Monoclonal antibodies to phosphatidylinositol 4-phosphate 5-kinase: distribution and intracellular localization of the C isoform

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We have raised a panel of monoclonal antibodies to PtdIns4*P* 5-kinase C purified from bovine brain [Divecha, Brooksbank and Irvine (1992) Biochem. J. **288**, 637–642]. This panel includes antibodies which specifically recognize PtdIns4*P* 5-kinase C both in a native catalytically active condition, and/or when presented on Western blots. Some of the former antibodies will also inhibit PtdIns4*P* 5-kinase C activity. We have used the blotting antibodies to study the bovine tissue distribution of PtdIns4*P* 5-kinase C and its distribution in mammalian species. We have also studied its localization in Jurkat cells and found it to be predominantly bound to membranes, with only a minority localized to the cytoskeleton. Neither PtdIns4*P* 5-kinase activity nor PtdIns4*P* 5-kinase C, as detected by Western blotting, were increased in the cytoskeleton after stimulation of Jurkat cells with OKT3. These antibodies should prove to be extremely useful tools with which to study the regulation of PtdIns4*P* 5-kinase C.

INTRODUCTION

One of the major mechanisms of activation of animal cells occurs via the receptor-stimulated hydrolysis of a minor membrane lipid, PtdIns(4,5)P_2, by a phosphoinositidase C. This yields the two second messengers Ins(1,4,5)P_3, which mobilizes intracellular Ca^{2+} (Berridge and Irvine, 1989), and diacylglycerol, which is an activator of protein kinase C (Nishizuka, 1988). A putative third second messenger, Ins(1,3,4,5)P_4, is synthesized by phosphorylating Ins(1,4,5)P_3 (Irvine et al. 1986). Production of the 3-phosphorylated inositol lipid PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2 is also believed to be a receptor-stimulated event (Stephens et al., 1991; Hawkins et al., 1992), although the second-messenger function of PtdIns(3,4,5)P_3, if any, is unknown.

Stimulation of these pathways causes depletion of the cellular levels of PtdIns(4,5)P_2, and this in turn necessitates an increase in the net synthesis of PtdIns(4,5)P_2 in order to maintain sufficient substrate for the continued production of second messengers. This has been shown to occur in several tissues, including T-cell lines (e.g. Thomas et al., 1983; Cockcroft et al., 1987; Rubin and Hoek, 1988; Inokuchi and Imboden, 1990; L. R. Stephens, unpublished work). The mechanisms controlling the concentration of PtdIns(4,5)P_2 are not known, but, because active PtdIns4*P* 5-kinases and PtdIns(4,5)P_2 5-phosphomonoesterases may continuously 'cycle' PtdIns(4,5)P_2, they could impinge on either its synthesis or degradation; hence an increase in net synthesis could be produced by an increase in the rate of PtdIns4*P* 5-kinase and/or a decrease in the rate of PtdIns(4,5)P_2 5-phosphomonoesterase. Suggested mechanisms for direct regulation of PtdIns4*P* 5-kinase include product inhibition (Van Rooijen et al., 1985; Lundberg et al., 1986), G-proteins (Smith and Chang, 1989; Urumow and Wieland, 1988, 1990b), tyrosine kinases (Gaudette and Holub, 1990; Payrastre et al., 1990) and translocation to the cytoskeleton (Payrastre et al., 1991) or to receptors (Cochet et al., 1991).

Studies on the regulation of PtdIns4*P* 5-kinase are complicated by the existence of several isoforms and by the confusion over their intracellular location. PtdIns4*P* 5-kinases of various molecular masses have been purified from both cytosolic and particulate fractions of a number of tissues, including rat brain (Van Dongen et al., 1984; Cochet and Chambaz, 1986), bovine brain (Moritz et al., 1990; Divecha et al., 1992), rat liver (Urumow and Wieland, 1990a), human red blood cells (Ling et al., 1989; Bazenet et al., 1990) and adrenal medulla (Husebye and Flatmark, 1989).

