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TOPICAL REVIEW

Phospholipid Asymmetry in Biological Membranes: Is the Role of Phosphatidylethanolamine Underappreciated?

Abhijit Chakrabarti[1,2]

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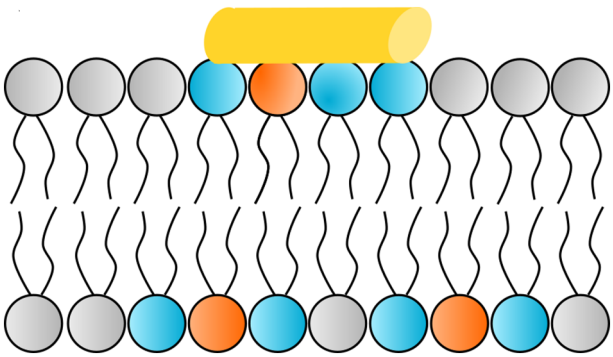
Abstract

The asymmetric distribution of phospholipids in cell membranes has been the focus of a lot of important research keeping its biological importance in mind. Most of this research is focused on phosphatidylserine (PS) since it is an apoptotic marker, and there is a robust and easy method available its selective quantification. The aim of this commentary is to argue in favour of another highly abundant membrane lipid, phosphatidylethanolamine (PE) almost always associated with PS. PE has one of the smallest headgroups and shows distinctly asymmetric transbilayer distribution. It is a neutral aminophospholipid and capable of a vastly wider range of interactions as seen in its unique ability to act as a molecular chaperone, implicated role in disease biology and its possible role as an anti-cancer target. There are ample evidences to the fact that PE may also bind to Annexin V (ANV), the PS-specific probe, at higher than 10 mol% PE concentrations and absence of Ca^{2+} ions. An update of the major takeaways from the literature regarding PE asymmetry is also provided.

Graphic Abstract



Keywords Aminophospholipids · Membrane asymmetry · Phosphatidylethanolamine · Annexin V

Abbreviations

PC	Phosphatidylcholine
SM	Sphingomyelin
PE	Phosphatidylethanolamine
PS	Phosphatidylserine
SUV	Small unilamellar vesicles
TNBS	2,4,6-Trinitrobenzenesulphonate
ANV	Annexin V
K_d	Apparent binding dissociation constant

✉ Abhijit Chakrabarti
abhijit.chakrabarti@saha.ac.in

[1] Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, HBNI, 1/AF Bidhannagar, Kolkata 700064, India

[2] Homi Bhabha National Institute, Mumbai 400094, India

Introduction

It is a well-established fact that the inner and outer leaflets of cellular and organellar membranes show differences in lipid composition, which is popularly called lipid asymmetry. In eukaryotic cell membranes, sphingomyelin (SM) and phosphatidylcholine (PC) along with other choline-containing

headgroups are predominantly located in the outer or exoplasmic leaflet of the bilayer membrane. Phosphatidylethanolamine (PE) and phosphatidylserine (PS), the amine-containing phospholipids, on the other hand, are largely confined to the inner or cytoplasmic leaflet, observed in numerous studies on red blood cells and other cell types (Bretscher 1972; Verkleji et al. 1973; Op den Kamp 1981; Devaux 1991; Williamson and Schlegel 1994; Devaux and Morris 2004; Son and London 2013; Shin and Takatsu 2019).

Two energy-dependent processes involving the enzymes—aminophospholipid translocase and ATP-dependent floppase, are found to be responsible for the maintenance of this asymmetric transbilayer distribution. Inhibition of either or both of these enzymes does not destroy the non-random distribution immediately. It rather takes several days in vitro for the complete loss of membrane asymmetry (Zwaal and Schroit 1997). There is also a third, Ca^{2+}-dependent enzyme—lipid scramblase, which mediates rapid transbilayer mixing of phospholipids across the membrane bilayer (Connors et al. 1992; Smeets et al. 1994). In an important MD simulation work, it has been shown that membrane electrostatics could affect asymmetric distribution in lipid membranes composed of zwitterionic PC or PE and the anionic PS resulting in a nonzero potential difference between the two leaflets (Gurtovenko and Vattulainen 2008).

