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ARTICLES

The Phosphatidylinositol Transfer Protein in 3T3 Mouse Fibroblast Cells Is Associated With the Golgi System

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Abstract By use of indirect immunofluorescence it was shown that the phosphatidylinositol transfer protein (PI-TP) in 3T3 mouse fibroblast cells is associated with the Golgi system. This was concluded from double-labeling experiments with TRITC-labeled Ricin which binds to sugar residues that are specifically processed in the Golgi system. Independent evidence for this association was provided by the fact that dissociation of the Golgi system by brefeldin A was reflected in an extensive redistribution of PI-TP labeling. In addition, PI-TP is localized in the cytoplasm and in the nucleus. In exponentially growing cells an enhanced labeling of PI-TP was observed in the cytosol and in the Golgi system in comparison with quiescent cells. By Western blot analysis and by transfer activity assays, it was confirmed that the concentration of PI-TP was increased in exponentially growing cells. These results strongly suggest that PI-TP fulfills a role in the functioning of the Golgi complex.

Key words: phosphatidylinositol transfer protein, immunofluorescence, Golgi system, nuclear localization

Proteins that catalyze the transport of phospholipids between membranes in vitro have been extensively studied (Wirtz, 1991). However, it is not yet clear whether or not the physiological function of these phospholipid transfer proteins (PL-TPs) is the actual transfer or binding of phospholipids. PL-TPs with different specificities towards the polar headgroups of phospholipids include the phosphatidylcholine (PC) transfer protein which specifically binds and transfers PC (Kamp et al., 1973; Lumb et al., 1976) and the phosphatidylinositol transfer protein (PI-TP) which binds and transfers both PI and, to a lesser extent, PC (Helmkamp et al., 1974; Demel et al., 1977; DiCorletto et al., 1979). In addition, a non-specific lipid transfer protein (identical to sterol carrier protein 2) has been identified which catalyzes the transfer of phospholipids and cholesterol between membranes (Bloj and Zilversmit, 1977; Crain and Zilversmit, 1980).

In mammalian cells most phospholipids, including PI, are synthesized on the endoplasmic reticulum. This implies that specific mechanisms of transport must operate to redistribute phospholipids from the site of synthesis to the proper location in the cell. It has been suggested that PI-TP is involved in this transport process, particularly directed to those membranes that have an active PI metabolism (Helmkamp et al., 1974; Van Paridon et al., 1987a; Cleves et al., 1991b). Transfer of PI to these membranes can be accomplished either by PI-TP directly or by a flow of membrane vesicles, the PI-content of which has been modulated by PI-TP. Recently it was shown that PI-TP from yeast is identical to the SEC14 protein which is involved in the secretion of proteins from a late Golgi compartment (Bankaitis et al., 1989, 1990). From studies on yeast mutants it was inferred that the capacity of PI-TP to bind PI or PC is essential for its function (Aitken et al., 1990; Cleves et al., 1991a). Due to its dual specificity, it was proposed that PI-TP is very well suited to regulate the PI/PC ratio in membranes (Wirtz et al., 1978; Van Paridon et al., 1987a). In agreement with this proposal PI-TP may be instrumental in maintaining the PI/PC ratio in the Golgi

Abbreviations used: PI-TP, phosphatidylinositol transfer protein; FCS, foetal calf serum; BFA, brefeldin A; FITC, fluorescein isothiocyanate; TRITC, tetramethyl rhodamine isothiocyanate; PI, phosphatidylinositol; PC, phosphatidylcholine; PA, phosphatidic acid.

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system which could be important for the proper movement of secretory vesicles through the Golgi stack (Cleves et al., 1991a,b).

Although the structure of PI-TP is highly conserved in mammalian tissues (Venuti and Helmkamp, 1988) and in yeast strains (Bankaitis et al., 1989), there is no homology between mammalian and yeast PI-TP (Dickeson et al., 1989; Salama et al., 1989). Therefore, it is not clear whether or not the physiological function of PI-TP in mammalian tissues is similar to that in yeast. In order to gain more insight into its cellular function, we have studied the localization of PI-TP in quiescent and exponentially growing 3T3 mouse fibroblast cells. We have found, that similar to yeast, part of the PI-TP is associated with the Golgi system. Furthermore, PI-TP is homogeneously distributed throughout the cytoplasm and is present in the nucleus.

MATERIALS AND METHODS

Cell Culture

Swiss 3T3 mouse fibroblast cells were cultured in Dulbecco's minimum essential medium (DMEM) containing 7.5% fetal calf serum (FCS) and buffered with $NaHCO_3$ (44 mM), at 7.5% CO_2 atmosphere. Cultures were made quiescent by incubation in DMEM containing 0.5% FCS for 48 h.

Preparation of the Antibody Against PI-TP

The antibody was raised in rabbits against synthetic peptides which are identical to the amino acid sequences of predicted epitopes in rat brain PI-TP (Dickeson et al., 1989). These peptide segments (residues 40–60 and 240–260) were synthesized with an additional cystein residue at the C-terminal end. The peptides (separate or mixed) were coupled under reducing conditions to keyhole limpet hemacyanin (KLH) as a carrier protein and mixed with Freund's complete adjuvant prior to immunization. The antibody IgG fraction was purified from the serum by DEAE-cellulose chromatography followed by chromatography on protein A sepharose at pH 8.9.

