Published in final edited form as:

Biochim Biophys Acta. 2010 August ; 1801(8): 896–905. doi:10.1016/j.bbalip.2010.03.014.

Isoprenoids, Small GTPases and Alzheimer's Disease

Gero P. Hooff¹, W. Gibson Wood², Walter E. Müller¹, and Gunter P. Eckert^{1,*}

¹Department of Pharmacology, Campus Riedberg, Biocenter N260, Goethe University, Max-von-Laue-St. 9, 60438 Frankfurt, Germany

²Department of Pharmacology, School of Medicine, University of Minnesota, Geriatric Research, Education and Clinical Center, VAMC, Minneapolis, MN 55417, USA

Abstract

The mevalonate-pathway is a crucial metabolic pathway for most eukaryotic cells. Cholesterol is a highly recognized product of this pathway but growing interest is being given to the synthesis and functions of isoprenoids. Isoprenoids are a complex class of biologically active lipids including for example, dolichol, ubiquinone, farnesylpyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Early work had shown that the long-chain isoprenoid dolichol is decreased, but that dolichylphosphate and ubiquinone are elevated in brains of Alzheimer's diseased (AD) patients. Until recently, levels of their biological active precursors FPP and GGPP were unknown. These short-chain isoprenoids are critical in the post translational modification of certain proteins which function as molecular switches in numerous, signaling pathways. The major protein families belong to the superfamily of small GTPases, consisting of roughly 150 members. Recent experimental evidence indicated that members of the small GTPases are involved in AD pathogenesis and stimulated interest in the role of FPP and GGPP in protein prenylation and cell function. A straightforward prediction derived from those studies was that FPP and GGPP levels would be elevated in AD brains as compared with normal neurological controls. For the first time, recent evidence shows significantly elevated levels of FPP and GGPP in human AD brain tissue. Cholesterol levels did not differ between AD and control samples. One obvious conclusion is that homeostasis of FPP and GGPP but not of cholesterol is specifically targeted in AD. Since prenylation of small GTPases by FPP or GGPP is indispensable for their proper function we are proposing that these two isoprenoids are up-regulated in AD resulting in an over abundance of certain prenylated proteins which contributes to neuronal dysfunction.

2. Introduction

The mevalonate- (MVA-) pathway is a crucial metabolic pathway for most eukaryotic cells, whose most recognized product is cholesterol as seen in Figure 1 [1]. This pathway also provides the cell with indispensable lipids such as isoprenoids. The long-chain isoprenoids dolichol and ubiquinone participate in membrane organization and mitochondrial oxygen consumption, respectively. The short-chain isoprenoids farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) covalently attach to small GTPases, which act as

© 2010 Elsevier B.V. All rights reserved.

*Corresponding author: Gunter P. Eckert Ph.D., Goethe University, Dept. of Pharmacology, Campus Riedberg, Biocenter N260, Max-von-Laue Str. 9, 60438 Frankfurt, Germany. Phone: +49 69 79829374; Fax: +49 69 798 293 74; g.p.eckert@em.uni-frankfurt.de; website: www.eckert-science.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

molecular switches in various biochemical pathways [2]. This attachment is necessary because it enables these proteins to be inserted into membranes resulting in activation of pathways involved in inflammation, oxidative stress, and cell proliferation [3–7]. The Rho family of GTPases are one of the major regulators in synaptic plasticity, both in dendrite morphogenesis and stability as well as in growth cone motility [8–11]. Another vitally important role of FPP is that it is the key branch point for cholesterol and GGPP (Fig. 1)

There is keen interest in the role of FPP and GGPP in post-translational modification of proteins and cell function in Alzheimer's Disease (AD) [3,12,13]. FPP and GGPP like cholesterol are derived from mevalonate (Fig. 1) but unlike cholesterol, there is little if any information on their regulation and abundance in the brain. The absence of such data is in stark contrast to the aforementioned interest in the role of those isoprenoids in protein prenylation and cell function. The present article builds on a comprehensive review by Cole et al. [3] and summarizes the actual knowledge on isoprenoids, recent findings concerning their regulation and targeted proteins with special focus on AD.

3. The mevalonate/isoprenoid/cholesterol pathway

Initial steps of the MVA-isoprenoid pathway involve the synthesis of 3-hydroxy-3-methylglutaryl- coenzyme A (HMG-CoA) from acetyl-CoA via acetoacetyl-CoA (figure 1). HMG-CoA reductase, the rate limiting step of the entire pathway then forms mevalonate [1]. Biosynthesis mainly occurs in the endoplasmic reticulum (ER) but also in peroxisomes [14]. After mevalonate production mevalonate kinase (MK) catalyzes the phosphorylation of mevalonic acid to phosphomevalonate. Phosphomevalonate kinase catalyzes the reaction of mevalonate 5-phosphate and adenosine triphosphate to mevalonate 5-diphosphate where the latter is subjected to decarboxylation and forms isopentenyl pyrophosphate (IPP), which equilibrates with its isomer dimethylallyl pyrophosphate (DMAPP) catalyzed by the isopentenyl pyrophosphate isomerase. IPP or DMAPP then undergo subsequent condensation reactions to produce a 10-carbon lipid GPP, the 15-carbon FPP and the 20-carbon GGPP. The synthesis of GPP and FPP is catalyzed by farnesylpyrophosphate synthase (FPPS) while GGPP is synthesized by geranylgeranylpyrophosphate synthase (GGPPS) [15]. FPP and GGPP are substrates for farnesyltransferase (FTase) and geranylgeranyltransferases (GGTase) involved in post-translational prenylation of small GTPases. Prenylation of these proteins is required for proper localization and function in cell membranes [16]. FPP is a key branch point of the pathway leading to GGPP synthesis and to squalene that is catalyzed by farnesyl diphosphate farnesyltransferase (FDFT), also known as squalenesynthase. From squalene two subsequent enzymatic reactions result in the production of lanosterol, which represents the structure of all steroids. Subsequent reactions lead to production of desmosterol and 7-dehydrocholesterol and finally to cholesterol (Fig. 1).

Mechanisms of cholesterol regulation are well understood and that topic has been covered in depth in many excellent reviews [17–19]. In the present review, we will only briefly discuss cholesterol regulation while mainly focusing on isoprenoid biosynthesis and cellular utilization. Knowledge on isoprenoid regulation lags behind that of cholesterol particularly regulation in brain.

When cellular cholesterol levels are low, a series of actions are initiated resulting in activation of the membrane-bound transcription factors, sterol regulatory element-binding proteins (SREBPs) [20]. The SREBP family consists of three proteins with SREBP-2 the principal protein activating cholesterol synthesis. SREBP cleavage-activating protein (SCAP) senses cholesterol levels in the cell and transports SREBPs to the Golgi complex where two proteases cleave the SREBPs and these products move to the nucleus. Once in the nucleus these transcription factors bind to nonpalindromic sterol response elements in the promoter regions

of several different target genes [21]. Genes regulated by SREBP-2 are for example HMG-CoA reductase, mevalonate kinase, IPP isomerase, and FPP synthase. GGPP synthase does not appear to be upregulated by SREBP-2.

4. Isoprenoids

4.1. Short-chain isoprenoids - Farnesyl- and geranylgeranylpyrophosphate

FPP is a 15-carbon lipid and the key branch point in the synthesis of ubiquinone, dolichol, GGPP and cholesterol (Fig. 1). FPP is, derived from the 5-carbon lipid isopentenyl diphosphate (IPP), dimethylallyl pyrophosphate and geranyl pyrophosphate (GPP) by FPPS a 80 kDa homodimer. GGPP contains an additional 5-carbon unit (isoprene) and is derived from FPP by GGPPS, a 195 kDa homo-oligomer [22]. Activity of FPPS (i.e., production of FPP) was reported to be several fold higher than activity of GGPPS (i.e., production of GGPP) in bovine brain cytosol [23]. Even though FPP is a substrate for GGPPS, there are data suggesting that synthesis of GGPP may be dependent on its distribution in the cytosol versus the membrane and it was reported that abundance of certain small GTPases might stimulate GGPP production [24]. In contrast to FPP, GGPP inhibited its own synthesis in rabbit reticulocytes [25]. Another study found similar results and it was also observed that GGPP could inhibit GGPPS activity [26].

It was concluded from structural studies that GGPP does bind in an inhibitory manner to GGPPS and may be a means of regulating GGPP levels [22]. Another potentially novel regulatory role of GGPP is stimulation of the ubiquitination and degradation of HMG-CoA reductase [27]. FPP did not have a similar effect and it was suggested that GGPP might be prenylating a protein that is responsible for regulation of HMG-CoA reductase [27].

There is evidence that SREBP-2 may activate FPP synthase gene expression. The promoter region of FPP synthase contains a binding site for SREBP-2, which is also present in other genes associated with cholesterol synthesis. What is not well-understood is if changes in FPP levels alone where cholesterol levels are unaffected alter SREBP function. The idea that SREBP-2 activation may not be responsive to FPP levels is that depletion of mevalonate in Caco-2 cells stimulated SREBP-2 activation but adding FPP did not reduce activation [28], which would be expected if FPP was acting in a similar manner as cholesterol. There is limited information on the transcriptional regulation of FPP synthase. FPP synthase gene expression was directly stimulated by the transcription factor LXR (liver X receptor) [29]. Activation of FPP synthase gene expression was stimulated by the LXR agonist, hypocholamide, in immortalized brain cell lines [29]. On the other hand, FPP synthase gene expression was significantly reduced in brain tissue of LXR-null mice [30].

4.2. Long-chain isoprenoids – Dolichol and ubiquinone

In mammals FPP is the precursor of dolichol and ubiquinone. Ubiquinone (coenzyme Q) as part of the electron transfer between complex I, II and III of the respiratory chain [31,32] is mainly localized in the inner mitochondrial membrane. Its synthesis proceeds upon conversion of GGPP, leading to chain elongation and predominately takes place in the ER–Golgi system [33]. The number of incorporated isoprene units and therefore the chain length varies among different organisms and is commonly indicated by the following number e.g. coenzyme Q₁₀ [15,34]. Ubiquinone is essential for normal cell function and misdirected biosynthesis results in pathological respiratory chain deficiencies [35–37].

