UDC 547.953:663.14
ISSN 1330-9862

review

Phospholipid Synthesis and Transport in Yeast

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Received: September 12, 1997
Accepted: May 28, 1998

Summary

The yeast Saccharomyces cerevisiae is a suitable system to study cell biological and molecular biological problems of organelle biogenesis. Synthesis and correct distribution of phospholipids are essential prerequisites for the assembly of functional membranes. The majority of enzymes involved in phospholipid biosynthesis is located in the endoplasmic reticulum. In addition, certain steps of phospholipid synthesis occur in mitochondria, Golgi and lipid particles. Interplay of these organelles is required to maintain a balanced cellular level of phospholipids. A prominent example of organelle collaboration is the sequence of biosynthetic steps of aminoglycerophospholipid synthesis, which occur in a concerted action of the endoplasmic reticulum, mitochondria and probably the Golgi. Intracellular flow of lipids is necessary for the flux of intermediates between organelles that contribute to lipid biosynthesis, and for the supply of lipids to membranes that are unable to synthesize their own lipids. Protein-catalyzed lipid transport, vesicle flux and lipid translocation via membrane contact are possible mechanisms of lipid migration within the cell. Experimental evidence suggests that contact between organelles appears to be most important for lipid transport between subcellular compartments.

Keywords: phospholipid, biogenesis, cellular membranes, cellular organelles

Introduction

Membranes of eukaryotic cells have two important functions. First, they serve as a diffusion barrier between the interior of the cell and its environment, and the lumen and the outside of organelles. Second, membranes harbor proteins that catalyze selective exchange of components between compartments, or act as enzymes in metabolic pathways. The basic structure of a membrane, a bilayer, is formed from lipids. Major lipid components of eukaryotic membranes are phospholipids, sterols, sphingolipids and glycoglycerolipids.

Within a cell, not all membranes have the capacity to synthesize their own lipids. The major site of lipid biosynthesis in eukaryotic cells is the endoplasmic reticulum, but other cellular membranes, e.g., mitochondria or the Golgi, harbor also activities of certain lipid-synthesizing enzymes (*1*). Other membranes such as the plasma membrane seem to lack the ability to synthesize lipids. Thus, lipids must migrate from their site of synthesis to the cellular membrane(s) of destination by efficient processes of transport from one hydrophobic compartment to another.

Several mechanisms of lipid transport between organelle membranes can be envisaged (Fig. 1): (1) transport of lipid monomers by diffusion through a hydrophilic compartment; (2) transfer of lipids through the aqueous phase between donor and acceptor membrane with the aid of lipid transfer proteins; (3) vesicular transport of lipids which involves budding of a vesicle from one and fusion with another membrane; the most prominent pathway governed by vesicle flux is protein secretion, and transport of lipids via secretory vesicles to the cell periphery along organelles of the secretory pathway may be anticipated; (4) lipid translocation through membrane contact which requires collision of organelle membranes followed by temporary point fusion events. Zones of membrane contact have been visualized by electron microscopy between the nucleus and the endoplasmic reticulum, the plasma membrane and the en-

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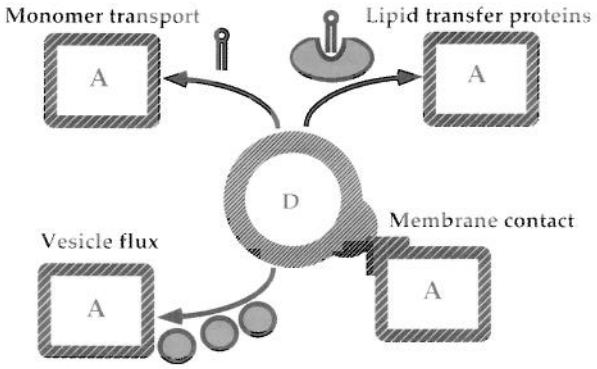

Fig. 1. Proposed mechanisms of intermembrane phospholipid translocation. D: donor membrane; A: acceptor membrane.

doplasmic reticulum, and the inner and outer mitochondrial membrane. Translocation of lipids from one to another hydrophobic compartment through a zone of membrane contact avoids passage across an aqueous phase.

One can anticipate that more than one of these mechanisms is active *in vivo*. It is, however, not easy to design appropriate experiments, which allow to distinguish between the various possibilities of lipid transport in the living cell. Most of the information available comes from experiments *in vitro*, and it is still a matter of dispute whether such results are relevant for the situation *in vivo*.

A convenient experimental system to study synthesis and intracellular transport of lipids is baker's yeast, *Saccharomyces cerevisiae*. This unicellular organism can serve as a convenient model because the subcellular structure of yeast is similar to that of higher eukaryotes, and its biochemistry and genetics of lipids have been well characterized. Availability of sequence information of the entire yeast genome has opened new aspects to search for homologies with higher eukaryotes. However, studies of lipid synthesis and transport are complicated by the fact that systems involved in these processes may exist in redundancy. As a consequence, mutants lacking one gene of phospholipid biosynthesis or transport do not necessarily exhibit a phenotypic defect. Therefore, it is difficult to design appropriate screening strategies to isolate such mutants.

