AIMS Molecular Science, 7(3): 211–228.
DOI: 10.3934/molsci.2020010
Received: 25 March 2020
Accepted: 15 June 2020
Published: 18 June 2020

http://www.aimspress.com/journal/Molecular

*Review*

# Phospholipid synthetic and turnover pathways elicited upon exposure to different xenobiotics

**Teresa M. Fonovich**[1,2,*]

[1] School of Science and Technology, National University of San Martín, San Martín, Buenos Aires Province, Argentina
[2] Laboratory of Ecotoxicology, Institute of Research and Environmental Engineering, National University of San Martín, San Martín, Buenos Aires Province, Argentina

* **Correspondence:** Email: tfonovich@iib.unsam.edu.ar; Tel: 541140061500.

**Abstract:** Phospholipids, neutral lipids and glycolipids metabolism take the place during cell responses to different stimuli. Phospholipase and acyl glycerol lipase activities have been demonstrated to release second messengers that trigger cascade responses. Studies on lipid droplets structure, formation and interaction with other organelles have recently been described in prokaryotes and eukaryotes. Remodeling of membrane phospholipids has also been reported. NMR studies performed on synthetic membranes allowed to postulate membrane lipids polymorphism and to explain how processes like cell division, endocytosis and exocytosis can take place, due to special arrangements of the membranes. Studies from our research group on the pesticide dieldrin and $Cu^{2+}$ effects through exposure of amphibian oocytes and embryos to sub-lethal and acclimation concentrations followed by toxic concentration challenges respectively, are discussed. Membrane phospholipids structure alterations allowing stabilization of bilayer arrangements were found for both stressors in cell and tissues. Metallothionein induction response that prevents oxidative stress was also found in acclimated embryo tissues. Probable connections between enzyme activities taking place on lysophospholipid and triacylglycerol substrates present in lipid droplets as well as phospholipid trafficking leading to modifications on membrane phospholipid ratios are discussed. Current new evidences in agreement with our findings allow us to suggest that our previously published results of dieldrin effects on amphibian cells correspond to joint coordinated activities, which probably had involved various metabolic pathways, in line with the acclimation experiment results and discussion. Independently of the cascade

responses each toxicant is able to elicit, lipids play an important role in cell responses, both through rapid turnover and final stabilization of membrane bilayers.

**Keywords:** phospholipid; triacylglycerol; lipid droplet; lipid polymorphism; membrane; dieldrin; $Cu^{2+}$

## 1. Introduction

The first approach to the study of lipids commonly divides them into two major categories, according to two major functions in the cell or tissue: phospholipids (glycerophospholipids and sphingomyelin (SPH)) as integral components of cell membranes and neutral glycerides as energy storage molecules. Other lipids that can be found in living organisms are often called "lipids containing other functional groups".

Membrane protein activities lay both on individual interactions with lipids according to their characteristics and on mechanical forces such as lateral pressure or membrane curvature stress, which depend on specific lipids content but not on direct interaction with them. Some reported examples are phosphatidylethanolamine (PE) induced physical curvature stress, which can fully activate conformational changes in rhodopsin [1] and local membrane curvature induced by surface topography resulting in actin reorganization, through curvature-sensing protein FBP17 activity [2].

In mammalian cell membranes, cholesterol is the key to their adaptation to the need for changes in fluidity, rather than rapid incorporation of more saturated or unsaturated fatty acids as occurs in bacteria [3]. Lipid rafts have more recently been described as specialized supramolecular structures containing lipids and integral proteins, with large amounts of SPH and cholesterol that also contribute to the best environment for proteins activity [4,5]. Phospholipid polymorphism has contributed in recent decades to the knowledge of changes in the structure of the membrane that take place during processes like cell division, endocytosis, exocytosis, etc.

De novo synthesis and turnover pathways had generally been studied as separated events and especially considering to design protocols according to the duration of the responses and the stimuli eliciting them. However, a variety of new evidences describe reactions that take place between many membrane phospholipids and other polar and non-polar lipids. In addition, de novo synthesis and turnover pathways do not appear to be as clearly separated events. The aim of the present review is to provide a renewed insight into various mechanisms of phospholipid and neutral lipid metabolism, with a special focus on the activities of phospholipases and acyltransferases as well as on lipid droplets and their roles. Additional attention is paid to explore the possible coordinated responses that take place on protein activities and lipid metabolism triggered to decrease different toxic effects of xenobiotic substances.

## 2. Phospholipids synthesis and turnover

Different authors have extensively described de novo synthesis of phospholipids and neutral glycerides from glycerol-3-phosphate, as well as common pathways between different organisms, from prokaryotes to multiple cell organisms, including plants and vertebrates, describing phosphatidic acid

(PA) as an intermediate of both types of lipids [6]. In fact, PA is a minor phospholipid in most of the living organisms, located in the middle of various synthetic routes, which hydrolysis can rapidly be activated in response to different stimuli. Yeasts share some lipid synthesis pathways with bacteria as PE and phosphatidylglycerol (PG) ones, which depend on cytidine diphosphate-diacylglycerol (CDP-DAG) formation, keep cardiolipin (CL) synthesis as the rest of eukaryotes and also utilize the mammalian pathways for the synthesis of phosphatidylinositol (PI), PE and phosphatidylcholine (PC). Modifications of the polar head functional groups from phosphatidylserine (PS) to render PE and afterwards PC, and a common pathway from PA to diacylglycerol (DAG), in *Saccharomyces* [7] as well as in mammalians cells, had been demonstrated in the past and recently corroborated [8]. The Kennedy pathway involves DAG formation from PA and its reaction with CDP-choline or CDP-ethaolamine to form PC and PE. On the other hand, the CDP-DAG pathway consists on the reaction between these molecules (also formed from PA) with polar head groups which were not previously activated, especially for PI and PS synthesis.

