 doi:10.20944/preprints201904.0210.v1

Acute regulation of the Glycerophospholipid Composition of The Membranes of Mammalian Cells. The First Comprehensive Model.

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Abstract

Is unclear how mammalian cells maintain the complex glycerophospholipid (GPL) compositions of their various membranes. Here we propose the first comprehensive model that suggests how this could be accomplished. The model is based on the idea that there are a limited number of GPL compositions that are energetically more favorable than the other compositions, i.e. those (optimal) compositions represent local free energy minima. Thus, the GPL composition of a membrane has a natural tendency to settle in one of the optimal composition. When the mole fraction of an GPL class exceeds that in an optimal composition, its chemical activity abruptly increases, which (i) increases its propensity to efflux from the membranes thus making it susceptible for hydrolysis by homeostatic phospholipases; (ii) increases its potency to inhibit its own biosynthesis via a feedback mechanism; (iii) enhances its conversion to another GPL class via “head group remodeling” or (iv) enhances its translocation to another membrane. These four processes may act separately or simultaneously to maintain GPL homeostasis.

Introduction

Glycerophospholipids (GPLs) are the most abundant lipids in virtually all mammalian membranes each of which contain more than 10 GPL classes varying in the structure of the polar head group. The GPL major classes are the phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylinositols (PI) phosphatidylserines (PS) phosphatidylglycerols (PG), phosphatidic acids (PA) and cardiolipins (CL). Mammalian cells maintain the relative concentrations of GPL classes in their different subcellular membranes within narrow limits, obviously because this is essential for the numerous membrane-associated functions. Despite the vital importance of such GPL homeostasis, information regarding the mechanisms underlying this crucial phenomenon in mammalian cells is limited. In particular, hardly anything is known about the coordination of the key processes underlying GPL homeostasis except that biosynthesis and degradation must be tightly coordinated.

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This has been demonstrated by many studies in which the rate of GPL synthesis was either boosted or inhibited. Thus, when the synthesis of PC was increased several-fold, its concentration in the cells remained essentially unchanged due to increased degradation (Baburina and Jackowski, 1999; Barbour et al., 1999; Jackowski, 1994; Lykidis et al., 2001; Walkey et al., 1994). Parallel evidence has been obtained for PE and PS (Baburina and Jackowski, 1999; Lykidis et al., 2001; Stone et al., 1998; Walkey et al., 1994). On the other hand, when the synthesis of PC, PE or PS was inhibited, their turnover decreased correspondingly (Fullerton and Bakovic, 2010; Fullerton et al., 2009; Nishijima et al., 1984; Polokoff et al., 1981; Steenbergen et al., 2006). However, there it is no information on how the coordination of synthesis and degradation is accomplished mechanistically, which must be a challenging task (***Fig. 1***) due to the presence of many GPL classes. Here, we present the so-called *Optimal Composition Model* (OC-model) which appears to represent the first attempt to explain how synthesis and degradation of GPLs could be accurately coordinated. This hypothesis was inspired by our recent findings on the processes involved in GPL homeostasis in mammalian cells.

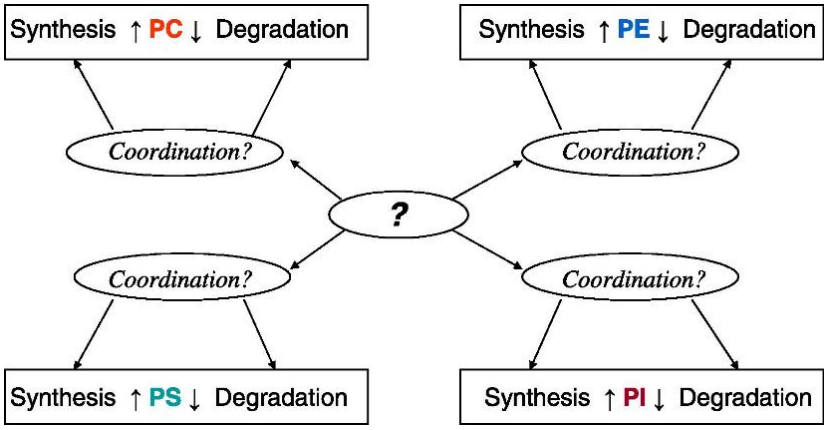

Figure 1. Complexity of regulation of GPL compositions of mammalian membranes. This scheme emphasizes the complexity of regulation of the GPL compositions of membranes consisting of many different lipid classes. All GPL classes present in mammalian cells are not shown here for simplicity.