In this review we summarize our knowledge about the relationship between phospholipid synthesis and transport in the yeast, *Saccharomyces cerevisiae*. Possible mechanisms of phospholipid transfer that may be relevant for membrane biogenesis in this eukaryotic microorganism will be discussed. The reader is also referred to recent reviews dealing with problems of lipid synthesis and transfer in yeast (2–4).

Synthesis of phospholipids in yeast

Phospholipids consist of a glycerol backbone esterified with fatty acids in *sn*–1 and *sn*–2 position, and contain a phosphate group in the *sn*–3 position. One hydroxyl group of phosphate is linked to a polar head group which is characteristic for the various phospho-

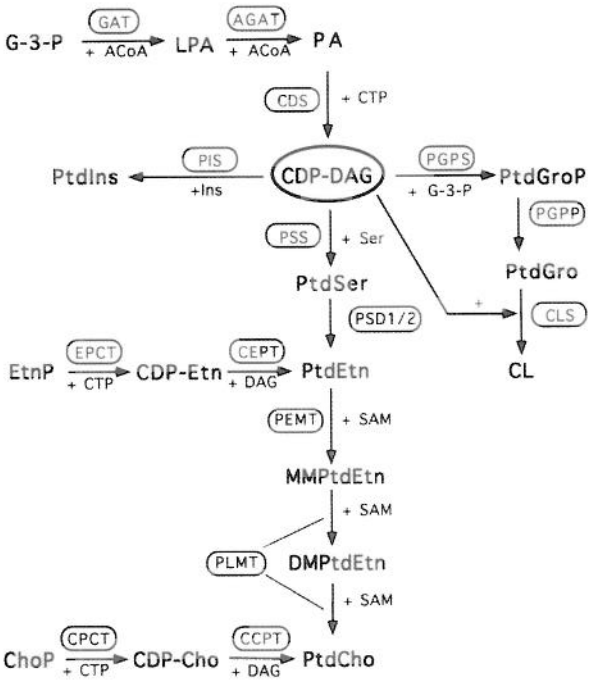

Fig. 2. Pathways of phospholipid synthesis in yeast.
Metabolites: G-3-P: glycerol-3-phosphate; ACoA: acyl-CoA; LPA: lysophosphatidic acid; PA: phosphatidic acid; CDP-DAG: CDP-diacylglycerol; DAG: diacylglycerol; Ins: inositol; Ser: serine; EtnP: ethanolaminephosphate; ChoP: cholinephosphate; CDP-Etn: CDP-ethanolamine; CDP-Cho: CDP-choline; PtdSer: phosphatidylserine; PtdIns: phosphatidylinositol; PtdEtn: phosphatidylethanolamine; MMPtdEtn: monomethyl phosphatidylethanolamine; DMPtdEtn: dimethyl phosphatidylethanolamine; PtdCho: phosphatidylcholine; SAM: *S*-adenosyl methionine; PtdGroP: phosphatidylglycerophosphate; PtdGro: phosphatidylglycerol; CL: cardiolipin.
Enzymes: GAT : glycerol-3-phosphate acyltransferase; AGAT : 1-acylglycerol-3-phosphate acyltransferase; CDS : CDP-diacylglycerol synthase (CTP : phosphatidic acid cytidylyltransferase); EPCT : ethanolaminephosphate : CTP cytidylyltransferase; CPCT : cholinephosphate : CTP cytidylyltransferase; CEPT : CDP-ethanolamine : 1,2-diacylglycerol ethanolaminephosphotransferase; CCPT : CDP-choline : 1,2-diacylglycerol cholinephosphotransferase; PSS : phosphatidylserine synthase; PIS : phosphatidylinositol synthase; PSD : phosphatidylserine decarboxylase; PEMT : phosphatidylethanolamine *N*-methyltransferase; PLMT : phospholipid *N*-methyltransferase; PGPS : phosphatidylglycerophosphate synthase; PGPP: phosphatidylglycerophosphate phosphatase; CLS : cardiolipin synthase.

lipid classes. Fatty acids in the *sn*–1 position are mostly saturated whereas those in the *sn*–2 position are unsaturated.