We have recently described the purification from bovine brain cytosol of a 53 kDa isoform of PtdIns4*P* 5-kinase, designated PtdIns4*P* 5-kinase C, and have partially purified two other isoforms, A and B, which appear to be immunologically distinct from C (Divecha et al., 1992). Here we describe the production and characterization of a panel of monoclonal antibodies to the different isoforms, in particular isoform C, and the use of these antibodies to study the tissue and species distribution of PtdIns4*P* 5-kinase C. We have also used the antibodies to study the intracellular localization of PtdIns4*P* 5-kinase C in Jurkat cells, a T-lymphocyte-derived cell line, and to show that PtdIns4*P* 5-kinase C does not translocate to the cytoskeleton on stimulation of the T-cell receptor via CD3.

MATERIALS AND METHODS

Materials

Microtitre plates were from Falcon, Oxnard, CA, U.S.A. Horseradish peroxidase-linked antibodies, anti-(rat Ig)–agarose and *o*-phenylenediamine tablets were from Sigma, Poole, Dorset, U.K. Supported nitrocellulose was from Sartorius, Göttingen, Germany. ECL reagents, prestained protein molecular-mass markers and [γ-^{32}P]ATP were from Amersham International, Amersham, Bucks., U.K. Iodogen was from Pierce, Warrington, Cheshire, U.K. Streptolysin-O was from Wellcome Diagnostics, Dartford, Kent, U.K. Alhydrogel (aluminium hydroxide gel) was from Superfos Biosector, Vedbaek, Denmark. All other reagents were of analytical grade.

Abbreviations used: TBS, Tris-buffered saline; TTM, TBS/0.05% Tween 20/5% non-fat dried milk.

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Preparation of antigen

PtdIns4*P* 5-kinase was purified as described previously (Divecha et al., 1992). The three purified PtdIns4*P* 5-kinase peaks (100–200 μg of protein per peak) were pooled and precipitated with ice-cold 10% trichloroacetic acid. The precipitated protein was collected by centrifugation in a microfuge and washed with ice-cold acetone, air-dried, then resuspended in phosphate-buffered saline.

Production of monoclonal antibodies

The PtdIns4*P* 5-kinase suspension (50–100 μg of each enzyme) was emulsified with Freund's complete adjuvant and Alhydrogel and used to inoculate one female LOU/C rat. The rat was given booster injections of antigen 40 and 110 days later, and bled for serum screening on day 50. On day 113 the rat was killed and spleen cells were fused with Y3Ag1.2.3 (Galfre et al., 1979) or IR983 F (Bazin, 1982) myeloma cells, as described by Galfre and Milstein (1981), and the resulting hybridomas were assayed by e.l.i.s.a. Those hybridomas which scored positive were then screened by e.l.i.s.a., Western blotting, immunoprecipitation and inhibition assay. Hybridomas which scored positive in one or more of these assays were cloned by limiting dilution, and the clones assayed by the most appropriate method for that hybridoma.

Screening procedures

E.l.i.s.a.

This was carried out essentially as in Gee et al. (1983). Purified PtdIns4*P* 5-kinases (25 ng of either a mixture of isoforms A and B, or isoform C) were bound to polyvinyl microtitre plates overnight (4 °C). After blocking in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4)/3% BSA, tissue culture supernatants diluted 1/2 with PBS/BSA were incubated at room temperature for 2 h. The plates were washed thoroughly with PBS/0.2% Tween-20 and incubated with anti-rat Ig conjugated to peroxidase (1:2000). After washing as above, e.l.i.s.a. plates were developed with *o*-phenylenediamine and H_2O_2, and the results were quantified by reading the A_{492} on an automatic plate-reader.

Western blots

Proteins were electrophoretically separated by the method of Laemmli (1970) in 10% gels, then transferred to supported nitrocellulose by electroblotting (Towbin et al., 1979).

