# Hypothesis: Chemical Activity Regulates and Coordinates the Process Maintaining Glycerophospholipid Homeostasis in Mammalian Cells.

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Running Title: Regulation of Membrane Phospholipid Composition

**Nonstandard abbreviations**

CL, cardiolipin

CCT, CTP:phosphocholine cytidylyltransferase

DAG, diacylglycerol

GPL, glycerophospholipid

OC-model, Optimal Composition Model

PA, phosphatidic acid

PC, phosphatidylcholine

PG, phosphatidylglycerol

PE, phosphatidylethanolamine

PI, phosphatidylinositol

PS, phosphatidylserine

PLA, phospholipase A

TAG, triacylglycerol

**Abstract**

Mammalian cells maintain the complex glycerophospholipid (GPL) class compositions of their various membranes within close limits because this is essential to their well-being or viability. Surprisingly, however it is still not understood how those compositions are maintained except that GPL synthesis and degradation closely coordinated. Here, we hypothesize that abrupt changes in the chemical activity of the individual GPL classes coordinate the synthesis and degradation, as well other homeostatic processes. A previously proposed model proposed that in cellular membranes only a limited number of "allowed" or optimal GPL glass compositions exist because they are energetically more favorable than the other compositions, i.e. they represent local free energy minima (Somerharju et al. 2009). This model, however, could not satisfactorily explain how the optimal compositions are sensed by the key homeostatic enzymes i.e., the rate-limiting synthetizing enzymes and the degrading enzymes (i.e., homeostatic phospholipases). We now propose that when the mole fraction of a GPL class exceeds an optimal one, its chemical activity abruptly increases, which (*i*) increases its propensity to efflux from the membrane 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* a novel process termed "head group remodeling" or (iv) enhances its translocation to other subcellular membranes. Accordingly, abrupt changes in the chemical activity of the individual GPL classes is proposed to regulate and coordinate those four processes maintaining GPL class homeostasis in mammalian cells.

**Key words:** Homeostasis, maintenance, coordination, set point

## Introduction

Glycerophospholipids (GPLs) form the backbone of all membranes in mammalian cells and there are 7 major GPL classes in those membranes, i.e. phosphatidylcholine (PC), -ethanolamine (PE), -inositol (PI), -serine (PS), -glycerol (PG), phosphatidic acid (PA) and cardiolipin (CL). The relative concentrations of these GPLs are kept within close limits in mammalian cells (Hermansson 2011) apparently because deviations from the "optimal" composition can have dire consequences (Sousa et al. 2014; Sohn and Balla 2016; van der Veen 2017; Zhao et al. 2019; Wang and Tontonoz 2019). Remarkably, however, despite the vital importance of GPL homeostasis, the mechanisms underlying this crucial phenomenon is poorly understood, except that biosynthesis and degradation are tightly coordinated. Such coordination is demonstrated by that when the synthesis of PC was increased several-fold, its concentration in the cells remained essentially unchanged due to increased degradation (1-5). Parallel evidence has been obtained for PE and PS (1, 3, 5, 6). Conversely. when the synthesis of PC, PE or PS was inhibited, their turnover decreased correspondingly (7-11). However, there it is no information on how the synthesis and degradation are coordinated, which is a challenging task due to the presence of many GPL classes in the same membrane (***Fig. 1***). The key challenge derives from the fact that when the mole fraction (relative concentration) of a single GPL class changes, the mole fractions of all other GPL classes are simultaneously altered. Accordingly, the mechanisms controlling the mole fractions of the individual GPL classes must be acutely and accurately coordinated to maintain homeostasis. As far as we are aware, no model or theory on how the coordination is accomplished has been put forward thus far.

Here, we present a hypothesis proposing that the abrupt, composition dependent changes in the chemical activity of the individual GPL classes regulate and coordinate their synthesis and degradation thus maintaining GPL homeostasis in mammalian cells. This hypothesis, inspired by our recent and novel findings on the processes involved in GPL homeostasis in mammalian cells, represents a major extension of the previously proposed Superlattice model.

### *Superlattice model and its shortcomings*

We have previously shown that the GPL compositions of the inner and outer leaflets of mammalian erythrocyte and platelet membranes are remarkably similar to compositions predicted by the so-called Superlattice (SL) Model which proposes that there is a limited number of "allowed" GPL class concentrations which (for a ternary system) are multiples of 11.1 mol%, i.e. 11.1, 22.2, 33.3, 44.4 mol% etc. (12, 13). Accordingly, the concentrations of the different phospholipid classes tend to settle in an "allowed" mole percentage because that provides the optimal interaction between proximal molecules, i.e., a free energy minimum. This model could not, however, adequately explain how the "allowed" compositions are achieved

and maintained in the membranes of nucleated cells where the GPLs are rapidly turning over *via* metabolic and interorganelle translocation processes.