Alterations in the pathways involving lipid polar head modifications have been reported as a consequence of the exposure of tissues or cells to different stressors.

Biocide tributyltin (TBT) effects on *Cunninghamella elegans* revealed modifications in phospholipids content originated both in shifts on the CDP-DAG pathway (increased PI/PE ratio) and in phospholipase D (PLD) activation rendering and increase in PA concomitantly with decreased PE content [7]. Carman and Han [9] have recently demonstrated that this last pathway is fully activated also for the synthesis of PE and PC in yeasts (used as a model of eukaryotic cells), which were grown without choline or ethanolamine. These authors also described how the synthesis of triacylglycerol (TAG) and phospholipids is regulated by the enzyme phosphatidic acid phosphatase (PAP), through directing the synthesis towards TAG. In turn, when PAP is not active and PA accumulates, the expression of PS synthase is activated through CHO1 expression, redirecting the synthetic process to the formation of PS, PE and PC. Zhang et al. [10] also studied PAP commonly called lipins (1, 2 and 3). They found that lipins 2/3 are responsible for enterocyte TAG formation and targeting to chylomicrons, and that their deficiency causes PA accumulation as well as altered membrane phospholipid composition.

Phosphatidylinositol 4,5-bisphosphate ($PIP_2$ or $PI4,5P_2$) is a lipid mediator whose rapid hydrolysis had been recognized to take place by phospholipase C (PLC) activation, after various stimuli on the plasmatic membrane of several living organisms, which generate the soluble second messenger inositol triphosphate ($IP_3$) and 1,2-diacylglicerol (1,2-DAG) [11]. We had studied in amphibians both $PIP_2$ and phosphatidylinositol 4-monophosphate (PIP or PI4-P) turnover, as well as their alteration due to exposure to pesticides [12,13]. Other localizations and functions have been recently discovered for different inositol lipids. Phosphatidylinositol 3-monophosphate (PI-3P), PI4-P and phosphatidylinositol 3,5-bisphosphate ($PI3,5\text{-}P_2$) are concentrated in endosomes and Golgi membranes. Binding PI4-P to GOLPH3 protein participates in Golgi function during cell migration processes. $PI3,5\text{-}P_2$ formation from PI3-P is regulated by myotubularin and a myotubularin related protein and plays an important role in autophagy. Not only $IP_3$ formed form $PI4,5\text{-}P_2$ but also $PI3,5\text{-}P_2$ response to external stimuli, producing PI, are involved in $Ca^{2+}$ mobilization from intracellular stores. Phosphatidylinositol 5-monophosphate (PI5-P) can be formed from $PI4,5\text{-}P_2$ and $PI3,5\text{-}P_2$ and is an important effector during nuclear membrane signaling and dynamics [11].

A minor pool of PC molecules can also be hydrolyzed by PLC activation allowing the formation of 1,2-DAG and soluble phosphocholine and by the activity of PLD liberating PA and choline. It was

demonstrated that different agonists were able to elicit those responses. As an example, we had demonstrated dieldrin effect on in vitro PLC mediated hydrolysis of PC [12]. Nishio et al. [14] had found a 200% increase in 1,2-DAG content in human lung adenocarcinoma cells treated with *cis*-Diamminedichloro,Platinum(II)(CDDP) and at the same time, 2,5 folds increase in PC specific PLC activity.

Although the replacement of phospholipids by sulpho and galactolipids had been demonstrated in plants, as adaptive processes on phosphate-limiting soil, and PA and DAG formation had also been described under such conditions, it was not clear how DAG was formed [15,16]. Sulfoquinovosyl diacylglycerol, ornithine-containing lipids, and diacylglyceryl trimethylhomoserine (DGTS) are phosphorus-free lipids formed in *Sinorhizobium meliloti*. These molecules replace phospholipids and allow inorganic phosphate released from this source, to be used for the synthesis of other molecules, under phosphorus deprivation conditions. Zavaleta-Pastor [17] have worked on this issue and has described membrane lipid remodeling from PE and PC in *Sinorhizobium meliloti*, due to PLC activity under phosphate limiting conditions, leading to the formation of diacylglyceryl-homoserine (DGHS) and DGTS.

## 3. Phospholipase, triacylglycerol lipase and acyltransferase activities exchanging fatty acid moieties

Phospholipases A (PLAs) activities and their physiological roles in signal transduction pathways had been extensively described. Early studies described phospholipase $A_2$ ($PLA_2$)-mediated release of arachidonic acid from the ester union at the sn-2 position of the glycerol moiety of phospholipids, and posterior synthesis and secretion of prostaglandins, leukotrienes or thromboxanes. These mediators secretion constitute well-known mechanisms involved in cell-cell communication [18]. Phospholipase $A_1$ ($PLA_1$)-mediated production of lysophospholipids and fatty acids have been shown to be conserved in diverse organisms and lysophospholipids have been demonstrated to have multiple functions in different tissues. Phospholipase B (PLB) is the name given to the enzyme that is capable of hydrolyzing fatty acids from both positions (sn-1 and sn-2). However, its activity is sometimes considered as the sum of a PLA activity followed by a lysophospholipase one. On the other hand, some activities described at the beginning as PLA ones, later have been shown to hydrolyze fatty acids from both sn-1 and sn-2 glycerol positions (outer-membrane PLA from *E. coli* and other bacteria) [19]. The activities of PLA and B in Fungi have been reported not only to participate in signal transduction, but also in cell-cell communication. $PLA_2$ released from cells can play roles in nutrient acquisition and tissue invasion and also in the modulation of host´s immune response [20].