Optimal composition model

We have previously shown that the phospholipid compositions of the inner and outer leaflets of mammalian erythrocyte and platelet membranes, the by far best characterized biological membranes in terms of GPL composition, are remarkably similar to compositions predicted by the so-called *Superlattic*^{i}*e Model* (Somerharju et al., 2009; Virtanen et al., 1998). The key elements of this model are that 1) the different GPLs *tend* to be regularly distributed locally and 2) structurally similar GPL molecules can be assigned to 3 classes, i.e. (i) choline lipids (PC and sphingomyelin), (ii) PE and (iii)

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negatively charged GPLs. From these assumptions it necessarily follows that there are only a limited number of allowed class compositions (Somerharju et al., 2009), which in ternary systems are multiples of 11.1 mole%, i.e. 11.1, 22.2, 33.3, 44.4 mol% etc. The allowed compositions is that they represent local *free energy minima* along the composition axis because they allow for the optimal or tightest interaction between neighboring molecules. Accordingly, the membrane GPL composition has a natural tendency to settle in one of allowed composition, rather than in an intervening one.

When the mole fraction of a GPL class exceeds that corresponding to an optimal one, the interaction of the molecules *in excess* with its neighbors will be weakened and thus the *chemical activity* is those molecules strongly increased. We propose that this increased chemical activity results in *(i)* increased propensity of the GPL molecules in excess to efflux from the membrane which makes them targets for homeostatic PLAs, and *(ii)* in an increased capacity to inhibit their own synthesis via a feed-back mechanism. Accordingly, chemical activity could be the "signal" regulating and thus coordinating biosynthesis and degradation, the processes critical for GPL homeostasis. Below we will discuss the recent data strongly supporting this model and also indicate two other, potentially important homeostatic process i.e., GPL class interconversion and intracellular transfer which both are likely to driven by chemical activity of the GPL molecules present "in excess".

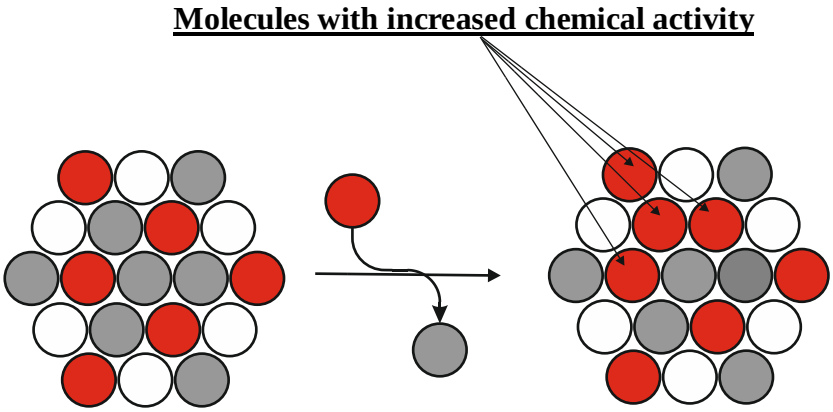

Figure 2. Deviation from an optimal composition brings about GPL molecules with an increased chemical activity. On the left: The GPL class composition is optimal as proposed previously for the erythrocyte membrane inner leaflet where PE (gray) is ~44 mol%, the choline lipids (white) are ~22 mol% and the negatively charged GPLs (red) are ~33 mol% (Virtanen et al., 1998). On the right: If a single (zwitterionic) PE molecule has been replaced by a negatively charged GPL, the chemical activity of several negatively charged GPL molecules is greatly increased due to electrostatic repulsion between the proximal negatively charged molecules.

