GDE transmembrane ecto-enzymes : novel signaling functions through GPI-anchor cleavage

Veen, T.P.M. van


QUERCUS

Chapter 1

General introduction: The family of GDPD enzymes and their involvement in GPI-anchor cleavage

Introduction

After having built some of the earliest microscopes, Robert Hooke first published his observations in his *Micrographia* in 1665 [1]. He described his observation of a common structure present in all organisms which he called "Cells" because of the resemblance to monastic cells. Roughly 200 years later the cell theory was formulated, which states that all living organisms are composed of cells, making them the most basic building block of life [2]. Today this statement is still valid and in addition we now know that all cells come from preexisting cells and carry the heredity information DNA. Although cells are independent units capable of self-replication, communication with their surroundings is vital and enables them to work together to form organs and ultimately the human body. To do so, mechanical or biochemical stimuli from outside the cell are transmitted across the membrane by specialized integral membrane proteins that span the lipid bilayer and activate intracellular signaling cascades. This process is called outside-in signaling. However, from within, cells can also control or adapt their response to extra-cellular stimuli, either by changing the conformation of transmembrane proteins or by pulling the specific protein inside the cell (internalization) to reduce their exposure to extracellular stimuli. But transmembrane proteins are not the only proteins that respond to extracellular stimuli. Another class of peripheral membrane proteins do not cross the membrane but are anchored to the outer leaflet via a lipid tail, so called glycosylphospatidylinositol (GPI) -anchored proteins. About 20% of all membrane proteins are GPI-anchored and play key roles in a wide variety of biological processes. However, because of their very nature, they are incapable of directly signaling inside the cell and thus need to interact with transmembrane co-receptors. Also, because they lack a transmembrane domain, control from within the cell is limited. Therefore, ever since the discovery of this complex protein modification in the 1970s the biological importance of the GPI anchor has been elusive. In this thesis we identify a family of transmembrane enzymes, glycerophosphodiesterases (GDEs), that specifically cleave GPI anchors. By doing so, GDEs release the protein from the membrane to control cell signaling, thereby adding a new role to the GPI-anchor as an enzyme substrate.

GPI-anchored proteins

Since their discovery approximately 40 years ago, we know that proteins can be covalently bound to a phospholipid anchor which is inserted into the outer lipid leaflet[3, 4]. Since then, increasing numbers of GPI-anchored proteins (GPI-APs) have been identified in eukaryotes ranging from fungi to mammals. Today we know that GPI-anchored proteins (predictions vary between 150 to 300 proteins carrying a GPI-anchor [5]) are functionally and structurally diverse, ranging from enzymes (uPAR) to cell-cell adhesion molecules (NCAM) and

complement regulatory factors (CD55). Consequently, proteins carrying a GPI anchor play fundamental roles in various (patho) physiological processes, such as adhesion, cellular differentiation, immune response and general cell signaling [6-11]. The importance of the lipid anchor is further demonstrated by early research showing that complete abrogation of this modification is embryonic lethal [10, 11]. In mammals, mutations in the promotor region of *PIG-M* or in the *PIG-C* gene that are involved in GPI-biosynthesis lead to seizures and are associated with intellectual disability [12, 13]. In addition, in human hematopoietic stem cells, somatic defects in genes involved in GPI-biosynthesis result in paroxysmal nocturnal hemoglobinuria, an acquired life-threatening hemolytic disease [14]. Although these examples clearly demonstrate the importance of GPI-anchored proteins in localization to the membrane, they fail to reveal a physiological role for the GPI anchor itself, which remains an outstanding question. The molecular structure of GPI anchors is complex and includes a phospholipid, a glycan core and a linker group. Although this modification is efficient to anchor proteins to the membrane, it seems too complex to serve only this purpose. Indeed, recent studies have revealed that GPI anchors can be further modified and released by GPI-specific enzymes, showing that the structural complexity of GPI-anchors could have multiple functions beyond simple membrane tethering [15-18].