Available literature on the role of other proteins, such as those of the membrane skeletal network in maintaining asymmetric distribution of PS and PE, is inconclusive and provides contradictory evidences. However, this is still noteworthy and perhaps unresolved since skeletal proteins like spectrin and band 4.1 are known to specifically bind to aminophospholipids (Haest et al. 1983; Dressler et al. 1984; Mohandas et al. 1985; Pradhan et al. 1991; Salomao et. al. 2008; Basu and Chakrabarti 2015). The asymmetric transbilayer distribution of phospholipids is crucial to the functioning of proteins embedded in or associated with the membrane and plays a key role in various physiological functions such as cell signalling, transport across membranes, cell–cell interactions and cellular adhesion. Loss of asymmetry, particularly the appearance of PS on the cell surface, is strongly associated with many physiological and pathological conditions (Zwaal and Schroit 1997; Balasubramanian and Schroit 2003; Fadeel and Xue 2009; Tan et al. 2017).

It is also worth noting that the existing literature on various aspects of membrane lipid asymmetry is heavily biased in favour of PS. Loss of PS asymmetry and appearance of this anionic lipid on the cell surface have received enormous attention, whereas the neutral aminophospholipid PE with a smaller headgroup size and a natural propensity to reside in the inner cytoplasmic leaflet, is much less well understood and has drawn little attention. The preferential localization of PE in the inner leaflet of the highly curved membranes of small unilamellar vesicles (SUV), microparticles from malignant cells, apical plasma membranes and red cell spicules in sickle cell disease have been demonstrated (Nordlund et al. 1981; Larson et al. 2012; Julien et al. 1993; Choe et al. 1986). The importance of the biological effects of PE distribution is seen in the membrane associations of protein kinase C and other proteins, which show selectivity for membranes containing PE over PS (Bazzi et al. 1992). Variation of the PS:PE ratio shows that membranes containing about 20% PS:60% PE provide optimum conditions for binding and are as effective as membranes composed of 100% PS (Bazzi et al. 1992; Ray and Chakrabarti 2004). In recent times, PE has also gained importance as a potential chemotherapeutic target due to its higher abundance in cancer cells (Tan et al. 2017).

This commentary aims to concentrate on the status of current knowledge on the membrane asymmetry of PE, which is potentially one of the best candidates for transbilayer movement across cell membranes, guided by lateral associations with other membrane lipids such as cholesterol and long-chain SM molecules (Vance 2008; Steck and Lange 2018). An updated list of literature regarding the status of PE asymmetry in eukaryotic cells is presented in Table 1, along with remarks about their major conclusions.

Why to Study PE?

The role of lipids as an important structure-forming and/or function-inducing environment for membrane proteins is well known, and PE plays its part in this. PE is asymmetrically distributed like PS in the membrane with about one third in the outer leaflet and two thirds in the inner leaflet. The size of the PE headgroup is one of the smallest among the phospholipids and can form a stable bilayer, particularly in presence of PC, SM and PS. PE with unsaturated fatty acyl chains is also capable of forming non-bilayer hexagonal phases (Gennis 1989a). PE is the second most abundant phospholipid in eukaryotic cell membranes, accounting for about 20% of the total phospholipids (Spector and Yorek 1985; Devaux 1991). PE participates in many important pathophysiological processes (Vance 2008; Calzada et al. 2016). Distribution and differential localization of PE play an important role in cell division, cell death and cytokinesis (Emoto et al. 1996, 1997; Emoto and Umeda 2000). The existence of PE at the cleavage sites during cell division may be attributed to the small headgroup of PE, supporting the seeding of local non-bilayer structures with uncontrolled transbilayer movement. Asymmetric distribution of sarcolemmal PE in neonatal rat cardiomyocytes was found to cause membrane damage after a prolonged period of ischemia (Musters et al. 1993). Many such diverse molecular

