IUBMB *Life*, 56(8): 467–475, August 2004

Taylor & Francis
healthsciences

Critical Review

Phosphatidylinositol Transfer Proteins: Emerging Roles in Cell Proliferation, Cell Death and Survival

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Summary

The actual cellular functions of the highly homologous small isoforms of the phosphatidylinositol transfer proteins, PI-TPα and PI-TPβ have been studied using many different experimental conditions varying from *in vitro* experiments with purified proteins and lipid vesicles to investigations in animals. In this review, the very diverse data of these investigations have been collected and joined to propose a model for the cellular functions of PI-TPα and PI-TPβ. The model is based on the suggested roles of PI-TPα and PI-TPβ in various lipid-mediated cellular signaling pathways and leads to the conclusion that both proteins have a regulating function in pathways involved in the proliferation, apoptosis as well as survival of cells.
IUBMB *Life*, 56: 467–475, 2004

Keywords Phosphatidylinositol transfer protein; phosphatidylinositol metabolism; arachidonic acid metabolites; endocannabinoids; G-protein coupled receptor; cell proliferation; apoptosis.

INTRODUCTION

Phosphatidylinositol transfer proteins constitute a family of highly conserved proteins sharing the ability to transfer phosphatidylinositol, phosphatidylcholine and/or sphingomyelin between membranes.

Recently, several excellent reviews have appeared describing in detail all members and pseudo members, differences as well as similarities, of the growing PI-TP family (*1–4*). Here I will describe PI-TPα and PI-TPβ, the two soluble isoforms in mammalian cells. For more than 30 years, many experiments with PI-TPα and after 1994 also with PI-TPβ, have been performed to get information about the cellular function. The experimental approach has been very diverse:

- In *in vitro* studies purified proteins and either natural or artificial membranes are used to investigate transfer and/or binding of radioactive or fluorescent phospholipids.
- Semi-intact/permeabilized cells have been used to identify the soluble proteins that are essential for cellular processes, e.g. PI-metabolism, other cellular signaling pathways involving phospholipids and secretion.
- In intact cells, organisms or animals the expression of the proteins has been manipulated.

In this review, we will summarize the data accumulated so far and come up with a proposal for the cellular functions for the PI-TPs based on this work.

PROPOSED FUNCTIONS OF PI-TPS: *IN VITRO* AND STRUCTURE STUDIES

What we now consider to be PI-TPα was described in 1973 (*5, 6*). The isoform, PI-TPβ was detected in 1994 (*7–9*). It is not always clear therefore whether data obtained before 1994 relate to PI-TPα or PI-TPβ. The homology between the two isoforms is very high (77% homology, 94% identity (*7*)).

The *in vitro* binding and transfer of lipids is determined by various techniques using either radioactive or fluorescently labeled phospholipids (*10–12*). The data from several laboratories using different assays are remarkably unanimous: PI-TPα and β are able *in vitro* to specifically bind and

Received 23 July 2004; accepted 21 September 2004

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Abbreviations: PI-TP: phosphatidylinositol transfer protein; PI: phosphatidylinositol; PC: phosphatidylcholine; SM: sphingomyelin; PLC: phospholipase C; PLA: phospholipase A; PIP-5-K: phosphatidylinositol phosphate-5-kinase; PI-3-K: phosphatidylinositol-3-kinase; PIP_2: phosphatidylinositol bisphosphate; PIP_3: phosphatidylinositol trisphosphate; LysoPC: lysophosphatidylcholine; LysoPI: lysophosphatidylinositol; GPI: glycerophosphoinositol; I(1)P: inositol-1-phosphate; I(2)P: inositol-2-phosphate; COX-1/2: cyclo-oxygenase 1 or 2; TNFα: tumor necrosis factor α; CB1/2: cannabinoid receptor 1 or 2; THC: Δ^9-tetrahydrocannabinol; GPCR: G-protein coupled receptor

ISSN 1521-6543 print/ISSN 1521-6551 online © 2004 IUBMB
DOI: 10.1080/15216540400012152

transfer PI and to a lesser extent PC. In addition, PI-TPβ purified from bovine brain, demonstrated the ability to transfer fluorescently labeled SM (*8*). However, the transfer of SM by PI-TPβ has led to some discrepancy. Using an assay with liposomes containing radioactive SM or (^{3}H)-choline labeled HL60 cells as donor membranes, no transfer of SM by PI-TPβ could be detected (*13*). On the other hand, it was also proposed that PI-TPα and PI-TPβ demonstrate equal SM transfer activities (*14*). However, none of these findings proves that binding and/or transfer of phospholipids is actually involved in the cellular functions of PI-TPα and PI-TPβ.