Pathways of phospholipid formation in yeast are shown in Fig. 2. Phospholipid biosynthesis and delivery to subcellular membranes are highly interconnected, because extensive transfer of intermediates and cross-compartment coordination of partial reactions are required. The most prominent example in that respect is the sequence of the aminoglycerophospholipid synthesis. Phosphatidylserine (PtdSer) is synthesized in the endoplasmic reticulum, transferred to mitochondria and converted to phosphatidylethanolamine (PtdEtn). Part of

the PtdEtn newly formed in mitochondria migrates to the endoplasmic reticulum where methylation to phosphatidylcholine (PtdCho) occurs. Besides the endoplasmic reticulum and mitochondria, the Golgi and lipid particles contribute to cellular formation of phospholipids in yeast (Fig. 3).

A general precursor of all glycerophospholipids is phosphatidic acid (PA). PA is synthesized by acylation of glycerol-3-phosphate or dihydroxyacetonephosphate (*1*). In the yeast *Saccharomyces cerevisiae* the highest specific activity of enzyme(s) that catalyze the acylation of glycerol-3-phosphate is found in the lipid particle fraction (*5, 6*). Lipid particles are a compartment consisting of a hydrophobic core of triacylglycerols and steryl esters that is enveloped by a phospholipid monolayer with a small amount of proteins embedded. Recently, it was shown in our laboratory (*7*) that lipid particles harbor two acyltransferases: the first, Gat1p, acylates glycerol-3-phosphate and forms lysophosphatidic acid as an intermediate; the second enzyme encoded by the *SLC1* gene (sphingolipid compensation) (*8*) is a 1-acylglycerol-3-phosphate acyltransferase which completes the biosynthesis of PA. Both enzymes were not only detected in lipid particles, but also in the endoplasmic reticulum. In addition to Gat1p and Slc1p the microsomal fraction seems to contain an additional system of PA synthesis which has not yet been characterized.

CDP-diacylglycerol synthase, Cds1p, converts PA to CDP-diacylglycerol (CDP-DAG), another major intermediate of phospholipid synthesis. The yeast *CDS1* structural gene was recently identified by its homology to the corresponding *Escherichia coli* and *Drosophila* genes (*9*). Consistent with its central role in *de novo* glycerophospholipid synthesis, disruption of *CDS1* is lethal, which suggests that *CDS1* encodes the only CDP-DAG synthase in yeast, or supplies an essential subcellular pool of CDP-DAG. As CDP-DAG synthase activity has been detected in the endoplasmic reticulum and mitochondria (*10*), the physiological relevance of the distribution of this enzyme could be significant. The availability of CDP-DAG in specific cellular compartments may contribute to the control of the synthesis of different phospholipids. For example, enrichment of CDP-DAG in mitochondria might stimulate synthesis of phosphatidylglycerol (PtdGro) and cardiolipin (CL) in this compartment, whereas formation of other phospholipids such as phosphatidylinositol (PtdIns) and phosphatidylserine (PtdSer) might be enhanced by an excess of CDP-DAG in the endoplasmic reticulum.

Phosphatidylserine (PtdSer) is only a minor component of whole cell phospholipids, but it is an important intermediate in the *de novo* synthesis of the two main yeast phospholipids, PtdEtn and PtdCho. Phosphatidylserine synthase (PSS) which is encoded by the *CHO1* gene is localized in the endoplasmic reticulum and forms PtdSer from CDP-DAG and serine. PtdSer in itself is not essential, and the disruption of the *CHO1* structural gene is not lethal. A *cho1* null mutant has no detectable PtdSer which suggests that there is only one copy of the *CHO1* gene in yeast. Cells that lack PSS need supplementation of choline and/or ethanolamine to form PtdEtn and PtdCho via the so called Kennedy pathway (see below).

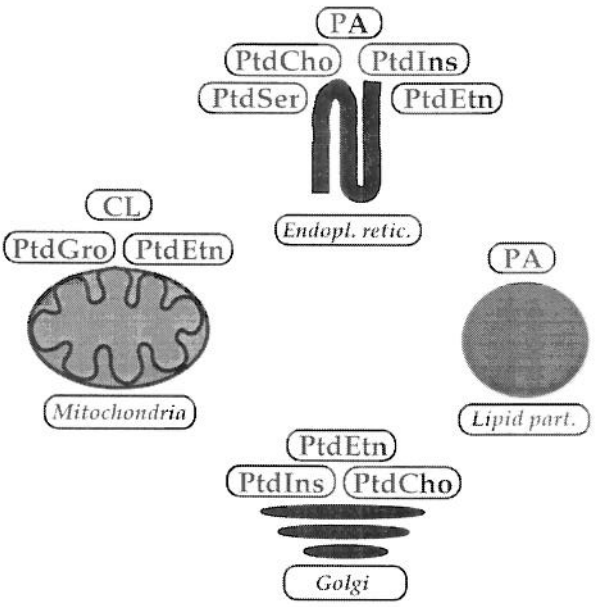

Fig. 3. Intracellular sites of phospholipid synthesis. For abbreviations see Fig. 2.