Immunofluorescence

The localization of PI-TP was determined by indirect immunofluorescence using the polyclonal anti PI-TP antibody. Cells grown on glass coverslips were fixed with methanol at −20°C for 5 min or in 3% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) containing 2% (w/v) sucrose for 30 min at room temperature. The paraformaldehyde-fixed cells were permeabilized with 1% Triton X-100 in PBS for 5 min at room temperature and the paraformaldehyde was quenched by incubation with 25 mM NH_4Cl for 10 min at room temperature. The cells, fixed by either method, were then rinsed with PBS without Ca^{2+} and Mg^{2+} (PBSO) and incubated with DMEM, buffered with Hepes (25 mM) and containing 0.1% bovine serum albumin (DBH), for 30 min at room temperature to block aspecific binding sites. The cells were exposed to the anti-PI-TP antibody or to control IgG isolated from pre-immune serum (both diluted 1:100 with DBH) for 1 h at room temperature. The cells were rinsed with PBSO, incubated for 1 h at room temperature with FITC-conjugated goat anti rabbit IgG (GAR-FITC, diluted 1:80 with DBH), rinsed with PBSO, and mounted in Mowiol. The Golgi system was visualized by labeling the fixed cells with TRITC-conjugated ricin (1:60 diluted with DBH). The labeled cells were viewed either with a Leitz inverted microscope equipped with barrier filters to prevent cross-over of FITC and TRITC fluorescence or with a confocal laser scanning microscope.

Preparation of the Cellular Fractions

3T3 cells were harvested by incubation with 8 mM EGTA in PBSO for 10 min at 37°C. The cells were collected by centrifugation (10 min, 600 rpm at room temperature) and resuspended in 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl pH 7.5 (SET buffer), and homogenized by 10 strokes in a Dounce homogenizer. Nuclei, debris and unbroken cells were removed by centrifugation for 5 min at 14,000*g*. The cytoplasmic and total membrane fraction were prepared by centrifugation of the 14,000*g* supernate for 1 h at 100,000*g* (Beckman, R50). Nuclei were purified by three established procedures for 3T3 cells (Cocco et al., 1988; Thomas et al., 1988; Bunce et al., 1988). The protein content of each fraction was determined by the method of Lowry (Lowry et al., 1951) after precipitation with 10% trichloroacetic acid and solubilization in 0.2 N NaOH.

PI-TP Transfer Assay

PI-transfer activity in the cytosolic fraction of 3T3 cells was estimated by measuring the transfer of phosphatidyl [^{3}H]inositol from microsomes to vesicles (Helmkamp et al., 1974). The donor microsomes contained 25 nmoles [^{3}H]-PI, the

acceptor vesicles consisted of 0.5 μmole PC and 0.01 μmoles PA. Cholesteryl 1-[^{14}C] oleate (0.06 nmole) was incorporated as a non-exchangeable marker in the acceptor vesicles to monitor recovery. The incubation was carried out in 0.3 ml of SET-buffer. After 30 min of incubation at 37°C the reaction was terminated by the addition of 0.06 ml of 0.24 M sodium acetate, pH 5.0, and chilling on ice to 0°C. The microsomes were pelleted by centrifugation for 10 min at 14,000*g* at 4°C and the liposomal lipids were extracted from the supernate (0.3 ml). Transfer activity was determined from the ^{3}H/^{14}C ratio. The amount of PI-TP per mg of total cytoplasmic protein was calculated from a standard curve of PI-TP transfer activity.

Gel Electrophoresis, Iso-Electric Focusing and Blotting

Cell fractions were analyzed by electrophoresis on 11% polyacrylamide gels in the presence of sodium dodecylsulfate and 2-mercaptoethanol. For immunological detection, proteins were electrophoretically transferred to nitrocellulose membranes at 1 mA/cm^2 for 75 min at room temperature. Gelatin (3% w/v) in Tris-buffered saline (TBS) was used as a blocking agent. The blot was incubated with the anti PI-TP antibody (1:100 diluted in TBS containing 1% (w/v) gelatin) and the PI-TP-antibody complex was identified with goat-anti rabbit IgG conjugated with alkaline phosphatase (GAR-AP, 1:1000 diluted with TBS containing 1% (w/v) gelatin). The GAR-AP was visualized with 5-bromo-4-chloro-3-indoyl phosphate p-toluidine salt and p-nitro blue tetrazolium chloride (BCIP/NBT) as color development substrate for alkaline phosphatase.

PI-TP I and II were separated by iso-electric focusing on polyacrylamide slab gel in the presence of 2% ampholytes. Focusing took place overnight at 200 V, followed by one h at 500 V. Before blotting the gel was incubated for 30 min in 62.5 mM Tris-HCl pH 6.8, 10% glycerol, 5% 2-mercaptoethanol, 2.3% sodium dodecyl sulphate. The proteins were transferred to nitrocellulose and visualized as described above.