The nitrocellulose was blocked overnight at 4 °C in Tris-buffered saline (TBS: 50 mM Tris/HCl, pH 7.5, 25 °C, 140 mM NaCl) containing 0.05% Tween 20 and 5% non-fat dried milk (Marvel) (TTM), followed by incubation for 2 h with antibodies diluted in the above solution. After washing in TTM, the blots were incubated with anti-rat Ig conjugated to peroxidase, washed and developed either with 3,3-diaminobenzidine and H_2O_2 or by using an ECL kit according to the protocol provided.

Inhibition assays

A modification of a rapid PtdIns4*P* 5-kinase assay was used, in which the lipid substrate is pre-bound to polyvinyl microtitre wells and incorporation of ^{32}P from [γ-^{32}P]ATP is measured (Divecha et al., 1992). Isoforms A + B or C [sufficient activity to incorporate approx. 1500 c.p.m. into PtdIns(4,5)P_2 over a 10 min assay] were diluted into 60 μl of kinase buffer (50 mM Tris/acetate, pH 7.4, 25 °C, 80 mM KCl, 10 mM magnesium acetate, 4 mM EGTA) + 3% fatty-acid-free BSA and preincubated for 1 h on ice with 60 μl of hybridoma supernatant in 0.5 ml microfuge tubes. The tubes were allowed to warm to room temperature for 10 min, then 100 μl portions were transferred into the wells of a microtitre plate which had been individually pre-coated with 500 pmol of sonicated PtdIns4*P* micelles in kinase buffer overnight and washed extensively to remove the free PtdIns4*P* (Divecha et al., 1992). The assays were started by adding 100 μl of kinase buffer containing 1 μCi of [γ-^{32}P]ATP and 5 μM ATP (final concn.), then quenched after 10 min by washing extensively with water. The plate was cut into individual wells and the amount of ^{32}P incorporated into the wells was quantified by Čerenkov counting. Inhibition was calculated as a percentage of the radioactivity (c.p.m.) incorporated into a sample preincubated with Dulbecco's modified Eagle medium + 20% fetal-calf serum.

Immunoprecipitation

A mixture of all three isoforms of PtdIns4*P* 5-kinase was labelled with ^{125}I by the Iodogen method (Fraker and Speck, 1978). A 10 μl portion of TBS/3% BSA containing 20000 c.p.m. of ^{125}I-labelled PtdIns4*P* 5-kinase was added to 40 μl of hybridoma supernatant in a 0.5 ml microfuge tube, and incubated on ice for 2 h. At this point 20 μl of a 50% slurry of goat anti-(rat Ig)–agarose in TBS/3% BSA was added, and the tubes were incubated on ice for a further 1 h. The pellets were washed with 2 × 0.5 ml of TBS/0.05% Tween-20/0.5 M NaCl, then counted for radioactivity in a γ-counter.

Antibody isotyping

The isotypes of the monoclonal antibodies were determined by double immunodiffusion (Ouchterlony and Nilsson, 1986) using isotype-specific anti-rat antisera (a gift from A. R. Bradwell, University of Birmingham, U.K.).

Streptolysin-O permeabilization of Jurkat cells

This was as described in Alexander et al. (1989). Briefly, cells were washed in PBS and resuspended at 5×10^6 cells/ml in buffer containing 12.5 mM Pipes (adjusted to pH 7.4 at 25 °C with KOH), 12.5 mM EGTA, 940 mM KCl, 5.16 mM $MgCl_2$, 8.18 mM $CaCl_2$. This gave a final free [Ca^{2+}] of 150 nm. Samples (200 μl) were pipetted into prewarmed tubes at 37 °C and equilibrated for 5 min. Cells were permeabilized by adding 50 μl of streptolysin-O (2 i.u./ml, reconstituted from dry with water). Permeabilization was stopped at various time points by brief centrifugation in a microfuge. The pellets and supernatants were processed for Western blotting as described in Figure 3.