The similarity between PI-TPα and PI-TPβ is very high. However, in addition to the binding/transfer of SM, a second important property of PI-TPβ is not shared by PI-TPα: PI-TPβ is the only member of the PI-TP-family that contains a unique, protein kinase C-dependent phosphorylation site, Ser^{262}, in addition to a protein kinase C-dependent phosphorylation site that is present in all members: S$^{165/166}$ (*15, 16*).

The crystal structure of PI-TPα without a phospholipid (the apo-form) as well as of the holo-form containing a PC or a PI molecule have been described (*17–20*). The structure of the apo-form is different from the PI- or PC-bound forms although the secondary structural elements are preserved. However, the overall fold of the PI- and PC-bound forms is highly similar, in contrast to previous suggestions (*20*). In all current structures the main structural feature is a concave β-sheet consisting of eight strands (*1–8*) and seven α helices (A–G). The part of the structure forming the lipid-binding site consists of the β-sheet and two α-helices, A and F facing the interior of the β-sheet. In the apo-structure, the lipid-binding core is open; when a lipid is bound, helix G and loop B are moved over the lipid-binding core. The apo- and PI/PC bound forms are shown in Fig. 1.

One of the putative membrane surface interaction sites of the molecule consists of a loop comprising two tryptophan residues, Trp203 and Trp204. The PI-TPα double mutant W203A/W204A is able to bind PI or PC but lacks PI/PC transfer activity and is unable to reconstitute PLC activity. The lipid head group cavity reveals a set of amino acids that can form H-bonds with the inositol moiety. The choline head group is less well H-bonded explaining the poor affinity for PC relative to PI (*17*).

The crystal structure of PI-TPβ has not yet been elucidated but because of the very high homology between PI-TPα and PI-TPβ, a good model structure for PI-TPβ can be derived from that of the α-species. Closer inspection of structural details reveals that only two amino acids are different in the phospholipid binding region: Ile^{84} and Phe^{225} in PI-TPα are replaced by a Phe and Leu in the β-species, respectively. Only minor differences in substrate preference are to be expected from such subtle differences between the two forms when

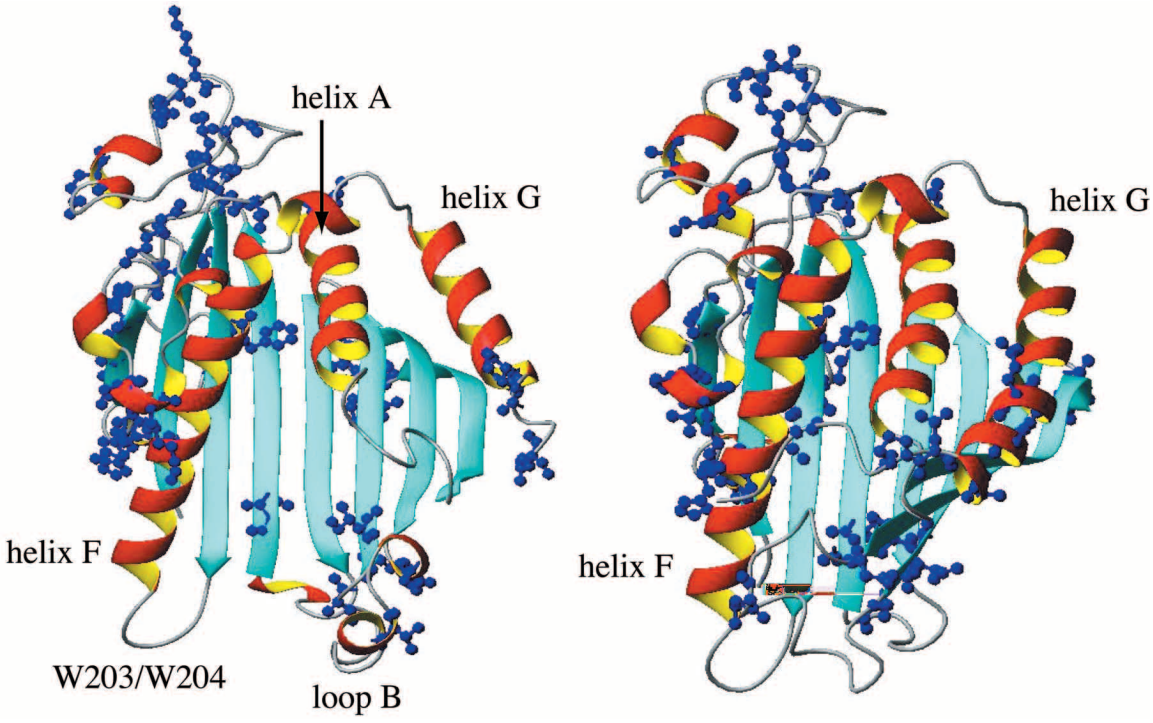

Figure 1. The structures of the apo-form (left) and the holo-form containing PI- or PC (right) of PI-TPα are shown indicating structural changes induced by phospholipid binding. Amino acid side chains (blue) are shown only for those 24 residues that are functionally different for the PI-TPα and PI-TPβ species (Courtesy of Prof. M. R. Egmond).

taking into account the properties of the substrate-binding region only.