In an attempt to understand most probable effects of the pesticide dieldrin both on de novo synthesis and turnover of major phospholipids, we had exposed amphibian oocytes to the pesticide, after they had been obtained from adult females injected one day before with $^3$H-glyceride or $^3$H-palmitic acid together with hormones that induced ovulation. Our results lead us to suggest that the pesticide inhibited de novo synthesis of all phospholipids, without affecting neutral lipids. At the same time, exposure to dieldrin was apparently responsible of a transacylation process that took place from TAG to phospholipids, producing a significant increase in PC and PE $^3$H-Palmitate labeling. An in vitro assay which demonstrated that lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) were efficiently acylated in the presence of the pesticide and microsomal fraction obtained from amphibian

oocytes, leads us to suggest that these lysophospholipids could be the substrates for the transacylation pathway involving the activity of a triacyl glycerol:phospholipid acyl transferase that we found after in vivo treatment of the oocytes [21]. Lysolipids are minor constituents in most cells. However, the presence of lysophosphaditic acid (LPA) has been reported to be essential during reproduction of different species; through activation of its G protein coupled receptor LPAR 1–6 [22,23]. The cellular activities controlled by LPA signaling are diverse, including proliferation, cell motility, chemotaxis, tumor invasion, gap-junction closure, tight junction opening, etc. [24]. Lysophospholipids importance in plants has also been described. Jasieniecka-Gazarkiewicz et al. [25] have recently demonstrated in *Arabidopsis* the contribution of lysophosphatidylethanolamine transferase 1 and 2 activities to plant growth, by supplying adequate amounts of PE and PC from LPE and ether analogs of LPC respectively.

## 4. Phospholipid polymorphism

The polar lipids polymorphism was described some decades ago, based on $^{31}$P-NMR spectra obtained from aqueous dispersions of different amounts PC and PE, subjected to variations in temperature, $Ca^{2+}$ concentration and other conditions [26], and confirmed lately in living cells. While PC, SPH, PS and PG were reported as molecules that favor the stabilization of the bilayer structure of artificial membranes; lysolipids favored micelle ones and PE, CL+$Ca^{2+}$ and PA+$Ca^{2+}$ (or these phospholipids at lower pH) lead to the formation of non-bilayer structures (HII hexagonal arrangements or cubic or rhombic ones). The emergence of these nonbilayer arrangements was postulated to take place in membranes from living organisms at the time when processes involving membrane invagination, such as cell division, membrane fusion and vesicle formation, occur.

Non-bilayer bacterial lipids such as PE with unsaturated alkyl chains and CL in the presence of divalent cations were also described. At the same time, the non-bilayer neutral lipid monoglucosyl diacyl glycerol (monoglucosyl-DAG) was reported, and diglucosyl diacyl glycerol (diglucosyl- DAG) was described to assume liquid ordered lα or lβ phase. In addition, wild type *E. coli* has been shown to adjust PE fatty acid content to increase or decrease its non-bilayer potential, being it higher when unsaturated fatty acids are more abundant [27].

Mitochondrial membranes contain all major classes of phospholipids. Inner membrane exhibits large amounts of non-bilayer phospholipids CL and PE and a highly conserved ratio of bilayer to non-bilayer phospholipids in most eukaryotic cells. The amounts of individual phospholipids vary according to different conditions; nevertheless, total amount of all of them does not change, allowing preservation of the membranes [28].

Following this line, Baker et al. [29] worked with yeasts mutants for PC and PE biosynthetic pathways and reported that, unlike CL, these phospholipids were not required for the formation of mitochondrial respiratory chain (MRC) supercomplex and that only non-bilayer forming PE but not bilayer forming PC was required for MRC complex III and IV activities. More recently, CL dependent non-bilayer structures that are formed in the inner membrane of mitochondria have been suggested to be generated and remodeled by cardiolipin-F0 (CL-F0) complexes in intact mitochondria [30]. Based on the $^{31}$P-NMR spectra obtained working on isolated mitochondrial fractions or multilamellar liposomes, and $^{1}$H-NMR spectra of unilamellar liposomes, these authors have proposed a new model for ATP synthase regulation by CL through non-bilayer structures which enhance proton translocation to the F0 sector.

All the enzymes involved in PE and CL synthesis and trafficking to mitochondrial inner membranes have been described. The classic Kennedy´s pathway taking place from cytosol and ER followed by translocation to mitochondria was described for PE. On the other hand, immature CL synthesis occurs in the mitochondria involving PA imported from ER through sequential activities of phosphatidylglicerol phosphate synthase Pgs1, phosphatidylglicerol phosphate phosphatase Gep4 and Crd1 cardiolipin synthase enzymes. Subsequently, the CL molecules reach maturity as the consequence of remodeling of its fatty acids moieties, through sequential activation of a CL-specific deacylase Cld1 and a transacylase Taz1 [28].