Detection of PtdIns4*P* 5-kinase C in cytoskeletons

Cytoskeleton preparation was as described in Payrastre et al. (1991). Briefly, cells were washed in PBS, resuspended at 10^7–10^8 cells/ml in modified Hanks medium [130 mM NaCl, 5 mM KCl, 20 mM Hepes (adjusted to pH 7.4 at 25 °C with NaOH), 0.5 mM $MgCl_2$, 0.2 mM $MgSO_4$, 1 mM $CaCl_2$, 0.8 mM Na_2HPO_4, 10 mM glucose] and 100 μl samples were lysed in 1 ml of lysis buffer [20 mM Hepes (pH 7.4 at 25 °C), 50 mM NaCl, 1 mM EGTA, 1 mM phenylmethanesulphonyl fluoride, 10 μg/ml leupeptin, 0.1 mM Na_3VO_4] plus 0.5% (v/v) Triton X-100, after stimulation with OKT-3 at a final concentration of 1 μg/ml in modified Hanks medium where indicated. Particulate matter was collected by centrifugation in a microfuge, and the pellets were washed with 1 ml of lysis buffer plus Triton X-100 and then with 2 × 1 ml

of lysis buffer without Triton X-100. The pellets were then dissolved in 6 M urea, subjected to SDS/PAGE and blotted as described above, and PtdIns4*P* 5-kinase C was detected by using monoclonal antibody MAC 344 and an ECL kit.

Measurement of PtdIns4*P* 5-kinase activity in Jurkat-cell cytoskeletons

Cytoskeletons from control and OKT-3-stimulated Jurkat cells (10^7/sample) were prepared as described above and finally resuspended in 100 μl of kinase buffer. The samples were warmed to room temperature, and 100 μl of kinase buffer containing 10 μM PtdIns4*P* micelles, 10 μM ATP and 2 μCi of [γ-^{32}P]ATP were added. After 10 min the samples were quenched with 750 μl of chloroform/methanol/conc. HCl (100:200:1, by vol.), and the lipids were extracted by the method of Bligh and Dyer (1959), then resolved by t.l.c., as described in Divecha et al. (1992).

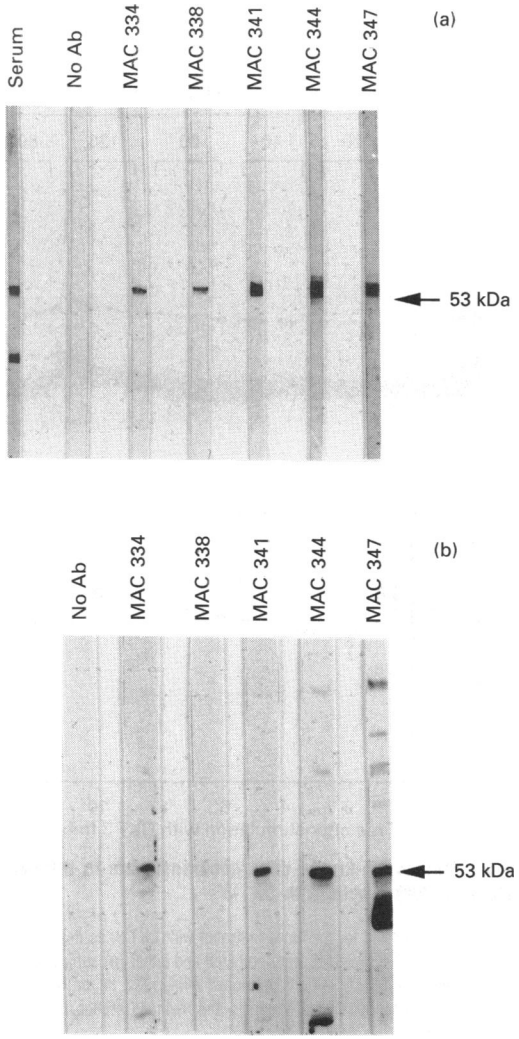

Figure 1 Western blots of purified PtdIns4*P* 5-kinase and Jurkat-cell homogenates with monoclonal antibodies to PtdIns4*P* 5-kinase C

A mixture of purified PtdIns4*P* 5-kinase isoforms (**a**) or Jurkat-cell homogenates (**b**) were subjected to SDS/PAGE and electroblotting as described in the Materials and methods section, and incubated with the supernatants indicated. The track marked 'Serum' is probed with immune serum from the rat which was used to make the hybridomas. Antibody binding was detected by using 3,3-diaminobenzidine (**a**) or an ECL kit (**b**). Ab, antibody.