PROPOSED FUNCTIONS OF PI-TPS: STUDIES IN RECONSTITUTED SYSTEMS AND IN PERMEABILIZED CELLS

In 1993, a first paper pointing to an important *in vivo* function of PI-TP appeared. Upon permeabilization of HL60 cells, the activity of phospholipase Cβ is diminished indicating the necessity of a cytosolic factor for its activity that was identified as PI-TPα (*21*). Either PI-TPβ or PI-TPα added to the reaction mixture stimulated the production of inositol phosphates to the same extent (*8*). In permeabilized PC12 cells, it was shown that one of the cytosolic factors required for the calcium-regulated fusion of secretory vesicles with the plasma membrane is PI-TPα. A second essential factor isolated from the cytosol was shown to be phosphatidylinositol phosphate-5-kinase (PIP-5-K) acting synergistically with PI-TPα leading to the conclusion that PIP_2 (and also PIP_3) is necessary for priming of the Ca^{++}-dependent secretion (*22, 23*). Involvement of PI-TPs in membrane traffic by vesicle flow was confirmed by the finding that PI-TPs stimulate the formation of secretory vesicles/granules from the trans Golgi network isolated from PC12 cells. Cytosolic activities stimulating (CAST) the formation of constitutive secretory vesicles as well as immature secretory granules were purified and identified. CAST1 was shown to be a mixture of PI-TPα and β (*24*). CAST2 was identified as a PIP-5-K (*23*).

In a cell free system prepared from hepatocytes containing stacked Golgi fractions budding of constitutive transport vesicles from the Golgi membranes was stimulated by PI-TPα and PI-TPβ (*25*). Under conditions of limiting cytosol, purified phosphatidylinositol-3-kinase (PI-3-K) and PI-TPα/β functionally cooperate to drive exocytotic vesicle formation in this system. A comparable conclusion was drawn from experiments with trans Golgi membranes that were isolated from Madin-Darby Canine Kidney (MDCK) cells transfected with Vesicular Stomatitis Virus (SVS). Antibodies recognizing mammalian PI-TPα and PI-TPβ did inhibit the capacity of cytosol to support normal vesicle generation. Also turkey erythrocyte ghosts have been used to study the cellular functions of PI-TPs. Exogenously added PI-TP from bovine brain was able to maintain agonist-stimulated PIP_2 degradation by PLC until 70% of the PI in the turkey erythrocyte ghosts was consumed. In the control ghosts without added PI-TP only 15% of the PI could be metabolized (*26*).

Only little information on possible molecular mechanisms involved in the reported observations are given but the general suggestion is that PI-metabolism, in particular the formation of phosphorylated phosphoinositides or water soluble inositolphosphates is involved in various stages of the intracellular membrane traffic. The identical conclusions in most of these papers is the involvement of PI-TPs in fission of vesicles from the Golgi membranes and/or fusion with the plasma membrane as well as the cooperation with PI-kinases indicating the involvement of phosphoinositides in vesicle dynamics.

An interesting observation is that in most investigations there is no difference in activation of the particular cellular processes by either both mammalian PI-TP isoforms and the structurally unrelated yeast PI-TP, SEC14p when matched for PI-transfer activity. It seems likely that in these studies it is the PI-transfer or binding activity (rather than any specific co-factor function), which is the critical component in the action of PI-TP. However, to stimulate the fission of vesicles from the Golgi membranes it seems obvious that PI-TP has to be present at the location where this occurs. As will be shown, the only PI-TP that is able to stably associate with the Golgi membranes in mammalian cells *in vivo* is PI-TPβ due to its unique phosphorylation site on Ser^{262} (*9, 16*).

PROPOSED FUNCTIONS OF PI-TPS: STUDIES IN INTACT CELLS

Approaches to gain insight into cellular functions of proteins include the determination of the cellular localization by (immuno) fluorescence techniques or electron microscopy. Further detailed understanding can be obtained from modified expression of proteins using DNA recombinant techniques.