The major route of phosphatidylethanolamine (PtdEtn) synthesis in yeast is the *de novo* pathway through decarboxylation of PtdSer. The majority of phosphatidylserine decarboxylase (PSD) activity was localized to the mitochondrial fraction (*10*). It came as a surprise that the function of the mitochondrial PSD, named Psd1p, is dispensable for the cell (*11, 12*) even in the absence of ethanolamine. Inactivation of the *PSD1* structural gene not only had no effect on cell viability, but also had hardly any impact on the cellular lipid composition. Subsequently, a second PSD named Psd2p was identified that is thought to reside in the Golgi and accounts for less than 5% of the cellular PSD activity *in vitro* (*13, 14*). Only inactivation of both PSD enzymes renders cells auxotrophic for ethanolamine. The relative roles of the two forms of PSD are not known. The probable localization of Psd2p to the Golgi, however, further emphasizes the role of this compartment as a site of phospholipid synthesis.

An alternative route for synthesis of PtdEtn in yeast is the Kennedy pathway (for a review see ref. *3*). This route is a salvage pathway for yeast cells which are not able to synthesize PtdSer provided that sufficient ethanolamine is present in the growth medium. The first step of this biosynthetic sequence is phosphorylation of ethanolamine by ethanolamine kinase which is located in the cytosol. Ethanolaminephosphate is then converted to CDP-ethanolamine by the ethanolaminephosphate cytidylyltransferase (EPCT). Finally, PtdEtn is formed in the endoplasmic reticulum by CDP-ethanolamine : 1,2--diacylglycerol ethanolaminephosphotransferase (CEPT).

In the absence of exogenous choline, phosphatidylcholine (PtdCho) is synthesized by a three-step methylation of PtdEtn. These reactions are catalyzed by two independent methyltransferases, Cho2p and Opi3p, which are localized to the endoplasmic reticulum. PtdEtn

formed in the endoplasmic reticulum can be converted to PtdCho *in situ*, whereas PtdEtn formed in mitochondria must migrate to the endoplasmic reticulum to ensure conversion to PtdCho.

As an alternative route of PtdCho synthesis the Kennedy pathway contributes to formation of this major cellular phospholipid. Reactions are similar to those described for the synthesis of PtdEtn. The enzymes involved are choline kinase, cholinephosphate cytidylyltransferase (CPCT) and CDP-choline: 1,2-diacylglycerol cholinephosphotransferase (CCPT) (*3*). The Kennedy pathway is not only active when exogenous ethanolamine or choline is present, but also functions continuously to recycle degradation products of PtdEtn and PtdCho (*15*).

Phosphatidylinositol (PtdIns) plays an important role in cellular signaling and serves as a physiological sensor in yeast cells. PtdIns is synthesized from CDP-DAG and inositol analogously to PtdSer. Phosphatidylinositol synthase (PIS) has been purified from *Saccharomyces cerevisiae* (*16*), and the structural gene has been cloned (*17*). In yeast, PtdIns is essential since disruption of *PIS1*, the gene encoding phosphatidylinositol synthase, is lethal (*17*).

Phosphatidylglycerol (PtdGro) is a minor phospholipid component of yeast subcellular membranes, whereas cardiolipin (CL) comprises 10–15% of mitochondrial phospholipids (*6*). PtdGro and CL are closely related biosynthetically. First, phosphatidylglycerophosphate (PtdGro-P) is synthesized from CDP-DAG and glycerol-3-phosphate. Then, PtdGro-P is dephosphorylated to PtdGro, and CL (diphosphatidylglycerol) is formed by reaction of PtdGro with a second molecule of CDP-DAG. Phosphatidylglycerophosphate synthase activity is present in both mitochondrial inner membrane and microsomal membranes (*10*), but it is not known whether the enzymes in the two locations are the same or different. Cardiolipin synthase has been localized to mitochondrial membranes (*18*). Genes encoding PtdGro-P synthase, *PGS1* (*19, 20*), and CL synthase, *CRD1* (*21*), were recently identified. The gene encoding PtdGro-P phosphatase has not yet been cloned nor has the gene product been characterized.

General aspects of phospholipid transport in yeast

Restriction of phospholipid-synthesizing enzymes to specific subcellular compartments (see Fig. 3) requires efficient intracellular transport of phospholipids to those compartments that are not able to synthesize their own membrane lipids. It is not only the supply of the quantitatively predominant phospholipids, PtdCho, PtdEtn and PtdIns from their sites of synthesis to all other organelles which necessitates such a process, but also the migration of precursor phospholipids to their site of metabolic conversion that is essential for the balanced synthesis of cellular phospholipids.