1. Structure of GPI anchors

The GPI anchor is a C-terminal protein modification with a highly conserved core structure consisting of an ethanolamine phosphate (EtNP) linker region directly attached to the protein, a glycan chain containing a minimum of 3 mannose residues (Man) and a glucosamine (GlcN) group [3]. Finally, an inositol group links the GPI anchor to a phospholipid tail which is inserted into the outer membrane leaflet. Attached to this highly conserved glycan core various additional modifications can be attached, which differ not only within organisms, but also within the protein attached to it. For instance, structural comparisons between the insect life-cycle and bloodstream *T. brucei* VSG (arguably the best charachterized GPI anchor) revealed alterations in carbohydrate side chains. This demonstrates that side chains are constantly modified depending on the cell state or its surroundings[3, 19]. In humans, intra-protein GPI-anchor heterogeneity is also found, as shown by mass spec analysis of CD59 where a fraction of the GPI anchors carry a β-N-acetylgalactosamine group (GalNAc) attached to the 4-position of Man-1 [20]. In general, GPI-anchor modifications include additional EtNP's or various linear or branched glycosyl groups including sialic acid, mannose or galactose (Figure 1)[21]. However, exactly why GPI anchors carry these modifications, to what extent they are reversible and how they are regulated are outstanding questions. Clues from lower eukaryotes suggests that the presence of specific intracellular enzymes together with the tertiary/quarternary structure dictates GPI anchor modifications [5, 22, 23]. Not only the GPI-sugar core is modified, also the inositol phospholipid moiety, typically D-*myo*-inositol-

1-*P*-3(*sn*-1,2-diacylglycerol), can differ between proteins and species. Inositol phospholipid variants can include diacylglycerol (the classic GPI-anchor), *lyso*acyl (in *Trypanosoma cruzi),* ceramide (in *Dictyostelium),* alkenylacyl (bovine) or alkylacylglycerol (in *Leishmania*) [24] (Figure 2). Yet, many of the above modifications are only found in lower eukaryotes. In mammals, only two variations of the inositol phospholipid are described: alkylacylglycerol (Figure 2B) and palmitoylation of the inositol group (erythrocyte AChE) (Figure 2A) [25-28]. The latter modification is especially interesting since it renders GPI-anchor cleavage resistant to bacterial phospholipase C (PLC)(Figure 2) and thus prevents PLC-induced release from the membrane. It is nevertheless unclear if inositol acylation is only present during biosynthesis or also in mature GPI-anchored proteins at the plasma membrane [27, 28]. Furthermore, inositol acylation was only shown in erythrocytes and may represent an exception [29].

2. GPI-anchor biosynthesis

During biosynthesis of GPI-anchored proteins, a C-terminal attachment sequence is recognized and a pre-assembled GPI anchor is then attached to the protein through an irreversible transamidation reaction at the luminal site of the endoplasmic reticulon (ER) [21] (Figure 3). Although the attachment signal is crucial, it is poorly conserved and does not contain a consensus sequence. However, it does contain common features including (i) an ω-site, the lipidation site where the GPI anchor is amine-bonded to the protein; this is generally a small amino acid with small side chains; (ii) two small amino acids in close proximity to the ω-site (ω+1, ω+2), followed by (iii) a short stretch of hydrophilic amino acids (linker region) and (iv), a carboxyl-terminal hydrophobic region [30]. The latter region displays similar properties to a classical transmembrane α-helix, however with marginal hydrophobicity. Consequently, an attachment signal that diffuses between the ER membrane and ER lumen prior to transamidation is thought to be a requisite for proper GPI-anchor attachment [31].

The synthesis of the GPI anchor itself requires at least 10 reactions and over 20 genes are involved in the process [22, 29, 32]. The assembly of the PI moiety occurs at the cytoplasmic side of the ER by stepwise addition of sugars and acylation of the inositol group. The latter modification targets the GPI precursor to the luminal side of the ER by an as yet unknown flippase [29]. Here, the protein is attached to the lipid anchor, which is then modified through removal of a EtNP group at the second Man by PGAP5 and acyl chain removal by PGAP1 [33, 34] (Figure 4). In mice, defective acyl removal through Pgap1 knockout results in normal GPI-AP cell-surface expression, but the mice suffer from severe defects during development and fertilization [9, 33]. One possible explanation for infertility is that acylated GPI-APs accumulate on the membrane because of their resistance to PLC attack. Intriguingly, GPI-anchored cleavage was shown to be essential for sperm maturation, hinting at a possible role

for GPI specific phospholipase [35].

After acyl removal in the ER, only correctly folded GPI-APs are loaded from the ER into Coat protein complex II (COPII) vesicles (Figure 3 and 4). Correctly folded and remodeled GPI-anchors act as an ER exit signal that guide GPI-APs to the Golgi and then to the plasma membrane [36, 37]. When sorted in vesicles, GPI-APs fail to bind cytosolic COPII proteins since they lack a transmembrane domain. Transmembrane adaptor proteins are therefore needed to ensure efficient transport to the plasma membrane. The family of p24 proteins represent such cargo receptors that recycle between the ER and the Golgi [38-40] (Figure 4). These proteins serve as gatekeepers by selectively recognizing GPI-APs modified by PGAP1 and PGAP5 [36, 41].