Intracellular Localization of PI-TPα and PI-TPβ

In early studies on the cellular localization of PI-TP it was shown that in an exponentially growing Swiss mouse 3T3 cells PI-TP were localized in the nucleus, in the cytosol and associated with the peri-nuclear Golgi membranes (*27*). By microinjection of a mixture of Cy3-labeled PI-TPα and Cy5-labeled PI-TPβ it was shown that PI-TPα was predominantly present in the nucleus and in the cytosol whereas PI-TPβ was preferentially associated with the Golgi membranes. The distinct cellular localization was confirmed by indirect immuno-fluorescence indicating that the fluorescently labeled PI-TPα and PI-TPβ were targeted to the same sites as their endogenous counterparts (*28*). An interesting feature was added to these findings using photobleach experiments of fluorescently labeled PI-TPα and PI-TPβ: PI-TPα could move rapidly from the cytosol to the nucleus and vice versa but PI-TPβ was immobilized on the Golgi membranes (V. Bankaitis, personal communication). The presence of PI-TPα in the nucleus could indicate a role for PI-TPα in the autonomous nuclear phosphoinositide signaling system either in the transfer of PI from the Golgi membranes to the nuclei or in the regulation of the nuclear phosphoinositide signaling system (*29–31*). Activation of protein kinase C by phorbol esters affected the cellular localization of the PI-TPs, increasing the association with the Golgi membranes. Both proteins could be phosphorylated *in vitro* but PI-TPβ was phosphorylated to a much higher level than PI-TPα (*15, 16, 32*). Actually, it was shown that *in vivo* 85% of PI-TPβ is already

phosphorylated on the unique phosphorylation site Ser^{262} and that this phosphorylation is essential for the association with the Golgi membranes. The distinct localization of PI-TPα and PI-TPβ as regulated by the protein kinase C-dependent phosphorylation, suggests distinct cellular functions *in vivo*.

Manipulation of the Expression of PI-TPα and PI-TPβ

The studies on biochemical and physiological processes in intact eukaryotic cells with increased or decreased expression of the proteins further widened the view on possible cellular functions of PI-TPα and PI-TPβ. Either increased or decreased expression or knock out by DNA recombinant techniques are not normally occurring physiological processes but do occur in pathological situations, diseases or carcinogenesis. Both methods can give significant information on the physiological function of a protein. Information obtained from knock out cells is limited when the mutation is lethal or when the function of the protein in the cell can be taken over by another protein.

Decreased expression or knockout of PI-TPα and PI-TPβ. The result of a decreased expression of PI-TPα was revealed in Rat WRK-1 mammary tumor cells transfected with a plasmid containing a full-length rat PI-TPα cDNA inserted in antisense direction (*33*). The expression of this cDNA leads to a 25% decrease in the concentration of PI-TPα. Two of the three established cell lines exhibited a decreased growth rate. PC synthesis was significantly decreased while PI synthesis was unchanged. Analysis of PC metabolites showed that levels of SM, LysoPC, choline and glycerophosphorylcholine are decreased in the antisense-transfected cell lines. In addition, the level of phosphorylcholine was increased two-fold and the level of CDPcholine is comparable in control and in the antisense-transfected cell lines. Based on these data it was proposed that PI-TPα is involved in the regulation of PC metabolism (*33*). Ablation of PI-TPα in murine embryonic stem cells had no effect on growth and pluripotency. There was no consequence for bulk phospholipid metabolism, in particular SM, choline metabolism and the steady state levels of inositol phospholipids and phosphoinositides were not affected in the PI-TPα-deficient cells. Moreover, the data suggested that PI-TPα does not play an obvious role in any of the cellular activities investigated in semi-intact cells. On the other hand, genetic ablation of PI-TPβ could not be established since PI-TP$\beta^{-/-}$ clones from murine embryonic stem cells could not be isolated (*34*). From these studies it is concluded that PI-TPα is a non-essential protein and that PI-TPβ is an essential protein in murine embryonic stem cells.