We have recently studied arachidonic acid incorporation into different phospholipids present in amphibian embryos which were previously acclimated to very low concentrations of copper and were then challenged with higher concentrations of the same metal [31]. Acclimated embryos showed low incorporation of arachidonic acid in the acidic phospholipid PA and in PE, which tend to allow the formation of hexagonal arrangements thus being classified as “non-bilayer” phospholipids, along with large incorporation in SPH, PS and PC, well-known “bilayer” ones. PC/PE radioactivity ratios increased from 0.49 ± 0.05 in Control embryos to 1.38 ± 0.24 and 1.03 ± 0.20 in two different acclimation conditions. Taking these phospholipid polymorphism results together with metallothionein (MT) induction, which we also found in the same experiments, we have concluded that metal buffering ability and the fatty acids exchange of membrane must had protected the acclimated organisms when they were challenged. Similar results were obtained by Bernat et al. [7], who found changes in PC/PE concentration ratios in *C. elegans* of 1.42 to 1.93 for control and TBT treated organisms, respectively.

Unlike specific transacylation with arachidonic acid into PC and not into non-bilayer phospholipids that we have recently described, due to acclimation to copper [31], our previous findings demonstrated unspecific transacylation from TAG to phospholipids, with increasing activity towards palmitic acid incorporation in PC, PA and PI, but also and to a lesser extent towards the formation of transacylated PE [21]. It should be useful to note, at this point of the discussion, that both fatty acids may certainly have opposite effects as the consequence of their incorporation in PE. Arachidonic acid is a poliunsaturated fatty acid which certainly enhances the non-bilayer potential of this phospholipid. On the contrary, palmitic acid is a saturated one and contributes to decrease PE participation in the formation of non-bilayer arrangements in membranes. At the same time PC bilayer potential may surely be reinforced due to palmitic acid incorporation. Both works published from our group, on major phospholipid fatty acids turnover [21,31], were based on experiments performed in whole cells and organisms; also the last one did not include neutral lipid analysis. Future studies should consist on experiments considering lipid status in different organelles, including lipid droplets (LDs).

## 5. Recent advances on the study of LDs

LDs had been recognized in different tissues by electron microscopy for decades and they were at first described to be formed exclusively by neutral lipids. Their real composition as well as their formation pathways have been recently studied and most precisely described [32–35]. A polar lipids coat surrounds LDs, which can be placed both at the cytoplasm and inside plastids present in photosynthetic organisms. Some proteins are integral component of that monolayer coat consisting predominantly on phospholipids, so they somewhat resemble the apolipoproteins present in mammalian

plasma lipoproteins. Storage of neutral lipids in LDs contributes not only to provide energy needed for survival, but also to cell division, growth and stress response.

### *5.1. Unicellular organisms*

While the majority of prokaryotes produces and stores polyhydroxyalkanoates (PHA) comprising specialized lipids such as poly(3-hydroxybutyrate) (P3HB), polyhydroxyvalerate (PHV), or copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate); some of them and eukaryote organisms accumulate TAG. Reactions leading to TAG de novo formation take place in different compartments of unicellular organisms.

In the case of yeasts, TAG is synthesized between the two membrane leaflets of endoplasmic reticulum, where LPA is transformed in PA due to incorporation of a fatty acid that was synthesized in the cytosol, in a reaction catalyzed by acyl-CoA:lysophosphatidic acyltransferase (LPAAT). PA loses its polar head and is then transformed in DAG. A new acylation step transforms DAG molecules into TAG ones, which are immediately accumulated in nascent lipid droplets, that are surrounded by phospholipids and recruit proteins as perilipin, adipocyte differentiation-related protein (PAT) and tail-interacting protein of 47 kD (TIP47). The abundance of phospholipids in LDs is different from the one in ER, due to a process called “demixing”. It has been confirmed that LDs contain more lysophospholipids and less SPH and PA compared to the total membrane [32]. PI and LPC are phospholipids that stimulate convex surfaces formation and are abundant in LDs, where they mix with the bilayer phospholipids PC and PS. On the other hand, phospholipids that stimulate concave surfaces formation (PA and PE) accumulate and form hexagonal arrangements at the point of droplet excision from the ER.

A remarkable characteristic of neutral lipid accumulation in microalgae is that these organisms synthesize TAG during the light period and utilize them in the dark, to obtain ATP. LD formation takes place both in the ER, as in yeasts, and within the plastid where they can grow facing the cytosol or toward the inside of the plastid, facing the stroma and are called plastoglobules (PTG). Specific family of proteins termed plastoglobulins, plastid lipid associated proteins, and fibrillins were found to be bound to PTG. Other proteins have been identified in microalgae LDs [32].

Bacteria accumulate neutral and polymeric lipids such as TAG, PHA, wax esters (WE) and sterol esters (SE). Most of them accumulate either TAG or PHA, but both neutral lipids are not found together. TAG is present only in aerobic heterotrophic bacteria and in cyanobacteria.

Alternative pathways for TAG synthesis do not involve DAG and the formation of TAG occurs from the transesterification of an acyl donor (e.g., acyl-CoA) to phospholipids. LD biogenesis may probably begin by the formation of very small triacylglycerol agglomerates that eventually apparently recruit phospholipids from the membrane to form a layer toward the cytoplasmic side. It has been reported in Rhodococcus sp LDs the presence of two major proteins, ro02104 and PspA, constituting about 15% of the total LD proteins [32].