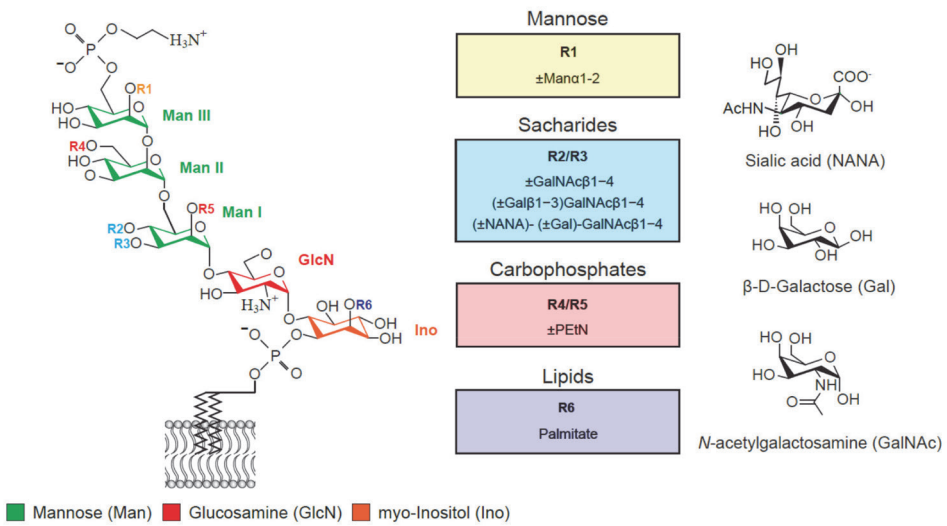


Figure 1. GPI-anchor molecular structure
(left) Minimum GPI-anchor core structure with sites for additional side chain modifications indicated as R^{site}. (right) Depicted modifications as found in human GPI-anchored proteins. PEtN, phosphoethanolamine.

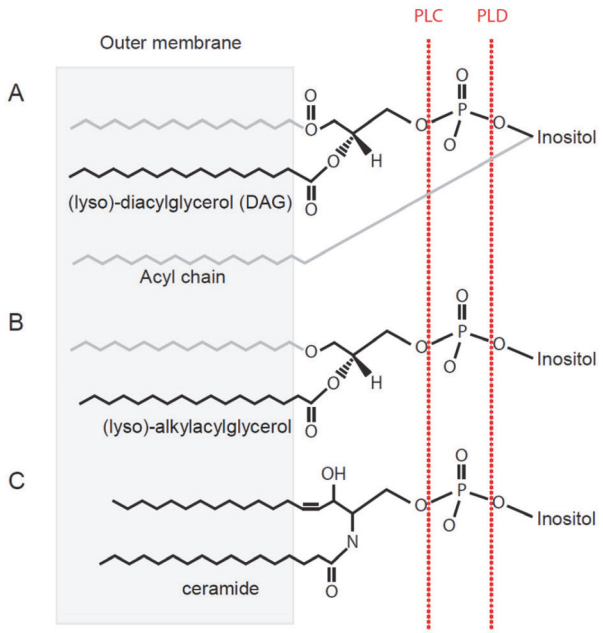


Figure 2. Phospholipid variants present in GPI anchors
Inositol phospholipids with the GPI-PLD and PLC cleavage sites indicated in red. A and B are only found in mammalian GPI anchors. Acylation of the inositol group (indicated in light grey) occurs during biosynthesis and in mature erythrocyte AChE. This modification confers resistance to GPI-PLC activity.

3. GPI membrane localization and recycling

From the Golgi GPI-APs are sorted in the trans-Golgi network (TGN) to so-called detergent resistant microdomains (DRMs) in the plasma membrane [42, 43]. These "rafts" (although their existence has been questioned) are thought to be sorting platforms for the TGN where GPI-anchors form signaling complexes. Through this mechanism, cells target various GPI-APs to the same membrane micro clusters, depending the lipid environment and the actin cytoskeleton [43-46].