Increased expression of PI-TPα and PI-TPβ. Mouse NIH3T3 fibroblast cells were transfected with cDNA encoding mouse PI-TPα and PI-TPβ. A 2–3-fold increase in PI-TPα significantly stimulated the rate of proliferation and cell density in confluent cultures but contact inhibition of the cells was maintained (*35*). A detailed analysis of more than 20 inositol metabolites showed that the levels of four metabolites is significantly increased when PI-TPα is over expressed: lysoPI, GPI, I(1)P and I(2)P. These metabolites are typical products of a PLA_2-stimulated degradation of PI (*35*). These intracellular metabolites did not demonstrate mitogenic activity towards quiescent NIH3T3 cells. The explanation for the increased growth rate was found when it was shown that the cells with increased expression of PI-TPα produce and secrete an arachidonic metabolite that is highly mitogenic (*36, 37*). Arachidonic acid, an additional major product of the PLA_2-mediated degradation of PI, was metabolized by cyclooxygenase-2 (COX-2) since specific inhibition of COX-2 blocked the production of the mitogenic factor. This factor, in turn, was able to increase proliferation and COX-2 expression in quiescent wtNIH3T3 cells (*37*). The high growth rate of the cells with increased expression of PI-TPα was accompanied by an increased survival upon UV- or TNFα-induced apoptosis. Similar to the mitogenic activity, the anti-apoptotic activity was secreted, COX-2 dependent and able to act autocrine as well as paracrine in protecting other cell against apoptosis. The identity of the mitogenic/anti-apoptotic factor is not yet known but it probably belongs to the family of endocannabinoids (metabolites of unsaturated fatty acids, e.g. arachidonic acid and oleic acid, sharing structural features with eicosanoids) which are the endogenous ligands of the cannabinoid receptors 1 or 2 (CB1 or CB2) that have been identified as the G-protein-coupled receptors (GPCRs) binding the psycho-active principle of marijuana, Δ^9-tetrahydrocannabinol (THC) (*38, 39*). This conclusion is based on the finding that the paracrine anti-apoptotic activity of the factor is inhibited by pertussis toxin (a general inhibitor of GPCRs) as well as by a specific antagonist of CB1: SR141716A (*37*).

PI-TPβ was 12–15-fold over expressed in NIH3T3 mouse fibroblast cells. The intracellular concentration of PI-TPβ is about a 100-fold lower than the concentration of PI-TPα in most mammalian tissues (*36*). It was shown that PI-TPβ is involved in the traffic of SM from the Golgi membranes to the plasma membrane (*40*). In contrast to the effect of PI-TPα, increased expression of PI-TPβ leads to an increase in cell cycle duration from 21 h to 34 h. The saturation density of confluent cultures decreased and the cells were much more sensitive to UV- and TNFα-induced apoptosis when compared to wtNIH3T3 cells (*36*). Medium conditioned by the cells with increased expression of PI-TPα, is able to fully protect cells with increased expression of PI-TPβ against apoptosis. This protection is accompanied by an up regulation of COX-2 expression (*36, 37*). Phosphorylation of Ser^{262} in PI-TPβ, responsible for its localization at the *Golgi* membranes, was shown to be essential for the specific effects in cells with increased expression of PI-TPβ. Cells stably transfected with a mutant PI-TPβ lacking the Ser^{262}, demonstrated properties comparable to those of wtNIH3T3 cells (*16, 36*).

Involvement of PI-TPα and PI-TPβ in the regulation of PC synthesis and/or SM transfer (*8, 33, 40*), was not confirmed in COS-7 cells that were transiently transfected with either PI-TPα or PI-TPβ (*13*). An explanation for this discrepancy might

be that the effects are strongly dependent on the cell type used for transfection or that transiently transfected cell cultures demonstrate different properties when compared to stable cell lines in particular when autocrine as well as paracrine stimulation processes are involved.

PROPOSED FUNCTIONS OF PI-TPS: STUDIES IN ANIMALS

The first data from intact animals about a physiological function of PI-TPα came from studies on the mouse *vibrator* mutation that causes an early-onset, progressive action tremor and degeneration by spongiform inclusions in the neurons of brain stem and spinal cord and juvenile death (*41*). The decrease in PI-TPα protein in the brain of these vibrator mice is specific: the level of PI-TPβ is unchanged. The authors suggest a defect in a PI-turnover pathway in specific neurodegeneration diseases. Analysis of lipids in brain, liver and mammary glands from mice that are homozygous for the vibrator mutation (vb/vb) showed a 2–4-fold increase in neutral lipids from livers when compared to neutral lipids from control livers. No significant changes were observed in the lipid content of the brain. Mammary glands of homozygous vibrator mice are underdeveloped. No differences were observed in any aspect of investigated lipid-mediated signal transduction pathways (*42*).

Deletion of PI-TPα was established by ablation of the gene in PI-TP$\alpha^{-/-}$ mice (*43*). As shown in mice expressing the vibrator mutation, the expression of PI-TPα was shown to be not necessary for prenatal development probably due to *in utero* supply of maternal nutrients. However, after birth the PI-TP$\alpha^{-/-}$ mice showed a lower body mass, many neurodegenerative symptoms, hypoglycemia and intestinal and hepatic steatosis. In the livers from PI-TP$\alpha^{-/-}$ mice the neutral lipids: triglycerides, diacylglycerols and free fatty acids are increased and the liver tissue showed microvesicular steatosis. Glucose and insulin levels in serum of PI-TP$\alpha^{-/-}$ were 9-fold decreased, resulting in severe hypoglycemia. The PI-TP$\alpha^{-/-}$ mice survive for not more than 15 days because of the neurodegeneration that might be significantly contributed by the hypoglycemic condition (*43*). However, neurodegeneration might as well be the main reason for many of the pathological conditions.