Dolichol is widely distributed in all tissues and membranes and besides carrying a terminal free hydroxyl group it exists in a phosphorylated, dephosphorylated or in an esterified form [15,38,39]. Although knowledge on dolichol function is limited, it has been proposed as a biomarker for ageing [39,40]. Numerous *in vitro* and *in vivo* experiments reported on

interactions of dolichol with cellular membranes. The unesterified dolichol was shown to modify the organization and packing of phospholipids in model membranes [41]. The free and the phosphorylated forms were found to be mediators of protein glycosylation or as sugar carriers [15,34]. The lipophilic molecule dolichol intercalates into the bilayer of cell membranes and interacts with the phospholipids [39]. A study by Wood et al. [42] showed that dolichol increased membrane fluidity of synaptic plasma membranes (SPM) isolated from brains of young and old mice. The same study revealed higher endogenous dolichol levels in isolated SPMs of old mice and it was concluded that dolichol might act as a regulator of membrane fluidity [42]. Several studies have reported higher dolichol levels with increasing age in peripheral tissue and in brain [43–45]. Besides dolichol potentially regulating membrane fluidity, another hypothesis is that membrane localization of free dolichol may act as an antioxidant and reduce cell toxicity [45]. It remains to be elucidated whether elevated dolichol levels with ageing are a consequence of a loss of enzymatic regulation in the MVA-pathway, as proposed by Pallottini et al. [46].

5. Protein prenylation - Small GTPases

Over 100 proteins in the human proteome are potential substrates to undergo prenylation [3, 12,47]. Many of those proteins belong to the super family of small GTPases, with molecular masses ranging from 20 – 40 kDa [48]. Small GTPases are integral components of complex signaling networks and control diverse cellular activities including intracellular vesicle transport, cell adhesion, endocytosis, cytoskeletal organization, receptor signaling, vesicle trafficking, cell cycle progression and gene expression [48–50].

5.1. Prenylation – a post translational modification

Prenylated proteins include Ras and related small GTP-binding proteins such as Rho, Rab and Rac, the subunits of trimeric G proteins, nuclear lamins and other proteins [12]. Prenylation of proteins by FPP and GGPP is critical for enabling those proteins to be inserted into membranes, thus determining their localization and function [12]. Prenylation is a lipid based post translational modification involving the covalent attachment of either FPP catalyzed by FTase or GGPP catalyzed by GGTase I or II to the cysteine residue in defined consensus motifs [6, 51–54]. Following prenylation, the prenylation motif containing proteins undergo two further processing steps. The diphosphate is cleaved off by the Ras converting enzyme (Rce1p) and the Ste24p endogenous proteases remove the terminal three amino acids (-aaX) [55,56] - [57]. Upon cleavage of the terminal tripeptide, the remaining prenylated cysteine residue undergoes carboxymethylation by a methyl group, delivered from S-adenosyl methionine (SAM) (Fig. 2). This conversion is catalyzed by isoprenylcysteine carboxyl methyltransferase (Icmt). Icmt is located in the Golgi apparatus, ER and nuclear membranes [58]. Under physiological conditions, the carboxymethylation is reversible [59]. It is assumed that the intermediates during these subsequent enzymatic reactions exist only transiently and are rapidly converted into the mature prenylated protein [54]. Overall, prenylation enhances lipophilicity and favors lipid-lipid interactions of small GTPases with cellular membranes [60].

Some proteins e.g. N-Ras, H-Ras or RhoB following prenylation are palmitoylated. This modification entails a thioester linkage of the palmitoleic acid chain to the sulphur group of a cysteine residue, which is in close proximity to the prenylated cysteine [6]. Similar to the previously described processes of protein prenylation and carboxymethylation, protein palmitoylation allows membrane binding and intracellular localization by increasing lipophilicity of certain proteins [61,62].

5.2. Small GTPases

Small GTPases are guanine nucleotide-binding molecules (G-proteins). Small GTPases play a fundamental role in a multitude of intracellular signal transduction pathways involving vesicle trafficking, cell growth, differentiation and cytoskeletal function. The small GTPases bind guanosine triphosphate (GTP) when activated and guanosine diphosphate (GDP) when inactivated. In response to an upstream signal as for example proposed for amyloid beta (A β) [63], GDP dissociates from GTPase and then binds a GTP molecule. Two types of regulatory proteins mainly execute the GDP/GTP exchange of all small GTPases. The guanine-nucleotide-exchange factors (GEFs) promote the GTP bound and therefore active form of the G-protein [64], while the GTPase activating proteins (GAPs) lead to the GDP bound inactive form by stimulating the weak intrinsic GTP hydrolysis capacity of the GTPases [65]. RhoA, Rac1, Cdc42 and Rab proteins possess the same GEFs and GAPs regulatory mechanisms while additionally regulated by a third class of proteins, the guanine nucleotide dissociation inhibitors (GDIs) [48,66]. When complex formation of GDI and a GTPase, GEF stimulated dissociation of GDP from its GTPase is inhibited and these GTPases cycle between the membrane and cytosol [48]. Binding of GTP leads to a conformational change in the downstream effector associated region and to activation of secondary effector pathways, such as the MEKK-JNK/p38 – pathway [63].

A classification of the small GTPase superfamily into at least five different families is generally accepted and briefly described below. The differentiation was based on sequence and functional similarities between the members of each branch [3,48,67].

The Ras family—The family name of this group of small GTPases originates from Ras sarcoma (Ras) and these GTPases have been the most extensively studied proteins due to their well-known role in oncogenesis [68]. About 30% of all human cancers have been associated with mutations in the Ras proto-oncogen [69]. H-Ras, K-Ras and N-Ras are the most renowned members of this family and they are constantly activated as a consequence of the mutation in the proto-oncogen [70]. Furthermore, the majority of the Ras subfamily members are known to be farnesylated and interestingly K- and N-Ras but not H-Ras can be geranylgeranylated when physiological farnesylation is inhibited [reviewed by [70,71]. Despite the fact that Ras proteins are associated with carcinogenesis, it is worth mentioning that other Ras family members, such as RhoB seem to act as tumor suppressors [69].

The Rho family—Extracellular stimuli activate Ras homologous (Rho) proteins, which are involved in proliferation, apoptosis, actin formation, adhesion, and motility [67,69]. RhoA, Rac1 and Cdc42 belong to the Rho GTPases [72], which effect on more than 30 secondary effector proteins, such as ROK α/β or MEKK1,4 [reviewed by [73]].

The Rab family—The 60 Ras-like proteins in the brain (Rab) represent the largest group within the superfamily of small GTPases [74]. These proteins and their effectors, such as Rabex-5 or Coronin 3 are mainly involved in intracellular vesicular transport, including vesicle formation, organelle motility and directing vesicles between different compartments [reviewed by [75–78].

The Ran family—The Ras-like nuclear proteins (Ran) function in nucleo-cytoplasmic transport, mitotic spindle and nuclear envelope assembly and DNA replication [79,80]. The transport mechanism relies on a unique gradient of Ran-GTP between the nucleus and the cytosol [67].

The Sar1/Arf family—The ADP-ribosylation factor (Arf) and secretion-associated and Ras-related (Sar) proteins are mainly involved in vesicle formation and intracellular trafficking [48,81].

For example, Arf1 regulates the formation of vesicle coats at different steps in the exocytic and endocytic pathways. Beside others, Arf1 controls the formation of coat protein I (COPI)-coated vesicles involved in retrograde transport between the Golgi and ER, of clathrin/adaptor protein 1 (AP1)-complex-associated vesicles at the trans-Golgi network (TGN) and on immature secretory vesicles, and of AP3-containing endosomes [67]. In addition to prenylation, some members of the Arf family are post-translationally modified with a myristate fatty acid at their N-terminus [67].

6. Isoprenoids in ageing and neurodegeneration

Normal cell function requires prenylated proteins and there is evidence that GGPP is required for long-term potentiation in mouse hippocampal slices [82]. However, it is becoming increasingly recognized that prenylated proteins are involved in certain diseases both within and outside of the central nervous system (CNS). Diseases largely outside the CNS in which FPP and GGPP potentially play a significant role are certain cancers, osteoporosis, Paget disease, and atherosclerosis [6,83–85]. Moreover, recent evidence suggested that inhibition of FPP-induced protein prenylation using a farnesyltransferase inhibitor significantly improved motor function and survival in a mouse model of Hutchinson-Gilford progeria syndrome or what has been referred to by some as premature ageing [86,87]. Diseases of the CNS in which isoprenoids and protein prenylation are drawing increasing attention are Parkinson disease, multiple sclerosis, and ischemic stroke [88–90]. Recent experimental evidence indicates that isoprenylated small GTPases are involved in Alzheimer Disease pathogenesis [3,91–94].

6.1. Isoprenoids and ageing

Ageing is a process which leads to specific structural, biochemical and functional changes in multiple organs including the brain [95–97] and represents the major risk factor for the development of the most common neurodegenerative diseases like AD, Parkinson Disease, cerebrovascular disease and amyotrophic lateral sclerosis [98]. Age-related processes in the brain have been extensively studied [98,99] and changes in various lipid have been examined with several studies on cholesterol homeostasis [100,101]. Isoprenoids on the other hand, have received only scant attention in ageing research. Age related changes in FPP and GGPP levels have not been reported which is in stark contrast to their important biological roles. Dolichol levels increase with ageing [46] and dolichols were identified as a potential biomarker of senescence [39]. Levels of dolichol were markedly higher in human brain tissue of aged individuals (e.g. approx. 19 vs. 26 $\mu\text{g/g}$ w.t. hippocampus tissue in young and aged humans, respectively) [43,102]. SPM of 18 and 28 month old C57BL/6 mice had a 5 fold and 11 fold increase, respectively, in dolichol levels when compared with 6 month old mice [42]. In the same study, it was found that dolichol had a greater fluidizing effect on SPM of young mice than aged mice. Increased dolichol content has been observed in brain tissue of individuals with AD and neuronal lipofuscinosis [103–105]. Neither has it been determined whether the elevated dolichol levels observed with increasing age and certain neurodegenerative diseases are injurious to cell function nor the mechanisms of such changes [100].

Ubiquinone levels were found to decrease with age in human brain tissue (e.g. approx. 14 vs. 8 $\mu\text{g/g}$ w.t. hippocampus tissue in young and aged humans, respectively) [106,107]. In rat brains however, the ubiquinone synthesis rate was shown to steadily increase with ageing and ubiquinone levels (approx. 20 $\mu\text{g/g}$ w.t.) were relatively constant throughout the entire live span [108].

