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Review

Phosphatidic acid- and phosphatidylserine-binding proteins

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Abstract

Phosphatidic acid and phosphatidylserine are negatively charged abundant phospholipids with well-recognized structural roles in cellular membranes. They are also signaling lipids since their regulated formation (or appearance) can constitute an important signal for downstream responses. The list of potential effectors for these lipids is expanding rapidly and includes proteins involved in virtually all aspects of cellular regulation. Because it is not always clear whether these effectors recognize the specific phospholipids or a general negatively-charged membrane environment, questions about specificity must be addressed on a case by case basis. In this review we present an up to date list of potential phosphatidic acid- and phosphatidylserine-binding proteins.

Keywords: Lipid signaling; Phosphatidic acid; Phosphatidylserine; Lipid recognition; Phospholipase D

1. Introduction

When considering possible effectors for phosphatidic acid (PA) and phosphatidylserine (PS), several important characteristics of these molecules must be kept in mind. PA and PS are abundant negatively charged phospholipids and it is likely that in many cellular settings their roles will be structural rather than as signaling molecules. As a rough approximation, PS can constitute 5–10% of total cellular lipid whereas PA is slightly lower at 1–4% [1]. In addition, PA is implicated (either directly or indirectly) in the biosynthesis of most other phospholipids and triacylglycerols [2] and this role as a biosynthetic intermediate must be kept distinct from its potential signaling functions.

Nevertheless, there is no doubt that both PA and PS are implicated in signaling pathways. PA can be produced rapidly following stimulated hydrolysis of phosphatidylcholine (PC) by phospholipase D (PLD) [3,4], whereas PS, which is normally enriched on the cytoplasmic side of the plasma membrane bilayer, can be exposed in a regulated way to the outer side of the bilayer thus constituting a signal for downstream responses [5] (Fig. 1). The search for proteins which interact specifically with PA or PS is of considerable interest and should help define the regulatory roles of these phospholipids.

The search for PA and PS effectors is complicated by the fact that it is not always clear whether a given protein has specific affinity for one of these phospholipids as opposed to a general affinity for a negatively-charged membrane surface. It is also not always clear whether PA/PS binding (or specificity) for the full-length protein can be deduced by constructing and analyzing shorter polypeptide fragments. Such an approach can sometimes lead to the isolation of extremely small peptides whose only feature relating to PA/PS binding is a short stretch of positively charged residues that ultimately proves to be an oversimplification. Recent work from Nakai and colleagues [6] illustrates these pitfalls: These workers tried to identify PS-specific proteins using a library of randomly generated 15-aminoacid peptides and isolated a peptide of the sequence "RSRRMTRRARAA" which bound to PS over other phospholipids with good specificity. Mutagenesis of this peptide indicated that either one of the two di-arginine motifs was required for strong PS binding. The authors then tried to identify *Drosophila* proteins containing this sequence and one such protein was identified. Further work on the intact protein indicated that it could bind equally well to PS and phosphatidylethanolamine (PE) and, in addition, mutagenesis of the di-arginine motif on the intact protein did not result in clear inhibition of PS binding. On the other hand, several studies have shown convincingly that binding to acidic phospholipids with good specificity *can* depend on short peptide sequences. The yeast Ste5 protein translocates to the plasma membrane following