Genetic ablation of PI-TPβ in murine embryonic cells resulted in non-viable cells and PI-TPβ deficiency in PI-TP$\beta^{-/-}$ resulted in catastrophic failure early in murine embryonic development (34). It can be concluded from these studies that expression of PI-TPα is not essential for normal embryonic development while the expression of PI-TPβ is essential. PI-TPα is necessary for normal development of newborn mice, for PI-TPβ this is not yet known.

MODEL

We propose a model for the cellular functions of PI-TPα (Fig. 1) and PI-TPβ (Fig. 2) based on the data described above.

PI-TPα

PI-TPα activates a PI-specific PLA_2 thereby increasing the intracellular concentration of lysoPI and arachidonic acid (Fig. 2). This arachidonic acid is metabolized by COX-2 into one (or more) yet unidentified survival and growth factors since a specific inhibitor of COX-2, NS398, prevents the production of the PI-TPα-dependent survival factor. The survival factor is secreted into the medium since conditioned medium of cells with increased expression of PI-TPα demonstrates paracrine growth stimulating and anti-apoptotic activity towards other cells, e.g. neurons and cells with increased expression of PI-TPβ. This paracrine stimulation is inhibited by pertussis toxin and suramin, specific inhibitors of GPCRs and by SR141716A, a specific inhibitor of the CB1. CB1 is a GPCR and activation of this family of receptors is related to activation of intracellular signal transduction cascades involving, e.g. phospholipase C (PLC), phosphatidylinositol-3-kinase, MAPkinase, akt/PKB (*37*). Activation of PLC via this pathway could lead to the PI-TPα-dependent production of inositolphosphates and the activation of PI-3-K that has been described (*21, 44*). The increased gene expression due to growth stimulation or induced cell survival leads to an increase in COX-2 expression thereby amplifying the stimulation process.

PI-TPβ

PI-TPβ (Fig. 3) is localized at the Golgi (*8, 28*), stimulates SM transport to the plasma membrane (*40*) , is able to stimulate the formation of secretory vesicles from the Golgi membranes (*24, 25*) and could be involved in various secretory events (*22*). NIH3T3 cells with increased expression of PI-TPβ grow very slowly and are extremely sensitive to induction of apoptosis (*36*). Medium conditioned by NIH3T3 cells with increased expression of PI-TPβ completely lacks the COX-2-dependent mitogenic or survival activity that is present in the medium conditioned by wtNIH3T3 cells. This is unexpected since NIH3T3 cells with increased expression of PI-TPβ and wtNIH3T3 cells contain equal amounts of PI-TPα. When the medium conditioned by NIH3T3 cells with increased expression of mutant PI-TPβ lacking the S^{262} phosphorylation site, the mitogenic and apoptotic activity increased to the level in medium conditioned by wtNIH3T3 cells (*36*). An explanation for this observation is that the NIH3T3 cells with increased expression of PI-TPβ produce and/ or secrete a factor (either a protein or a lipid) that is able to inhibit the mitogenic and anti-apoptotic activity that is produced by wtNIH3T3 cells. The inhibition could be established by interference either with the PI-TPα-dependent survival factor itself or with the receptor involved. This could explain why the NIH3T3 cells with increased expression of PI-TPβ lack the mitogenic and survival activity, demonstrate a decreased expression of COX-2, grow very slowly and are more sensitive for induction of apoptosis.