Regarding synthesis, it was proposed that when the mole fraction of a particular GPL reaches a "critical" value predicted by the model, the lateral order of the membrane increases abruptly which leads to aggregation of the respective synthetizing enzyme thus inactivating it (Somerharju 2009). Correspondingly, when the mole fraction of the particular GPL class falls below its critical value, the superlattice would collapse partially and the membrane order would drop abruptly thus reactivating of the enzyme synthetizing the particular GPL. As far a we are aware, such a model is not supported by the data published obtained thus far and, consequently, it remains speculative.

Regarding degradation, the SL-model proposed that when the mole fraction of a particular GPL exceeds a critical value, segregated lateral domains would appear and then homeostatic phospholipases, activated by poorly packed domain boundaries, would hydrolyze the GPL molecules in excess. Once the GPL in excess had been degraded, the segregated domains and the boundaries would disappear thus rendering the phospholipases inactive. A serious shortcoming of this model is that it could not explain why only the molecules in excess would be degraded by homeostatic phospholipases? In summary, it remained speculative in the SL-model proposed in 2009 how the synthesis and degradation of GPLs are regulated and coordinated so that homeostasis is maintained in growing cells in which GPLs are continuously synthetized and degraded.

Due to the shortcomings indicated above as well as recent novel data of the synthesis and degradation of GPLs (Batchu 2015, Hermansson 2016; ref 19; unpublished data) we have extensively revised the SL-model to what is now is referred to as the *Optimal Composition Model* (OC-model). The key novel aspect of the OC-model is that the *chemical activities* of the different GPLs regulate and coordinate their metabolism thus maintaining the GPL homeostasis. We hypothesize (*i*) that when the mole fraction of one GPL class deviates from that in an optimum composition, its chemical activity changes abruptly due to weakened interactions with the proximal GPLs (***Fig. 2***) and (*ii*) such abrupt changes in the chemical activities of the different GPLs regulate and coordinate multiple homeostatic process including synthesis, degradation, interconversion (head group remodeling) and interorganelle translocation of GPLs (***Fig. 3***). Below we will discuss in more detail how chemical activity could regulate each of these processes to maintain GPL homeostasis in mammalian cells.

### *Chemical activity regulates GPL biosynthesis*

Excluding PS and PC, it is poorly established what regulates the biosynthesis of GPLs in mammalian cells. Kuge and coworkers have demonstrated that PS strongly inhibits its own synthesis in CHO cells and that this

inhibition is most probably mediated by the interaction of PS with a specific arginine in PS synthase 1 or 2 (reviewed in (14)). In the synthesis of PC the rate limiting, and thus the regulatory step, is the binding of CTP:phosphocholine cytidylyltransferase (CCT) to the ER or nuclear membrane (15). The binding is inhibited by lyso-PC and stimulated by PE, diacylglycerol (DAG) and negatively charged lipids, and it has been proposed that the ratio of those lipids differently modulate the membrane packing (or curvature elastic stress) or charge of the ER membrane thus regulating CCT binding to the membrane (16, 17). Early studies have also indicated that, beside PS and PC, the synthesis of PI may also be regulated by a feed-back mechanism in rat pituitary cells (18), but the details of this process remain unclear. Recently, we have shown that loading of any common GPL to Hela, BHK-21 or CHO cells strongly inhibited the synthesis of the corresponding GPL class (19); unpublished data). The GPL molecules now present in excess in the membrane should have an increased chemical activity, which would promote their binding to the active (or putative regulatory) site in the synthetizing enzyme thus inhibiting its activity. In conclusion, the chemical activity of GPLs is proposed to be the key factor regulating the rate limiting enzymes of GPL biosynthesis via a feed-back mechanism. Previously, chemical activity of cholesterol has been suggested to regulate its biosynthesis (20, 21).

***Increased chemical activity renders GPLs susceptible to hydrolysis by homeostatic phospholipases***

There is good evidence that $Ca^{2+}$-independent PLAs (iPLAs alias PNPLAs) are the key players in homeostatic degradation of GPLs in mammalian cells (3, 4, 22-24) as discussed in more detail elsewhere (25). Consistently, we have recently shown that PNPLA9, -6 and -4 catalyze homeostatic degradation of PC, PE and PS in human cells (26). More importantly, 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 (27), consistent with the prediction that the active site of PNPLA9 resides well above the membrane surface (28). Since the efflux propensity of a GPL molecule should be proportional to its chemical activity, we propose that homeostatic degradation of GPLs in mammalian cells depends on their chemical activity.