6.2. Isoprenoids and neurodegeneration

The MVA-pathway and its several products are involved in a wide range of critical cell functions. Biosynthesis of different products involves a complex and not fully understood regulation of transcription, translation, activation and degradation. Abnormal functioning or misdirected regulation of genes and protein products associated with the MVA-pathway will lead to severe consequences for the cell. A defective cholesterol homeostasis is causal for the Smith-Lemli-Opitz syndrome. Other disorders that have been at least partly linked to cholesterol are Huntington's Disease, Alzheimer's disease and the lysosomal storage disease Niemann-Pick type C [109–113].

6.2.1. Isoprenoids in Alzheimer Disease—There is some evidence that dolichol and ubiquinone levels are altered in AD [106]. Dolichol levels were decreased in all brain regions of AD patients (e.g. approx. 199 vs. 111 $\mu\text{g/g}$ w.t. frontal cortex tissue from controls and Alzheimer's disease, respectively) [114]. In contrast, dolichyl phosphate levels increased in those regions that exhibited morphological changes (e.g. approx. 21 vs. 32 $\mu\text{g/g}$ w.t. frontal cortex tissue from controls and Alzheimer's disease, respectively). In the same study, a significant elevation in ubiquinone content was observed (e.g. approx. 13 vs. 17 $\mu\text{g/g}$ w.t. frontal cortex tissue from controls and Alzheimer's disease, respectively). It was concluded that the increase in the sugar carrier dolichyl phosphate might reflect an increased rate of glycosylation in the diseased brain whereas the increase in ubiquinone may be an attempt to protect the brain from oxidative stress [114]. A recent study reported increased oxidized ubiquinone levels in the cerebrospinal fluid of AD patients [115]. Experimental studies in animal AD models suggest that ubiquinone may protect against neuronal damage [116]. However, a synthetic analogue of ubiquinone (Idebenone) clinically failed to slow cognitive decline in AD [117]. However, the exact roles of dolichol and ubiquinone in AD pathogenesis have not been established yet.

There is evidence that prenylated small GTPases are involved in AD pathogenesis [94,118]. A straightforward prediction was that FPP and GGPP levels would be elevated in AD brains as compared with normal neurological controls [3,119] however, due to methodological problems such data had not been reported. Using a newly developed and validated HPLC fluorescence (HPLC-FLD) method [120], we recently determined FPP and GGPP levels and gene expression of their respective synthases in the frontal cortex of AD patients as compared with control samples [93]. We showed for the first time FPP and GGPP levels in brain tissue of AD patients and normal neurologic controls. GGPP levels were significantly higher in brain tissue of AD patients (56%) as compared with control samples. FPP levels were also significantly higher (36%) in the AD brain tissue. In both AD patients and controls, GGPP levels were markedly higher than FPP levels and that finding was consistent with two recent reports in mouse brain and normal human brain (Table 1) [120,121].

Gene expression of FPP synthase and GGPP synthase were determined in AD and control brain samples by qRT-PCR [93]. The increase in FPP levels in the frontal cortex of AD brain was associated with a significant up-regulation of FPP synthase gene expression. GGPP synthase mRNA levels were increased but differences did not reach significance [93].

FPP is a precursor of both GGPP and cholesterol (Figure 1). We found significantly increased levels of FPP and GGPP in brain tissue of AD patients (Table 1) but cholesterol levels and HMGCR gene expression were similar in AD and control samples [93]. An obvious conclusion of these results is that homeostasis of FPP and GGPP but not cholesterol is specifically targeted in brain tissue of AD patients [93].

Tong et al. described the first method for the simultaneous determination of FPP and GGPP in cultured cells [122]. Up to now, FPP and GGPP were determined in mouse embryonic

fibroblasts and human cancer cells such as immortalized colorectal adenocarcinoma, myelogenous leukaemia and multiple myeloma cells (Table 2) [28,121–123]. Recently, levels of FPP and GGPP in cultured neuronal cells were shown [124]. Cellular levels of FPP and GGPP are generally in the picomolar range as depicted in Table 2, however significant differences in isoprenoid abundance can be observed between different cell lines (Table 2). Moreover, the relative distribution between FPP and GGPP varies between different cell lines. Such variances were also observed in mammalian tissue. Compared to FPP, GGPP levels are lower in mouse kidney and liver tissue, equal in mouse heart tissue and higher in mouse and human brain tissue (Table 1) [93,120,121]. The differences in FPP and GGPP distribution may be due to the demand for intermediates of the MVA-pathway in specialized cells. There is a dearth of data on FPP and GGPP regulation and no more so than in brain [125]

It is well established that APP processing is influenced by cholesterol abundance [126–129] and that the reduction of cellular cholesterol levels using inhibitors of the MVA-pathway or extraction by methyl- β -cyclodextrin (M β CD) results in lower A β levels in vivo and in vitro [reviewed in [130–133]]. However, evidence exists that FPP and GGPP are also involved in the cellular production of A β . It has been previously reported that γ -secretase is stimulated by geranylgeraniol [92] and incubation of H4 neuroglioma cells with FPP or GGPP were found to increase A β levels [91]. In those studies, APP processing was modified after incubation of cells with an extraordinarily high concentration of geranylgeraniol, FPP or GGPP (10 μ M). It is noteworthy, that endogenous FPP and GGPP levels are in the picomolar range [124] and interpretation must be guarded when high isoprenoid concentrations are employed. The data presented in this work demonstrated that modulation of the mevalonate/isoprenoid/cholesterol pathway results in changes in endogenous FPP, GGPP and cholesterol levels in human neuroblastoma cells. A β levels were specifically reduced when cholesterol levels were lowered by inhibitors of cholesterol synthesis, while selective inhibition of either farnesylation or geranylgeranylation did not affect A β production [124].

6.2.2. Prenylated proteins in Alzheimer's Disease—Prenylation of specific small GTPases has been associated with the processing of APP. Activation of effectors downstream of prenylated small GTPases, such as the protein kinases ROCK (Rho associated coiled-coil forming protein kinase) have been implicated in APP metabolism [134,135]. GGPP stimulates γ -secretase to increase cleavage of APP and A β secretion [136] and inhibition of the Rho/Rock pathway reduced A β production [137,138].

Several small GTPases have been associated with AD in other pathways than those discussed in the preceding paragraph. There is evidence that Rab6 may be involved in APP processing and intracellular trafficking [139], Rab11 may contribute to vesicular transport [140] and Rac1 as a participant in A β induced generation of reactive oxygen species (ROS), which could damage neurons [141]. Moreover, Rac1 was shown to down-regulate transcriptional activity of the APP gene [142] and additionally, Rac1 and Rho-A appear to be involved in the production of actin-based morphological plasticity in dendrites and spines in hippocampal neurons [143]. Rab3 as a presynaptic protein was significantly reduced in AD brains [144]. Protein level of Ras and Rac1 in both the cytosolic and membranous fractions and that of Rap2 in the cytosolic fraction was significantly decreased, while that of Rab8 in the membranous fraction was significantly increased in AD brains compared with controls.[145].

6.2.2.1. Prenylated Proteins, Synaptic Plasticity and Alzheimer's Disease: Long-term storage of information is, at least in part, achieved by structural changes in neuronal connectivity, particularly changes in the structure and number of synapses. As a consequence, attention has focused on the rearrangement of specialized structures, termed dendritic spines, found at many synapses in the mammalian CNS. Increased synaptic activity has been shown to lead to long-term-potential (LTP) of synaptic efficiency via the formation of new spines

[146]. Pronounced loss of neurites and synapses represents one of the most relevant histopathological lesions in AD brain with respect to neuronal function [147–149]. Failures of synaptic plasticity are thought to represent early events in AD [150,151]. Synaptic plasticity is dependent on the structural regulation of the actin cytoskeleton in dendritic spines [8,9, 152]. The Rho family of GTPases such as RhoA, Rac1, and Cdc42 monomeric G-proteins, are the major regulators in synaptic plasticity, both in dendrite morphogenesis and stability as well as in growth cone motility and collapse [7–11], affecting neuronal architecture and synaptic connectivity [143].

Small Rho proteins act as molecular switches between inactive GDP-bound and active GTP-bound forms under the regulation of several Rho GEFs (guanine-nucleotide-exchange factors) and Rho GAPs (GTPase activating proteins) [7,153]. Representative of all Rho-proteins, the role of Rac1 (Ras related C3 botulinum toxin substrate) in synaptic function is highlighted: Rac1 is expressed in the adult mouse hippocampus [154], a brain area that exhibits robust synaptic plasticity and is crucial for the acquisition of associative memories [155]. In neurons, Rac1 is associated with neuronal development, participating in the morphological changes required for migration of newborn neurons to extension of axons and dendrites into proper target regions, and formation of synapses with appropriate partners. Its participation in neuronal morphogenesis is a contributor to synaptic plasticity and synapse formation [156]. *In vitro* studies in hippocampal slices indicated that activation of NMDA receptors results in membrane translocation and activation of Rac1 [154,157]. Furthermore, *in vivo* studies in adult mice revealed that Rac1 activation of NMDA receptors is linked with associative fear learning. Thus, Rac1 is recognized as an important molecule required for synaptic plasticity and involved in the morphological changes observed at neuronal synapses during hippocampal learning and memory [154,157]. Recently, a small G protein Rac-dependent forgetting mechanism, which contributes to both passive memory decay and interference-induced forgetting was identified in *Drosophila* and it was suggested that Rac's role in actin cytoskeleton remodeling may contribute to memory erasure [158]. Recent studies described the Rac-specific GEF Tiam1 as a critical mediator of NMDA receptor-dependent spine developments. Tiam1 regulates spine and dendrite development by activating Rac1-dependent signaling pathways which promote actin cytoskeletal re-organization and protein synthesis [159]. This process is triggered by the brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor B (TrkB), which binds and phosphorylates Tiam 1, leading to activation of Rac-1 and induction of changes in cellular morphology [160]. TrkB activity is increased in ageing and correlates with changes in cholesterol [161]. TrkB activates GGTase I, which mediates geranylgeranylation of Rac 1 [162] and the GGTase I substrate GGPP acts on NMDA receptors and restores LTP dysfunction [163]. Hence, GGPP seems to be directly involved in Rac1 related actin cytoskeletal remodeling, which mediate dendritic spine formation, motility, and morphology, which are thought to be important for functional synaptic plasticity. FPP and GGPP levels in grey and white matter are elevated in male AD brain tissue (Table 1) [93] and the findings suggest that abundance of prenylated small GTPases may be increased in AD [3,118]. Phospho-Rac/Cdc42 protein levels were increased in hippocampal membrane fractions isolated from AD brain and phospho-Rac/Cdc42 labeled only weakly and diffusely in hippocampal slices from normal controls, but appear as abnormal granular structures in AD brain [164]. The antibody used in that study detects endogenous levels of Rac1/cdc42 only when phosphorylated at serine 71. Phosphorylation at that site may inhibit GTP binding of Rac1, attenuating the signal transduction pathway downstream of Rac1 [165]. These findings are indicative for RhoA/Rac/Cdc42 dysfunction in AD brain. The brain derived neurotrophic factor (BDNF) and its receptor TrkB also play important roles in synaptic plasticity and findings that BDNF and TrkB levels are severely decreased in the hippocampus and some cortical areas of AD patients [166–168] further support synaptic dysfunction in AD brain. Further, *in vitro* and *in vivo* findings support the idea that synaptic plasticity goes awry when levels of small GTPases are abnormally enhanced. Alzheimer related A β peptide increased the levels of the active GTP-bound form of

RhoA in SH-SY5Y cells and increased levels of RhoA were found in neurons surrounding amyloid plaques in the cerebral cortex of APP(Swe) Tg2576 mice [169]. It was suggested in that study that A β -induced neurite outgrowth inhibition could be initiated through increased Rho GTPase activity that, in turn, phosphorylates the collapsin response mediator protein-2 and interferes with tubulin assembly in neurites [169].