Table 1 Properties of monoclonal antibodies to PtdIns4*P* 5-kinase

Clones were evaluated by four different assays, as described in the Materials and methods section. E.l.i.s.a. results are expressed as A_{492} readings, inhibition assays are expressed as % of the control activity, and immunoprecipitations (I.P.) are expressed as c.p.m. bound after subtraction of the control value.

	E.l.i.s.a.		Western blot		Inhibition assay			
Antibody	A/B	C	A/B	C	A/B	C	I.P.	Isotype
AFRC MAC 334	0.3	3.0	–	+	70	100	250	γ2c
AFRC MAC 335	1.5	1.0	–	–	100	100	300	μ
AFRC MAC 336	0	0	–	–	90	70	800	μ
AFRC MAC 337	0	0	–	–	70	100	800	γ
AFRC MAC 338	0.25	1.0	–	+	100	90	500	μ
AFRC MAC 339	0	0	–	–	60	40	0	μ
AFRC MAC 340	0	0	–	–	70	60	0	μ
AFRC MAC 341	0.5	2.5	–	+	90	90	250	μ
AFRC MAC 342	0	0	–	–	90	90	300	μ
AFRC MAC 343	0.5	0.5	–	–	80	60	0	μ
AFRC MAC 344	1.5	3.0	–	+	70	70	250	μ
AFRC MAC 345	0.5	0	–	–	90	80	400	μ
AFRC MAC 346	0	0	–	–	40	100	0	μ
AFRC MAC 347	1.0	3.0	–	+	40	60	200	μ

RESULTS

The panel of monoclonal antibodies contained five antibodies which recognized PtdIns4*P* 5-kinase C on Western blots (see Figure 1). Three of these (MAC 334, MAC 338 and MAC 341) were specific for PtdIns4*P* 5-kinase C on Western blots; MAC 344 recognized two other bands at 66 kDa and 29 kDa, and MAC 347 recognized a number of proteins. All five antibodies also recognized PtdIns4*P* 5-kinase C by e.l.i.s.a. and could immunoprecipitate it. Two of the antibodies, MAC 344 and MAC 347, also recognized a mixture of the A and B isoforms by e.l.i.s.a., and could inhibit the enzymic activity of both isoform C and a mixture of A and B to between 40 and 70% of control values (see Table 1). It is possible that the concentration of the tissue-culture supernatants from these clones would yield complete inhibition.

Western blots of the (bovine) tissue and species distribution of PtdIns4*P* 5-kinase C (Figure 2) reveal that it is present in a wide range of tissues, skeletal and cardiac muscle being notable exceptions. It is well conserved among mammalian species, appearing as a 53 kDa band in all the species that we have tested. Of the cell lines screened, T-lymphocyte-derived cell lines contain particularly large amounts of PtdIns4*P* 5-kinase C. This led us to study the intracellular distribution of PtdIns4*P* 5-kinase C in Jurkat cells.

Gentle permeabilization of Jurkat cells with streptolysin-O caused all of the lactate dehydrogenase activity, which is cytoplasmic, to diffuse out of the cells within 10 min (results not shown). Over the same time course, however, PtdIns4*P* 5-kinase C, as detected by Western blotting, remains cell-associated (Figure 3a). Triton extraction of the cells solubilizes a large proportion of the PtdIns4*P* 5-kinase C (Figure 3b). Thus PtdIns4*P* 5-kinase C is located mostly in a particulate Triton-extractable compartment in the cell, which is compatible with it being membrane-associated. A small proportion is not Triton-extractable, which is compatible with it being associated with the cytoskeleton, or possibly the nucleus (Payrastre et al., 1992; Divecha et al., 1993). Measurement of PtdIns4*P* 5-kinase activity in Jurkat cells gave parallel results, with 14% of the