Materials

Brefeldin A was a gift from Prof. F.T. Wieland (Heidelberg). Protein A sepharose and Ricin-TRITC (TRITC-Toxin RCA60) were obtained from Sigma, GAR-FITC was from Nordic, and Mowiol 4-88 was a gift from Hoechst. GAR-AP and the substrates BCIP/NBT were from Bio-Rad. [^{3}H]inositol and cholesteryl 1-[^{14}C]oleate were obtained from the Radiochemical Centre, Amersham. PI-TP was purified from bovine brains as described by Van Paridon et al. (1987b).

RESULTS

Characterization of the Anti PI-TP Antibody

A mixture of the two peptides representing the predicted epitopes of PI-TP (residues 40–60 and 240–260), coupled to KLH, we found to be the most effective in raising antibodies. Upon purification of the IgG fraction, it was determined whether or not the antibody recognized PI-TP. PI-TP occurs in two forms: PI-TP I containing one molecule of PI (iso-electric point of 5.5) and PI-TP II containing one molecule of PC (iso-electric point of 5.7) (van Paridon et al., 1987b). Purified PI-TP I and II were submitted to iso-electric focusing followed by Western blotting. Incubation of the immunoblots with the antibody demonstrated that the antibody was cross-reactive with both PI-TP I (Fig. 1, lane 4)

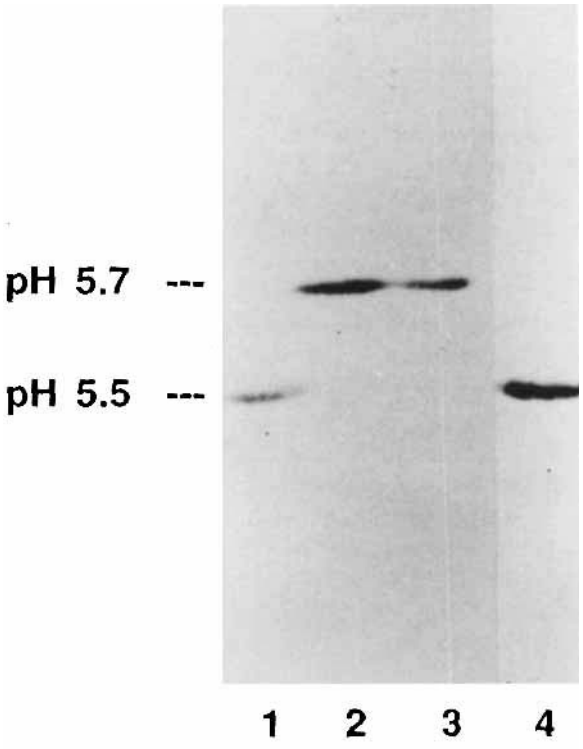

Fig. 1. Immunoblots of PI-TP I and PI-TP II upon iso-electric focusing. PI-TP II was converted into PI-TP I by incubation with PI-containing vesicles. As a control, PI-TP II was incubated with PC-containing vesicles. **Lane 1:** PI-TP II incubated with PI-vesicles (12 nmole PI/0.2 nmole PI-TP, 15 min at room temperature); **lane 2:** PI-TP II; **lane 3:** PI-TP II incubated with PC-vesicles (12 nmole PC/0.2 nmole PI-TP, 15 min at room temperature); **lane 4:** PI-TP I.