6.2.2.2. Prenylated proteins, oxidative stress and Alzheimer's Disease: AD elevated abundance of prenylated Rac1 could possibly also contribute to Rac1-NADPH oxidase-regulated generation of ROS causing oxidative stress, which has been reported in AD brain [170–172]. In the brain, microglia cells are prominent sources of ROS through expression of the phagocyte oxidase, which generates superoxide ions. However, a recent study showed that microglia also express NADPH oxidases (NOX) and Rac1 [173]. Immunohistochemical evaluations of NOX expression in human post-mortem tissue indicate that while microglia express high levels of the regulatory subunit gp91phox, moderate levels of gp91phox are also expressed in neurons [174]. Activation of NOX via Rac1 is a source of ROS generation in the brain [175], which plays a role in cerebral dysfunctions, such as stroke, blood-brain barrier damage or seizure induced hippocampal damage [176–178]. A recent study found that NOX-associated redox pathways might participate in the early pathogenesis of AD. NOX expression and activity are up-regulated specifically in the temporal gyri of mild-cognitive-impaired (MCI) patients as compared to controls, but not in preclinical or late stage AD samples, and not in the cerebellum [174]. Moreover, *in vitro* studies identified Rac1 as an essential component of the beta-amyloid signaling cascade leading to generation of ROS [141].

7. Conclusion

Early studies had shown that levels of the long-chain isoprenoid dolichol were decreased in post-mortem AD brain tissue, while ubiquinone levels were elevated (Fig. 3). We have recently reported for the first time that FPP and GGPP levels are significantly higher in brain tissue of AD patients, while cholesterol levels are unchanged. Gene expression of FPP synthase and GGPP synthase were stimulated in AD brain tissue while expression of HMG-CoA reductase was similar to control levels. These data indicate specific targeting of isoprenoid regulation within the mevalonate/isoprenoid/cholesterol pathway. FPP and GGPP play a fundamental role in prenylation of small GTPases, which function as molecular switches in various signaling pathways. In AD pathology, small GTPases have been connected with oxidative stress, the generation of A β and synaptic dysfunction. There is a great need for expanding the very limited knowledge base on regulation of FPP and GGPP in brain due to the potential significance of small GTPases in AD.

Acknowledgments

This work was supported by the Alzheimer Forschung Initiative e.V. (Grant AFI #08823), the National Institutes of Health, National Institute on Ageing (Grants AG23524, AG18357) and the Department of Veterans Affairs.

References

1. Brown MS, Goldstein JL. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *Journal of Lipid Research* 1980;21:505–517. [PubMed: 6995544]
2. Hall A. Rho GTPases and the control of cell behaviour. *Biochem Soc Trans* 2005;33:891–895. [PubMed: 16246005]
3. Cole SL, Vassar R. Isoprenoids and Alzheimer's disease: a complex relationship. *Neurobiol.Dis* 2006;22:209–222. [PubMed: 16406223]
4. Pfeffer S, Aivazian D. Targeting Rab GTPases to distinct membrane compartments. *Nat.Rev.Mol.Cell Biol* 2004;5:886–896. [PubMed: 15520808]

5. Konstantinopoulos PA, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov* 2007;6:541–555. [PubMed: 17585331]
6. Gelb MH, Brunsfeld L, Hrycyna CA, Michaelis S, Tamanoi F, Van Voorhis WC, Waldmann H. Therapeutic intervention based on protein prenylation and associated modifications. *Nat Chem Biol* 2006;2:518–528. [PubMed: 16983387]
7. Linseman DA, Loucks FA. Diverse roles of Rho family GTPases in neuronal development, survival, and death. *Front Biosci* 2008;13:657–676. [PubMed: 17981578]
8. Sekino Y, Kojima N, Shirao T. Role of actin cytoskeleton in dendritic spine morphogenesis. *Neurochem Int* 2007;51:92–104. [PubMed: 17590478]
9. Schubert V, Dotti CG. Transmitting on actin: synaptic control of dendritic architecture. *J Cell Sci* 2007;120:205–212. [PubMed: 17215449]
10. Ramakers GJ. Rho proteins, mental retardation and the cellular basis of cognition. *Trends Neurosci* 2002;25:191–199. [PubMed: 11998687]
11. Govek EE, Newey SE, Van Aelst L. The role of the Rho GTPases in neuronal development. *Genes Dev* 2005;19:1–49. [PubMed: 15630019]
12. McTaggart SJ. Isoprenylated proteins. *Cell Mol. Life Sci* 2006;63:255–267. [PubMed: 16378247]
13. Cordle A, Koenigsknecht-Talboo J, Wilkinson B, Limpert A, Landreth G. Mechanisms of statin-mediated inhibition of small G-protein function. *Journal of Biological Chemistry* 2005;280:34202–34209. [PubMed: 16085653]
14. Kovacs WJ, Faust PL, Keller GA, Krisans SK. Purification of brain peroxisomes and localization of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Eur. J. Biochem* 2001;268:4850–4859. [PubMed: 11559353]
15. Holstein SA, Hohl RJ. Isoprenoids: remarkable diversity of form and function. *Lipids* 2004;39:293–309. [PubMed: 15357017]
16. Buhaescu I, Izzedine H. Mevalonate pathway: a review of clinical and therapeutical implications. *Clin. Biochem* 2007;40:575–584. [PubMed: 17467679]
17. DeBose-Boyd RA. Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG CoA reductase. *Cell Res* 2008;18:609–621. [PubMed: 18504457]
18. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990;343:425–430. [PubMed: 1967820]
19. Espenshade PJ, Hughes AL. Regulation of sterol synthesis in eukaryotes. *Annu Rev Genet* 2007;41:401–427. [PubMed: 17666007]
20. Sato R. SREBPs: protein interaction and SREBPs. *FEBS J* 2009;276:622–627. [PubMed: 19143831]
21. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res* 2001;40:439–452. [PubMed: 11591434]
22. Kavanagh KL, Dunford JE, Bunkoczi G, Russell RG, Oppermann U. The crystal structure of human geranylgeranyl pyrophosphate synthase reveals a novel hexameric arrangement and inhibitory product binding. *J Biol Chem* 2006;281:22004–22012. [PubMed: 16698791]
23. Runquist M, Parmryd I, Thelin A, Chojnacki T, Dallner G. Distribution of branch point prenyltransferases in regions of bovine brain. *J. Neurochem* 1995;65:2299–2306. [PubMed: 7595519]
24. Ericsson J, Runquist M, Thelin A, Andersson M, Chojnacki T, Dallner G. Distribution of prenyltransferases in rat tissues. Evidence for a cytosolic all-trans-geranylgeranyl diphosphate synthase. *J. Biol Chem* 1993;268:832–838. [PubMed: 8419360]
25. Lutz RJ, McLain TM, Sinensky M. Feedback inhibition of polyisoprenyl pyrophosphate synthesis from mevalonate in vitro. Implications for protein prenylation. *J Biol Chem* 1992;267:7983–7986. [PubMed: 1569056]
26. Sagami H, Morita Y, Ogura K. Purification and properties of geranylgeranyl-diphosphate synthase from bovine brain. *J. Biol. Chem* 1994;269:20561–20566. [PubMed: 8051156]
27. Sever N, Song BL, Yabe D, Goldstein JL, Brown MS, DeBose-Boyd RA. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. *J Biol Chem* 2003;278:52479–52490. [PubMed: 14563840]

