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The differential regulation of phosphatidylinositol 4-phosphate 5-kinases and phospholipase D1 by ADP-ribosylation factors 1 and 6

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Abstract

Phosphatidylinositol 4-phosphate 5-kinases [PtdIns4*P*5Ks] synthesise the majority of cellular phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] and phospholipase D1 (PLD1) synthesises large amounts of phosphatidic acid (PtdOH). The activities of PtdIns4*P*5Ks and PLDs are thought to be coupled during cell signalling in order to support large simultaneous increases in both PtdIns(4,5)P_2 and PtdOH, since PtdOH activates PtdIns4*P*5Ks and PLD1 requires PtdIns(4,5)P_2 as a cofactor. However, little is known about the control of such a system. Membrane recruitment of ADP-ribosylation factors (Arfs) activates both PtdIns4*P*5Ks and PLDs, but it is not known if each enzyme is controlled in series by different Arfs or in parallel by a single form. We show through pull-down and vesicle sedimentation interaction assays that PtdIns4*P*5K activation may be facilitated by Arf-enhanced membrane association. However PtdIns4*P*5Ks discriminate poorly between near homogeneously myristoylated Arf1 and Arf6 although examples of all three known active isoforms (mouse $\alpha>\beta$, γ) respond to these G-proteins. Conversely PLD1 genuinely prefers Arf1 and so the two lipid metabolising enzymes are differentially controlled. We propose that isoform selective Arf/PLD interaction and not Arf/PtdIns4*P*5K will be the critical trigger in the formation of distinct, optimal triples of Arf/PLDs/PtdIns4*P*5Ks and be the principle regulator of any coupled increases in the signalling lipids PtdIns(4,5)P_2 and PtdOH.

Keywords: Arf; ADP-ribosylation factor; Kinase; Phospholipase; Phosphatidylinositol; Membrane; GTPase; PtdIns4*P*; PLD

1. Introduction

The minor membrane phospholipid PtdIns(4,5)P_2 is principally synthesised by phosphatidylinositol 4-phosphate 5-kinases [PtdIns4*P*5Ks] while many transient changes in the levels of phosphatidic acid (PtdOH) arise from the activation of phospholipases D (PLDs). Both PtdIns4*P*5Ks and PLDs are implicated in the control of many of the same cellular processes, since it is well established that many events that trigger the metabolism of PtdIns(4,5)P_2 also stimulate PLD and *vice versa* [1–4]. PtdIns(4,5)P_2 is the precursor of the three second messengers 1,2-diacylglycerol (DAG), inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] and also controls membrane channel activity [5], vesicular trafficking [6], Golgi structure and function [7,8] actin dynamics [9], nucleic acid and nuclear activities [10,11]. These roles correlate with the distribution of PtdIns(4,5)P_2 in cellular membranes [12,13]. PLDs most likely function through their phospholipid product phosphatidic acid to control membrane ruffling, endocytosis, exocytosis, the induction of stress fibres, respiratory burst, and possibly Golgi transport [4]. Since PtdIns(4,5)P_2, the product of PtdIns4*P*5Ks, is an activator of PLD and conversely PtdOH, the product of

Abbreviations: PtdInsP, phosphatidylinositol phosphate; DAG, 1,2 diacylglycerol; Ins(1,4,5)P3, inositol 1,4,5-trisphosphate; PtdIns(3,4,5)P3, phosphatidylinositol 3,4,5-trisphosphate; GEF, guanine nucleotide exchange factor; GST, reduced glutathione; GST, glutathione-S-transferase; HA, haemagluttinin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PIPES, Piperrazine-*N*,*N*′-bis(2-ethanesulfonic acid); EDTA, ethyenediamine tetraacetic acid; GTPγS, guanosine 5′-O-(thio)triphosphate; DTT, dithiothreitol; Arf, ADP-ribosylation factor; PBS, phosphate buffered saline; PMSF, phenlymethysulphonylfluoride

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PLD, is an activator of PtdIns4*P*5Ks, (Fig. 1) then reciprocating, explosive co-activation is an attractive feature in models of lipid-mediated cell regulation [14–18]. Even a simple system of two enzymes coupled in this way provides a novel network architecture that can be expected to have complicated kinetic properties. However, very little is known about any possible restraints that might exist. For example, are the two enzymes necessarily controlled individually or can they be co-activated by a single common regulator to guarantee their coupling?