Table 1 Phosphatidylethanolamine asymmetry in eukaryotic cells

Sl. no.	Cell type	Remarks	References
1	Chinese hamster ovary cells	Cells express outer leaflet PE in cleavage furrow at late telophase	Emoto et al. (1996); Emoto and Umeda (2000)
2	PC-12 cells	Differentiation-dependent decrease in outer leaflet PE	Ikemoto et al. (1999)
3	Myoblasts, diffentiating myotubes in vivo	Large fraction of both PE and PS in outer leaflet of chick embryo cells	Sessions and Horwitz (1983); van den Eijnde et al. (2001)
4	Bovine aortic endothelial cells	Transbilayer and lateral motions of fluorescent analogues of PC and PE in the apical plasma membrane	Julien et al. (1993)
5	Rat liver mitochondrial outer membrane	Large amounts of PE on the outer membrane and sealed vesicles in intact mitochondria	Hoviusa et al. (1993)
6	Chick embryo neurons	Outer leaflet PE increases during development of interneuronal contacts	Lelong et al. (1991)
7	Sheep platelets	PE fatty acyl chains across the plasma membrane	Sánchez-Yagüe et al. (1991)
8	Human blood platelet	Asymmetric distribution of PE in platelet membranes	Spangenberg et al. (1985)
9	Yeast plasma membrane	Protein-mediated transbilayer movements of PE	Balasubramanian and Gupta (1996)
10	Liposomal membranes	PE asymmetry in highly curved small unilamellar vesicles	Nordlund et al. (1981)
11	Plasmodium infected erythrocytes	Loss of PE asymmetry in *Plasmodium knowlesi*-infected rhesus monkey erythrocytes	Gupta and Mishra (1981)
12	Neonatal rat cardiomyocytes	Loss of asymmetry of sarcolemmal PE during simulated Ischaemia	Musters et al. (1993), (1996)
13	Human breast cancer	Greater loss of PE asymmetry than PS, in microparticles from malignant cells	Larson et al. (2012)
14	Diabetes	Shown in patient's samples and in an in vitro experimental system	Wali et al. (1988); Wilson et al. (1993)
15	Sickle cell disease	Loss of PE asymmetry in red cell spicules	Choe et al. (1986)

properties PE, thus, represent a rich target of study among the phospholipids.

PE has been also established as a non-protein molecular chaperone in the folding and maturation process of some proteins in prokaryotes (Bogdanov et al. 1999; Bogdanov and Dowhan 1998, 1999). PE acts as an endogenous cofactor for prion propagation in vitro (Supattapone 2012). All these indicate the potential of PE to play an important role in pathogenic and/or proteopathic disease progression in eukaryotes and could even act as an anti-cancer target (Tan et al. 2017).

Measurement of PS & PE Asymmetry

The rate of phospholipid transbilayer diffusion is slow, taking time from hours to days; thus, steady state measurements are possible for phospholipids—following the order PS > PE ≫ PC > SM. Classical biochemical techniques are still used for the study of lipid asymmetry in membranes (Op den Kamp 1979, 1981; Etemadi 1980; Gennis 1989b).

In a hallmark study, both PE and PS were estimated in platelet plasma membrane using 2,4,6-trinitrobenzenesulphonate (TNBS) which does not penetrate intact cells. Results indicated that PE was partly and PS was completely inaccessible to TNBS in intact platelets (Schick et al. 1976). They concluded that "PS and probably PE are located primarily in the inner lipid bilayer of the platelet plasma membrane". Schick et al. could estimate a total amount of 26.5% PS and 71% PE in the extracted phospholipids; however, only 6.9% PE of platelet membranes was accessible to TNBS after 30 min. The levels of PS and PE in different eukaryotic membranes are assessed to be around 10% and 20% of the total phospholipids, respectively (Stuart et al. 1998; Spector and Yorek 1985). A sizable fraction of both PS and PE exists in the outer leaflet; amounts were shown to vary among different cell types in the ranges of 0–40% for PS and 0–70% for PE, respectively (Devaux 1991; Zachowski 1993).