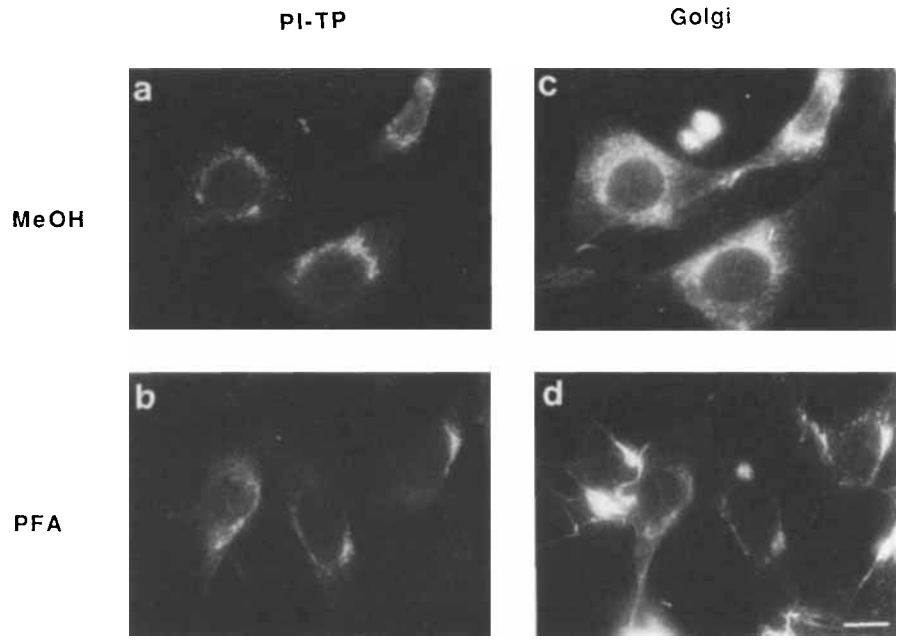

Fig. 2. Immunofluorescence localization of PI-TP and sugar residues which are specifically processed in the Golgi-system in 3T3 cells fixed in methanol (MeOH; **a,c**) and in paraformaldehyde (PFA; **b,d**). Cells were grown in 7.5% FCS and fixed as described in Materials and Methods. Incubation with anti-PI-TP antibody, followed by incubation with GAR-FITC (a,c). Incubation with Ricin-TRITC (panel b,d). Bar: 5 μm.

and PI-TP II (Fig. 1, lane 2). In addition, PI-TP II was converted into PI-TP I by incubating the protein with PI-containing vesicles. The antibody recognized the converted protein as shown by the appearance of one band shifted to a lower pH (Fig. 1, lane 1). Incubation of PI-TP II with PC containing vesicles did not result in a shift of the immuno-reactive band (Fig. 1, lane 3). From these results it was inferred that the antibody raised against the synthetic peptides binds to PI-TP.

The specificity of the antibody was demonstrated on Western blots of the total cytosolic fractions of bovine brain and 3T3 cells. With either fraction one major band (35 kDa) was observed. Sometimes an additional minor band (36 kDa) was observed (see also Fig. 5). The major band corresponds to PI-TP and the minor band corresponds to one of the precursor proteins of PI-TP (Van den Akker et al., 1991).

Intracellular Localization of PT-TP

The localization of PI-TP was studied in 3T3 cells that were fixed in methanol or paraformaldehyde. PI-TP is homogeneously distributed throughout the cytoplasm, localized in the nucleus, and associated with structures around the nucleus (Fig. 2a,c). No specific labeling is found in the plasma membranes of the cells. Incubation of the cells with purified pre-immune IgG shows a negligible labeling; incubation of the cells with antibody which has been pre-incubated with PI-TP, or with GAR-FITC alone also failed to give any labeling (results not shown). The pattern of labeling around the nucleus is representative for Golgi structures. Additional evidence was obtained from double-labeling experiments using Ricin as a marker. This lectin binds specifically to N-acetyl-D-galactosamine (D-gal-NAc) and to the β-d-galactosyl (β-D-gal) residues that are specifically processed in the Golgi compartment. As shown in Figure 2b,d, the structures around the nucleus, that are labeled by PI-TP antibodies, are also labeled by Ricin-TRITC.

Comparison of methanol- and paraformaldehyde-fixed cells demonstrated only small differences in labeling by the anti-PI-TP antibody (Fig. 2a,c). However, in double-labeling experiments where GAR-FITC is combined with Ricin-TRITC, paraformaldehyde fixation leads to an increased background labeling by Ricin-TRITC (Fig. 2d). For this reason we only show results obtained with methanol-fixed cells. However, all