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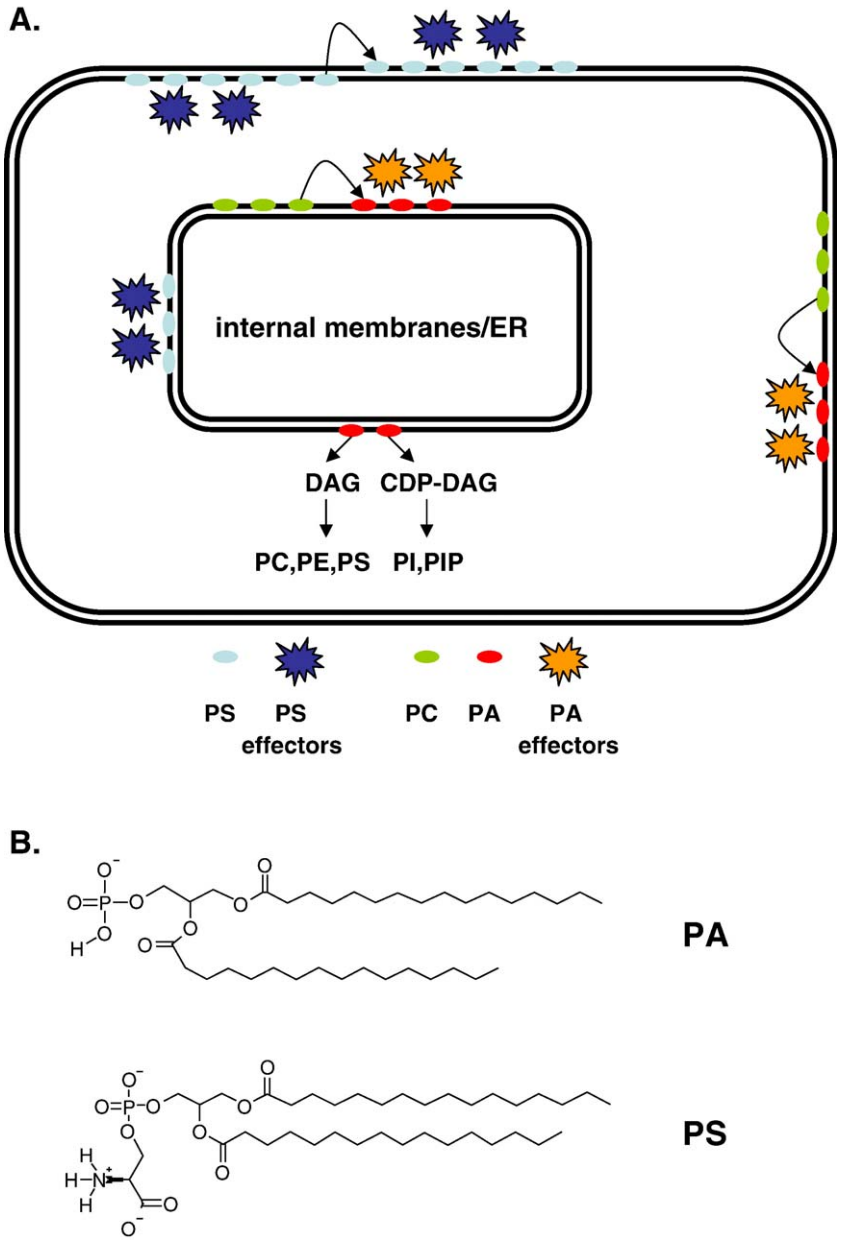

Fig. 1. Mechanisms for the regulated formation of PA and PS. (A) In addition to their role as structural/biosynthetic phospholipids, PA and PS can be formed inducibly. PA levels can increase after hydrolysis of PC by PLD whereas PS, which is normally enriched in the inner membrane bilayer, can appear on the outer side of the plasma membrane. (B) Chemical structures of PA and PS are shown. For simplicity, the di-palmitoyl (C:16/C:16) species are shown; note that, in reality, cellular membranes contain numerous species of these phospholipids, differing in the size of the acyl chain and the degree of saturation. These differences may have profound effects on how these lipids function during signaling but the information on this is too incomplete at present.

pheromone stimulation where it in turn serves as a scaffold for signaling during the mating reaction. Recent work [7] has shown that membrane targeting of Ste5 depends on a short alpha-helical segment that binds to acidic phospholipids such as PA, PI(4)P and PI(4,5)P_2 and overlaps with a nuclear import signal. For Ste5, the sequence LSRGKK*W*TEK*L*AR*F*QRSS*A*KKKR serves both as a phospholipid effector (residues in italics) and for entering the nucleus (underlined residues).

In reviewing the literature on potential PA/PS effectors, we have tried to focus on proteins for which phospholipid binding was shown by more than one approach and was put in a physiological context consistent with the protein's function. With these criteria in mind, the list of potential effectors is actually small and we consider it likely that, as this area of research advances, many more effectors will be identified. We also believe that some of the properties of the current effectors with respect to phospholipid specificity will be modified. Especially for PA targets, we consider our current understanding to be at a very early stage.

2. How PA and PS signals work

Most lipid signaling ultimately concerns a binding reaction between a – usually cytosolic – protein and a membrane surface