28. Murthy S, Tong H, Hohl RJ. Regulation of fatty acid synthesis by farnesyl pyrophosphate. *J Biol Chem* 2005;280:41793–41804. [PubMed: 16221687]
29. Fukuchi J, Song C, Ko AL, Liao S. Transcriptional regulation of farnesyl pyrophosphate synthase by liver X receptors. *Steroids* 2003;68:685–691. [PubMed: 12957674]
30. Wang L, Schuster GU, Hultenby K, Zhang Q, Andersson S, Gustafsson JA. Liver X receptors in the central nervous system: from lipid homeostasis to neuronal degeneration. *Proc.Natl.Acad.Sci.U.S.A* 2002;99:13878–13883. [PubMed: 12368482]
31. Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim.Biophys.Acta* 1995;1271:195–204. [PubMed: 7599208]
32. Lenaz G, Genova ML. Mobility and function of coenzyme Q (ubiquinone) in the mitochondrial respiratory chain. *Biochim.Biophys.Acta* 2009;1787:563–573. [PubMed: 19268424]
33. Kalen A, Appelkvist EL, Chojnacki T, Dallner G. Nonaprenyl-4-hydroxybenzoate transferase, an enzyme involved in ubiquinone biosynthesis, in the endoplasmic reticulum-Golgi system of rat liver. *J.Biol.Chem* 1990;265:1158–1164. [PubMed: 2295606]
34. Houten SM, Frenkel J, Waterham HR. Isoprenoid biosynthesis in hereditary periodic fever syndromes and inflammation. *Cell Mol.Life Sci* 2003;60:1118–1134. [PubMed: 12861380]
35. Valnot I, Kleist-Retzow JC, Barrientos A, Gorbatyuk M, Taanman JW, Mehaye B, Rustin P, Tzagoloff A, Munnich A, Rotig A. A mutation in the human heme A:farnesyltransferase gene (COX10) causes cytochrome c oxidase deficiency. *Hum.Mol.Genet* 2000;9:1245–1249. [PubMed: 10767350]
36. Rotig A, Appelkvist EL, Geromel V, Chretien D, Kadhon N, Edery P, Lebeideau M, Dallner G, Munnich A, Ernster L, Rustin P. Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency. *Lancet* 2000;356:391–395. [PubMed: 10972372]
37. Distelmaier F, Koopman WJ, van den Heuvel LP, Rodenburg RJ, Mayatepek E, Willems PH, Smeitink JA. Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease. *Brain* 2009;132:833–842. [PubMed: 19336460]
38. Chojnacki T, Dallner G. The biological role of dolichol. *Biochem.J* 1988;251:1–9. [PubMed: 3291859]
39. Parentini I, Cavallini G, Donati A, Gori Z, Bergamini E. Accumulation of dolichol in older tissues satisfies the proposed criteria to be qualified a biomarker of aging. *J.Gerontol.A Biol.Sci.Med.Sci* 2005;60:39–43. [PubMed: 15741281]
40. Marino M, Girelli AM, Leoni S, Trentalance A. Variations of hepatic dolichols during rat development. *Biochim.Biophys.Acta* 1990;1047:192–194. [PubMed: 2174268]
41. Vigo C, Grossman SH, Drost-Hansen W. Interaction of dolichol and dolichyl phosphate with phospholipid bilayers. *Biochim.Biophys.Acta* 1984;774:221–226. [PubMed: 6547619]
42. Wood WG, Sun GY, Schroeder F. Membrane properties of dolichol in different age groups of mice. *Chem Phys Lipids* 1989;51:219–226. [PubMed: 2611963]
43. Andersson M, Appelkvist EL, Kristensson K, Dallner G. Age-dependent changes in the levels of dolichol and dolichyl phosphates in human brain. *Acta Chem.Scand.B* 1987;41:144–146. [PubMed: 3604517]
44. Carroll KK, Guthrie N, Ravi K. Dolichol: function, metabolism, and accumulation in human tissues. *Biochem.Cell Biol* 1992;70:382–384. [PubMed: 1449704]
45. Bergamini E, Bizzarri R, Cavallini G, Cerbai B, Chiellini E, Donati A, Gori Z, Manfrini A, Parentini I, Signori F, Tamburini I. Ageing and oxidative stress: a role for dolichol in the antioxidant machinery of cell membranes? *J Alzheimers Dis* 2004;6:129–135. [PubMed: 15096696]
46. Pallottini V, Marino M, Cavallini G, Bergamini E, Trentalance A. Age-related changes of isoprenoid biosynthesis in rat liver and brain. *Biogerontology* 2003;4:371–378. [PubMed: 14739708]
47. Sebt SM. Protein farnesylation: implications for normal physiology, malignant transformation, and cancer therapy. *Cancer Cell* 2005;7:297–300. [PubMed: 15837619]
48. Takai Y, Sasaki T, Matozaki T. Small GTP-binding proteins. *Physiol Rev* 2001;81:153–208. [PubMed: 11152757]
49. Boettner B, Van Aelst L. The role of Rho GTPases in disease development. *Gene* 2002;286:155–174. [PubMed: 11943472]

50. Luo L. Rho GTPases in neuronal morphogenesis. *Nat Rev Neurosci* 2000;1:173–180. [PubMed: 11257905]
51. Casey PJ, Seabra MC. Protein prenyltransferases. *J.Biol.Chem* 1996;271:5289–5292. [PubMed: 8621375]
52. Leung KF, Baron R, Ali BR, Magee AI, Seabra MC. Rab GTPases containing a CAAX motif are processed post-geranylgeranylation by proteolysis and methylation. *J Biol Chem* 2007;282:1487–1497. [PubMed: 17114793]
53. Leung KF, Baron R, Seabra MC. Thematic review series: lipid posttranslational modifications. geranylgeranylation of Rab GTPases. *J Lipid Res* 2006;47:467–475. [PubMed: 16401880]
54. Appels NM, Rosing H, Stephens TC, Hughes A, Schellens JH, Beijnen JH. Absolute quantification of farnesylated Ras levels in complex samples using liquid chromatography fractionation combined with tryptic digestion and electrospray tandem mass spectrometry. *Anal Biochem* 2006;352:33–40. [PubMed: 16564488]
55. Tam A, Nouvet FJ, Fujimura-Kamada K, Slunt H, Sisodia SS, Michaelis S. Dual roles for Ste24p in yeast a-factor maturation: NH₂-terminal proteolysis and COOH-terminal CAAX processing. *J.Cell Biol* 1998;142:635–649. [PubMed: 9700155]
56. Porter SB, Hildebrandt ER, Breevoort SR, Mokry DZ, Dore TM, Schmidt WK. Inhibition of the CaaX proteases Rce1p and Ste24p by peptidyl (acyloxy)methyl ketones. *Biochim.Biophys.Acta* 2007;1773:853–862. [PubMed: 17467817]
57. Manandhar SP, Hildebrandt ER, Schmidt WK. Small-molecule inhibitors of the Rce1p CaaX protease. *J.Biomol.Screen* 2007;12:983–993. [PubMed: 17942791]
58. Dai Q, Choy E, Chiu V, Romano J, Slivka SR, Steitz SA, Michaelis S, Philips MR. Mammalian prenylcysteine carboxyl methyltransferase is in the endoplasmic reticulum. *J.Biol.Chem* 1998;273:15030–15034. [PubMed: 9614111]
59. Chelsky D, Ruskin B, Koshland DE Jr. Methyl-esterified proteins in a mammalian cell line. *Biochemistry* 1985;24:6651–6658. [PubMed: 4084550]
60. Wright LP, Philips MR. Thematic review series: lipid posttranslational modifications. CAAX modification and membrane targeting of Ras. *J Lipid Res* 2006;47:883–891. [PubMed: 16543601]
61. Smotrys JE, Linder ME. Palmitoylation of intracellular signaling proteins: regulation and function. *Annu.Rev.Biochem* 2004;73:559–587. [PubMed: 15189153]
62. Dietrich LE, Ungermann C. On the mechanism of protein palmitoylation. *EMBO Rep* 2004;5:1053–1057. [PubMed: 15520806]
63. Otth C, Mendoza-Naranjo A, Mujica L, Zambrano A, Concha II, Maccioni RB. Modulation of the JNK and p38 pathways by cdk5 protein kinase in a transgenic mouse model of Alzheimer's disease. *Neuroreport* 2003;14:2403–2409. [PubMed: 14663200]
64. Schmidt A, Hall A. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev* 2002;16:1587–1609. [PubMed: 12101119]
65. Bernardis A, Settleman J. GAP control: regulating the regulators of small GTPases. *Trends Cell Biol* 2004;14:377–385. [PubMed: 15246431]
66. Olofsson B. Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. *Cell Signal* 1999;11:545–554. [PubMed: 10433515]
67. Wennerberg K, Rossman KL, Der CJ. The Ras superfamily at a glance. *J Cell Sci* 2005;118:843–846. [PubMed: 15731001]
68. Repasky GA, Chenette EJ, Der CJ. Renewing the conspiracy theory debate: does Raf function alone to mediate Ras oncogenesis? *Trends Cell Biol* 2004;14:639–647. [PubMed: 15519853]
69. Appels NM, Bolijn MJ, Chan K, Stephens TC, Hochtin-Boes G, Middleton M, Beijnen JH, de Bono JS, Harris AL, Schellens JH. Phase I pharmacokinetic and pharmacodynamic study of the prenyl transferase inhibitor AZD3409 in patients with advanced cancer. *Br J Cancer* 2008;98:1951–1958. [PubMed: 18506143]
70. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat.Rev.Cancer* 2003;3:11–22. [PubMed: 12509763]
71. Brunner TB, Hahn SM, Gupta AK, Muschel RJ, McKenna WG, Bernhard EJ. Farnesyltransferase inhibitors: an overview of the results of preclinical and clinical investigations. *Cancer Res* 2003;63:5656–5668. [PubMed: 14522880]

72. Ridley AJ. Rho family proteins: coordinating cell responses. *Trends Cell Biol* 2001;11:471–477. [PubMed: 11719051]
73. Bishop AL, Hall A. Rho GTPases and their effector proteins. *Biochem.J* 2000;348(Pt 2):241–255. [PubMed: 10816416]
74. Pereira-Leal JB, Hume AN, Seabra MC. Prenylation of Rab GTPases: molecular mechanisms and involvement in genetic disease. *FEBS Lett* 2001;498:197–200. [PubMed: 11412856]
75. Zerial M, McBride H. Rab proteins as membrane organizers. *Nat.Rev.Mol.Cell Biol* 2001;2:107–117. [PubMed: 11252952]
76. Bergbrede T, Chuky N, Schoebel S, Blankenfeldt W, Geyer M, Fuchs E, Goody RS, Barr F, Alexandrov K. Biophysical analysis of the interaction of Rab6a GTPase with its effector domains. *J Biol Chem* 2009;284:2628–2635. [PubMed: 19019823]
77. Zhu H, Liang Z, Li G. Rabex-5 is a Rab22 effector and mediates a Rab22-Rab5 signaling cascade in endocytosis. *Mol Biol Cell* 2009;20:4720–4729. [PubMed: 19759177]
78. Kimura T, Kaneko Y, Yamada S, Ishihara H, Senda T, Iwamatsu A, Niki I. The GDP-dependent Rab27a effector coronin 3 controls endocytosis of secretory membrane in insulin-secreting cell lines. *J Cell Sci* 2008;121:3092–3098. [PubMed: 18768935]
79. Li HY, Cao K, Zheng Y. Ran in the spindle checkpoint: a new function for a versatile GTPase. *Trends Cell Biol* 2003;13:553–557. [PubMed: 14573347]
80. Weis K. Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. *Cell* 2003;112:441–451. [PubMed: 12600309]
81. Memon AR. The role of ADP-ribosylation factor and SAR1 in vesicular trafficking in plants. *Biochim.Biophys.Acta* 2004;1664:9–30. [PubMed: 15238254]
82. Kotti T, Head DD, McKenna CE, Russell DW. Biphasic requirement for geranylgeraniol in hippocampal long-term potentiation. *Proc Natl Acad Sci U S A* 2008;105:11394–11399. [PubMed: 18685105]
83. Alegret M, Silvestre JS. Pleiotropic effects of statins and related pharmacological experimental approaches. *Methods Find Exp Clin Pharmacol* 2006;28:627–656. [PubMed: 17200729]
84. Russell RG, Watts NB, Ebetino FH, Rogers MJ. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. *Osteoporos Int* 2008;19:733–759. [PubMed: 18214569]
85. Swanson KM, Hohl RJ. Anti-cancer therapy: targeting the mevalonate pathway. *Curr Cancer Drug Targets* 2006;6:15–37. [PubMed: 16475974]
86. Capell BC, Olive M, Erdos MR, Cao K, Faddah DA, Tavaréz UL, Conneely KN, Qu X, San H, Ganesh SK, Chen X, Avallone H, Kolodgie FD, Virmani R, Nabel EG, Collins FS. A farnesyltransferase inhibitor prevents both the onset and late progression of cardiovascular disease in a progeria mouse model. *Proc Natl Acad Sci U S A* 2008;105:15902–15907. [PubMed: 18838683]
87. Capell BC, Erdos MR, Madigan JP, Fiordalisi JJ, Varga R, Conneely KN, Gordon LB, Der CJ, Cox AD, Collins FS. Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A* 2005;102:12879–12884. [PubMed: 16129833]
88. Rajanikant GK, Zemke D, Kassab M, Majid A. The therapeutic potential of statins in neurological disorders. *Curr.Med.Chem* 2007;14:103–112. [PubMed: 17266571]
89. Paintlia AS, Paintlia MK, Khan M, Vollmer T, Singh AK, Singh I. HMG-CoA reductase inhibitor augments survival and differentiation of oligodendrocyte progenitors in animal model of multiple sclerosis. *FASEB J* 2005;19:1407–1421. [PubMed: 16126908]
90. Dunn SE, Youssef S, Goldstein MJ, Prod'homme T, Weber MS, Zamvil SS, Steinman L. Isoprenoids determine Th1/Th2 fate in pathogenic T cells, providing a mechanism of modulation of autoimmunity by atorvastatin. *J Exp Med* 2006;203:401–412. [PubMed: 16476765]
91. Kukar T, Murphy MP, Eriksen JL, Sagi SA, Weggen S, Smith TE, Ladd T, Khan MA, Kache R, Beard J, Dodson M, Merit S, Ozols VV, Anastasiadis PZ, Das P, Fauq A, Koo EH, Golde TE. Diverse compounds mimic Alzheimer disease-causing mutations by augmenting Abeta42 production. *Nat.Med* 2005;11:545–550. [PubMed: 15834426]