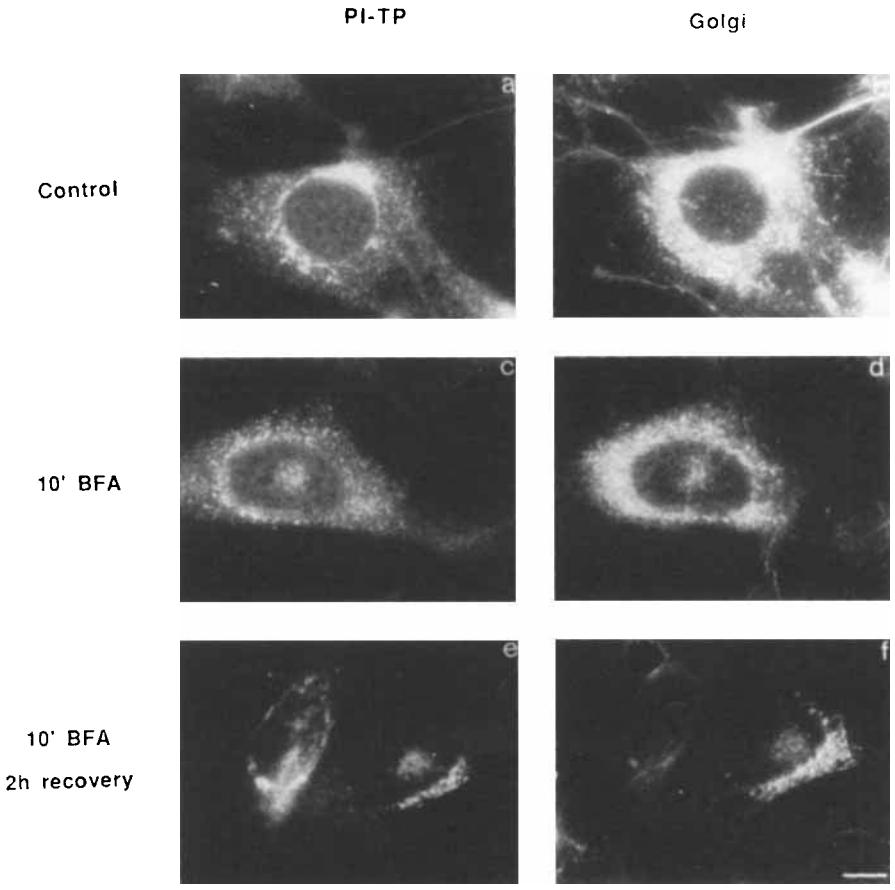

Fig. 3. Immunofluorescence localization of PI-TP (**a,c,e**) and sugar residues which are specifically processed in the Golgi-system (**b,d,f**) in exponentially growing cells. Labeling was carried out with control and BFA-treated cells and with cells recovered from BFA-treatment. Cells were incubated for 10 min at 37°C in DMEM (+7.5% FCS) in the absence (a,b) or presence (c,d,e,f) of BFA (5 μg/ml). After removal of the media the cells were fixed as described in Materials and Methods (a,b,c,d). Recovery from BFA-treatment was achieved by incubating the cells for 2 h in DMEM (+7.5% FCS) prior to fixation (e,f). Bar: 3 μm.

experiments have been carried out with both fixation methods yielding comparable results in PI-TP labeling.

Localization and Activity of PI-TP in Quiescent and Exponentially Growing Cells

3T3 cells were fixed during exponential growth (Fig. 3) or after 48 h of serum deprivation (Fig. 4). It can be seen that in exponentially growing cells the total amount of PI-TP labeling is increased in the cytoplasm and in the Golgi system (Fig. 3a) in comparison to the quiescent cells (Fig. 4a). In quiescent cells, PI-TP is mostly found in the perinuclear Golgi system in a closely packed stack pattern. The structures labeled by the PI-TP antibody are also labeled by Ricin-TRITC both in exponentially growing cells (Fig. 3b) and in quiescent cells (Fig. 4b).

Further evidence for the localization of PI-TP in/on the Golgi system was obtained from experiments using brefeldin A (BFA). Incubation of exponentially growing and quiescent cells with BFA, a drug that induces a rapid and reversible dissociation of the Golgi structures (Lippincott-Schwartz et al., 1989), leads to changes in the fluorescence patterns. After 10 min of BFA treatment (5 μg/ml), PI-TP labeling has virtually disappeared from the perinuclear region (Figs. 3c, 4c). Since the action of BFA is reversible, the Golgi structures recover when these cells are incubated with fresh medium for 2 h, leading to a reappearance of PI-TP labeling around the nucleus (Figs. 3e, 4e). Ricin-TRITC labels similar structures as the anti-PI-TP antibody in control cells (Figs. 3b, 4b), in BFA-treated cells (Figs. 3d, 4d), as well as in cells where the Golgi system has recovered (Figs. 3f, 4f).