***Chemical activity drives GPL glass interconversion***

We have recently found that exogenous PE, PS, PI, PG and PA are rapidly and effectively converted to PC when loaded to HeLa cells (29); unpublished data). Notably, blocking of fatty acyl-CoA formation with Triacsin C had no effect on the conversion to PC thus excluding the possibility that deacylation/reacylation of the GPL precursor is involved in the process. Extensive knock-down studies indicated that different enzymes (e.g. PLCs) catalyze the initial, committing step of the interconversion or ”head group remodeling”. Since loading of an exogenous GPL to the cells should greatly increase the chemical activity of the respective GPL, it is most likely that chemical activity drives the conversion. A particular benefit of such novel homeostatic

process is that it requires far less input of cellular energy than biosynthesis *de novo,* simply because the fatty acids need not to be activated to CoA derivatives.

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

As suggested above, when the molar fraction of a GPL class increases above its optimal or critical 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 (30, 31). While spontaneous intermembrane translocation of lipids is often considered negligible, this does not apply to all lipids as their hydrophobicity varies by orders of magnitude (32). Notably, efflux from the donor membrane seems to be the rate limiting step in protein-mediated translocation processes as well (33, 34). It is also worthy to note that interorganelle translocation of a GPL is coupled to its biosynthesis, since the translocation affects the concentration of that GPL both in the donor and acceptors membranes and thus the efficiency of feed-back inhibition of biosynthesis in the donor or acceptor membrane. In conclusion, if the mole fraction of a GPL in a membrane increases, its chemical activity and, consequently, its intracellular translocation is also likely to increase as has been previously proposed for cholesterol (20, 35).

***Other modes of regulation***

Finally, we stress that beside the one proposed here, there are also other mechanisms that regulate GPL composition of mammalian cells, such as those based on altered gene expression or translation. However, those mechanisms are far too slow to acutely regulate the GPL composition without energy-wasting fluctuations (hysteresis). Those "coarse" mechanisms are rather involved when a change in GPL composition is required as e.g., during mitosis, cell differentiation or by altered cellular environment (2, 36-38). In principle, protein phosphorylation (or other modifications) could play a role in acute regulation of GPL compositions since those processes can take place rapidly and can influence protein activity (39-42). However, this mechanism requires the existence of proteins that accurately "sense" the change in the GPL class composition of the membrane. As far as we are aware, no such proteins have been identified in mammalian cells so far. Notably, even if such sensor proteins do exist, they as well are likely to respond to variations in the chemical activity of the different GPL classes. In conclusion, abrupt variations in the chemical activity of the individual GPL classes is most probably the primary factor regulating GPL homeostasis.

***Acknowledgements***

We acknowledge the funding from Finnish Academy and Sigrid Juselius Foundation to P.S.

***Author contribution***

P. Somerharju, J. Virtanen and M. Hermansson wrote the paper.

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## Figure Legends

***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.

***Figure 2. Deviation from an optimal composition brings about several 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% (12). Note that at this composition there are no proximal (strongly repelling) negatively charged GPLs. On the right: If a (zwitterionic) GPL molecule is replaced by a negatively charged one, the chemical activity of 3 or 4 negatively charged GPL molecules is greatly increased due to electrostatic repulsion between the proximal negatively charged GPLs. If the mole fraction of an zwitterionic GPL increases above its optimal value (not shown here), its chemical activity is predicted to increase due to weakened van der Waals or hydrogen bonding interactions with its neighbors, or steric strain.

***Figure 3. Multiple homeostatic events can be driven by increased chemical activity of the GPLs 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 with a different head group (= head group remodeling) or (*iv*) enhance its translocation to another membrane. All these events are may occur simultaneously to maintain GPL class homeostasis in mammalian cells.

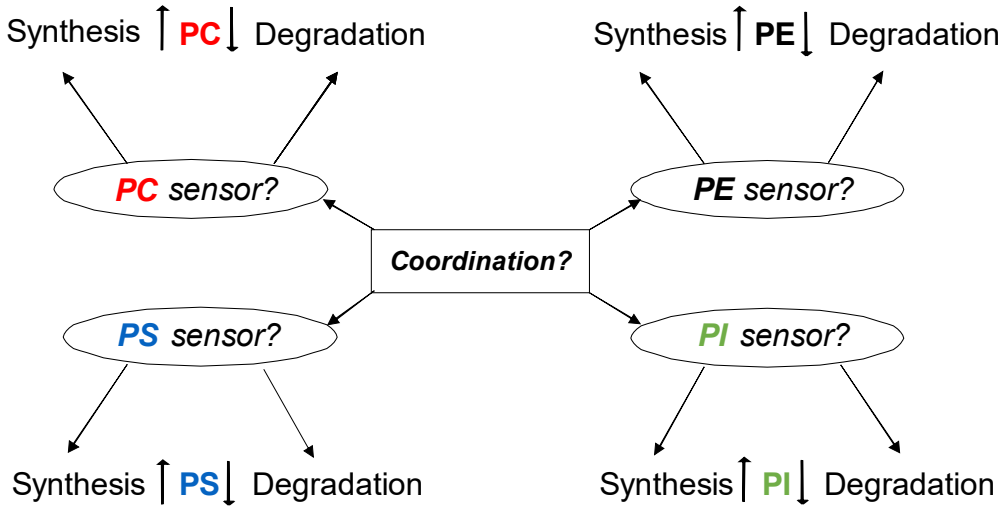


Figure 1.