In polarized endothelial and neuronal cells, GPI-APs are selectively transported to the apical membrane or axonal region, respectively [42, 47-50]. Although the underlying mechanism is unclear, perturbation of the cholesterol or sphingolipid biosynthesis machinery interrupts GPI-AP-specific sorting. This again demonstrates the importance of the lipid microenvironment [51]. However, lipid composition alone is not sufficient for apical sorting. GPI-AP oligomerization and *N*-glycosylation are also required for correct vesicle sorting [52-54]. While apical sorting is the preferred route for GPI-anchors, they also localize to

basolateral membranes. Fusing the GPI anchor motif from different GPI-APs to a GFP dictates basolateral or apical sorting [48]. In conclusion, it is clear that different mechanisms are in play to sort GPI-APs.

Once on the membrane, GPI-APs can diffuse between heteroclusters in DRMs or as monomers. From here they can recycle through clathrin-independent mechanisms. One such route, the "caveolae pathway", is dependent on dynamin and uses specialized membrane domains (caveolae) [29, 55]. However, most GPI-APs use another clathrin- and dynamin-independent pathway. In this pathway, GPI-APs are internalized by clathrin-independent carriers (CLIC) and transported to a compartment termed "GPI-APs enriched at early endosomal compartment" (GEEC) [56, 57] . From there GPI-APs recycle through early and late endosomes [58, 59] (Figure 4).

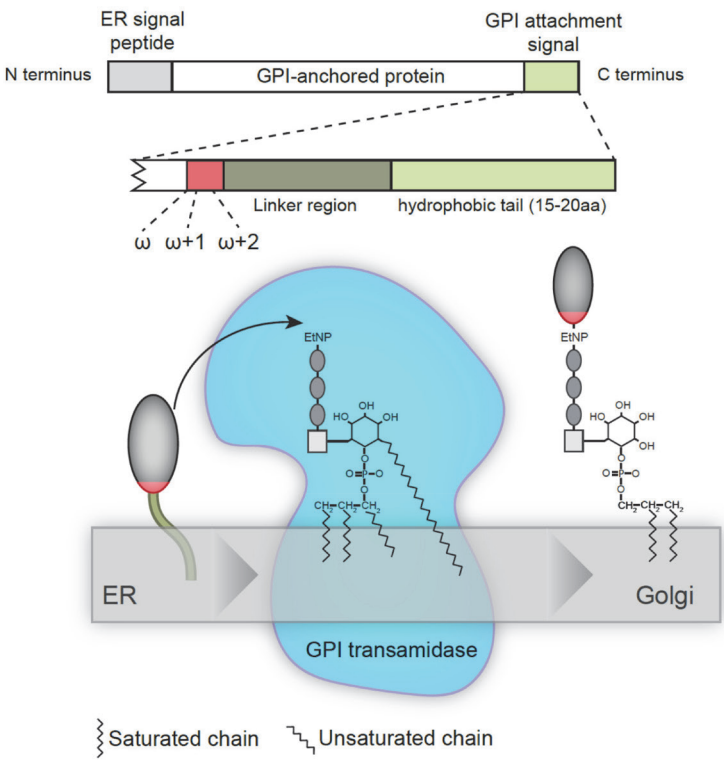


Figure 3: GPI-anchor addition by transamidase
All GPI-anchored proteins have an N-terminal ER signal peptide and a C-terminal GPI attachment signal that consists of a marginal hydrophobic region and a lipidation site (Omega). In the ER, the GPI anchor is preassembled and attached to the protein by transamidases. Thereafter the GPI-AP is transported to the Golgi.

4. GPI-anchor cleavage and release from the membrane

A unique feature of GPI-APs is that they can be cleaved and released from the membrane by phospholipases. Through this mechanism, cells not only regulate cell surface expression, but also generate a soluble protein which in some cases can function as a ligand for neighboring cells and as biomarker of various diseases [35, 60-62]. Of note, cleaving the GPI-anchor can have profound effects on the conformation and hence the function of the attached protein [23, 63]. Early research showed that GPI-APs are efficiently released by a bacterial GPI-specific PLC [64]. In mammals, the release of GPI-APs and its physiological relevance is not understood, however. A GPI-specific phospholipase D (GPI-PLD) was the first characterized enzyme capable of hydrolyzing various GPI-APs in vitro [65-67]. Secreted GPLD1 is able to cleave inositol-acylated GPI-anchors, in contrast to bacterial PI-PLC [68]. However, unlike bacterial PI-PLC, GPI-PLD fails to release GPI-anchors from native membranes, requiring detergents for its activity [4, 69]. Thus, its physiological role remains elusive to date. Another secreted protein, called Notum, was shown to regulate Wnt signaling by cleaving the GPI