Upon fractionation of the exponentially grow-

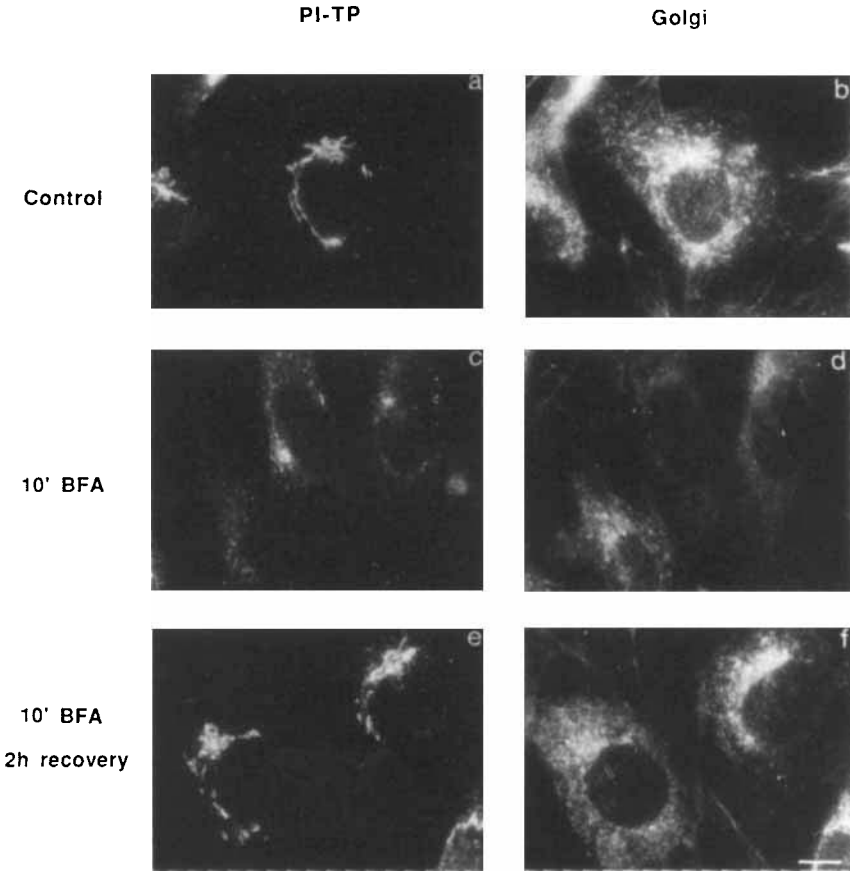

Fig. 4. Immunofluorescence localization of PI-TP (**a,c,e**) and sugar residues which are specifically processed in the Golgi-system (**b,d,f**) in quiescent cells. Labeling was carried out with control and BFA-treated cells and with cells recovered from BFA-treatment. Quiescent cells were prepared as described in Materials and Methods. Cells were incubated for 10 min at 37°C in DMEM (+0.5% FCS) in the absence (a,b) or presence (c,d,e,f) of BFA (5 μg/ml). After removal of the medium, the cells were fixed immediately as described in Materials and Methods (a,b,c,d). Recovery from BFA-treatment was achieved by incubating the cells for 2 h in DMEM (+0.5% FCS) prior to fixation (e,f). Bar: 3 μm.

ing and quiescent cells in a cytosol and a total membrane fraction, the distribution of PI-TP was determined by Western blot analysis. All PI-TP appeared to be present in the cytosol fractions (Fig. 5a, lanes 1, 2). In the cytosolic fraction of exponentially growing cells a second, minor band (36 kDa) is found. This band most likely corresponds to a precursor protein of PI-TP as described before (Van den Akker et al., 1991). Since no PI-TP could be detected in the membrane fractions (Fig. 5a, lanes 4, 5), it appears likely that PI-TP is loosely associated with the Golgi structures. These blots also indicate that the amount of PI-TP in exponentially growing cells is increased (Fig. 5a, lanes 1, 2), which is in agreement with the elevated fluorescence labeling observed (Fig. 3a). This was confirmed by measuring PI-transfer activity in the cytosolic fractions of exponentially growing and quiescent cells. The amount of PI-TP as calculated from the transfer activity is doubled under conditions of cell growth (Fig. 5b).

Nuclear Localization of PI-TP by Confocal Laser Scanning Microscopy

In exponentially growing cells, nuclear labeling of PI-TP can be detected (Fig. 6a). Apart from the cytosol and the perinuclear Golgi system, the nuclei are homogeneously labeled, except for the nucleoli. However, the intensity of the labeling can vary between nuclei in one cell culture. Analysis of a cross-section of the labeled cells shows a distinct difference between Golgi-associated labeling (G) and nuclear labeling (N) (Fig. 6b). We have not been able to confirm the presence of PI-TP in purified nuclei, neither by