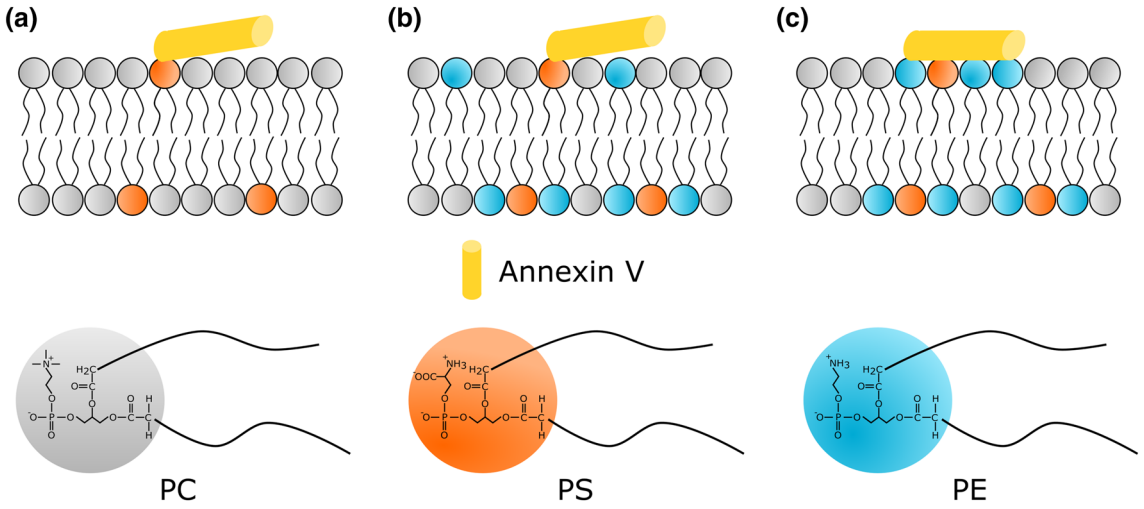


Fig. 1 The schematic diagram of membranes shows the binding of the Annexin V (ANV), to a phospholipid bilayer. In all three panels, the top leaflet represents the extracellular face, and the bottom leaflet represents the intracellular face. Panel **a** shows the binding of ANV to the PS headgroups in a PC/PS bilayer and Panel **b** shows the same in the presence of PE, below 10 mol%. Panel **c** shows the scenario when the content of PE is greater than 10 mol% showing ANV binding to PE along with PS, particularly in the absence of Ca^{2+} ions

Similar observations were made in intact rat liver mitochondria and derived sealed vesicles where 55% and 77% of PC and PE, respectively, were localized in the outer membrane (Hoviusa et al. 1993). However, questions were raised on the accuracy of those measurements (Op den Kamp 1979; Etemadi 1980; Zachowski 1993; Fujimoto and Parmryd 2017). But the divergent results could not be explained in terms of methodological inaccuracy alone, indicating the presence of residual, non-negligible amounts of both PS and PE on the outer leaflet of the plasma membrane in most cell types.

Annexin V (ANV) binding in the presence of Ca^{2+} has remained the most popular flow cytometry-based method of studying PS asymmetry (Koopman et al. 1994). ANV belongs to the family of proteins that binds with high affinity, almost solely to PS, preferably in the presence of Ca^{2+}. Early work showed that ANV bound to model membranes containing PS:PC (20:80)% with an estimated binding dissociation constant (K_d) of $< 10^{-10}$ M at physiological concentration of Ca^{2+} (Tait et al. 1989; Andree et al. 1990). Subsequent studies of binding of ANV conjugates to platelets and erythrocytes produced widely different K_d values ranging from 10^{-11} to 10^{-8} M (Thiagarajan and Tait 1990; Yen et al. 2010).