Chemical activity regulates the biosynthesis

In general, it is poorly established what regulates the biosynthesis of GPLs in mammalian cells except for PS and PC. Kuge and coworkers have demonstrated that exogenous PS strongly inhibits the synthesis of PS in CHO cells and that this inhibition is most probably mediated by the interaction of PS, the product, with specific arginine in PS synthase 1 or 2 (Reviewed in (Kuge and Nishijima,

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2003). In case of PC synthesis, the rate limiting and thus the regulatory step is the binding of cytidyltransferase (CT) to the ER or nuclear membrane (Cornell and Ridgway, 2015). The binding is inhibited by PC and stimulated by addition of PE, diacylglycerol (DAG) ad negatively charged lipids presumably by modulating the membrane packing or curvature elastic stress or charge (Arnold and Cornell, 1996; Dymond, 2015). Notably, addition of PE, DAG or negatively charged lipids should also decrease the chemical activity of PC in the membrane which thus could the actual regulating factor in CT binding. Beside PS and PC, there is evidence that PI synthesis is inhibited by PI in rat pituitary cells (Imai and Gershengorn, 1987).

Recently, we have shown than all common GPLs when loaded to HeLa cells strongly inhibit the synthesis of the corresponding GPL (Hermansson, 2010); our unpublished data). In these studies the concentration of a GPL class was increased above its normal level, which would thus increase the chemical activity of that GPL class. In conclusion, increased chemical activity is most probably the key factor regulating the rate limiting enzymes of GPL biosynthesis. This is analogous as what has been suggested for cholesterol biosynthesis (Radhakrishnan et al., 2000; Sokolov and Radhakrishnan, 2010).

Increased chemical activity renders GPLs susceptible to hydrolysis by homeostatic phospholipases

There is strong evidence that Ca^{2+}-independent PLAs (iPLAs) are key players in homeostatic degradation of GPLs in mammalian cells (Baburina and Jackowski, 1999; Barbour et al., 1999; Mancuso et al., 2007; Manguikian and Barbour, 2004; Zaccheo et al., 2004) as discussed in more detail elsewhere (Hermansson et al., 2011). Consistently, we have recently shown that PNPLA9, -6 and -4 catalyze homeostatic degradation of PC, PE and PS in human cells (Hermansson et al., 2016). In addition, we have provided strong evidence that the activity of PNPLA9 in vitro is proportional to the propensity of its GPL substrate to efflux from the membrane (Batchu et al., 2015), which is consistent with the prediction that the active site of PNPLA9 resides well above the membrane surface (Bucher et al., 2013). Notably, the efflux propensity of a GPL molecule is proportional to its chemical activity as suggested previously for cholesterol (Lange and Steck, 2008).

GPL glass interconversion: a novel homeostatic mechanism

We have recently found that exogenous PE, PS, PI, PG and PA are rapidly and effectively converted to PC and triacylglycerol (TAG) in HeLa cells (Hermansson et al., 2010); our unpublished data). The initial step of such conversion is probably catalyzed by a PLC (-type) enzyme and the driving force is most likely an increased chemical activity of the GPL which has been loaded to the cells.