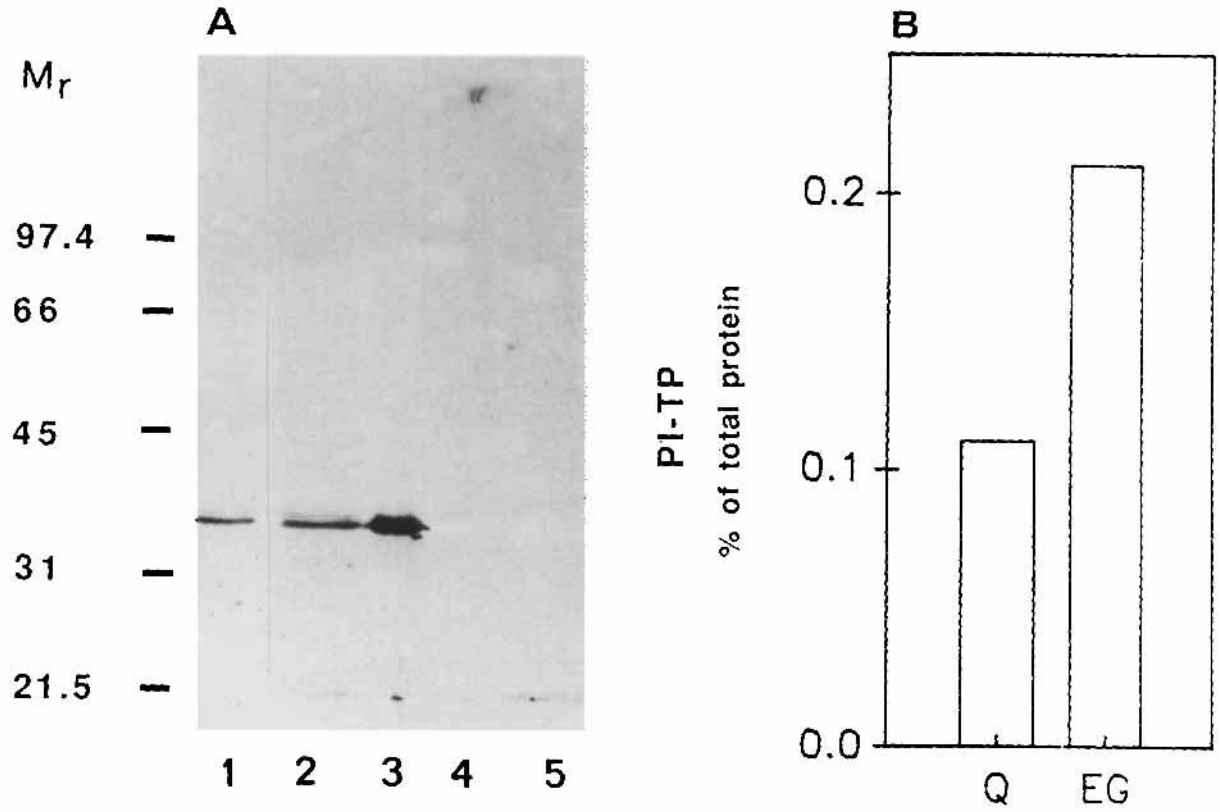

Fig. 5. Distribution of PI-TP between the cytosol and total membrane fraction from 3T3 cells. **A:** Western blots of cytoplasmic (**lanes 1, 2**) and total membrane fractions (**lanes 4, 5**) isolated from quiescent and exponentially growing 3T3 cells, respectively. In each lane 200 μg of protein was applied. Standard bovine PI-TP (**lane 3**). **B:** The amount of PI-TP in cytosolic fractions of quiescent (Q) and exponentially growing 3T3 cells (EG). Transfer activity was converted into PI-TP as percentage of total cytosolic protein (see Materials and Methods). The results are the average values of triplicates in four experiments; the overall variation between values was 8–12%.

Western blots analysis nor by transfer activity assays. Similar results were obtained with nuclei purified by three established procedures for 3T3 cells. To investigate the possibilities of artifacts, we have determined antibody-binding to DNA by incubating a Southern blot with PI-TP antibody. When this blot was developed as described for a Western blot, no binding of antibody to DNA could be detected (data not shown).

DISCUSSION

Recently it was reported that PI-TP in yeast is identical to the SEC14 protein and that this protein is specifically associated with the Golgi system (Bankaitis et al., 1989, 1990). Since this intriguing discovery it has been of great interest whether or not the mammalian PI-TP has a similar function. Except for the molecular weight of 35 kDa and the capacity to bind in vitro PI and PC, yeast and mammalian PI-TP show remarkable differences. The proteins lack immunological cross-reactivity (Szolderits et al., 1989), as well as any homology in their amino acid sequence (Dickeson et al., 1989; Salama et al., 1990). In yeast it was shown that the deficiency in PI-TP is lethal (Aitken et al., 1990) and that the requirement for PI-TP can be bypassed by a mutation in the CDP-choline pathway of PC synthesis (Cleves et al., 1991a,b). These observations were interpreted to indicate that the capacity to bind and/or transfer PI and PC is important for the physiological function of PI-TP, thereby regulating the PI/PC ratio in intracellular membranes, in particular that of the Golgi system. Cleves et al. (1991a,b) have proposed that the maintenance of the PI/PC ratio of the Golgi system by PI-TP is essential for the functioning of this membrane system in secretory processes. In previous studies it has been suggested that in view of its dual specificity, PI-TP is well suited to maintain the PI/PC ratio of membranes (Van Paridon et al., 1987a). PI-TP