92. Urano Y, Hayashi I, Isoo N, Reid PC, Shibasaki Y, Noguchi N, Tomita T, Iwatsubo T, Hamakubo T, Kodama T. Association of active gamma-secretase complex with lipid rafts. *J Lipid Res* 2005;46:904–912. [PubMed: 15716592]
93. Eckert GP, Hooff GP, Strandjord DM, Igbavboa U, Volmer DA, Muller WE, Wood WG. Regulation of the brain isoprenoids farnesyl- and geranylgeranylpyrophosphate is altered in male Alzheimer patients. *Neurobiol Dis* 2009;35:251–257. [PubMed: 19464372]
94. Ostrowski SM, Wilkinson BL, Golde TE, Landreth G. Statins reduce amyloid-beta production through inhibition of protein isoprenylation. *J Biol Chem* 2007;282:26832–26844. [PubMed: 17646164]
95. Loerch PM, Lu T, Dakin KA, Vann JM, Isaacs A, Geula C, Wang J, Pan Y, Gabuzda DH, Li C, Prolla TA, Yankner BA. Evolution of the aging brain transcriptome and synaptic regulation. *PLoS.One* 2008;3:e3329. [PubMed: 18830410]
96. Yankner BA, Lu T, Loerch P. The aging brain. *Annu.Rev.Pathol* 2008;3:41–66. [PubMed: 18039130]
97. Caserta MT, Bannon Y, Fernandez F, Giunta B, Schoenberg MR, Tan J. Normal brain aging clinical, immunological, neuropsychological, and neuroimaging features. *Int.Rev.Neurobiol* 2009;84:1–19. [PubMed: 19501710]
98. Farooqui T, Farooqui AA. Aging: an important factor for the pathogenesis of neurodegenerative diseases. *Mech Ageing Dev* 2009;130:203–215. [PubMed: 19071157]
99. Mattson MP, Magnus T. Ageing and neuronal vulnerability. *Nature Reviews Neuroscience* 2006;7:278–294.
100. Wood, WG.; Igbavboa, U.; Eckert, GP.; Müller, WE. Cholesterol - A Janus Face molecule in the central nervous system. In: Lajtha; Abel; Reith; Marten, editors. *Neural Membranes and Transport*. Vol. vol. XIV. New York: Plenum Press; 2007. p. 513
101. Dietschy JM, Turley SD. Thematic review series: Brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J.Lipid Res* 2004;45:1375–1397. [PubMed: 15254070]
102. Pullarkat RK, Reha H. Accumulation of dolichols in brains of elderly. *J Biol Chem* 1982;257:5991–5993. [PubMed: 7076658]
103. Wolfe LS, Ng Ying Kin NM, Palo J, Bergeron C, Kotila M, Varonen S. Dolichols are elevated in brain tissue from Alzheimer's disease, but not in urinary sediment from Alzheimer's disease and Down's syndrome. *Neurochem Pathol* 1985;3:213–221. [PubMed: 2938031]
104. Ng Ying Kin NM, Palo J, Haltia M, Wolfe LS. High levels of brain dolichols in neuronal ceroid-lipofuscinosis and senescence. *J Neurochem* 1983;40:1465–1473. [PubMed: 6834069]
105. Edlund C, Soderberg M, Kristensson K. Isoprenoids in aging and neurodegeneration. *Neurochem Int* 1994;25:35–38. [PubMed: 7950967]
106. Edlund C, Soderberg M, Kristensson K, Dallner G. Ubiquinone, dolichol, and cholesterol metabolism in aging and Alzheimer's disease. *Biochem.Cell Biol* 1992;70:422–428. [PubMed: 1449707]
107. Soderberg M, Edlund C, Kristensson K, Dallner G. Lipid compositions of different regions of the human brain during aging. *J.Neurochem* 1990;54:415–423. [PubMed: 2299344]
108. Andersson M, Aberg F, Teclebrhan H, Edlund C, Appelkvist EL. Age-dependent modifications in the metabolism of mevalonate pathway lipids in rat brain. *Mech.Ageing Dev* 1995;85:1–14. [PubMed: 8789251]
109. Porter FD. Smith-Lemli-Opitz syndrome: pathogenesis, diagnosis and management. *Eur.J.Hum.Genet* 2008;16:535–541. [PubMed: 18285838]
110. Valenza M, Rigamonti D, Goffredo D, Zuccato C, Fenu S, Jamot L, Strand A, Tarditi A, Woodman B, Racchi M, Mariotti C, Di Donato S, Corsini A, Bates G, Pruss R, Olson JM, Sipione S, Tartari M, Cattaneo E. Dysfunction of the cholesterol biosynthetic pathway in Huntington's disease. *J Neurosci* 2005;25:9932–9939. [PubMed: 16251441]
111. Schweitzer JK, Krivda JP, D'Souza-Schorey C. Neurodegeneration in Niemann-Pick Type C disease and Huntington's disease: impact of defects in membrane trafficking. *Curr.Drug Targets* 2009;10:653–665. [PubMed: 19601769]
112. Karten B, Peake KB, Vance JE. Mechanisms and consequences of impaired lipid trafficking in Niemann-Pick type C1-deficient mammalian cells. *Biochim.Biophys.Acta* 2009;1791:659–670. [PubMed: 19416638]

113. Yanagisawa K. Cholesterol and pathological processes in Alzheimer's disease. *J.Neurosci.Res* 2002;70:361–366. [PubMed: 12391598]
114. Soderberg M, Edlund C, Alafuzoff I, Kristensson K, Dallner G. Lipid composition in different regions of the brain in Alzheimer's disease/senile dementia of Alzheimer's type. *J Neurochem* 1992;59:1646–1653. [PubMed: 1402910]
115. Isobe C, Abe T, Terayama Y. Increase in the oxidized/total coenzyme Q-10 ratio in the cerebrospinal fluid of Alzheimer's disease patients. *Dement Geriatr Cogn Disord* 2009;28:449–454. [PubMed: 19907182]
116. Yang X, Yang Y, Li G, Wang J, Yang ES. Coenzyme Q10 attenuates beta-amyloid pathology in the aged transgenic mice with Alzheimer presenilin 1 mutation. *J Mol Neurosci* 2008;34:165–171. [PubMed: 18181031]
117. Thal LJ, Grundman M, Berg J, Ernstrom K, Margolin R, Pfeiffer E, Weiner MF, Zamrini E, Thomas RG. Idebenone treatment fails to slow cognitive decline in Alzheimer's disease. *Neurology* 2003;61:1498–1502. [PubMed: 14663031]
118. Scheper W, Hoozemans JJ, Hoogenraad CC, Rozemuller AJ, Eikelenboom P, Baas F. Rab6 is increased in Alzheimer's disease brain and correlates with endoplasmic reticulum stress. *Neuropathol Appl Neurobiol* 2007;33:523–532. [PubMed: 17573808]
119. Cole SL, Grudzien A, Manhart IO, Kelly BL, Oakley H, Vassar R. Statins cause intracellular accumulation of amyloid precursor protein, beta-secretase-cleaved fragments, and amyloid beta-peptide via an isoprenoid-dependent mechanism. *J.Biol.Chem* 2005;280:18755–18770. [PubMed: 15718241]
120. Hooff GP, Volmer DA, Wood WG, Muller WE, Eckert GP. Isoprenoid quantitation in human brain tissue: a validated HPLC-fluorescence detection method for endogenous farnesyl- (FPP) and geranylgeranylpyrophosphate (GGPP). *Anal Bioanal Chem* 2008;392:673–680. [PubMed: 18690423]
121. Tong H, Wiemer AJ, Neighbors JD, Hohl RJ. Quantitative determination of farnesyl and geranylgeranyl diphosphate levels in mammalian tissue. *Anal Biochem* 2008;378:138–143. [PubMed: 18457649]
122. Tong H, Holstein SA, Hohl RJ. Simultaneous determination of farnesyl and geranylgeranyl pyrophosphate levels in cultured cells. *Anal.Biochem* 2005;336:51–59. [PubMed: 15582558]
123. Holstein SA, Tong H, Hohl RJ. Differential activities of thalidomide and isoprenoid biosynthetic pathway inhibitors in multiple myeloma cells. *Leuk Res* 2010;34:344–351. [PubMed: 19646757]
124. Hooff GP, Peters I, Müller WE, Wood WG, Eckert GP. Modulation of Cholesterol, Farnesyl- and Geranylgeranylpyrophosphate in neuronal SH-SY5Y–APP965 cells – Impact for Amyloid-Beta Production. *Mol Neurobiol*. 2010 *in press*.
125. Wood WG, Eckert GP, Igbavboa U, Müller WE. Statins and neuroprotection: A prescription to move in the field forward. *Ann N Y Acad Sci*. 2010 *in press*.
126. Kojro E, Gimpl G, Lammich S, Marz W, Fahrenholz F. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. *Proc.Natl.Acad.Sci.U.S.A* 2001;98:5815–5820. [PubMed: 11309494]
127. Peters I, Igbavboa U, Schutt T, Haidari S, Hartig U, Rosello X, Bottner S, Copanaki E, Deller T, Kogel D, Wood WG, Muller WE, Eckert GP. The interaction of beta-amyloid protein with cellular membranes stimulates its own production. *Biochim Biophys Acta* 2009;1788:964–972. [PubMed: 19366591]
128. Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, Keller P, Runz H, Kuhl S, Bertsch T, von Bergmann K, Hennerici M, Beyreuther K, Hartmann T. Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proc.Natl.Acad.Sci.U.S.A* 2001;98:5856–5861. [PubMed: 11296263]
129. Frears ER, Stephens DJ, Walters CE, Davies H, Austen BM. The role of cholesterol in the biosynthesis of beta-amyloid. *Neuroreport* 1999;10:1699–1705. [PubMed: 10501560]
130. Eckert GP, Wood WG, Müller WE. Statins: drugs for Alzheimer's disease? *J Neural Transm* 2005;112:1057–1071. [PubMed: 15682268]
131. Eckert GP, Wood WG, Müller WE. Cholesterol lowering drugs and Alzheimer's disease. *Future Lipidology* 2007;2:423–432.

