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Phosphatidic acid binding proteins display differential binding as a function of membrane curvature stress and chemical properties

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ABSTRACT Phosphatidic acid (PA) is a crucial membrane phospholipid involved in *de novo* lipid synthesis and numerous intracellular signaling cascades. The signaling function of PA is mediated by peripheral membrane proteins that specifically recognize PA. While numerous PA-binding proteins are known, much less is known about what drives specificity of PA-protein binding. Previously, we have described the ionization properties of PA, summarized in the electrostatic-hydrogen bond switch, as one aspect that drives the specific binding of PA by PA-binding proteins. Here we focus on membrane curvature stress induced by phosphatidylethanolamine and show that many PA-binding proteins display enhanced binding as a function of negative curvature stress. This result is corroborated by the observation that positive curvature stress, induced by lyso phosphatidylcholine, abolishes PA binding of target proteins. We show, for the first time, that a novel plant PA-binding protein, Arabidopsis Epsin-like Clathrin Adaptor 1 (ECA1) displays curvature-dependence in its binding to PA. Other established PA targets examined in this study include, the plant proteins TGD2, and PDK1, the yeast proteins Opi1 and Spo20, and, the mammalian protein Raf-1 kinase and the C2 domain of the mammalian phosphatidylserine binding protein Lact as control. Based on our observations, we propose that liposome binding assays are the preferred method to investigate lipid binding

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