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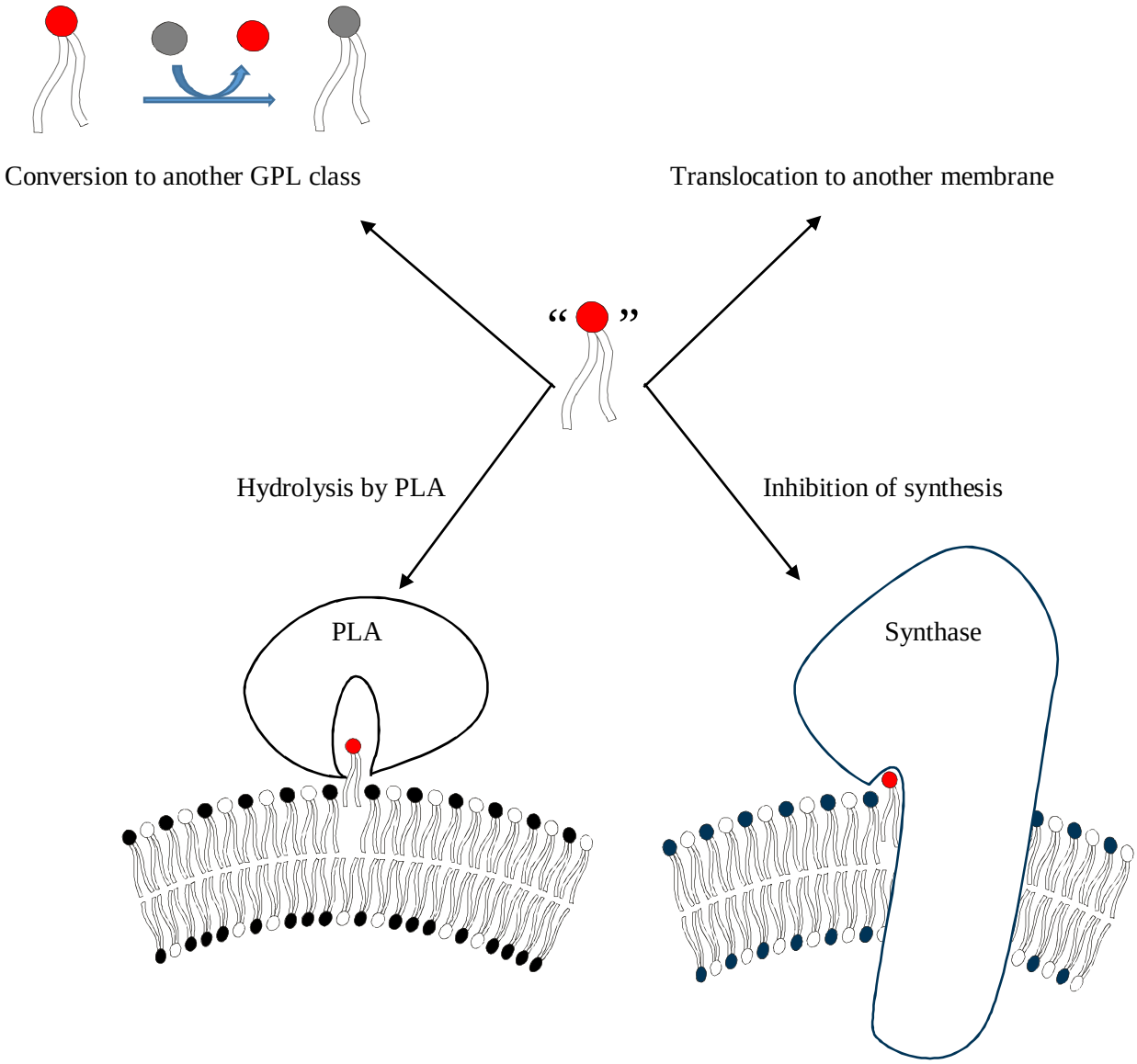

Figure 3. Multiple homeostatic events can be driven by the increased chemical activity of the GPL present in excess. As discussed in the text, the GPL molecules present in excess (red) has increased chemical activity which is predicted to (*i*) increase its hydrolysis by a PLA, (*ii*) inhibit its own biosynthesis, (*iii*) enhance its conversion to another GPL class and (*iv*) enhance its translocation to another membrane. All these events are proposed to collaborate to maintain GPL class homeostasis in mammalian cells.

Interorganelle translocation of GPLs, yet another process affected by the chemical activity

As suggested above, when the molar percentage of a GPL class increases above its optimal value, its chemical activity and thus its propensity to efflux from a membrane increases abruptly. It has been previously shown that the rate limiting step in spontaneous intermembrane translocation of a lipid is its efflux from the donor membrane (McLean and Phillips, 1984; Nichols, 1985). Notably, the efflux seems to be the rate limiting step in some protein-mediated translocation processes as well (Huuskonen et al., 1996; van Amerongen et al., 1989). In conclusion, the increase of the mole percentage of a GPL class in a membrane can result in abrupt increase of its chemical activity and, consequently, its

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intracellular translocation as has been proposed previously for cholesterol (Lange and Steck, 2008; Radhakrishnan et al., 2000).

Finally, we stress that there are also other levels of GPL compositional regulation such as those based on altered gene expression, translation or protein phosphorylation, but these mechanisms are far too slow to maintain the GPL composition in a steady state without major energy-wasting fluctuations (hysteresis). Such "coarse" mechanisms rather play role when a shift in the GPL composition is required as e.g. during mitosis, cell growth and differentiation or by altered environment (Jackowski, 1994; Murakami et al., 1992; Sanchez-Alvarez et al., 2015; Sugimoto et al., 2008).

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