Is PRG-1 a new lipid phosphatase?

To the Editor:

Brauer *et al.*[1] recently described an integral membrane protein, PRG-1, with partial sequence similarity to the lipid phosphate phosphatases (LPPs)—cell surface–localized 'ecto phosphatases' that dephosphorylate lysophosphatidic acid (LPA) and related substrates[2]. The title identifies PRG-1 as a "new lipid phosphatase," and the article says it contains "highly conserved phosphatase sequences," but these were not identified explicitly[1]. Alignment with the invariant tripartite consensus sequence (denoted C1, C2 and C3) that defines LPPs and related phosphatases reveals non-conservative substitution in PRG-1 of three residues that are critical in catalysis (**Supplementary Fig. 1** online) and directly involved in substrate hydrolysis[2–6]. The lysine and arginine residues from the C1 motif are critical in substrate/transition state orientation in the active site, and the imidazole group of the missing C3 histamine residue functions as a nucleophile in the first step of the hydrolysis reaction[2–6]. Thus PRG-1 could not catalyze a phosphatase reaction using the reaction chemistry shared by these enzymes.

Intact N1E 115 neuroblastoma cells expressing GFP-PRG-1 and a membrane fraction prepared from these cells were reported to exhibit a 5-fold increase in the conversion of LPA added extracellularly to monoacylglycerol (MAG)[1]. We incubated intact N115E cells or membranes from cells expressing GFP, GFP-LPP3 (a *bona fide* LPP) or GFP-PRG-1 with oleyl-LPA that was double-labeled with [^{32}P] in the phosphate group and [^{3}H] in the acyl chain. Although GFP-LPP3 produced approximately twice as much [^{3}H]MAG and [^{32}P]PO_4^{2-} as control cells, GFP-PRG-1 did not increase the formation of any radiolabeled lipid product (**Supplementary Table 1**). LPPs are most active against substrates dispersed by binding to albumin or solubilized with nonionic detergent[2,6–8]. Membranes from HEK293 and N1E 115E cells expressing GFP-LPP3 showed 7-and 3-fold increases in LPA phosphatase activity against Triton X-100 solublilized LPA, but membranes from cells expressing GFP-PRG-1 or GFP–lipid-phosphatase-related protein 1 (LPR1) which, like PRG-1, also has an incompletely conserved phosphatase catalytic motif (**Supplementary Fig. 1** online), showed no increase in activity. Whereas overexpression of GFP-LPP3 produced 2- and 1.5-fold increases in ecto LPA phosphatase activity in HEK293 and N115E cells, respectively, measured using substrate bound to BSA, no increases in ecto LPA phosphatase activity were observed in cells expressing GFP-LPR1 or GFP-PRG-1 (**Supplementary Table 1**). Although some heterogeneity was apparent, particularly in the case of PRG-1, which we presume resulted from proteolytic degradation, expression of GFP-tagged LPP3, LPR1 and PRG-1 was approximately equal (**Supplementary Fig. 2**). We therefore conclude that PRG-1 and LPR1 do not have significant LPA phosphatase activity under conditions that readily support activity of LPP3 and other LPPs[5–8].

Given that LPA promotes neurite collapse, it is attractive to suggest that overexpression of PRG-1 may increase neurite formation in N1E 115 cells via PRG-1 catalyzed dephosphorylation of LPA[1]. Our observations indicate that this suggestion is not viable. Several experiments Brauer *et al.* used to test this idea lack important controls and do not preclude an alternative mechanism. Conservative substitution of the C2 serine residue of PRG-1 abolished its effects on morphology[1]. The paper[9] cited to show that this mutation "inactivates" the proposed phosphatase activity of PRG-1 describes regulation of germ cell migration by a *Drosophila* LPP and includes no mutagenesis studies or LPP assays. Although other work identifies a critical role for this residue in catalysis by bona fide LPPs[5,6], Brauer *et al.* did not show that the mutant PRG-1 was expressed to the same levels as wild-type PRG-1 or (without appropriate high-resolution microscopy images) localized like the wild-type protein, which could account for its apparent inability to alter neurite extension. Furthermore, the concentrations of LPA used to elicit neurite collapse were extremely high (up to 100 μM), and could cause lysis in several cell types (particularly without a protein carrier[10]). No controls were shown for cellular viability following treatment.

The most parsimonious explanation for these observations is that PRG-1 is not a "new lipid phosphatase." PRG-1 must have alternate biological activities that are responsible for its effects on the morphology of neuronal cells.

Note: Supplementary information is available on the Nature Neuroscience website.

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Bräuer *et al.* reply:

In our recent paper[1], we described a family of integral membrane proteins called plasticity-related genes (PRGs). Database analysis of these molecules revealed a similar membrane topology and significant sequence homology to lipid phosphate phosphatases (see also ref. 2). We performed an enzymatic assay using N1E-115 cells transfected with a GFP control construct and an LPP-1 or PRG-1 fusion construct, and measured LPA degradation