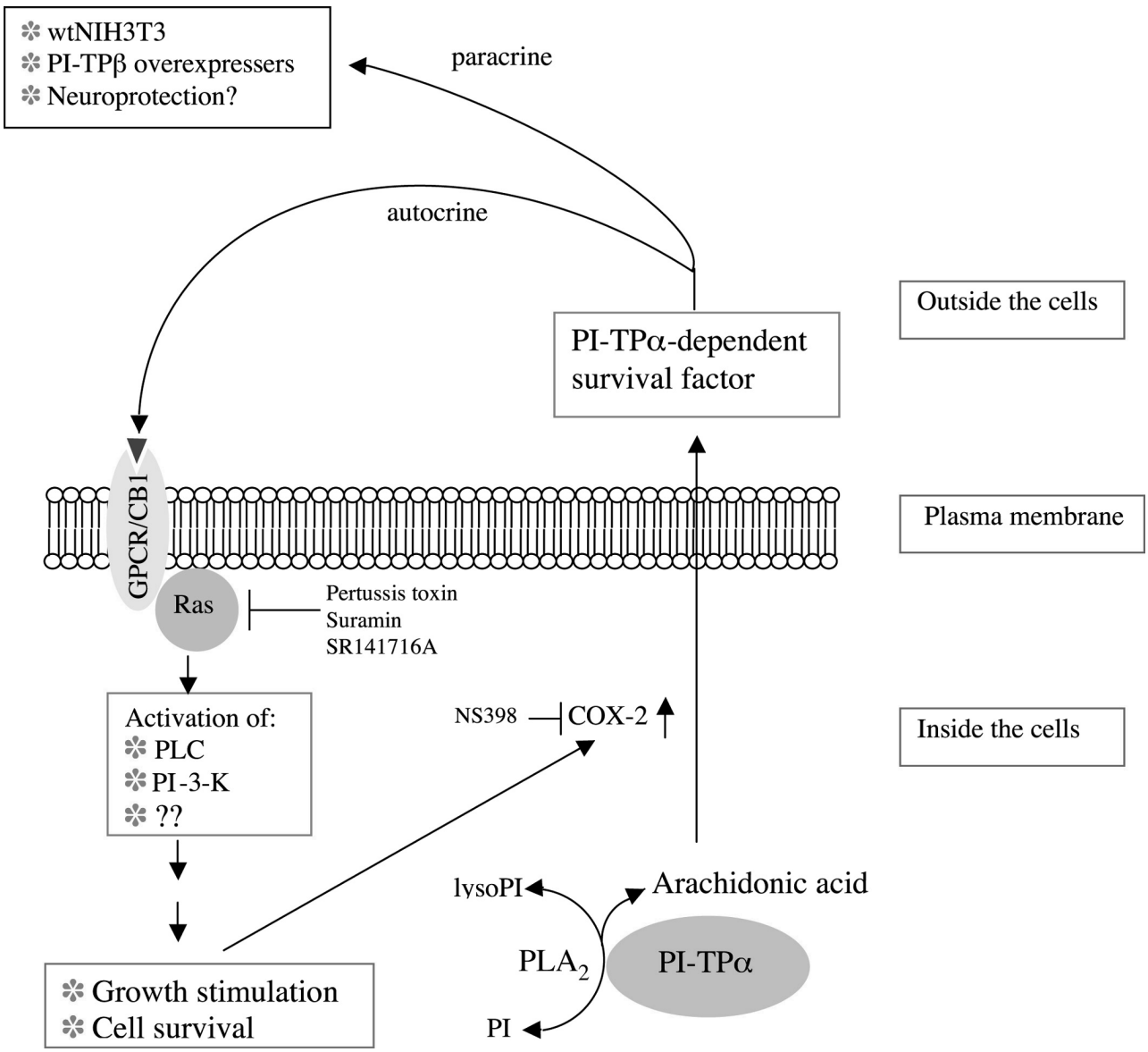

Figure 2. Model of the cellular function of PI-TPα. Description of the scheme is given in the text. PI-TP: phosphatidylinositol transfer protein; PI: phosphatidylinositol; PLC: phospholipase C; PLA_2: phospholipase A_2; PI-3-K: phosphatidylinositol-3-kinase; LysoPI: lysophosphatidylinositol; COX-2: cyclo-oxygenase 2; CB1: cannabinoid receptor 1; NS398: specific inhibitor of COX-2; SR141716: specific inhibitor of CB1.

CONCLUDING REMARKS

The clearly distinct intracellular localization of PI-TPα and PI-TPβ already suggested different physiological functions. The strong binding of PI-TPβ to the Golgi membranes, dependent on the phosphorylation of Ser^{262}, makes this isoform the likely candidate to be involved in vesicle fission from the Golgi membranes. On the other hand, PI-TPα, moving between nucleus and cytosol is involved in reactions leading to the production of bioactive lipid metabolites.

Expression of PI-TPα or PI-TPβ in intact cells changes opposite cellular effects: survival vs. cell death and increased vs. decreased rates of proliferation.

Data on the severe neurological degeneration when the expression of PI-TPα is decreased and the data on the production of a survival factor by PI-TPα and COX-2 with mitogenic and anti-apoptotic activity support the hypothesis that PI-TPα is important to maintain a normal, healthy physiology of many tissues, in particular nerve tissue. The PI-TPα-dependent survival factor probably belongs to the