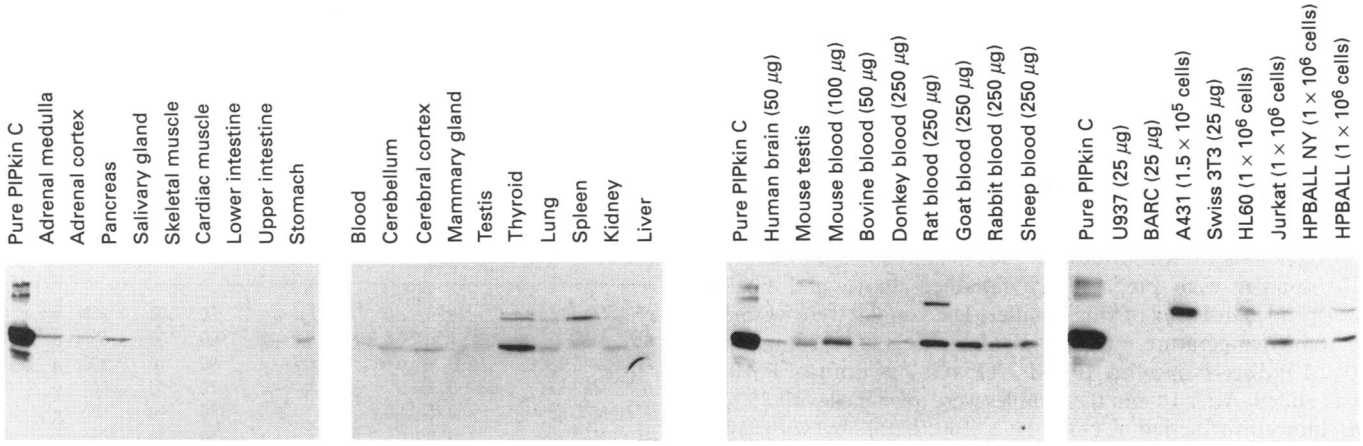

Figure 2 Bovine tissue and species distribution of PtdIns4*P* 5-kinase C (PIPkin C)

Tissues were homogenized in the presence of protease inhibitors, and low-speed supernatants were prepared. Equivalent amounts of protein (50 μg) were loaded on to 10% polyacrylamide gels (Laemmli, 1970) except where otherwise indicated, and subjected to SDS/PAGE followed by electroblotting. Blood samples from several species, and various cell lines, were prepared by the same method. Blots were probed with a mixture of three monoclonal antibodies, MAC 334, MAC 341 and MAC 344, and developed with an ECL kit.

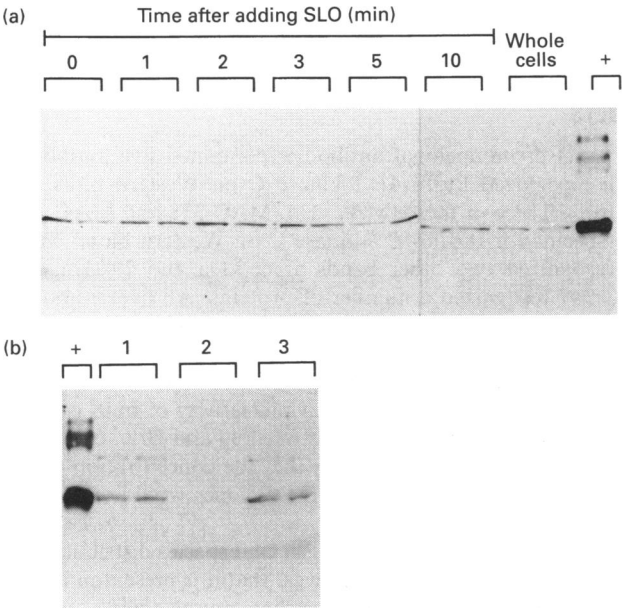

Figure 3 Location of PtdIns4*P* 5-kinase C in the particulate fraction of Jurkat cells