In an earlier work, binding of ANV to phospholipid bilayers adsorbed onto glass beads was found to increase with increasing PS concentrations, only up to 6 mol% PS (Stuart et al. 1998). Calcium concentrations below 3 mM were found to reduce the efficiency of the ANV binding. Interestingly, the addition of 30 mol% PE in the presence of 1–4 mol% PS in the bilayer, significantly increased the maximum binding of ANV over that in the absence of PS, thus, indicating the binding of ANV to PE at lower PS concentrations. Binding of ANV to phospholipid bilayers containing PE has also been reported by others (Meers and Mealy 1994). Validity of ANV as a probe for loss of membrane asymmetry or exposure of PS is thought to be apparently unhampered in the presence of up to 10 mol% PE in the outer membrane (Stuart et al. 1998). However, with a further increase in the PE content of the outer leaflet in combination with even a marginal increase in the PS level, a strong difference in ANV binding could result. This is shown in Fig. 1 as a schematic model.

Cell surface localization of PE was studied in dividing Chinese hamster ovary cells using cinnamycin (Ro 09-0198), a tetracyclic peptide antibiotic that binds specifically to PE with a 1:1 stoichiometry. PE was found exposed on the cell surface, at the cleavage furrow, only during late telophase. PE distribution was otherwise not found to be altered in the plasma membrane (Emoto et al. 1996). K_d values for Ro 09-0198-PE complex ranged from 10^{-7} to 10^{-8} M in liposome membranes (Machaidze and Seelig 2003). It has been shown by a few groups, including ours, which the membrane skeletal protein spectrin binds both PS and PE and possesses a high affinity-binding site for PE at its ankyrin-binding domain (Ray and Chakrabarti 2004; Grzybek et al. 2006; Giri et al. 2017; Bose and Chakrabarti 2019).

Conclusion

Classically phospholipids were only treated as the matrix that provides the right orientation to the more functional and active membrane proteins. Importance started to be

attributed to membrane phospholipid asymmetry, after the discovery of its loss as a marker of cellular apoptosis—using Ca^{2+}-dependent high affinity binding of ANV as a tool to assess the level of surface-exposed PS. However, like in other phospholipid-binding proteins, binding of ANV is also found to be associated with PE as well as PS. The size of the PE headgroup, its higher abundance, zwitter ionic nature, natural propensity for faster transbilayer diffusion, and its unique functional properties in maturation of prokaryotic membrane proteins, indicate that adequate attentions are to be paid in studying the asymmetric distribution of PE.

Only a few reports are available till date on the asymmetric distribution of PE alone. It is envisioned that this commentary could help to establish that most of the research related to membrane asymmetry is a bit biased towards PS, when considerable evidence is available in the literature in favour of PE being at least a co-partner of PS in driving the biological functions due to membrane phospholipid asymmetry. As such PE must receive greater attention in the ever-growing literature on membrane phospholipid asymmetry and its pathophysiological outcome.

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Compliance with Ethical Standards

Conflict of interest The author declares no conflict of interest.

References

Andree HAM, Reutelingsperger CPM, Hauptmann R, Hemker HC, Hermens WT, Willems GM (1990) Binding of vascular anticoagulant α (VAC α) to planar phospholipids bilayers. J Biol Chem 265:4923–4928

Balasubramanian K, Gupta CM (1996) Transbilayer phosphatidylethanolamine movements in the yeast plasma membrane. Evidence for a protein-mediated, energy-dependent mechanism. Eur J Biochem 240:798–806

Balasubramanian K, Schroit AJ (2003) Aminophospholipid asymmetry: a matter of life and death. Annu Rev Physiol 65:701–734

Basu A, Chakrabarti A (2015) Defects in erythrocyte membrane skeletal architecture. Adv Exp Med Biol 842:41–59

Bazzi MD, Youakim MA, Nelsestuen GL (1992) Importance of phosphatidylethanolamine for association of protein kinase C and other cytoplasmic proteins with membranes. Biochemistry 31:1125–1134

Bogdanov M, Dowhan W (1998) Phospholipid-assisted protein folding : phosphatidylethanolamine is required at a late step of the conformational maturation of the polytopic membrane protein lactose permease. EMBO J 17:5255–5264

