdc42) have also been identified in patients with MRX [105, 106].

Vascular Disease

Although initially developed as lipid-lowering agents, the clinical availability of the HMG-CoA reductase inhibitors ('statins') as drugs that inhibit the rate-limiting enzyme in cholesterol production via the MVA pathway has also focused attention on the role of prenylated proteins in vascular biology. The statins improve or restore endothelial function, enhance the stability of atherosclerotic plaques, decrease oxidative stress and inflammation, and inhibit the thrombogenic response in the vascular wall [reviewed in refs. 107, 108]. Inhibition of Rho and its downstream targets by statins has been shown to increase bioavailability of endothelium-derived nitric oxide (NO), resulting in improved NO-dependent vasorelaxation [109, 110]. Statins also have multiple additional effects on fibrinolysis that include blockade of thrombin-induced endothelial tissue factor [111], increased expression of tissue-type plasminogen activator (tPA) [112] and inhibition of expression of the potent vasoconstrictor endothelin-1 [113].

However, the role of prenylated proteins in contributing to these effects is debated, as improvement in endothelial function, decreased inflammation and increased plaque stability could all be due to lipid lowering alone. In addition, *in vitro* experiments typically use a much higher concentration of statins than is used clinically and both hydrophilic and lipophilic statins appear to exert similar cholesterol-independent effects, despite the relative impermeability of hydrophilic statins in vascular tissues. Despite these limitations, recent clinical trials appear to confirm *in vitro* observations of a beneficial antiinflammatory effect of statins. Two recent clinical trials of statin treatment in patients with atherosclerosis and coronary artery disease showed that reduction in inflammation (as measured by C-reactive protein level) improved clinical outcome independently of the reduction in serum cholesterol levels [114, 115].

Cholesterol-independent effects of the statins have also been noted in studies on Alzheimer disease (AD). Epidemiological evidence [116] suggested that hyperlipidaemia was important in the pathogenesis of AD and was supported by experimental data in cell culture [117–119] and transgenic mice [120] that showed that cholesterol influences the generation and aggregation of amyloid β peptide (Aβ), the pathological hallmark of AD. However, in one prospective study, the development of AD was independent of lipid and lipoprotein levels [121]. Recently, Cole et al. [122] have shown *in vitro* that the effects of low cholesterol are independent of the effects of low isoprenoids, but both appear to contribute to decreasing Aβ accumulation. Preliminary results from one double-blind randomized controlled trial on the effects of statin treatment in AD suggest benefits in both depressive symptoms and cognitive function [123]. While these results appear promising, data on the clinical benefit of statins in patients with AD are conflicting. Statins have been associated with memory loss, depression, sleep disorders and global amnesia [124] and a recent randomized trial documented minor decrements in cognitive functioning [125]. The mechanism by which statins may affect cognitive function is unknown. However, further study to define the particular isoprenylated target proteins that are involved may allow the development of more specific therapeutic agents for the treatment of AD, that lack the apparent cognitive side-effects of current statins.

Summary

In summary, the biochemical labyrinth of prenylated small G protein signalling pathways is only just beginning to be appreciated. The human genome is estimated to contain ~100 genes with the known CaaX box sequence for protein farnesylation and while not all proteins containing a CaaX motif are farnesylated, we can anticipate that many other isoprenylated proteins have yet to be identified. A recently developed proteomics strategy has identified a number of previously unknown farnesylated proteins, including some with potentially novel farnesylation motifs [126]. Extending this approach to other post-translational modifications such as geranylgeranylation is likely to yield further novel insights into protein farnesylation and further clarify the roles of prenylated proteins in a multitude of cellular signalling pathways. Aside from the complex regulatory framework of GDP-GTP cycling, post-prenylation processing is also emerging as a key regulator of the function of many prenylated proteins. Detailed understanding of isoprenylated protein function and regulation will likely remain a challenge to investigators for many years to come.

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