To demonstrate the routes of lipid migration in living cells experiments *in vivo* have been designed. A convenient method is labeling of cells with radioactive precursors of phospholipids, *e.g.*, inositol, serine, methionine, fatty acids, glycerol or phosphate, and following the routes of labeled phospholipids within the cell. During a short pulse newly made phospholipids accumulate in the organelle of synthesis; in the chase period migration to other cellular membranes can be observed. Subcellular localization of radiolabeled lipids requires efficient techniques of subcellular fractionation. For this purpose various methods of yeast organelle preparation were developed and adapted in our laboratory, which allow the isolation of highly enriched subcellular fractions with a low degree of cross-contamination (*22*).

In some cases the routes of lipids within the cell can be followed without cell fractionation. If metabolic conversion of a lipid occurs in a compartment which is different from the organelle of synthesis, the migration of the precursor lipid can easily be correlated with the appearance of the product. The best example for such an experimental strategy is the measurement of intracellular PtdSer transfer. As mentioned above, PtdSer is synthesized in the endoplasmic reticulum, whereas its conversion to PtdEtn occurs mainly in mitochondria through the action of Psd1p (*11, 12*), and to a smaller extent in the Golgi by catalysis of Psd2p (*13, 14*). Thus, the appearance of radiolabeled PtdEtn after labeling cells with radioactive serine indicates export of PtdSer from the endoplasmic reticulum. Investigations employing *psd1* and *psd2* null mutants will provide evidence for the molecular requirements of the specific routes of PtdSer migration.

An elegant alternative to the *in vivo* methods described above is the use of fluorescently labeled lipids for microscopic inspection of intracellular lipid migration. This technique has been successfully employed for studies with mammalian cells (*23*) but is less amenable to the much smaller yeast cells. Kean *et al.* (*24*) were able to demonstrate that uptake and internalization of fluorescently labeled PtdCho into yeast cells takes place both through a *SEC*-dependent endocytotic pathway to the vacuole and *SEC*-independent routes to the nuclear envelope and mitochondria.

Although experiments *in vitro* must always be interpreted with caution regarding their relevance for the situation in the living cell, this type of experiments has provided a large body of evidence concerning lipid transport processes. Assays of lipid transport between isolated organelles or artificial membrane vesicles were designed to mimic specific steps of intermembrane migration of lipids. Radioactively, fluorescently or spin-labeled lipids were largely employed for such experiments *in vitro*. Fluorescence and ESR methods offer the advantage of continuous measurement of the lipid transport process. Pyrene-labeled phospholipids used in our laboratory to study yeast lipid transfer proteins (*25*) turned out to be especially convenient probes. Donor membranes which contain a high concentration of the fluorophore exhibit high excimer fluorescence intensity; transfer of fluorescent phospholipids to the acceptor results in a »dilution» of the fluorophore in the unlabeled membrane, which can be measured by an increase of monomer fluorescence intensity. In contrast to fluorescence assays, radioassays are discontinuous techniques for lipid transfer measurements. Addition of non-transferable markers, e.g., radiolabeled triacylglycerol or steryl esters,

to donor membranes is required to test unspecific fusion between donor and acceptor membranes.

Transport of phospholipids in living yeast cells

Intracellular migration of aminoglycerophospholipids in yeast was measured using [^{3}H]serine as a precursor (*26, 27*). Within a few minutes not only radiolabeled PtdSer but also [^{3}H]PtdEtn and [^{3}H]PtdCho appeared and were found distributed over mitochondria and the endoplasmic reticulum. The appearance of the radioactivity in PtdEtn and PtdCho indicated that import of PtdSer into mitochondria and export of PtdEtn from mitochondria had occurred. Energy depletion of cells by poisoning with CN^- and F^-, or N_3^- and F^- led to an equilibration of [^{3}H]PtdCho between the endoplasmic reticulum and mitochondria. These results indicated that the uneven distribution of phospholipids among organelles created by synthesis was balanced by rapid energy independent transport.

Intracellular movement of PtdSer and PtdEtn in yeast was also blocked by inhibition of cellular energy production. This dependence, however, seems to be indirect because the first enzyme in the biosynthetic sequence of aminoglycerophospholipid biosynthesis, phosphatidylserine synthase (PSS), requires CDP-DAG formed from PA and CTP. Nevertheless, these results suggest that synthesis, metabolism and transfer of aminoglycerophospholipids may be linked. Similar results were obtained when transport of PtdSer, PtdEtn and PtdCho to the plasma membrane was measured. Also in this case energy depletion of cells which stopped the synthesis of aminoglycerophospholipids caused their retention in internal membranes. Disruption of the cytoskeleton network using nocodazole had no effect on phospholipid translocation processes.

Import of proteins into mitochondria requires energy and a membrane potential (*28, 29*). Does phospholipid transport across mitochondrial membranes have similar requirements? Experiments *in vivo* can answer this question only in part. Experiments mentioned above had shown that a linkage between the energy state of yeast cells, the synthesis of phospholipids and their translocation exists. The presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) neither inhibits synthesis nor intracellular transport of aminoglycerophospholipids (*27*). This result indicates that transport of PtdSer across the outer and to the inner mitochondrial membrane, and of PtdEtn from the inner membrane across the outer mitochondrial membrane to the endoplasmic reticulum, do not require a membrane potential. These results were confirmed by experiments *in vitro* and led to the conclusion that import of proteins and lipids into mitochondria are independent events.