Bogdanov M, Dowhan W (1999) Lipid-assisted protein folding. J Biol Chem 274:36827–36830

Bogdanov M, Umeda M, Dowhan W (1999) Phospholipid-assisted refolding of an integral membrane protein. Minimum structural features for phosphatidylethanolamine to act as a molecular chaperone. J Biol Chem 274:12339–12345

Bose D, Chakrabarti A (2019) Localizing the chaperone activity of erythroid spectrin. Cytoskeleton 76:383–397

Bretscher MS (1972) Asymmetrical lipid bilayer structure for biological membranes. Nat New Biol 236:11–12

Calzada E, Onguka O, Claypool SM (2016) Chapter two—phosphatidylethanolamine metabolism in health and disease. Int Rev Cell Mol Biol 321:29–88

Choe HR, Schlegel RA, Rubin E, Williamson P, Westerman MP (1986) Alteration of red cell membrane organization in sickle cell anaemia. Brit J Haematol 63:761–773

Connor J, Pak CH, Zwaal RFA, Schroit AJ (1992) Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. J Biol Chem 267:19412–19417

Devaux PF (1991) Static and dynamic lipid asymmetry in cell membranes. Biochemistry 30:1163–1173

Devaux PF, Morris R (2004) Transmembrane asymmetry and lateral domains in biological membranes. Traffic 5:241–246

Dressler V, Haest CWM, Plasa G, Deuiticke B, Erusalimsky JD (1984) Stabilizing factors of phospholipid asymmetry in the erythrocyte membrane. Biochim Biophys Acta 775:189–196

Emoto K, Umeda M (2000) An essential role for a membrane lipid in cytokinesis. Regulation of contractile ring disassembly by redistribution of phosphatidylethanolamine. J Cell Biol 149:1215–1224

Emoto K, Kobayashi T, Yamaji A, Aizawa H, Yahara I et al (1996) Redistribution of phosphatidylethanolamine at the cleavage furrow of dividing cells during cytokinesis. Proc Natl Acad Sci USA 93:12867–12872

Emoto K, Toyama-Sorimachi N, Karasuyama H, Inoue K, Umeda M (1997) Exposure of phosphatidylethanolamine on the surface of apoptotic cells. Exp Cell Res 232:430–434

Etemadi AH (1980) Membrane asymmetry. A survey and critical appraisal of the methodology II. Methods for assessing the unequal distribution of lipids. Biochim Biophys Acta 604:423–475

Fadeel B, Xue D (2009) The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease. Crit Rev Biochem Mol Biol 44:264–277

Fujimoto T, Parmryd I (2017) Inter leaflet coupling, pinning, and leaflet asymmetry—major players in plasma membrane nanodomain formation. Front Cell Dev Biol 4:155

Gennis RB (1989a) The structures and properties of membrane lipids. In: Cantor CR (ed) Biomembranes: molecular structure and function. Springer-Verlag, New York, pp 63–65

Gennis RB (1989b) Lateral and transverse asymmetry in membranes. In: Cantor CR (ed) Biomembranes: molecular structure and function. Springer-Verlag, New York, pp 151–158

Giri RP, Mukhopadhyay MK, Mitra M, Chakrabarti A, Sanyal MK et al (2017) Differential adsorption of a membrane skeletal protein, spectrin, in phospholipid membranes. Europhy Lett 118:58002

Grzybek M, Chorzalska A, Bok E, Hryniewicz-Jankowska A, Czogalla A, Diakowski W, Sikorski AF (2006) Spectrin-phospholipid interactions. Existence of multiple kinds of binding sites ? Chem Phys Lipids 141:133–141

Gupta CM, Mishra GC (1981) Transbilayer phospholipid asymmetry in *Plasmodium knowlesi*-infected host cell membrane. Science 212:1047–1049

Gurtovenko AA, Vattulainen I (2008) Membrane potential and electrostatics of phospholipid bilayers with asymmetric transmembrane distribution of anionic lipids. J Phys Chem B 112:4629–4634