132. Wood WG, Eckert GP, Igbavboa U, Müller WE. Amyloid beta-protein interactions with membranes and cholesterol: causes or casualties of Alzheimer's disease. *Biochim.Biophys.Acta* 2003;1610:281–290. [PubMed: 12648781]
133. Wood WG, Igbavboa U. Cholesterol trafficking and amyloid beta peptides. *Pharmacopsychiatry* 2003;2 Suppl 2:S144–S148. [PubMed: 14574629]
134. Zhou Y, Su Y, Li B, Liu F, Ryder JW, Wu X, Gonzalez-DeWhitt PA, Gelfanova V, Hale JE, May PC, Paul SM, Ni B. Nonsteroidal anti-inflammatory drugs can lower amyloidogenic Abeta42 by inhibiting Rho. *Science* 2003;302:1215–1217. [PubMed: 14615541]
135. Pedrini S, Carter TL, Prendergast G, Petanceska S, Ehrlich ME, Gandy S. Modulation of Statin-Activated Shedding of Alzheimer APP Ectodomain by ROCK. *PLoS.Med* 2005;2:e18. [PubMed: 15647781]
136. Zhou Y, Suram A, Venugopal C, Prakasam A, Lin S, Su Y, Li B, Paul SM, Sambamurti K. Geranylgeranyl pyrophosphate stimulates gamma-secretase to increase the generation of Abeta and APP-CTFgamma. *FASEB J* 2008;22:47–54. [PubMed: 17666454]
137. Leuchtenberger S, Kummer MP, Kukar T, Czirr E, Teusch N, Sagi SA, Berdeaux R, Pietrzik CU, Ladd TB, Golde TE, Koo EH, Weggen S. Inhibitors of Rho-kinase modulate amyloid-beta (Abeta) secretion but lack selectivity for Abeta42. *J Neurochem* 2006;96:355–365. [PubMed: 16300630]
138. Salminen A, Suuronen T, Kaarniranta K. ROCK, PAK, and Toll of synapses in Alzheimer's disease. *Biochem Biophys Res Commun* 2008;371:587–590. [PubMed: 18466762]
139. Scheper W, Zwart R, Baas F. Rab6 membrane association is dependent of Presenilin 1 and cellular phosphorylation events. *Brain Res Mol Brain Res* 2004;122:17–23. [PubMed: 14992812]
140. Dumanchin C, Czech C, Campion D, Cuif MH, Poyot T, Martin C, Charbonnier F, Goud B, Pradier L, Frebourg T. Presenilins interact with Rab11, a small GTPase involved in the regulation of vesicular transport. *Hum.Mol.Genet* 1999;8:1263–1269. [PubMed: 10369872]
141. Lee M, You HJ, Cho SH, Woo CH, Yoo MH, Joe EH, Kim JH. Implication of the small GTPase Rac1 in the generation of reactive oxygen species in response to beta-amyloid in C6 astroglia cells. *Biochem.J* 2002;366:937–943. [PubMed: 12038964]
142. Wang PL, Niidome T, Akaike A, Kihara T, Sugimoto H. Rac1 inhibition negatively regulates transcriptional activity of the amyloid precursor protein gene. *J Neurosci Res* 2009;87:2105–2114. [PubMed: 19267423]
143. Pilpel Y, Segal M. Activation of PKC induces rapid morphological plasticity in dendrites of hippocampal neurons via Rac and Rho-dependent mechanisms. *Eur.J.Neurosci* 2004;19:3151–3164. [PubMed: 15217371]
144. Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W Jr, Kaye J, Manczak M. Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. *J Alzheimers Dis* 2005;7:103–117. discussion 173–180. [PubMed: 15851848]
145. Shimohama S, Kamiya S, Taniguchi T, Sumida Y, Fujimoto S. Differential involvement of small G proteins in Alzheimer's disease. *Int J Mol Med* 1999;3:597–600. [PubMed: 10341289]
146. Pak DT, Sheng M. Targeted protein degradation and synapse remodeling by an inducible protein kinase. *Science* 2003;302:1368–1373. [PubMed: 14576440]
147. Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science* 2002;298:789–791. [PubMed: 12399581]
148. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol* 1991;30:572–580. [PubMed: 1789684]
149. DeKosky ST, Scheff SW, Styren SD. Structural correlates of cognition in dementia: Quantification and assessment of synapse change. *Neurodegeneration* 1996;5:417–421. [PubMed: 9117556]
150. Arendt T. Synaptic degeneration in Alzheimer's disease. *Acta Neuropathol* 2009;118:167–179. [PubMed: 19390859]
151. Arendt T. Neurodegeneration and plasticity. *Int J Dev Neurosci* 2004;22:507–514. [PubMed: 15465280]
152. Carlisle HJ, Kennedy MB. Spine architecture and synaptic plasticity. *Trends Neurosci* 2005;28:182–187. [PubMed: 15808352]

153. Schiller MR. Coupling receptor tyrosine kinases to Rho GTPases--GEFs what's the link. *Cell Signal* 2006;18:1834–1843. [PubMed: 16725310]
154. Tejada-Simon MV, Villasana LE, Serrano F, Klann E. NMDA receptor activation induces translocation and activation of Rac in mouse hippocampal area CA1. *Biochem Biophys Res Commun* 2006;343:504–512. [PubMed: 16546126]
155. Gruart A, Munoz MD, Delgado-Garcia JM. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *J Neurosci* 2006;26:1077–1087. [PubMed: 16436593]
156. Martinez LA, Klann E, Tejada-Simon MV. Translocation and activation of Rac in the hippocampus during associative contextual fear learning. *Neurobiol Learn Mem* 2007;88:104–113. [PubMed: 17363298]
157. Tejada-Simon MV, Serrano F, Villasana LE, Kanterewicz BI, Wu GY, Quinn MT, Klann E. Synaptic localization of a functional NADPH oxidase in the mouse hippocampus. *Mol. Cell Neurosci* 2005;29:97–106. [PubMed: 15866050]
158. Shuai Y, Lu B, Wang D, Kun K, Zhong Y. Forgetting Is Regulated through Rac Activity in *Drosophila*. *Cell*. 2010 *in press*.
159. Um, KM.; Niu, F.; Liu, KR.; Tolia, KR. Role of the Rac-GAP Bcr in dendritic and synapse development. Program No. 408.13, Neuroscience Meeting Planner; Society for Neuroscience. Online; Chicago, IL: 2009.
160. Miyamoto Y, Yamauchi J, Tanoue A, Wu C, C W. Mobley, TrkB binds and tyrosine-phosphorylates Tiam1, leading to activation of Rac1 and induction of changes in cellular morphology. *Proc Natl Acad Sci U S A* 2006;103:10444–10449. [PubMed: 16801538]
161. Martin MG, Perga S, Trovo L, Rasola A, Holm P, Rantamaki T, Harkany T, Castren E, Chiara F, Dotti CG. Cholesterol Loss Enhances TrkB Signaling in Hippocampal Neurons Aging in Vitro. *Mol Biol Cell* 2008;19:2101–2112. [PubMed: 18287532]
162. Zhou XP, Wu KY, Liang B, Fu XQ, Luo ZG. TrkB-mediated activation of geranylgeranyltransferase I promotes dendritic morphogenesis. *Proc Natl Acad Sci U S A* 2008;105:17181–17186. [PubMed: 18957540]
163. Kotti, T.; Ramirez, DMO.; Russel, DW. Geranylgeraniol acts on NMDA receptor to affect synaptic plasticity. Program No. 318.5, Neuroscience Meeting Planner; Society for Neuroscience. Online; Chicago, IL: 2009.
164. Ma QL, Yang F, Calon F, Ubeda OJ, Hansen JE, Weisbart RH, Beech W, Frautschy SA, Cole GM. p21-activated kinase-aberrant activation and translocation in Alzheimer disease pathogenesis. *J Biol Chem* 2008;283:14132–14143. [PubMed: 18347024]
165. Kwon T, Kwon DY, Chun J, Kim JH, Kang SS. Akt protein kinase inhibits Rac1-GTP binding through phosphorylation at serine 71 of Rac1. *J Biol Chem* 2000;275:423–428. [PubMed: 10617634]
166. Fumagalli F, Racagni G, Riva MA. The expanding role of BDNF: a therapeutic target for Alzheimer's disease? *Pharmacogenomics J* 2006;6:8–15. [PubMed: 16314887]
167. Phillips HS, Hains JM, Armanini M, Laramie GR, Johnson SA, Winslow JW. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. *Neuron* 1991;7:695–702. [PubMed: 1742020]
168. Siegel GJ, Chauhan NB. Neurotrophic factors in Alzheimer's and Parkinson's disease brain. *Brain Res. Brain Res. Rev* 2000;33:199–227. 2000.Sep.1.;33.(2.-3.):199.-227. [PubMed: 11011066]
169. Petratos S, Li QX, George AJ, Hou X, Kerr ML, Unabia SE, Hatzinisiriou I, Maksel D, Aguilar MI, Small DH. The beta-amyloid protein of Alzheimer's disease increases neuronal CRMP-2 phosphorylation by a Rho-GTP mechanism. *Brain* 2008;131:90–108. [PubMed: 18000012]
170. Markesbery WR, Carney JM. Oxidative alterations in Alzheimer's disease. *Brain Pathol* 1999;9:133–146. [PubMed: 9989456]
171. McGrath LT, McGleenon BM, Brennan S, McColl D, Mc IS, Passmore AP. Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. *QJM* 2001;94:485–490. [PubMed: 11528012]
172. Sultana R, Butterfield DA. Role of Oxidative Stress in the Progression of Alzheimer's Disease. *J Alzheimers Dis* 2010;19:341–353. [PubMed: 20061649]