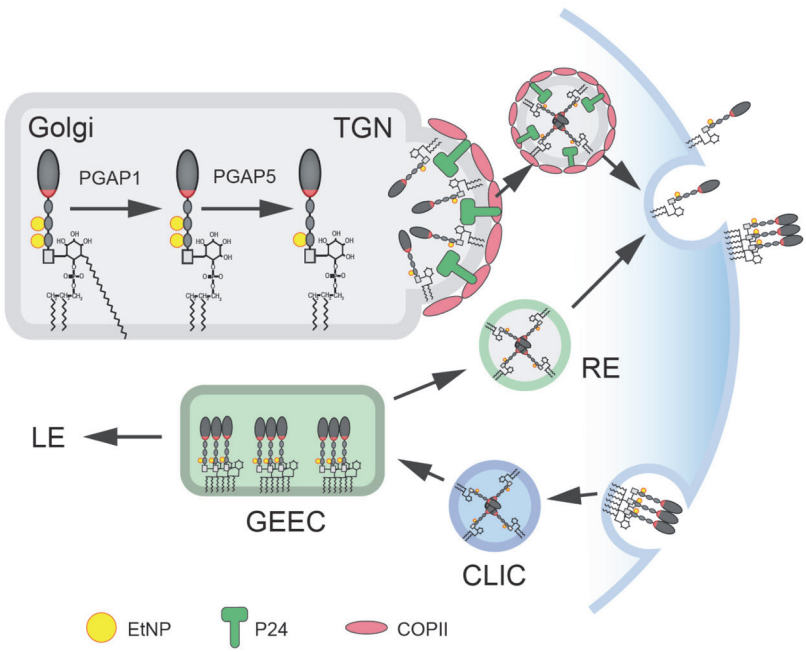


Figure 4: GPI-AP biosynthesis and trafficking

After acyl and EtNP removal in the Golgi, the GPI-AP is sorted and clustered in the TGN into p24- and COPII-positive vesicles. From there, GPI-APs are transported to the membrane where they localize to specific domains. GPI-APs undergo non-clathrin-mediated internalization and recycling following the CLIC-GEEC pathway. RE, recycling endosome; LE, late endosome; TGN, Trans Golgi Network; CLIC, clathrin-independent carrier; GEEC, GPI-AP-enriched early endosomal compartment.

anchor of Dally-like protein in Drosophila [70]. Notum releases multiple GPI-APs from mammalian cells in culture [16]. However, purified Notum was unable to hydrolyze GPI anchors [71]. Instead, recent studies revealed that Notum deacylates the Wnt protein which is required during brain development [15, 71].

During fertilization, spermatozoa express specific GPI-anchored proteins (a.o. TEX101) that are released during transport through the epididymis [72, 73]. Mice lacking germ-cell TEX101 or Angiotensin-converting enzyme (ACE) are subfertile because spermatozoa fail to migrate into the epididymis [74, 75]. Analysis showed that ACE was able to release GPI-anchored TEX101 from HEK293 cells, revealing an in vivo role for GPI-anchor release by ACE [18, 74]. However, caution should be taken when interpreting these results since GPI-releasing activity was weak and could not be reproduced by others [76]

As discussed above, during the last decade, several enzymes capable of releasing GPI-anchored proteins in vitro have been identified. Yet, these enzymes are either secreted or show very limited or even questionable activity. This suggests that more effective GPI-releasing enzymes must exist. Recent research has identified two other attractive candidates; the family of GDPDs and PGAP6.

PGAP6, a GPI-specific phospholipase A2

Bioinformatic analysis in search of PGAP3 (an enzyme involved in GPI-anchor lipid remodeling) family members revealed an uncharacterized gene, termed PGAP6 [77]. This transmembrane protein cleaves the GPI-anchored CRIPTO protein in a phospholipase A2-like manner resulting in a lysophosphatidylinositol-bearing GPI-AP. Although phospholipase A2 cleavage itself does not release GPI-APs into the medium, increased soluble CRIPTO was found in the medium after PGAP6 over-expression, possibly through an unidentified secondary effect. Interestingly, *Pgap6* KO mice exhibit severe defects in the anterior-posterior axis during development, reminiscent of a *Cripto* knockout phenotype.