In contrast to the endoplasmic reticulum and mitochondria the plasma membrane of yeast is devoid of phospholipid-synthesizing enzymes and relies completely on the supply with all the constituent phospholipids. Flux of secretory vesicles governs the supply of proteins to the plasma membrane, and it has been a matter of dispute for a long time whether these vesicles might also be vehicles for lipid translocation to the cell periphery. We used temperature-sensitive secretory mutants (sec mutants) (*30*) to investigate the linkage between protein secretion and transport of PtdCho and PtdIns to the plasma membrane. The experimental design was such that we measured the release of water-soluble products of phospholipid deacylation, glycerophosphocholine and glycerophosphoinositol, generated by the plasma membrane associated phospholipase B (*31*), into the growth medium. Using this approach we demonstrated that transport of PtdIns and PtdCho from internal membranes to the plasma membrane did not cease after a shift from the acceptable (24 °C) to the non-acceptable temperature (37 °C). This result is in line with the postulate of Atkinson (*32*) that different types of vesicles are responsible for protein export and membrane growth. The existence of specific phospholipid transporting vesicles, however, still needs to be verified experimentally.

Cloning of the gene encoding the yeast phosphatidylinositol transfer protein (PITP) and the observation that the gene product is identical to Sec14p (*33–35*) made it possible to test for the first time whether a lipid transfer protein is involved in the intracellular translocation of phospholipids *in vivo*. Pulse-chase experiments with a temperature-sensitive *sec14* strain (*27*) demonstrated that the transport rates of PtdIns and PtdCho to the plasma membrane were not altered by the mutation. This result supports the view that functions of lipid transfer proteins in the living cell may be different from their lipid transporting activity *in vitro*. PITP/Sec14p is required for protein secretion and acts at the stage of the Golgi (*36*). Remarkably, the lethality of a *sec14* deletion is suppressed by second-site mutations that block PtdCho biosynthesis through the salvage pathway (*15*). This observation and the fact that PITP/Sec14p inhibits CTP : cholinephosphate cytidylyltransferase activity *in vitro* (*37*) suggest that PITP/Sec14p regulates the PtdIns : PtdCho ratio of Golgi membranes. Most recently it was suggested that PITP/Sec14p may be required to maintain a sufficient pool of diacylglycerol in the Golgi to support the production of secretory vesicles (*38*). Alternatively, Sec14p may be involved in formation of phosphatidic acid (PA) by regulating phospholipase D catalyzed degradation of PtdCho (*39*).

Phospholipid transport *in vitro*

Many efforts to elucidate mechanisms of lipid transport focused on the design of appropriate *in vitro* assays. The advantage of such assays is that single components of interest can be easier controlled than in the complex system of a living cell. However, results obtained *in vitro* should be interpreted with caution regarding their relevance *in vivo*.

- One of the best examples demonstrating the discrepancy between the *in vivo* and *in vitro* systems is the observation that phospholipid transfer proteins (named so due to their function *in vitro*) do most likely not act as lipid transporters in the living cell (see above). One can roughly distinguish between three types of phospholipid transfer proteins, namely phosphatidylinositol transfer

proteins (PITP), phosphatidylcholine transfer proteins (PCTP) and non-specific lipid transfer proteins (nsLTP) (for a review see ref. *40*). Whereas PCTP and PITP are more or less specific for their substrates, nsLTP is able to transfer a large variety of lipid classes. In *Saccharomyces cerevisiae* two cytosolic phospholipid transfer proteins have been identified and characterized so far: a PITP (*25, 35, 41*) and an nsLTP, which was named phosphatidylserine transfer protein (PSTP) (*42*) due to its preference for PtdSer as a substrate. Both proteins were isolated to near homogeneity but only PITP was studied by molecular biological means. As mentioned above PITP was found to be identical to Sec14p. Although the majority of this protein can be isolated from the cytosol the more important cellular site of action of PITP/Sec14p is the Golgi (*36*). Similar to PITP/Sec14p the PSTP can be isolated from yeast cytosol. Interestingly, proteins with lipid transfer activity similar to that of the cytosolic PSTP were isolated from the surface of organelles, especially from peroxisomes (*43*). It is not known at present whether there is a relationship between yeast PSTP and the membrane bound lipid transfer proteins similar to mammalian forms of nsLTP in the cytosol and in peroxisomes (*40*).