PtdIns(4,5)P_2 is synthesised by both PtdIns4*P*5Ks and PtdIns5*P*4Ks (historically called type I and II correspondingly) from PtdIns4*P* and PtdIns5*P* respectively [19]. By mass the main pathway for PtdIns(4,5)P_2 synthesis is through PtdIns4*P*5Ks (Type I) which are found as splice variants of three active isoforms α, β and γ.[2] PtdIns4*P*5Ks are regulated by diverse factors including G-proteins (Arfs and Rho families) [20,21], protein phosphorylation (by PKA or autophosphorylation) [22], protein–protein interactions [23] and by the anionic phospholipids phosphatidylserine (PtdSer) and PtdOH [24,25]. Phospholipase D, on the other hand, is found as splice variants of each of two widely expressed forms, PLD1 and 2 [26–28]. The mammalian PLD1's are very similar and can be regulated by PtdIns(4,5)P_2 and also by many agents known to control PtdIns4*P*5Ks e.g. Arf and Rho-family G-proteins and PKCs. PLD2's also depend on PtdIns(4,5)P_2 and can be activated by Arfs [29] but are otherwise thought to be regulated differently. Consequently, it is essential to determine the true regulatory network architecture since separate activation of PtdIns4*P*5Ks and PLDs by different upstream agents might facilitate precise control of each enzyme, even if they are located in the same membrane, and this would provide for fine control of synergy. Alternatively, co-activation by a single common activator, in one membrane, should guarantee positive reinforcement and explosive amplification through synchronisation and cross-talk mediated by each enzyme product. To explore these mechanisms we have studied the regulation of both PtdIns4*P*5Ks and PLD1 by both Arf1 and Arf6, two maximally structurally divergent members of one family of common activators. In general both Arfs share a common mechanism of activation through GTP for GDP exchange and recruitment onto membrane surfaces. Currently it is difficult to anticipate the properties of Arf/PLD/PtdIns4*P*5K triples from the pair-wise studies of possible components e.g. Arf/PLD, Arf/PtdIns4*P*5Ks and PLD/PtdIns4*P*5Ks, because few of these interactions have been described in comparable systems. Here we show that by eliminating the usual differences in recombinant Arf protein preparations and running assays under comparable conditions, we can observe preferential activation of PLD1 but not PtdIns4*P*5Ks by Arfs 1 and 6, especially once differences in nucleotide exchange are accounted for. Co-sedimentation and vesicle pull-down assays indicate that membrane recruitment of PtdIns4*P*5Ks is the likely mechanism underlying activation. We propose that as a single activator Arf1 should be better than Arf6 at driving reciprocating positive feedback cycles between PtdIns4*P*5K and PLD1.

[2] Due to un-reconciled differences in nomenclature, the mouse PtdIns4*P*5K alpha (GenProt BAA13030) corresponds to human beta and mouse beta (GenProt BAA13031) corresponds to human alpha. Here we give priority to the mouse nomenclature to reflect historical primacy. Both nomenclatures agree on the gamma isoforms (e.g. mouse GenProt BAA25664).

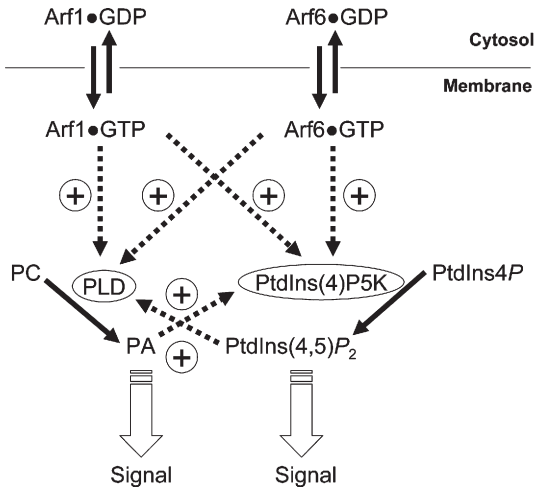

Fig. 1. Graphical illustration of possible connectivity in the regulation of PtdIns4*P*5Ks and PLD. Enzyme catalysed reactions are shown in solid arrows and regulatory interactions are shown in broken arrows.

2. Materials and methods

2.1. Materials

Baculovirus for GST-hPLD1b was a generous gift from M.J.O. Wakelam, University of Birmingham, Birmingham, UK. Gerenyl-gerenyl-GST-RhoA was a kindly given by R. Baron and M. Seabra, Imperial College, London, UK. Plasmids pMon5840 and pBB131 were a generously provided by J.I. Gordon, Washington University Medical School, MI, USA. Julie Donaldson, NHLBI, Bethesda, MD, USA kindly gave pXs plasmids coding for HA-tagged, Arf1, (T31N)Arf1, (Q71L)Arf1, Arf6, (T27N)Arf6, and (Q67L)Arf6. Ni-NTA agarose and Polyfect reagent were from Qiagen. GSH-sepharose, [*methyl*-^{3}H]choline chloride, anti-GST monoclonal antibody and [γ-^{32}P]ATP were from Amersham Pharmacia, UK. Naladixic acid, IPTG, myristic acid, L-α-dimyristoyl-phosphatidylcholine, phosphatidylinositol 4,5-bisphosphate and all cell culture materials were from Sigma, Poole, Dorset, UK. GTPγS was from Boerhinger and [^{35}S] GTPγS was from NEN Dupont. Anti-Arf1 antibody 1D9 was from Alexis Corporation and anti-Arf6 monoclonal antibody was from Santa Cruz.

2.2. Cell culture

HL-60 cells were cultured and labelled as described previously [30] except that medium was supplemented with dialysed, heat-inactivated foetal calf serum and where required grown in the presence of [*methyl*-^{3}H]choline (1 μCi/ml) for 48 h. Sf9 cells were in IPL-40 medium with 10% (v/v) foetal calf serum, 1% (v/v) amphotericin B, 8% (v/v) tryptose phosphate broth solution and 2% (v/v) yeastolate ultrafiltrate. For protein expression the cells were grown as monolayers in IPL-40 with all supplements except for fungizone.

2.3. Preparation of recombinant Arf proteins

PCR products produced from authentic Arf cDNA with forward and reverse primers corresponding to the termini of the sequences and any desired extensions e.g. polyhistidine tags, were cloned in to pMon5840 (ampicillin resistance) between the Nco1 and *Hin*dIII sites. For example, the Arf1 primers 5′-ATATACC·ATG·GGG·AAT·ATC·TTT·GCA·AAC·CTC-3′ (sense) and 3′-ATTATTAAGCT TCA·GTG·ATG·ATG·ATG·ATG·ATG·TTT·CTG·GTT·CCG·GAG·CTG·ATT·GGA·CAG-3′ (antisense) were used and analogous primers for Arf6 were also constructed. [Δ17]Arf1 primers exploited the in-frame ATG corresponding to Met17 in the Arf1 primary amino acid sequence. Plasmids were