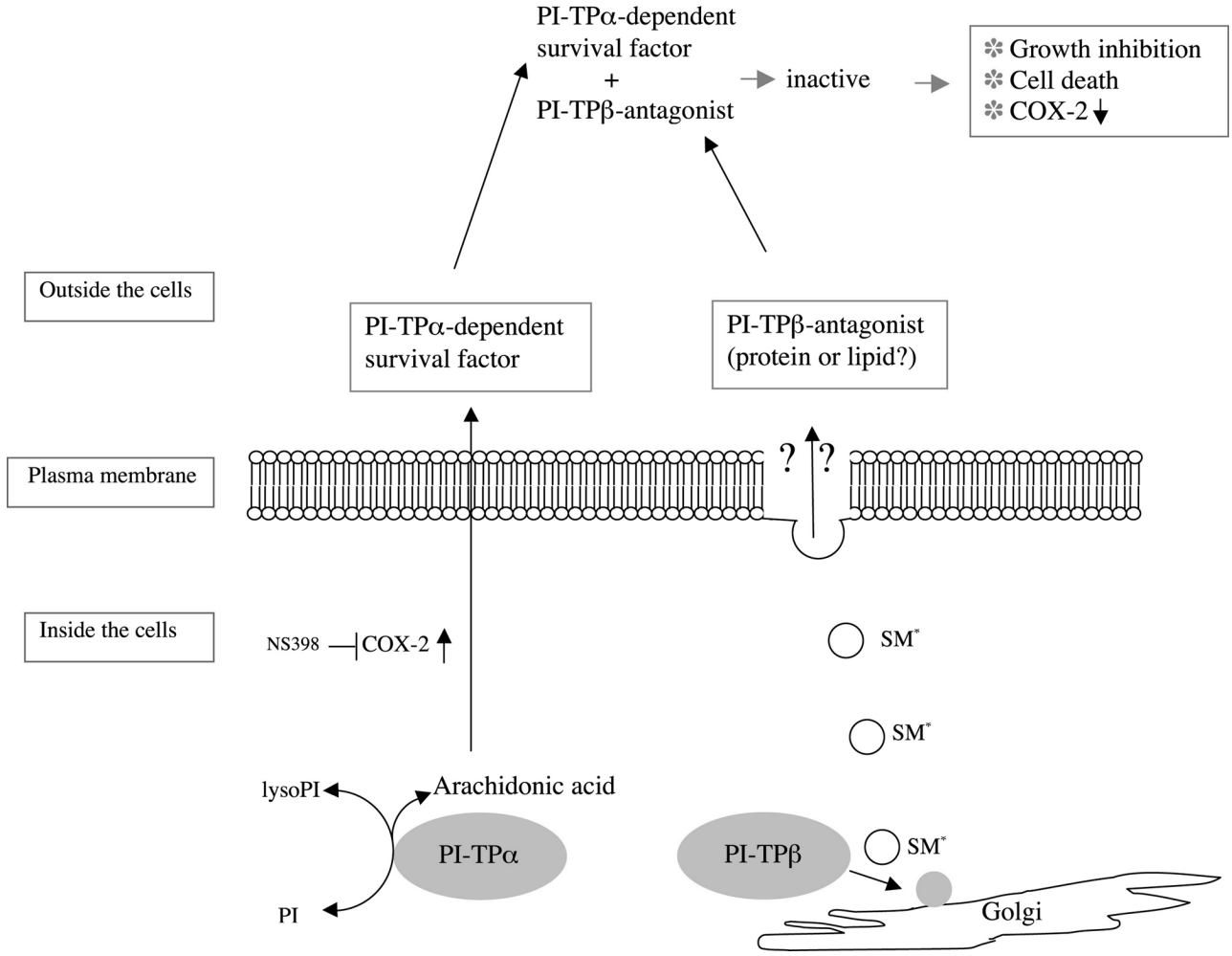

Figure 3. Model of the cellular function of PI-TPβ. Description of the scheme is given in the text. PI-TP: phosphatidylinositol transfer protein; PI: phosphatidylinositol; SM: sphingomyelin; LysoPI: lysophosphatidylinositol; COX-2: cyclo-oxygenase 2; NS398: specific inhibitor of COX-2.

family of endocannabinoids that have been shown to be also involved in protection of neurons against apoptosis (*45*). The normal development of murine embryos is probably possible due to the *in utero* supply of the PI-TPα-dependent mitogenic and survival factor circulating after secretion in the maternal blood. The observations that PI-TPβ is able to interfere with the mitogenic and anti-apoptotic effects mediated by PI-TPα by decreasing the rate of proliferation as well as cell survival, suggest that the relative expression levels of PI-TPα and PI-TPβ might be a critical condition involved in the determination of the life cycle of particular cells. Disturbance of the relative expression of PI-TPα and PI-TPβ might lead to modification of the cellular life cycle, carcinogenesis or degenerative processes by, on the one hand, the increased rate of proliferation and the prevention of apoptosis by PI-TPα and, on the other hand, the decreased rate of proliferation and the stimulation of apoptosis by PI-TPβ.

ACKNOWLEDGEMENTS

I wish to thank Prof M.R. Egmond and Prof K.W.A. Wirtz for critically reading the manuscript.

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