RdgB-beta, an interacting partner of the Angiotensin II Receptor-Associated Protein, ATRAP, binds and transfers Phosphatidic Acid

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ATRAP, the Angiotensin II Receptor (AT1R)-associated protein, down-regulates angiotensin II signalling by binding to the AT1R, uncoupling it from G_q protein and promoting its internalisation (Daviet *et al*., 1999; Lopez-Ilasaca *et al*., 2003). ATRAP interacts with a phosphatidylinositol (PI) transfer protein, RdgBβ (*PITPNC1*), when cells are stimulated with phorbol myristate acetate (PMA) (Garner *et al*., 2011), and the present study was undertaken to establish the significance of this interaction for angiotensin II signalling.

RdgBβ binds and transfers phosphatidic acid (PA) in addition to PI. Using a permeabilised [^{14}C]acetate-labelled HL60 cell assay with recombinant transfer proteins and thin layer chromatography (Segui *et al*., 2002), we observed that RdgBβ binds PI and PA in equal quantities (*n*=5), in contrast to another PI transfer protein, PITPα (*PITPNA*), which binds PI and phosphatidylcholine (PC) equally (*n*=6). A dequenching assay (Somerharju *et al*., 1987) was used to examine PA transfer by RdgBβ: RdgBβ transfers pyrene-labelled PA robustly, across a range of recombinant protein concentrations (5-25μg per assay, *n*=3) from quenched donor vesicles (pyrene-PA/ *sn*1-palmitoyl-*sn*2-oleoyl-PC (POPC)/ *N*-trinitrophenyl-phosphatidylethanolamine (TNPE) (2:88:10; mol/mol)) to POPC/POPA (98:2 mol/mol) acceptor vesicles.

Signalling downstream of G_q protein relies on the hydrolysis of $PI(4,5)P_2$ by phospholipase Cβ (PLCβ) to generate the second messengers inositol trisphosphate (IP_3), and diacylglycerol (DAG), which can be phosphorylated to PA. In addition, phospholipase D (PLD) produces PA by the hydrolysis of PC. Angiotensin II (100 nM, 30 min) stimulates the incorporation of [^{3}H]inositol into PI ~3-fold over basal (2.99 ±0.11 s.e.m., *n*=3) in HEK-293 cells stably expressing AT1R (HEK-293-AT1R), yet this incorporation was not significantly changed upon over-expression of RdgBβ (2.32 ±0.29 s.e.m, *n*=3; *t* test: *p*=0.0969), indicating that RdgBβ does not facilitate flux through the PI cycle when cells are stimulated with angiotensin II.

Mass spectrometric analysis (ESI-MS/MS, Hunt *et al*., 2004) of the phospholipids bound by RdgBβ indicated that whereas RdgBβ is not selective in its binding of PI, it chooses short-chain saturated or mono-unsaturated PA species (*n*=6), consistent with those generated by hydrolysis of PC by PLD, not by PLC. Stimulation of HL60 cells with GTPγS at 10μM Ca^{2+} for 20mins promoted an increase in PA binding by RdgBβ (1.09 ±0.59 s.d. (pCa7) to 3.34 ±0.18 s.d. (100μM GTPγS, pCa5) relative PA binding per μg protein, *n*=2) in contrast to PITPα, whose lipid binding was unaffected by stimulation.

In summary, RdgBβ binds and transfers PLD-derived PA in response to cellular stimulation, and we hypothesise that PA binding stabilises protein expression and provides the signal for binding to ATRAP. Since ATRAP promotes AT1R down-regulation, recruitment of RdgBβ may enable ATRAP to prepare the local lipid environment for receptor endocytosis.

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