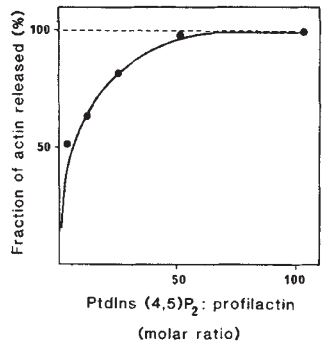

Fig. 3 Concentration dependence of the effect of PtdIns(4,5)P_2 on profilactin. Profilactin-β was mixed with PtdIns(4,5)P_2 at different molar ratios in the viscometer, and filament formation was followed as in Fig. 1. The fractions of the profilactin dissociated by the phospholipid were determined from the maximal viscosities reached, compared with that reached after addition of $MgCl_2$ (2 mM; see Fig. 1 legend).

by electrophoresis and scanning the gels (Fig. 2*d*). Gel scanning (Fig. 2*c*) indicated that the major part (88%) of the profilin co-chromatographed with the phospholipid and that 88% of the actin appeared as G-actin in the second peak. All the protein applied to the column was recovered. Thus, PtdIns(4,5)P_2 produced efficient dissociation of the profilactin complex, which apparently resulted from a relatively strong binding of profilin to the PtdIns(4,5)P_2 particles. The monomeric actin released, on the other hand, seemed unaffected by the phospholipid.

As PtdIns(4,5)P_2 clearly exerts its effect by binding to profilin, and as PtdIns(4,5)P_2 seems to have little effect on the polymerization of actin itself, it should be possible to estimate the stoichiometry of the PtdIns(4,5)P_2/profilin interaction indirectly by measuring the fraction of actin released using the viscosimetric assay at limiting amounts of PtdIns(4,5)P_2. For this, the fraction of actin released was determined by recording the maximum viscosity reached at varying concentrations of PtdIns(4,5)P_2. We found a direct relationship between the amount of PtdIns(4,5)P_2 added and the fraction of actin converted to filaments at low concentrations of phospholipid (Fig. 3), and from the initial part of the dose-response curve, calculated that dissociation of the protein complex required ~10 molecules of PtdIns(4,5)P_2 per molecule of profilactin. Considering the geometry of the PtdIns(4,5)P_2 micelles (spherical particles with a Stokes radius of 39 Å and a packing number of 82)[12] and of profilactin (Stokes radius 29 Å), this result indicates the possibility of a specific binding site for PtdIns(4,5)P_2 on profilin.

Clearly, binding of PtdIns(4,5)P_2 to profilactin causes the release of actin which is then available for polymerization in suitable conditions. Thus, the possibility should be considered that this phospholipid is involved in inducing actin filament formation in the cell. It has been realized for some time that ligand-induced turnover of phosphatidylinositol and mobilization of intracellular calcium may be causally related in many systems[13]. It was shown recently that a very early event following such receptor-mediated stimulation is the hydrolysis of PtdIns(4)P and PtdIns(4,5)P_2, resulting in the intracellular release of inositol 1,4-bisphosphate ($InsP_2$) and inositol 1,4,5-trisphosphate ($InsP_3$)[14-19]. The primary product, $InsP_3$, in turn seems to act as a second messenger for the induction of Ca^{2+} translocation[20-23]. Unstimulated cells contain a preponderance of PtdIns over the phosphorylated forms PtdIns(4)P and PtdIns(4,5)P_2, suggesting that the stimulated phosphorylation of PtdIns to PtdIns(4)P and PtdIns(4,5)P_2 through the action of membrane-associated kinase activities may be an equally early event (for review see ref. 24).

It is interesting that the increased activity in the phosphatidylinositol cycle induced by ligand-receptor interactions in turn seems to be closely correlated with an increased formation of microfilaments (polymerization of actin). Direct analysis of the G/F-actin ratio in cell extracts using the DNase I inhibition assay has shown that there is a rapid polymerization of actin following stimulation[25-30] of, for example, platelets with thrombin or ADP[25-27] and pancreatic β-cells with glucose[28-30]. In such cases, the ligand-receptor interaction has been shown to be linked to an increased turnover in the PtdIns cycle and Ca^{2+} mobilization[31-36]. Furthermore, ultrastructural studies suggest that one of the immediate events following stimulation of serum-starved glia-like cells and fibroblasts by platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) is the polymerization of actin, causing an outgrowth of new membrane lamellae and microspikes[8]; this phase is followed by increased cell motility. Both PDGF and EGF cause increased PtdIns turnover[37,38]. Finally, similar morphological changes, although less well characterized, have been observed after activation of *src* gene in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus[39]; it is now known that the *src* gene product can phosphorylate PtdIns(4)P to PtdIns(4,5)P_2, suggesting its involvement in the PtdIns cycle[40].

The results presented here provide biochemical evidence for a specific effect of PtdIns(4,5)P_2 on the putative precursor of microfilaments, profilactin. This may indicate that the formation of microfilaments and thus cell motility is regulated through the PtdIns cycle.

We thank Thomas Hult and Janusz Garbalinsky for technical assistance. This work was supported by a grant from the Swedish Cancer Society.

Received 17 August 1984; accepted 16 January 1985.

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Erratum

Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor

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ON page 805, Figures 2 and 3 were transposed.