An alternative to lipid translocation mediated by phospholipid transfer proteins is lipid transport through membrane contact. Permeabilized cells can be used to study such a process because the spatial arrangement of organelles is largely kept intact during preparation. A technique to prepare permeabilized yeast cells with a high degree of permeabilization was developed in our laboratory (*44*) for studies of intracellular transport of aminoglycerophospholipids. Biosynthetic steps from PtdSer to PtdEtn (via decarboxylation) and then to PtdCho (via methylation) are linked to the intracellular transport of these components between the endoplasmic reticulum and mitochondria. Using a [^{3}H]serine precursor it was possible to follow the synthesis of each of the aminoglycerophospholipids and examine the requirements for their interorganelle transport. This experimental approach revealed that in permeabilized yeast cells newly synthesized PtdSer is readily translocated to the locus of phosphatidylserine decarboxylase 1 (Psd1p) in the mitochondria. PtdSer transport to the mitochondria is ATP-independent and exhibits no requirements for cytosolic factors. PtdEtn formed in the mitochondria is exported to the locus of the methyltransferases (principally the endoplasmic reticulum) and converted to PtdCho. The requirement of ATP for the export of PtdEtn to the endoplasmic reticulum (*44*) is still a matter of dispute. In permeabilized yeast cells treated with apyrase PtdEtn was retained in mitochondria; more recent experiments from our laboratory (Achleitner *et al.*, submitted for publication) demonstrated that this effect may be due to an unspecific inhibition caused by apyrase. Neither cytosolic factors nor ongoing methylation of PtdEtn to PtdCho are required for the export of PtdEtn from mitochondria to the endoplasmic reticulum.

Lipid transport of aminoglycerophospholipids between the endoplasmic reticulum and mitochondria also occurs in permeabilized cells that have been disrupted by homogenization indicating that the processes are extremely efficient and may be dependent upon stable structural elements between organelles. This hypothesis led us to search for cellular structures that form a bridge between the site of PtdSer synthesis, the endoplasmic reticulum, and mitochondria. In the yeast *Saccharomyces cerevisiae* similar to higher eukaryotes a subfraction of the endoplasmic reticulum was detected that exhibits high specific activities of enzymes involved in phospholipid biosynthesis, especially phosphatidylserine and phosphatidylinositol synthase (*45*). This specialized fraction of the endoplasmic reticulum is associated with mitochondria and was named MAM (mitochondria associated membrane) in agreement with the nomenclature known from mammalian cells (*46*). PtdSer synthesized in MAM can be imported into mitochondria and converted to PtdEtn *in vitro*. Reassociation of MAM with purified mitochondria led to reconstitution of the import of PtdSer into mitochondria suggesting that organelle contact is sufficient for this process.

Import of PtdSer into mitochondria can also be tested in a more artificial *in vitro* system using unilamellar vesicles as donor membranes (*47*). In contrast to the above mentioned MAM/mitochondria system, import of PtdSer into mitochondria from vesicles requires a lipid transfer protein. Neither a membrane potential across the inner mitochondrial membrane nor ATP is necessary for this translocation process, which is in agreement with results obtained with permeabilized yeast cells. Adriamycin, which interacts with negatively charged phospholipids and was shown to inhibit protein import into mitochondria (*48*), had no effect on the import of PtdSer from vesicles into mitochondria.

Proteins of the mitochondrial surface can be envisaged to mediate the assembly of phospholipids into the membranes of this organelle. In a vesicle/mitochondria transport assay system mild proteolytic treatment of intact mitochondria led to a decrease of the PtdSer import rate (*49*). This effect, however, can be regarded as a disturbance of the interaction of the lipid transfer protein with the mitochondrial surface. Recent experiments employing a MAM/mitochondria reconstituted system demonstrated that also in this case proteolytic treatment of mitochondria negatively affected translocation of PtdSer (*50*). Fusogenic proteins similar to those described by Rakowska *et al.* (*51*) that are partially inactivated by exogenous proteases may be involved in this process.

Synthesis of PtdSer is not a driving force for the import of this phospholipid into mitochondria, because co- and post-synthetic import of PtdSer from MAM into mitochondria occur at comparable rates (*50*). In contrast, PtdIns synthesized in MAM with [^{3}H]inositol as a precursor was imported into mitochondria depending on its ongoing synthesis (*52*). When PtdIns synthesis was stopped after a short pulse, the percentage of [^{3}H]PtdIns in the outer leaflet of the outer mitochondrial membrane remained constant during the chase period.

Migration of PtdSer from its site of synthesis to its site of decarboxylation by Psd1p in the inner mitochondrial membrane requires not only transport of the lipid from the endoplasmic reticulum to the mitochondrial surface, but also intramitochondrial translocation. Phospholipid transfer between the outer and inner mitochon-

drial membranes of yeast does not involve phospholipid transfer protein(s) of the intermembrane space; no such protein could be detected when intermembrane space contents were tested in appropriate phospholipid transfer assays *in vitro* (*52*). This finding confirms previous results by Blok *et al.* (*53*) who failed to detect phospholipid transfer activity in rat liver mitochondria.