173. Cheret C, Gervais A, Lelli A, Colin C, Amar L, Ravassard P, Mallet J, Cumano A, Krause KH, Mallat M. Neurotoxic activation of microglia is promoted by a nox1-dependent NADPH oxidase. *J Neurosci* 2008;28:12039–12051. [PubMed: 19005069]
174. Bruce-Keller AJ, Gupta S, Parrino TE, Knight AE, Ebenezer PJ, Weidner AM, Levine H, Keller J, R W. Markesbery, NOX Activity is Increased in Mild Cognitive Impairment. *Antioxid Redox Signal*. 2010 *in press*.
175. Hirooka Y. Role of reactive oxygen species in brainstem in neural mechanisms of hypertension. *Auton Neurosci* 2008;142:20–24. [PubMed: 18650132]
176. Patel M, Li QY, Chang LY, Crapo J, Liang LP. Activation of NADPH oxidase and extracellular superoxide production in seizure-induced hippocampal damage. *J.Neurochem* 2005;92:123–131. [PubMed: 15606902]
177. Kahles T, Luedike P, Endres M, Galla HJ, Steinmetz H, Busse R, Neumann-Haefelin T, Brandes RP. NADPH oxidase plays a central role in blood-brain barrier damage in experimental stroke. *Stroke* 2007;38:3000–3006. [PubMed: 17916764]
178. Nozoe M, Hirooka Y, Koga Y, Sagara Y, Kishi T, Engelhardt JF, Sunagawa K. Inhibition of Rac1-derived reactive oxygen species in nucleus tractus solitarius decreases blood pressure and heart rate in stroke-prone spontaneously hypertensive rats. *Hypertension* 2007;50:62–68. [PubMed: 17515454]

Fig. 1. Abbreviated mevalonate/isoprenoid/cholesterol pathway

The mevalonate- (MVA-) pathway is a crucial metabolic pathway in almost all eukaryotic cells, which converts mevalonate into cholesterol. It is most recognized for the biosynthesis of cholesterol, while it provides the cell with further indispensable substrates, such as squalene and the isoprenoids farnesyl- (FPP) and geranylgeranylpyrophosphate (GGPP). Initial steps of the MVA-isoprenoid pathway involve the synthesis of 3-hydroxy-3-methylglutaryl- coenzyme A (HMG-CoA) from acetyl-CoA by acetoacetyl-CoA. Subsequently, the HMG-CoA reductase forms mevalonate. Subsequently, isopentenyl pyrophosphate (IPP) is formed, which equilibrates with its isomer dimethylallyl pyrophosphate (DMAPP). IPP or DMAPP then undergo subsequent condensation reactions to produce GPP, FPP and GGPP. The synthesis of

GPP and FPP is catalyzed by the farnesylpyrophosphate synthase (FPPS) and the geranylgeranylpyrophosphate synthase (GGPPS), respectively. FPP and GGPP are substrates for post-translational prenylation of the small GTPases by the farnesyltransferase (FTase) and the geranylgeranyltransferases (GGTase), respectively. In general, prenylation of these proteins assists in proper localization in internal cell membranes as well as in their correct function. After isoprenylation, the CaaX motif containing proteins undergo two further sequential enzymatic reactions. Upon the enzymatic attachment of the isoprenoid, the diphosphate is cleaved off and endoproteases remove the terminal three amino acids (-aaX). Upon cleavage of the terminal tripeptide, the remaining prenylated cysteine residue undergoes carboxymethylation by a methyl group, delivered from S-adenosyl methionine. The enzymatic conversion is catalyzed by the isoprenylcysteine carboxyl methyltransferase (Icmt). Moreover, FPP and GGPP serve as precursors for longer chain isoprenoids like dolichol or ubiquinone. FPP is the branching point of the pathway leading to squalene catalyzed by the farnesyl diphosphate farnesyltransferase (FDFT). Two subsequent enzymatic reactions result in the production of lanosterol, which basically represents the structure of all steroids. Subsequent reactions lead to production of desmosterol and 7-dehydrocholesterol and finally to cholesterol.

Fig. 2. Prenylation of Rac1

Prenylation of Rac1 represents a lipid based post translational modification involving the covalent attachment of GGPP catalyzed by GGTase I to the cysteine residue in the CaaX box consensus motif at the C- terminus through a thioether linkage. In case of Rac1, the 'a' in CaaX represents the aliphatic amino acid leucine (L) and 'X' a serine residue. Upon the enzymatic attachment of GGPP by GGTase I, the diphosphate is cleaved off by the Ras converting enzyme (Rce1p) and the Ste24p endogenous proteases remove the terminal three amino acids (-aaX). After cleavage of the terminal tripeptide, the remaining prenylated cysteine residue undergoes carboxymethylation by a methyl group, delivered from S-adenosyl methionine. The enzymatic conversion is catalyzed by the isoprenylcysteine carboxyl methyltransferase (Icmt). Isoprenylation enhances the lipophilicity and thus favors lipid-lipid interactions of Rac1 with the cellular membranes.

Isoprenoids in Alzheimer's Disease

Fig. 3. Isoprenoids, small GTPases and Alzheimer's Disease

Levels of the isoprenoids ubiquinone, dolichol, dolichyl-phosphate (dolichyl-P) farnesyl-(FPP) and geranylgeranylpyrophosphate (GGPP) were reported to be modified in AD brain. It is known for many years that the long-chain isoprenoid dolichol is decreased in post-mortem AD brain tissue, while dolichyl-P and ubiquinone are elevated. Recent data showed that FPP and GGPP levels were significantly higher in brain tissue of AD patients, while cholesterol levels were unchanged. These data indicate distinct alterations of isoprenoid metabolism within the mevalonate/isoprenoid/cholesterol pathway. FPP and GGPP play a fundamental role in prenylation of small GTPases, which function as molecular switches in various signaling pathways. In AD pathology, small GTPases have been connected with oxidative stress, the generation of A β and synaptic dysfunction (for details, please refer to the text).

Table 1

Leves of FPP and GGPP in different tissues.

Tissue	FPP	GGPP	unit	Reference
Mouse ¹ kidney ^a	0.320 ± 0.019	0.293 ± 0.035	nmol/g w.t.	Tong et al. [140]
Mouse ¹ liver ^a	0.326 ± 0.064	0.213 ± 0.029	nmol/g w.t.	Tong et al. [140]
Mouse ¹ heart ^a	0.364 ± 0.015	0.349 ± 0.023	nmol/g w.t.	Tong et al. [140]
Mouse ¹ brain ^a	0.355 ± 0.030	0.827 ± 0.082	nmol/g w.t.	Tong et al. [140]
Mouse ² brain ^b	25.27 ± 3.06	65.69 ± 9.59	pmol/ mg protein	Eckert et al. [114]
Human brain ^b				
white matter	2.88 ± 1.04	14.34 ± 8.40	ng/mg protein	Hooff et al. [139]
grey matter	3.02 ± 1.32	9.66 ± 7.72	ng/mg protein	
Human brain ^b				
white matter	5.53 ± 0.63	11.88 ± 1.26	pmol/ mg protein	Eckert et al. [114]
grey matter	2.95 ± 0.25	7.43 ± 0.81	pmol/ mg protein	
Alzheimer brain ^b				
white matter	7.93 ± 0.91	18.61 ± 3.00	pmol/ mg protein	Eckert et al. [114]
grey matter	4.03 ± 0.38	11.81 ± 1.69	pmol/ mg protein	

FPP and GGPP levels were determined in different tissues using validated fluorescence HPLC methods [139,140].

Mouse tissue was isolated from ¹athmic nu/nu or ²C57/BJ6 mice.

Human brain tissue was obtained from the Human Brain and Spinal Fluid Resource Center, West Los Angeles, USA.

Mean, ± ^aSD, ^bSEM.

Tabel 2

Leves of FPP and GGPP in different cell lines.

Cell line	FPP	GGPP	unit	Reference
NIH3T3	0.125 ± 0.010	0.145 ± 0.008	pmol/ 10^{-6} cells	Tong et al. [141]
NIH3T3	0.131 ± 0.008	0.133 ± 0.003	nmol/g w.t.	Tong et al. [140]
K562	0.112 ± 0.008	0.238 ± 0.003	nmol/g w.t.	Tong et al. [140]
Caco-2	0.65 ± 0.02	-	pmol/well	Murthy et al. [143]
RPMI-8226	0.19 ± 0.001	0.29 ± 0.02	pmol/ 10^{-6} cells	Holstein et al. [142]
H929	0.16 ± 0.001	0.16 ± 0.02	pmol/ 10^{-6} cells	Holstein et al. [142]
U266	1.4 ± 0.001	0.58 ± 0.03	pmol/ 10^{-6} cells	Holstein et al. [142]
SY5Y-APP695	17.2 ± 1.2	13.4 ± 1.3	pmol/mg protein	Hooff et al. [144]

FPP and GGPP levels were determined using a fluorescence HPLC method [141] in mouse embryonic fibroblast cells (NIH3T3), in human immortalized myelogenous leukaemia cells (K562), in human immortalized colorectal adenocarcinoma cells (Caco-2), in human multiple myeloma cells (RPMI-8226, H929, U266) and in human neuroblastoma cells (SH-SY5Y-APP695). Mean $\pm$ SD.