Jurkat cells were washed into intracellular buffer and duplicate samples were permeabilized with streptolysin-O (SLO) for increasing amounts of time, as described in the Materials and methods section. (**a**) Whole cells or particulate material from the same number of cells was rapidly collected by centrifugation in a microfuge and lysed in SDS/PAGE sample buffer. Samples were subjected to SDS/PAGE and blotted, then probed with antibody MAC 334; '+' contains molecular-mass markers and pure PtdIns4*P* 5-kinase C. (**b**) Cells were lysed in lysis buffer containing 0.5% Triton X-100 for 20 min, followed by centrifugation. The pellets were washed and dissolved in 6 M urea. The supernatants were precipitated with trichloroacetic acid and dissolved in urea. Samples (in duplicate) were subjected to SDS/PAGE and blotting, then probed with antibody MAC 344: 1, whole cells; 2, pellets; 3, supernatants; '+' contains molecular-mass markers and pure PtdIns4*P* 5-kinase C.

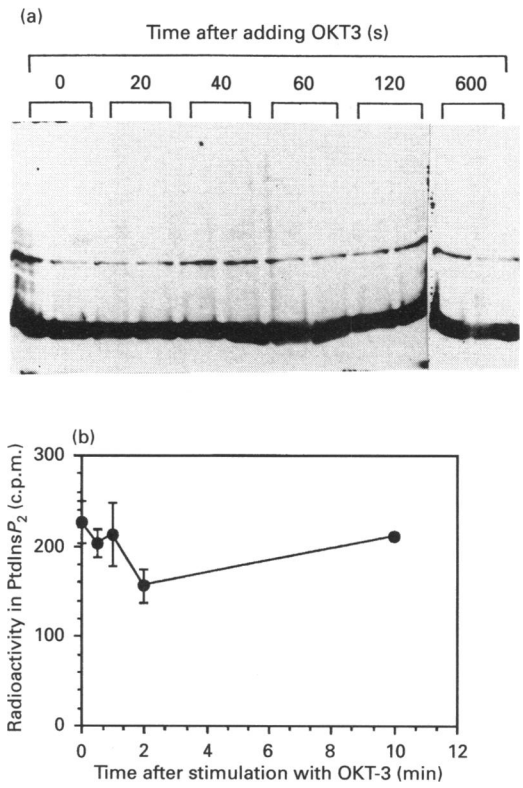

Figure 4 PtdIns4*P* 5-kinase does not translocate to the cytoskeleton on stimulation of Jurkat cells with OKT-3

Jurkat cells were stimulated for the times indicated with OKT-3, as described in the Materials and methods section. Cytoskeletons were prepared and either (**a**) subjected to SDS/PAGE and blotting, then probed with MAC 344 and developed with an ECL kit, or (**b**) assayed for PtdIns4*P* 5-kinase activity as described in the Materials and methods section.

PtdIns4*P* 5-kinase activity being Triton-non-extractable (results not shown). The amount of Triton-non-extractable PtdIns4*P* 5-kinase C in Jurkat cells did not change after stimulation of the T-cell receptor with 1 μg/ml OKT-3 (Figure 4a), which caused a 5-fold increase in $Ins P_3$ in these cells (results not shown). Triton-non-extractable PtdIns4*P* 5-kinase activity, measured by adding exogenous PtdIns4*P* micelles (no PtdIns4*P* 5-kinase activity could be measured without addition of exogenous PtdIns4*P*, presumably because endogenous PtdIns4*P* had been extracted by

the Triton; results not shown), decreased slightly over the same time period (Figure 4b).

DISCUSSION

PtdIns4*P* 5-kinase is a family of several distinct isoenzymes. We have recently purified one isoform, PtdIns4*P* 5-kinase C, to homogeneity and partially purified two other forms that are immunologically distinguishable from C. In this study we describe the production and initial characterization of a panel of monoclonal antibodies to the three different PtdIns4*P* 5-kinases (see Table 1), which will be useful tools for studying the distribution and regulation of each of the isoforms.