### *5.2. Interactions between LDs and nuclei and LDs and mitochondria*

New evidence for LDs present in the nucleus has been reported. Both the presence of them inside the nucleus as well as LDs from the cytoplasm exerting activities through sequestering transcription factors, enzymes, and chromatin components were suggested to be involved in the control of different

molecules concentration in the nucleus. The LD protein Fsp27, expressed in adipocytes, promotes fusion between droplets, causing the formation of a single droplet per cell and interacts with the transcription factor NFAT5 (Nuclear factor of activated T cells). These recent investigations suggest the need for evaluation of the interaction between Fsp27 and NFAT5 regulation, for example by signaling pathways controlling lipolysis [33]. In Drosophila embryos, LDs are associated with large amounts of specific histones. It has been proposed that this sequestration allows the organism to build up histone stores during oogenesis and keep them available for later chromatin assembly [36]. Li et al. [37] have also reported LDs to affect histone metabolism in the short term, by buffering the histone supply. These findings may probably be confirmed in other species as histones have been detected on LDs in housefly embryos, rat sebocytes, and mouse oocytes [33].

Oxidation of fatty acids in the mitochondria of mammalian cells was postulated to occur through the utilization of fatty acids present in LDs and/or in membranes. Recent studies have demonstrated that LDs are the principal source for these fatty acids oxidation inside the mitochondria. The rapid relocalization of fatty acids to mitochondria is thought to take place through direct transfer as LDs and mitochondria display close physical associations. Moreover, direct channeling of fatty acids from their site of release (droplets) to the site of consumption (mitochondria) may probably minimize the risk of toxic effects in the cells [33]. A promoter of mitochondrial fusion is important for LD formation and steroid signaling in Drosophila. Efficiency of lipid exchange between mitochondria, ER, and LDs is related to mitochondrial associated regulatory factor (Marf), the fly ortholog of mammalian mitofusins activity, which is a small GTPase that promotes fusion of the outer mitochondrial membrane. The ring gland, an endocrine tissue responsible for hormone secretion, contains LDs. Ring gland LDs receive sterols from the ER and store them as steryl esters; which are the precursors for the production of the steroid hormone ecdysone, in the mitochondrial matrix [38].

## 6. Signaling pathways involving TAG and phospholipids. Cell responses to the highly hidrofobic pesticide dieldrin and to oxidative stress caused by heavy metals

LDs have recently been reported to have vast roles in a number of cellular processes [34,35], including lipid trafficking between organelles inside the cell, lipid exchange between cells in the same tissue and production of lipid-derived molecules such as lipoproteins or hormones [33,39].

Besides transesterification reactions taking place from phospholipids to TAG that have recently been described in bacteria [32], we have described above our findings on transacylation reactions taking place to form phospholipids from lysophospholipids and TAG, in oocytes exposed to the pesticide dieldrin [21]. Additionally, both the concentration of fatty acids (evidenced through separation under thin layer chromatography) as well as $^{3}$H-palmitate radioactivity present in MAG fraction increased in dieldrin treated oocytes, thus indicating important TAG hydrolysis that exceeds transacylation processes. Also, in our studies on phospholipids esterification with arachidonic acid, not only did the PC/PE radioactivity ratio in Cu acclimated embryos rise, but also the sum of them (PC+PE) had increased, when compared to control embryos. That analysis of the results allows us now to suggest that not only could a possible transcacylation process between PE and PC has occurred, but also the synthesis of these phospholipids from lysophospholipids present in LDs could have occurred, which denotes the involvement of TAG in the remodeling of phospholipids [31]. A new insight on those hydrolysis and transacylation reactions between neutral and polar lipids together with recent advances, conduct us to

suggest LDs as the specialized organelle where these reactions can take place. Those results are also consistent with the trafficking of newly synthetized LD phospholipids to different cell membranes, according to the need of cells and tissues to prevent more damages.

On the other hand, MAG and DAG formed during that process as well as excess of released fatty acids may certainly act as second messengers through activation of transcription cascades. Peroxisome proliferator-activated receptors (PPARs) had been reported to be the receptors involved in a signaling cascade that plays important roles for lipid homeostasis, upon their activation by natural ligands fatty acids and MAG. Their roles are limited to specific tissue types through differential expression of isoforms PPARα, PPARγ and PPARβ/δ [33,40]. PPARα has been reported to decrease lipid levels while PPARγ regulates lipid biosynthesis and PPARβ/δ is involved in fatty acid oxidation as well as the regulation of glucose and cholesterol levels in blood [40]. On the other hand, 2-arachidonoylglycerol, which is a 2-MAG has been reported as an endocannabinoid ligand, interacting with CB1/2 receptors from the endocannabinoid system (ECS), and other cellular targets such as mammalian unc13-1 (Munc13-1), PPARs and the target for anti-diabetic drugs GPR119 [41]. Both endocannabinoid ligands 2-MAG and anandamide are rapidly formed on demand for cellular processes. Regulation of 2-MAG concentration is carried out by serine hydrolase α/β-hydrolase domain 6 (ABHD6) activity [41]. Nevertheless, some authors suggest that some tissues may contain preexisting amounts of both of them accumulated in LDs [42]. ECS is also known to impact the female reproductive system affecting folliculogenesis, oocyte maturation, and ovarian endocrine secretion [42]. Fan et al. [43] worked with *Xenopus* oocytes as a model for nuclear microinjection and expression. They reported that hawthorn flavonoids and prostaglandin E1 (PE1) increased the expression of endogenous lipoprotein lipase in microinjected oocytes and they also found that the effect was markedly enhanced by injection of exogenous PPARγ. The authors then concluded that there is an endogenous peroxisome proliferator response element regulatory system in *Xenopus* oocytes, which could respond to exogenous PPARγ. Nevertheless, it must be noted that PPARγ is naturally absent during early embryonic development of amphibian fertilized oocytes. On the contrary, PPARβ has been originally identified in that species and stages. Rotman et al. [44] detected PPARβ protein in all cell nuclei throughout embryogenesis, with a strong increase during gastrulation. They analyzed the impact of PPARβ on the transcriptome by RNA-seq and identified PPARβ as a major promoter of differentiation *in vivo* and reported this transcription factor as the one controlling the expression of the majority of the genes, whose RNA level varies between the cleavage and gastrula stages. Michalik et al. [45] had previously described the absence of PPARγ mRNA in early stages of development and its presence in significant amounts in post-embryonic stages. They found a restricted pattern of this transcription factor expression mainly in the adipose tissue, and also in the kidney, and the liver. At the same time, they reported that PPARα and PPARβ are expressed ubiquitously in adult *Xenopus* organs (testes, liver, kidney, fat body, muscle, brain, spleen). Michalik et al. [45] also described rodent and human PPARs functions both during embryogenesis and in adult organisms, and discussed their importance in several vertebrate physiological pathways, such as the control of the inflammatory response and the maintenance of energy homeostasis.