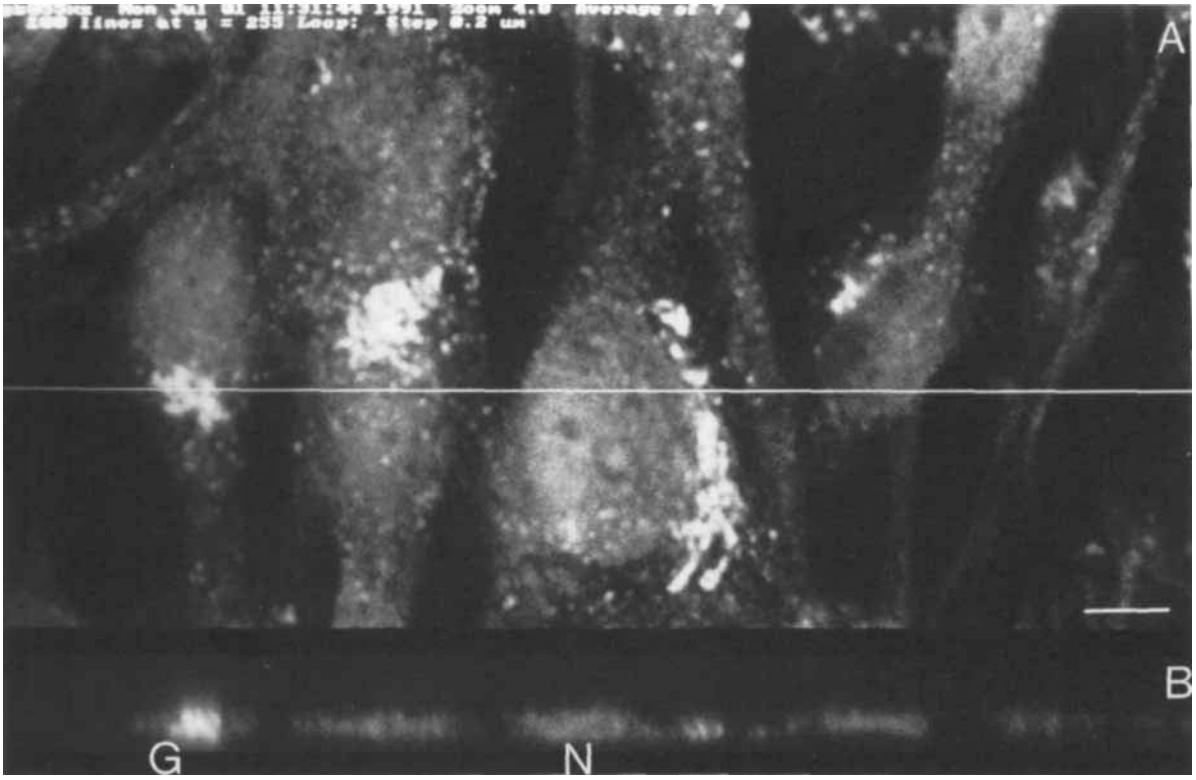

Fig. 6. Immunofluorescent labeling of PI-TP in the nucleus of 3T3 cells analyzed by confocal laser scanning microscopy. Exponentially growing cells were fixed and labeled with anti-PI-TP antibody and GAR-FITC as described in Materials and Methods. **A:** Scan of PI-TP labeling of 3T3 cells. **B:** Scan of PI-TP labeling in a plane perpendicular to that of panel A along the line drawn. Bar: 3 μm.

could be involved in replenishing the PI content of the plasma membrane upon agonist-stimulated phosphatidylinositol 4,5-bisphosphate breakdown (Wirtz et al., 1978; Helmkamp, 1986; Van Paridon et al., 1987a).

In the present study we have investigated the localization and transfer activity of PI-TP in quiescent and exponentially growing mammalian cells. By immunofluorescent labeling it was demonstrated that in analogy with yeast, PI-TP is associated with the Golgi structures in the cell. The localization was confirmed in double-labeling experiments using Ricin as a marker that binds to sugar residues which are specifically processed in the Golgi system. In addition, BFA-treatment of the cells gave rise to a redistribution of PI-TP labeling throughout the cell as a result of dissociation of the Golgi system (Donaldson et al., 1990; Lippincott-Schwarz et al., 1989; Orci et al., 1991; Hsu et al., 1991). Apart from being associated with the Golgi system, a distinct localization of PI-TP was observed in the cytoplasm and in the nucleus. In exponentially growing cells the extent of labeling of PI-TP associated with the Golgi system was clearly increased. Upon cell fractionation, Western blot analysis demonstrated that PI-TP was only present in the cytosol. Apparently, homogenisation of the cells leads to PI-TP release from the Golgi structures. The increased fluorescent labeling of PI-TP in exponentially growing cells was in agreement with results obtained by Western blot analysis and by measuring the PI-transfer activity in the cytoplasmic fractions.

The nuclear localization of PI-TP is particularly evident in exponentially growing cells. However, labeling of the nuclei in one cell culture is not homogeneous. This may reflect a cell cycle-dependent localization of PI-TP. The nuclear localization of PI-TP may be related to the presence of proteins that are involved in the PI-dependent signal transduction pathway in the nucleus (e.g., growth factor receptors, PI- and PIP-kinase, and protein kinase C) (Smith and Jarret, 1987; Cocco et al., 1987, 1988; Thomas et al., 1988; Jiang and Schindler, 1990; Capitani et al., 1990). On the other hand, we cannot yet exclude the possibility that the nuclear labeling

of PI-TP is an artifact because the nucleus is a well-known organelle for non-specific immunological effects (Nigg, 1988). We have not been able to detect PI-TP in isolated nuclei by Western blot analysis or by transfer activity assays. An explanation for this absence could be that proteins may "leak" from the nucleus through the nuclear pores because of their size (<65 kDa) or because of ATP-depletion (Silver, 1991).

The association of PI-TP with the Golgi system suggests that similar to what is observed in yeast, PI-TP in mammalian cells is part of the secretory pathway. However, it is striking that in quiescent cells, PI-TP is found only associated with the Golgi system while in exponentially growing cells PI-TP is also clearly visible in the cytoplasm. This redistribution between Golgi and cytoplasm may be related to the active membrane vesicle flow in exponentially growing cells. Incubation of quiescent cells with the calcium ionophore A23187 or phorbol 12-myristate, 13-acetate, well-known stimuli of secretion in 3T3 cells, leads to increased cytoplasmic localization of PI-TP (manuscript in preparation). It remains to be established what factors regulate the distribution of PI-TP between the Golgi system and the cytoplasm. The predominant association of PI-TP with the Golgi system suggests the possibility that specific "receptors" for PI-TP are present in this organelle (Cleve et al., 1991a,b). The dynamics of the association of PI-TP with the Golgi system are currently being investigated.

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