Family of GDPDs

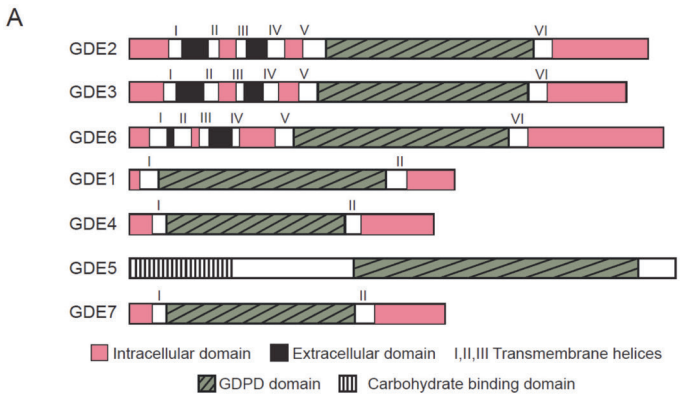
Glycerophosphodiester phosphodiesterases (GDPDs or GDEs) are a family of proteins present in many organisms, ranging from prokaryotes to protozoa and mammals. Although their primary sequence and catalytic sites are well conserved, their enzymatic features and biological significance are diverse. In *Escherichia coli*, the GDPDs, *GplQ* and *UgpQ* are well characterized and hydrolyze de-acylated glycerophospholipids into glycerol and phosphate [78-80]. Located in the periplasm and cytosol respectively, these enzymes serve a metabolic

function by generating essential building blocks such as choline, ethanolamine, inositol and serine. Likewise, in *H. Influenzae*, a homologue of *GlpQ*, protein D, is localized in the membrane and also hydrolyses deacylated phospholipids [81]. Protein D damages ciliated epithelial cell membranes in a GDPD-dependent manner, thereby contributing to *H. Influenzae* virulence [82]. Because of their high conservation in prokaryotes, the essential role in metabolism and their membrane localization, GDPDs from various organisms are currently exploited to target conjugate vaccines [83-86]. GDPDs isolated from Gram-positive bacteria also generate glycerol-3-phosphate, but in addition have a broad substrate specificity, including teichoic acid and di(glycerophospho)glycerol (deacylated) cardiolipin [87]. Because GDPDs exhibit a broad substrate specificity and can even break down organophosphates, they are also utilized for detoxification of pesticides and nerve agents [88, 89]. Bacterial GDPDs share a common tertiary structure, namely the classical triosephosphate isomerase barrel (TIM barrel) [90]. The general structure of a TIM barrel is composed of alternating α helices and β-strands, together forming a donut shaped (toroid) barrel, where beta strands form the inner lining surrounded by the α helices.

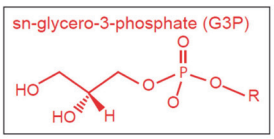
1. Mammalian GDPDs

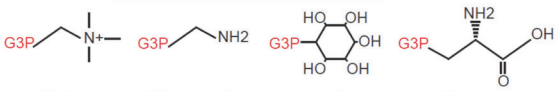
Bacterial GDPDs have long been studied and although some questions remain to be answered, their function and structure are known. By contrast, the family of mammalian GDPDs is understudied and only recently their role in glycerophospholipid signaling is emerging. The first identified mammalian GDPD, GDE1 (previously MIR16), was isolated from a two-hybrid screen in search of interactors with regulator of G-protein signaling (RGS16) [91, 92]. GDE1 is an integral membrane protein that localizes to internal membranes with the enzymatic domain facing the extracellular space [91] (Figure 5A). Structural modeling and sequence analysis of the enzymatic domain showed a conserved catalytic site with strong sequence and structural similarities to bacterial GDPDs [92, 93]. Consistent with this, GDE1 could hydrolyse glycerophosphoinositol (GroPIns) in a phospholipase D-like manner, producing glycerol-3-phosphate (Gro3P) and inositol (Figure 5B). Its activity was enhanced by magnesium and calcium [91]. MS-based metabolomics of mice lacking GDE1 revealed that GDE1 also hydrolyses GroPSer as well as an anandamide precursor glycerophospho-*N*-arachidonoylethanolamine [91, 94, 95]. A second yeast-two-hybrid screen identified GDE1 as a binding partner of yet another interactor of G-protein coupled signaling (PRAF2) [93], suggesting that GDE1 is involved in or regulated by G-protein signaling. In line with this, GDE1 activity was increased by isoproterenol and inhibited by lysophosphatidic acid (LPA). Taken together, these studies revealed GDE1 as the first mammalian glycerophosphodiester with a potential role in G-protein signaling, although the exact mechanism remains elusive. After GDE1, an additional six mammalian GDEs were cloned that are phylogenetically clustered together with bacterial UgpQ [96, 97] (Figure 5A). Within this family, one branch

is occupied by the six-fold transmembrane members GDE2, GDE3, and GDE6. The second group is formed by GDE4 and GDE7 with two putative trans membrane domains, leaving GDE5 as a unique cytosolic enzyme [96]. GDE5 is mainly expressed by skeletal muscle cells. Overexpression suppressed myogenic differentiation, while knockdown reversed this phenotype [98]. GDE5 selectively hydrolyses GroPCho *in vitro*. Unexpectedly, its myogenic activity was independent of the enzymatic domain, suggesting that other mechanisms are in play during myogenic differentiation [98].