Results from our laboratory demonstrated a similar step in cell responses to completely different stimuli, like dieldrin and $Cu^{2+}$, through rapid changes in phospholipid structures. Oocytes exposed to dieldrin incorporated the pesticide [46] and underwent to PLC activation and the formation of fertilization membrane [47], thus probably blocking sperm entry and leading to a decreased fertilization

rate [48,49]. On the other hand, heavy metals also produced alterations on reproduction of amphibian oocytes probably due to oxidative stress.

We have also investigated Cd and Cu effects on the inhibition of pentose phosphate pathway enzymes [50] and Zn effect [51–53] on the same enzymes, also modifying glutathione content in amphibian ovary, at concentrations able to inhibit oocytes fertilization. We have concluded that high-molecular-weight proteins, like glucose-6-phosphate dehydrogenase, should be able to bind Zn, leading to oxidative stress that conducted to the observed increase in endogenous glutathione content [52].

In accordance to our those previous findings, we decided to evaluate the possible alterations that take place on lipid polymorphism such as the changes in the fatty acyl phospholipids residues described above, as well as MT content during acclimation of toad embryos to Cu. Our findings could explain at least in part the protective effects exerted after challenging acclimated embryos to lethal concentrations of metals. These results also allow us to suggest that the aforementioned responses occur also at oxidative stressor concentrations that acclimate and protect embryos instead of producing adverse effects [31]. They also are in consonance with the ones demonstrated by Rotiskaya et al. [54] on the protective effect of double bonds in lipids on oxidative damage to membrane proteins caused by boronated chlorine e6 amide and tert-butyl hydroperoxide and Kim et al. [55] on antioxidant effect of PC supplemented in vivo to *Caenorhabditis elegans.*

## 7. Lipid mediators in multiple cell responses to single toxic challenges

Summarized cellular responses involving lipid mediators can be seen in the figures. Both oocytes that were exposed to a single toxic challenge with the organochlorinated pesticide dieldrin [21] (Figure 1), and embryos subjected to acclimation protocols to $Cu^{2+}$ followed by unique toxic challenges at different concentrations, with the same heavy metal (Figure 2), are illustrated. Results from our research works as well as bibliography whose findings are in agreement with ours are presented. Based on our results reported in Fonovich et al. [31] we have previously discussed the possible existence of different molecule responses to acclimation, providing protection to embryos against unique toxic challenges, through changes in phospholipid polymorphism [26,27] and MT induction, as could be observed through the increased survival rates. A variety of published articles reinforce our findings [7,10,40–42,55]. In the light of them, we can now suggest that those seemingly unconnected cellular responses may be part of coordinated joints, involving lipid and carbohydrate metabolism, glutathione and MTs protective effects as well as transcription and translation events elicited by second messenger's generation in LDs. The participation of membrane lipid molecules as mediators in response to exposure to xenobiotic substances can also be observed now as the sum of processes taking place from the beginning of the insult, as rapid lipid turnover, to the later events preventing greater cell damages, as transacylation reactions leading to the stabilization of membrane bilayers.