Haest CW, Erusalimsky J, Dressler V, Kunze I, Deuticke B (1983) Transbilayer mobility of phospholipids in the erythrocyte membrane. Influence of the membrane skeleton. Biomed Biochim Acta 42:S17-21

Hoviusa R, Thijssen J, van der Linden P, Nicolay K, de Kruijff B (1993) Phospholipid asymmetry of the outer membrane of rat

liver mitochondria: evidence for the presence of cardiolipin on the outside of the outer membrane. FEBS Lett 330:71–76
Ikemoto A, Kobayashi T, Emoto K, Umeda M, Watanabe S et al (1999) Effects of docosahexaenoic and arachidonic acids on the synthesis and distribution of aminophospholipids during neuronal differentiation of PC12 cells. Arch Biochem Biophys 364:67–74
Julien M, Tournier JF, Tocanne JF (1993) Differences in the transbilayer and lateral motions of fluorescent analogs of phosphatidylcholine and phosphatidylethanolamine in the apical plasma membrane of bovine aortic endothelial cells. Exp Cell Res 208:387–397
Koopman G, Reutelingsperger CPM, Kuijten GA, Keehnen RM, Pals ST, van Oers MH (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood 84:1415–1420
Larson MC, Woodliff JE, Hillery CA, Kearl TJ, Zhao M (2012) Phosphatidylethanolamine is externalized at the surface of microparticles. Biochim Biophys Acta 1821:1501–1507
Lelong I, Frechard E, Cremel G, Langley K, Rebel G et al (1991) Expression of plasma membrane and cell surface phospholipids and gangliosides of chick embryo neurons grown in primary cultures: developmental studies. Dev Neurosci 13:54–60
Machaidze G, Seelig J (2003) Specific binding of cinnamycin (Ro 09-0198) to phosphatidylethanolamine. Comparison between micellar and membrane environments. Biochemistry 42:12570–12576
Meers P, Mealy T (1994) Phospholipid determinants for annexin V binding sites and the role of tryptophan 187. Biochemistry 33:5829–5837
Mohandas N, Rossi M, Bernstein S, Ballas S, Ravindranath Y et al (1985) The structural organization of skeletal proteins influences lipid translocation across erythrocyte membrane. J Biol Chem 260:14264–14268
Musters RJ, Otten E, Biegelmann E, Bijvelt J, Keijzer JJ et al (1993) Loss of asymmetric distribution of sarcolemmal phosphatidylethanolamine during simulated ischemia in the isolated neonatal rat cardiomyocyte. Circ Res 73:514–523
Musters RJ, Pröbstl-Biegelmann E, van Veen TA, Hoebe KH, Op den Kamp JA et al (1996) Sarcolemmal phosphatidylethanolamine reorganization during simulated ischaemia and reperfusion: reversibility and ATP dependency. Mol Membr Biol 13:159–164
Nordlund JR, Schmidt CF, Dicken SN, Thompson TE (1981) Transbilayer distribution of phosphatidylethanolamine in large and small unilamellar vesicles. Biochemistry 20:3237–3241
Op den Kamp JAF (1979) Lipid asymmetry in membranes. Annu Rev Biochem 48:47–71
Op den Kamp JAF (1981) The asymmetric architecture of membranes. In: Finean JB, Michell RH (eds) Membrane structure. Elsevier, New York, pp 83–126
Pradhan D, Williamson P, Schlegel RA (1991) Bilayer/cytoskeleton interactions in lipid-symmetric erythrocytes assessed by a photoactivable phospholipid analogue. Biochemistry 30:7754–7758
Ray S, Chakrabarti A (2004) Membrane interaction of erythroid spectrin: surface-density-dependent high-affinity binding to phosphatidylethanolamine. Mol Membr Biol 21:93–100
Salomao M, Zhang X, Yang Y, Lee S, Hartwig JH et al (2008) Protein 4.1R-dependent multiprotein complex: new insights into the structural organization of the red blood cell membrane. Proc Natl Acad Sci USA 105:8026–8031