B





	Choline	Ethanolamine	Inositol	Serine	
GDE1	-	+	+	+	PLD
GDE2/GDPD5	+	N/K	N/K	-	N/K
GDE3/GDPD2	-	N/K	+	N/K	PLC
GDE4/GDPD1	-	-	-	-	LysoPLD
GDE5/GDPD6	+	+/-	-	-	PLD
GDE6/GDPD4	N/K	N/K	N/K	N/K	N/K
GDE7/GDPD3	-	-	-	-	LysoPLD

Figure 5. Mammalian GDPD structure and enzymatic activity
(A) schematic representation of the mammalian GDE/GDPD family. The closest family members GDE2, GDE3 and GDE6 have six transmembrane helices, an extracellular enzymatic domain and intracellular N- and C- termini. **(B)** Table illustrating the enzymatic activity towards various deacylated glycerophosphodiesters by the indicated GDE enzymes. + activity reported; - no activity reported; +/- minor activity; N/K, activity unknown; PLC, phospholipase C; PLD, phospholipase D; lyso-PLD, lyso-phospholipase D [91, 94, 98-101].

2. GDE4 and GDE7, two lyso-phospholipases D

GDE4 and GDE7 are differentially expressed and have a unique function compared to the classical GDPDs. Recent studies showed that the C-terminus of both proteins is conserved with that of *L. laeta* SMaseD, a lysophospholipase D [102]. Following activity studies revealed that both enzymes also have lysoPLD activity without activity towards deacylated phospholipids [101, 103]. Overexpressed GDE4 localizes to perinuclear membranes with no clear phenotypical changes [104]. Both enzymes could generate lysophosphatidic acid (LPA) from lysophosphatidylcholine (LPC) [101]. These studies reveal a novel pathway for LPA production. However, the significance of intracellular LPA production is not understood.

3. Transmembrane ecto-enzymes GDE2, GDE3 and GDE6

GDE2, GDE3 and GDE6 are of special interest because of their six transmembrane helices and enzymatic domain located on the luminal side. The less-studied, and arguably least interesting of the three, GDE6, solely expresses in the testis and localizes to the ER [105] (unpublished data). In contrast, GDE2 and GDE3 localize to the plasma membrane with the enzymatic domain pointing towards the extracellular space. Like other GDPDs, GDE2 and GDE3 hydrolyze deacylated glycerophospholipids GroPCho and GroPIno respectively [99, 100]. However, its physiological significance is ambiguous since GDE2 silencing did not alter choline concentration and had minimal effect on Gro3P levels [106]. Park et al., reported that GDE2 cleaves and releases the GPI anchored protein Reversion-inducing-cysteine-rich *protein* with kazal motifs (RECK). This suggests that these GDEs are the long-sought enzymes that release GPI-APs from the membrane.

GDE2 is a retinoic acid-inducible gene whose expression is sufficient to drive neurite formation *in vitro* [97, 107]. GDE2 is primarily expressed in the developing spinal cord during motor neuron differentiation [108]. Retinoic acid induced GDE2 expression drives differentiation, while GDE2 ablation results in decreased post-mitotic motor neurons, accompanied by increased cell death [108, 109]. The underlying mechanism involves non-cell-autonomous cleavage of RECK by GDE2 [110]. GPI-anchored RECK activates NOTCH signaling by inhibiting metalloprotease-mediate shedding of the NOTCH ligand Delta-like 1 [111, 112]. When expressed in neighboring post-mitotic motor neurons, GDE2 cleaves RECK and thereby inactivates NOTCH signaling in pre-motor neurons, thereby regulating the timing of cortical progenitor differentiation in the developing spinal cord [110]. GDE2 does not cleave GPI-anchored RECK in a classical PLC- or PLD-like manner, suggesting a different attack of the phosphodiester bond compared to bacterial GPI-PLC [100]. Postnatally, GDE2 is continuously expressed and mice lacking GDE2 exhibit slow and progressive neurodegeneration [113]. The observed cellular phenotypes, including vacuolization and lipofuscin depositions, closely resemble human neurodegenerative pathologies, signifying GDE2's role not only in neuronal differentiation but also in neuronal survival.