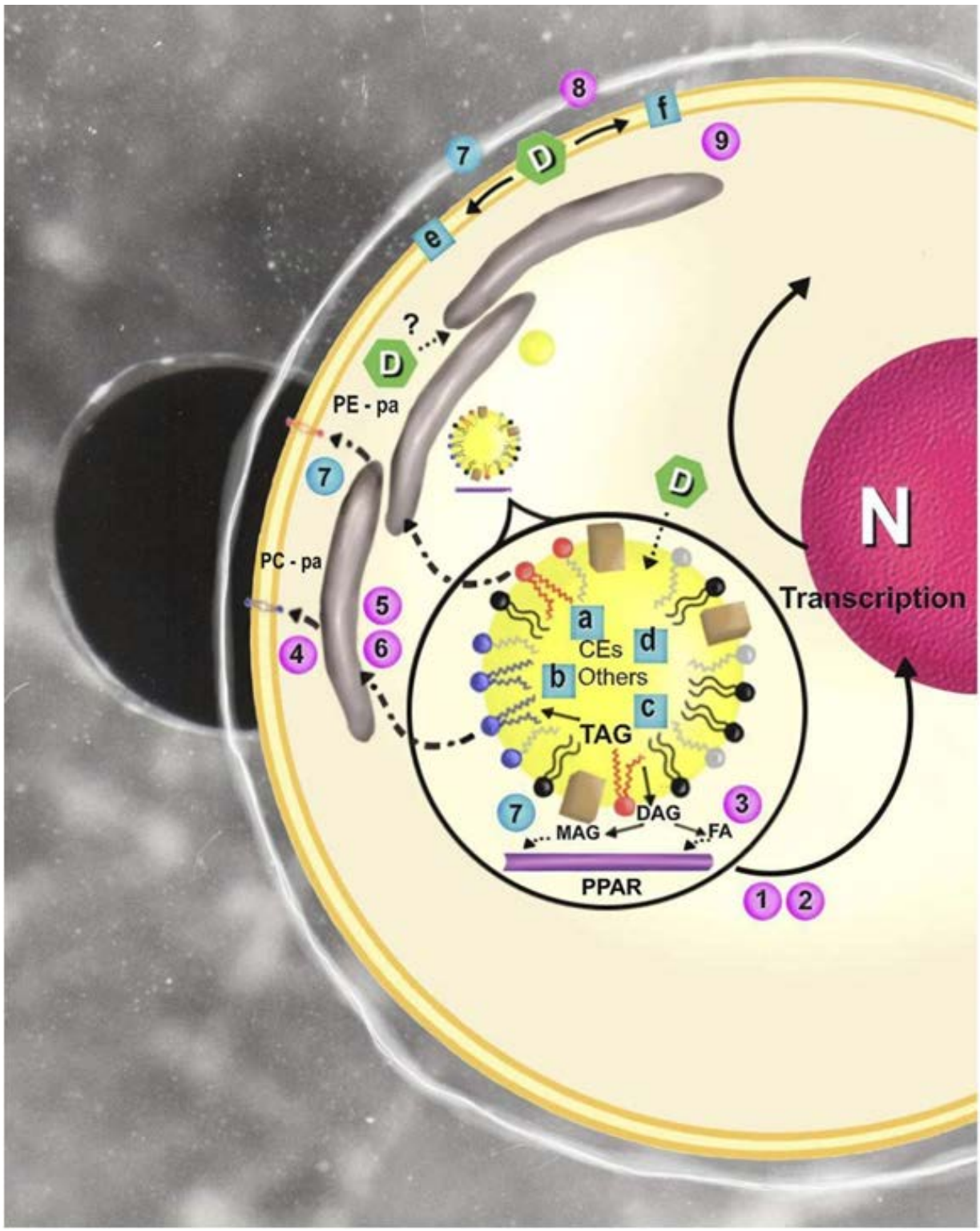


**Figure 1.** Illustration showing the molecular responses involving lipid reactions in *Rinella arenarum* oocytes exposed to the organochlorinated pesticide Dieldrin. An enlarged lipid droplet (LD) formed in the ER is shown.

Notes: Abbreviations: PE-pa: $^{3}$H-palmitic acid containing PE; PC-pa: $^{3}$H-palmitic acid containing PC; CEs: cholesterol esters; TAG: triacylglycerols; DAG: Diacylglycerols MAG: monoacylglycerols; PPAR: peroxisome proliferator-activated receptor.

The blue circles and squares respectively show an outline of the effects reported by the author on fatty acids exchange in phospholipids and glycerides and enzymatic activities according with them: 7: Fonovich de Schroeder and Pechén de D´Angelo [21]. a: acyl-CoA: acylglycerophosphocholine transferase; b : acyl-CoA:acylglycerophospho-ethanolamine transferase; c : TAG:phospholipid acyltransferase specific for the sn-2 position; d : TAG lipase; e: PC specific phospholipase C; f: $PIP_2$ specific phospholipase C.

The violet circles show reference cites in agreement with the effects reported in 7:

1: Poursharifi et al. [41]; 2: Walker et al. [42]; 3: Grygiel-Górniak [40]; 4: Zhang et al. [10]; 5: Kim et al. [55]; 6: Bernat et al. [7]; 8: Fonovich de Schroeder and Pechén de D´Angelo [47]; 9: Fonovich de Schroeder [13].

Symbols and arrows: : LD; : proteins; D : Dieldrin; : phospholipids; : lysophospholipids; : LysoPC; : PE; : LysoPE; : PE; : reactions leading to the effects described by the authors or other researchers; : possible stimulation; :possible phospholipid trafficking.