Sánchez-Yagüe J, Cabezas JA, Llanillo M (1991) Fatty acid composition of subcellular particles from sheep platelets and topological distribution of phosphatidylethanolamine fatty acids in the plasma membrane. Lipids 26:878–883
Schick PK, Kurica KB, Chacko GK (1976) Location of phosphatidylethanolamine and phosphatidylserine in the human platelet plasma membrane. J Clin Invest 57:1221–1226
Sessions A, Horwitz AF (1983) Differentiation-related differences in the plasma membrane phospholipid asymmetry of myogenic and fibrogenic cells. Biochim Biophys Acta 728:103–111
Shin H-W, Takatsu H (2019) Substrates of P4-ATPases: beyond aminophospholipids (phosphatidylserine and phosphatidylethanolamine). FASEB J 33:3087–3096
Smeets EF, Comfurius P, Bevers EM, Zwaal RFA (1994) Calcium-induced transbilayer scrambling of fluorescent phospholipid analogs in platelets and erythrocytes. Biochim Biophys Acta 1195:281–286
Son M, London E (2013) The dependence of lipid asymmetry upon polar headgroup structure. J Lipid Res 54:3385–3393
Spangenberg P, Heller R, Wagner C, Till U (1985) Localization of phosphatidylethanolamine in the plasma membrane of diamide-treated human blood platelets. Biomed Biochim Acta 44:1335–1341
Spector AA, Yorek MA (1985) Membrane lipid composition and cellular function. J Lipid Res 26:1015–1035
Steck TL, Lange Y (2018) Transverse distribution of plasma membrane bilayer cholesterol: picking sides. Traffic 19:750–760
Stuart MCA, Reutelingsperger CPM, Frederik PM (1998) Binding of Annexin V to bilayers with various phospholipid compositions using glass beads in a flow cytometer. Cytometry 33:414–419
Supattapone S (2012) Phosphatidylethanolamine as a prion cofactor: potential implications for disease pathogenesis. Prion 6:417–419
Tait JF, Gibson D, Fujikawa K (1989) Phospholipid binding properties of human placental anticoagulant protein-I, a member of the lipocortin family. J Biol Chem 264:7944–7949
Tan LT, Chan KG, Pusparajah P, Lee WL, Chuah LH et al (2017) Targeting membrane lipid a potential cancer cure? Front Pharmacol 8:12
Thiagarajan P, Tait JF (1990) Binding of Annexin V/Placental anticoagulant protein I to platelets. J Biol Chem 265:17420–17423
van den Eijnde SM, van den Hoff MJ, Reutelingsperger CPM, van Heerde WL, Henfling ME et al (2001) Transient expression of phosphatidylserine at cell–cell contact areas is required for myotube formation. J Cell Sci 114:3631–3642
Vance JE (2008) Thematic review series: glycerolipids. Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids. J Lipid Res 49:1377–1387
Verkleji AJ, Zwaal RFA, Roelofsen B, Comfurius P, Kastelijin D et al (1973) The asymmetric distribution of phospholipids in the human red cell membrane. Biochim Biophys Acta 323:178–193
Wali RK, Jaffe S, Kumar D, Kalra VK (1988) Alterations in organization of phospholipids in erythrocytes as factor in adherence to endothelial cells in diabetes mellitus. Diabetes 37:104–111
Williamson P, Schlegel RA (1994) Back and forth: the regulation and function of transbilayer phospholipid movement in eukaryotic cells. Mol Membr Biol 11:199–216
Wilson MJ, Richter-Lowney K, Daleke DL (1993) Hyperglycemia induces a loss of phospholipid asymmetry in human erythrocytes. Biochemistry 32:11302–11310
Yen T-C, Wey S-P, Liao C-H, Yeh C-H, Shen D-W et al (2010) Measurement of the binding parameters of annexin derivatives-erythrocyte membrane interactions. Anal Biochem 406:70–79
Zachowski A (1993) Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem J 294(Pt1):1–14
Zwaal RFA, Schroit AJ (1997) Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. Blood 89:1121–1132