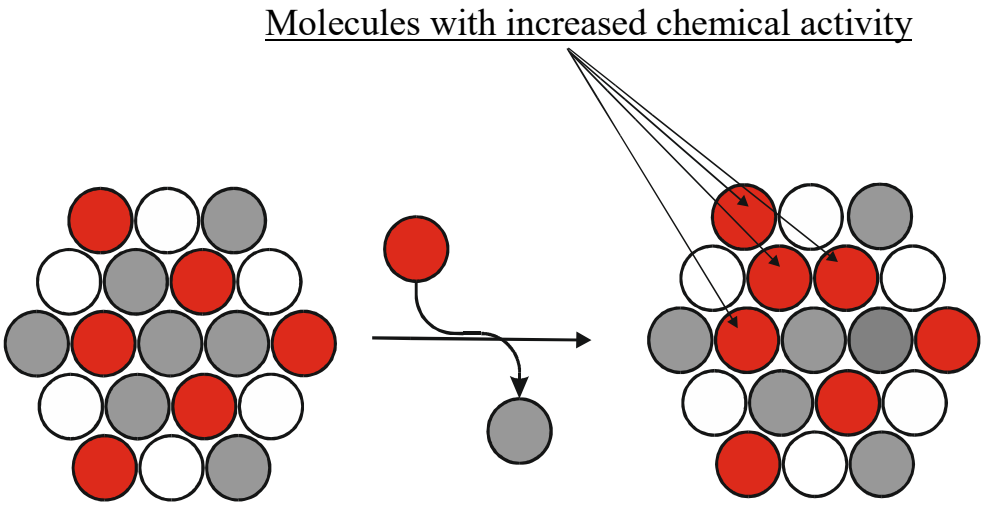


Figure 2.

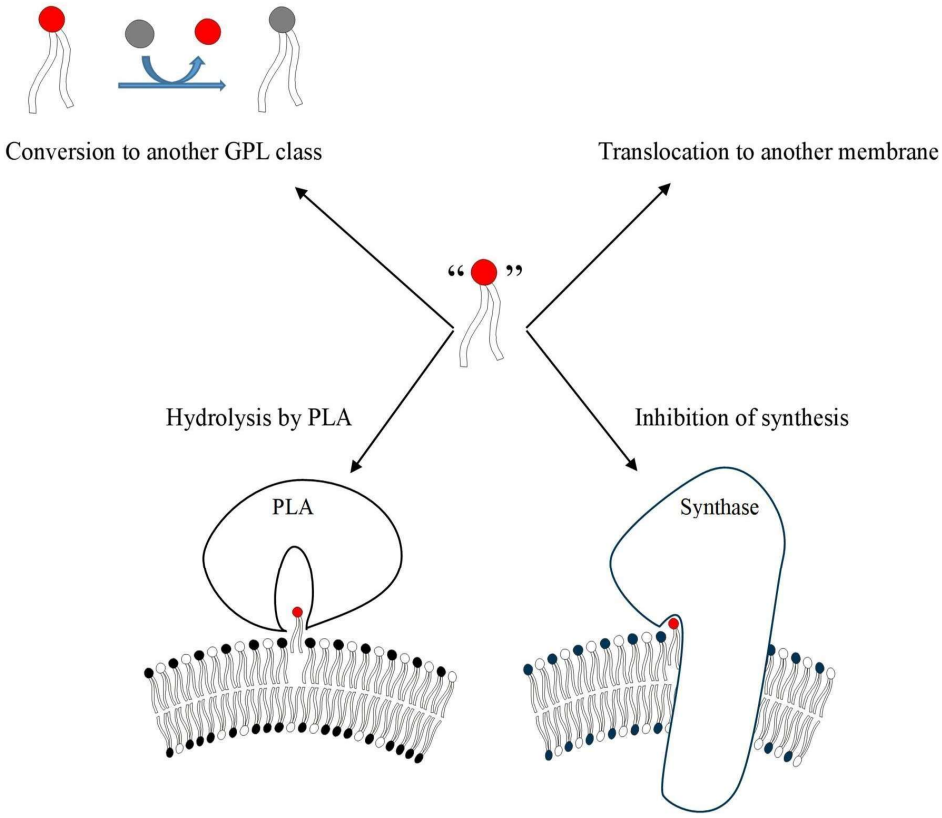


Figure 3.