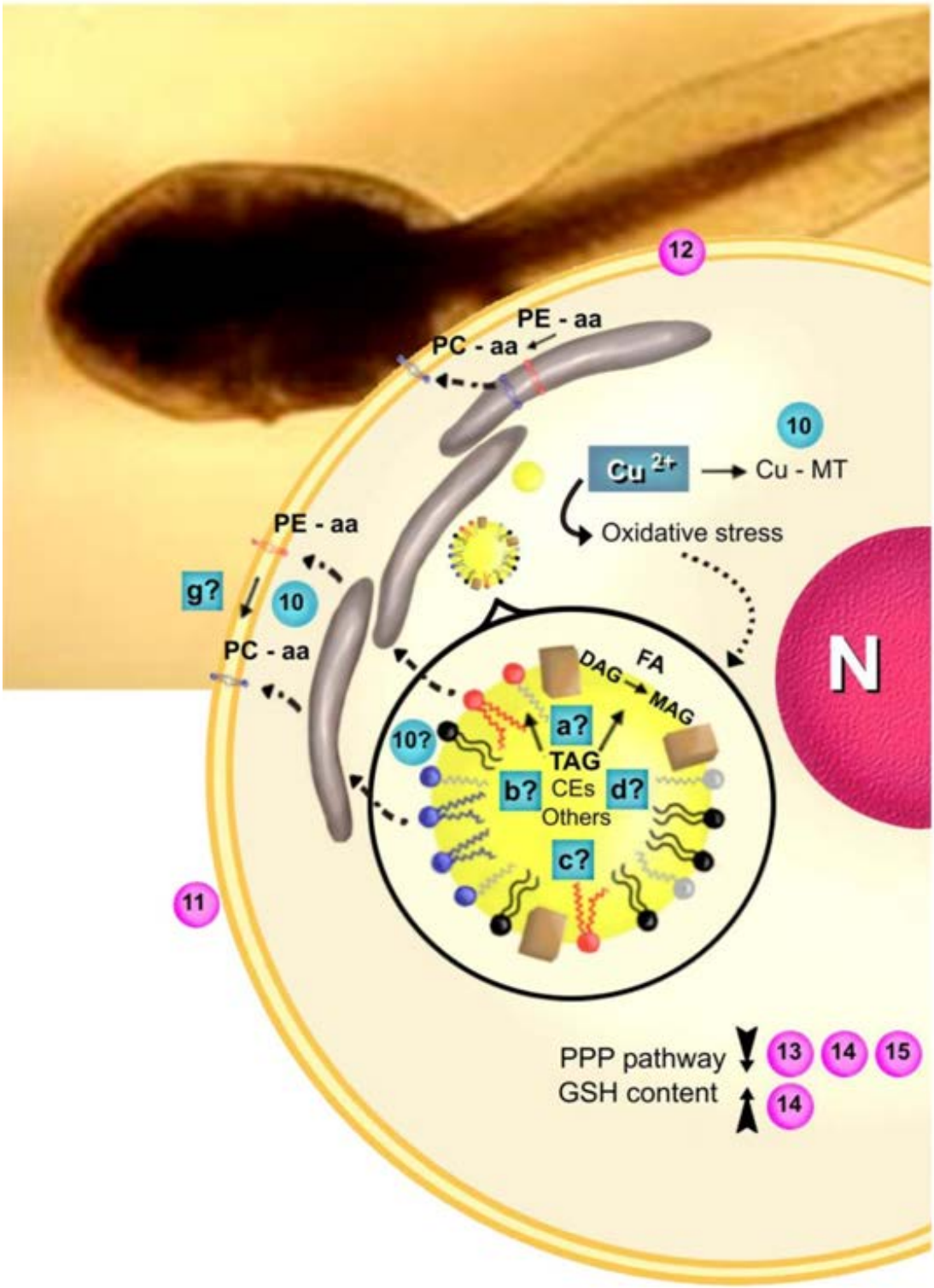


**Figure 2.** Illustration showing the molecular responses involving lipid reactions in *Rinella arenarum* embryos subjected to acclimation to $Cu^{2+}$. An enlarged lipid droplet (LD) formed in the ER is shown.

Notes: Abbreviations: PE-aa: $^{3}$H-arachidonic acid containing PE; PC-aa: $^{3}$H-arachidonic acid containing PC; Cu-MT: Methallotionein bound to $Cu^{2+}$; CEs: cholesterol esters. PPP pathway: pentose phosphate pathway; GSH content: glutathione content.

The blue circles and squares respectively show an outline of the effects reported by the author on fatty acids exchange in phospholipids or suspected in these molecules and glycerides (?), as well as enzymatic activities according with them: 10: Fonovich et al. [31].

a: acyl-CoA:acylglycerophosphocholine transferase; b: acyl-CoA:acylglycerophospho-ethanolamine transferase; c: TAG:phospholipid acyltransferase specific for the sn-2 position; d: TAG lipase; g: phospholipid transacylase activity.

The violet circles show reference cites in agreement with the effects reported in 10. 11: Rotiskaya et al. [54]; 12: Kim et al. [55]; 13: Carattino et al. [50]; 14: Naab et al. [52]; 15: Fonovich de Schroeder [53].

Symbols and arrows: : LD; : proteins; $Cu^{2+}$: copper ions; : phospholipids; : lysophospholipids; : LysoPC; : PE; : LysoPE; : PE; : reactions leading to the effects described by the authors or other researchers; : possible stimulation; :possible phospholipid trafficking; : pentose phosphate pathway enzymes decreased activity; : endogenous glutathione content increase.

## 8. Conclusion

The present work consists of describing and updating various aspects of lipid metabolism, as well as the protection of cells and tissues against different xenobiotics by modifying membrane phospholipids. It shows a description of the interaction between neutral lipids and lysophospholipids, in addition to the enzymatic activities responsible for the exchange of fatty acids in phospholipids of oocytes and amphibian embryos. The alterations previously reported are part of the early responses to exposure to toxic and acclimatization concentrations of dieldrin and copper ions, respectively. Some of them suggest transitions from non-bilayer to bilayer arrangements of major phospholipids as a consequence of the remodeling of PE and PC. New elements are provided to discuss the possible participation of LD as the organelle where these processes take place and also to discuss the joint responses of phospholipids, MT, glutathione content and the activity of the enzymes of the pentose phosphate pathway.

## Acknowledgments

This work was supported in part by the Science, Technology and Innovation Ministry (under grant FONCyT PICT 2017-0706) and by the School of Science and Technology from National University of San Martín. Figures 1 and 2 were illustrated by Silvina Mariel Schroeder. The author appreciates Cristina Pérez Coll kindly provision of embryo photograph shown in Figure 2. Photograph shown in Figure 1 belongs to the author.

## Conflict of interest

The author declares no conflict of interest for the contributions in this manuscript.

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