Of the five antibodies which recognize PtdIns4*P* 5-kinase C on Western blots, two (MAC 344 and MAC 347) recognize a 66 kDa band and a 29 kDa band (Figure 1b). The 66 kDa band is also apparent in some of the bovine tissues, which were screened with a mixture of antibodies including MAC 344, and the 29 kDa band is present in Jurkat cells, where it appears to be cytoskeletal (this is most apparent in Figure 4a, which was overexposed to facilitate location of the small amount of cytoskeletal PtdIns4*P* 5-kinase C). We do not know whether these bands are PtdIns4*P* 5-kinases or proteins which share an epitope with PtdIns4*P* 5-kinase C. It is interesting that many of the inhibitory antibodies do not recognize PtdIns4*P* 5-kinase by e.l.i.s.a.; differences in the presentation of antigen in solution compared with in an e.l.i.s.a. may account for this.

The C isoform of PtdIns4*P* 5-kinase has a distinctive tissue distribution, being relatively abundant in endocrine tissues, but absent (within our detection limits) from muscle. We also screened a number of cell lines, and were particularly interested in the distribution of this isoform in T-cell-derived cell lines such as Jurkat and HPBALL, which contain large amounts of PtdIns4*P* 5-kinase C, in sharp contrast with promyelocytic lines such as U937 and HL60 (and freshly isolated human neutrophils; results not shown), which have extremely low levels. Jurkat cells show increased turnover of PtdIns(4,5)P_2 on stimulation through the T-cell receptor (Inokuchi and Imboden, 1990) and should therefore be a good model system in which to study the regulation of PtdIns4*P* 5-kinase C.

Investigation of the intracellular location of PtdIns4*P* 5-kinase C in Jurkat cells revealed that, although it can be purified from bovine brain soluble fraction, it is entirely particulate in Jurkat cells, and the ability to extract it with Triton X-100 suggests that it is probably membrane-associated. Bazenet et al. (1990) have previously purified the human homologue of PtdIns4*P* 5-kinase C (which they call type II PtdIns4*P* 5-kinase) from both cytosol and membrane fractions of human erythrocytes. Interestingly, addition of the purified type II enzyme to stripped erythrocyte membranes yielded very low activity compared with the activity obtained with the type I isoform, suggesting that other components may be required for full activation of the type II enzyme *in vivo*: both in A431 cells stimulated with epidermal growth factor and in platelets stimulated with thrombin, there is an increase in the PtdIns4*P* 5-kinase activity associated with the cytoskeleton (Nahas et al., 1989; Grondin et al., 1991; Payrastre et al., 1991). It is an exciting possibility that the increase in PtdIns4*P* 5-kinase activity is due to interaction with cytoskeletal components, leading to activation of the enzyme. However, although there is good evidence for stimulation of PtdIns4*P* 5-kinase activity in Jurkat cells stimulated through the T-cell receptor (Inokuchi and Imboden, 1990), we found no evidence for a T-cell-receptor-stimulated increase in cytoskeletal PtdIns4*P* 5-kinase C or cytoskeletal PtdIns4*P* 5-kinase activity. This is suggestive of distinctive mechanisms of PtdIns4*P* 5-kinase regulation operating in Jurkat cells as compared with platelets and A431 cells.

With evidence accumulating that PtdIns4*P* 5-kinases can be stimulated by a G-protein (Urumow and Wieland, 1988, 1990a,b; L. R. Stephens, P. T. Hawkins and T. R. Jackson, unpublished work), and by association with either the cytoskeleton (Payrastre et al., 1991) or the epidermal-growth-factor receptor (Cochet et al., 1991), the possibility arises that the family of PtdIns4*P* 5-kinases may be similar to the phosphoinositidase C family (Rhee, 1991), such that different PtdIns4*P* 5-kinase isoforms are stimulated by distinct receptor–effector coupling mechanisms. Our antibodies to these enzymes should enable us to examine this possibility.

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