The GPI-anchored proteins Glypican 4 and 6 were identified as candidate substrates for GDE2, however, a direct role for the release of these GPI-APs in neuronal survival was not shown [113]. Exactly how GDE2 prevents neurodegeneration remains an outstanding question. In chapter 3 we show that Glypican 6 release by GDE2 drives neuroblastoma differentiation [17].

GDE2's closest homologue, GDE3, was isolated in a differential display screen comparing the sequential stages during osteoblast differentiation in culture [114]. GDE3 was highly expressed at the onset of differentiation and remained high thereafter. Tissue GDE3 expression was not only detected in developing bones but also in the spleen, skin and adult brain [115]. *GDE3* overexpression induced osteoblast differentiation [99]. In contrast, GDPD ecto-domain neutralizing antibodies as well as active site mutations reversed this phenotype, suggesting a possible role for the enzymatic domain in glycerophospholipid hydrolysis. Subsequent studies showed that GDE3 specifically hydrolyses GroPIno to glycerol and Ins1*P*. Hence, GDE3 cleaves GroPIno in a PLC dependent manner, unlike other mammalian GDPDs (Figure 5B). It was suggested that GDE3, by cleaving GroPIno, inhibits proliferation and accelerates differentiation. More recent studies demonstrated that GDE3 as well as GDE6 can also cleave GPI-anchors similar to GDE2 [110, 116]. In chapter 2 we show that GDE3 cleaves GPI-anchored uPAR in a phospholipase C-like manner[116]. Altogether these studies reveal a potential role for the serpentine GDE proteins in targeted cleavage and release of GPI-anchored proteins from the membrane.

4. Regulatory mechanisms involved in GDE2 and GDE3 activity

The regulation of GDE enzymatic activity is for the most part unknown. Their secondary structure and variation in intra-cellular N- and C-terminal lengths suggests that GDE2, GDE3 and GDE6 are differentially regulated by intracellular components. In support of this and similar to GDE1 binding to a regulator of G protein signaling, it was shown that the C-terminus of GDE2 binds to Gαi, with unknown consequences [92, 117]. Also, Yan and colleagues identified the antioxidant enzyme peroxiredoxin 1 (Prdx1) as an interactor of GDE2 [118]. They showed that Prdx1 drives motor neuron differentiation by a mechanism that activates GDE2 through the reduction of an intra-cellular disulfide bond, bridging the N- and C- terminus of GDE2. Prdx1 knockdown was reminiscent of GDE2 knockdown and mutant GDE2 variants, deficient in disulfide bond formation, were hyperactive [118]. Later, the same group proposed a second redox dependent mechanism. In this model, Prdx4 dimers in the ER, which are generated after metabolizing H_2O_2, oxidize a disulfide bond in the enzymatic domain of GDE2 [119]. As a consequence, GDE2 trafficking to the plasma membrane is inhibited. Thus, two opposing redox dependent mechanisms that regulate GDE2 cell-surface expression and activity are suggested. Interestingly, the cysteines involved in this process are conserved in other GDE family members. However, their role and the requirements for these thiol-oxidative mechanisms are unknown.

Thesis outline

The studies described in this thesis focus on the role of GDE2 and GDE3 on GPI-anchor hydrolysis to increase our understanding of their physiological functions. **Chapter 1** is a general introduction giving an overview of the current knowledge and progress in GPI-anchored bio-synthesis and signaling, together with an overview of the developing understanding of the mammalian GDPD protein family. **Chapter 2** identifies GDE3 as the first mammalian GPI-specific PLC that selectively cleaves and releases the urokinase receptor (uPAR) from the membrane. By doing so, GDE3 negatively regulates uPAR signaling in breast cancer cells. Its closest family member, GDE2, associates with a positive clinical outcome in neuroblastoma, a childhood disease characterized by defective neuronal differentiation. The molecular mechanism involving neuronal differentiation through selective release of Glypican 6 by GDE2 is presented in **Chapter 3**. **Addendum Chapter 3** comments on the release of GPI-anchored proteins during neuronal differentiation. Next, **Chapter 4** explores the involvement of GDE2 in zebrafish development and describes a potential role for GDE2 in the development of the pancreas. **Chapter 5** compares the enzymatic activity towards specific GPI-anchored proteins and localization of GDE2 and GDE3. In **Chapter 6**, the results presented in this thesis are summarized and discussed.