In intact mitochondria, the outer and the inner membrane are associated through contact sites, which are most likely involved in the transport of proteins into the inner membrane and the matrix space (*54–56*). These membrane junctions are also good candidate sites for the transfer of phospholipids between the two mitochondrial membranes. PtdSer synthesized in MAM can be efficiently translocated to the inner membrane of intact mitochondria and converted to PtdEtn. This translocation of PtdSer from MAM to the inner mitochondrial membrane also occurred with mitoplasts (*52*). In yeast mitoplasts, the outer mitochondrial membrane with the MAM fraction attached is disrupted but still linked to the inner membrane, most likely through original contact sites. Thus PtdSer synthesized in MAM appears to reach the inner mitochondrial membrane through membrane contact sites. This view was supported by experiments with isolated contact site preparations (*57*), which consist of a membrane contact zone and portions of the outer and inner mitochondrial membranes. In these preparations MAM containing phosphatidylserine synthase remains associated with the outer mitochondrial membrane. The reporter enzyme of PtdSer import to the inner mitochondrial membrane, Psd1p, cofractionates with the inner membrane portion of contact sites. In a metabolic transport assay using [^{3}H]serine as a precursor PtdSer was translocated efficiently from MAM across zones of mitochondrial membrane contact to the inner membrane.

In a vesicle/mitochondria assay PtdSer on its way to the inner mitochondrial membrane was detected at significant amounts in contact sites (*47*). When metabolic conversion to PtdEtn was inhibited by hydroxylamine, PtdSer accumulated rather in the inner mitochondrial membrane than in contact sites. This result indicates that intramitochondrial translocation and metabolic conversion of PtdSer are not strictly linked. Similar to PtdSer, [^{3}H]labeled PtdIns and PtdCho seem to be transported to the inner mitochondrial membrane through membrane contact sites in a vesicle/mitochondria assay system (*58*). The uncoupler CCCP, the antibiotic adriamycin, and energy depletion caused by oligomycin and apyrase did not inhibit the import of both phospholipids into mitochondria.

Conclusion and future perspectives

The similarity in subcellular organization of yeast and higher eukaryotes, the ease of molecular biological manipulation of the microorganism, and finally the availability of the sequence information of the entire genome make yeast a suitable model to study all kinds of cell biological processes. These prerequisites also stimulated studies of phospholipid transport between organelles of this unicellular eukaryote *in vivo* and *in vitro*. A good example for the value of the yeast system is the progress that was made to investigate the cellular function of PITP/Sec14p. The availability of mutants and the design of genetic experiments has led to a new understanding how lipid synthesis and transport are related to other cellular processes.

Studies with yeast demonstrated that lipid transfer proteins must not be longer regarded as lipid carriers *in vivo*. Vesicle flux as a mechanism of lipid translocation may still be relevant, although protein secretory vesicles are apparently not the vehicles for bulk lipid transport. Membrane contact and membrane fusion are more likely candidate mechanisms of interorganelle lipid translocation. Many unanswered questions, however, remain. How does membrane association occur? Is the association of membranes determined by proteins, lipids and/or membrane physical properties? Which regulatory mechanisms stimulate or prevent membrane contact? How are lipids of the donor membrane selectively captured and inserted into the acceptor membrane, and how is the specificity of the membrane lipid composition maintained during this process? One assumes that membrane contact and lipid translocation between associated membranes occur through protein catalysis. However, the molecular details of none of these factors are known at present. More than one mechanism of lipid transport might be relevant and several processes might function in parallel *in vivo*. A combination of molecular biological, cell biological and biochemical methods using yeast as a model will certainly contribute to our knowledge in this field within the next years.

Acknowledgments

Work in our laboratory was financially supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich (projects S 5811, 11491 and 12076), the Jubiläumsfonds der Österreichischen Nationalbank (project 5644), the Eurofan project BIO4–CT95–0080, and the Austrian Ministry of Science and Transportation (project 950080).

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Sinteza i transport fosfolipida u kvascu

Sažetak

Kvasac Saccharomyces cerevisiae pogodan je sustav za proučavanje staničnog i molekularno-biološkog tijeka biogeneze organela. Sinteza i ispravna razdioba fosfolipida bitni su preduvjeti za nastajanje funkcionalnih membrana. Većina enzima, koji sudjeluju u biosintezi fosfolipida, smještena je u endoplazmatskom retikulumu. Nadalje, pojedini stupnjevi sinteze fosfolipida provode se u mitohondrijima, Golgiju i pojedinim česticama lipida. Međureakcija tih organela potrebna je da bi se održala uravnotežena razina staničnih fosfolipida. Izrazit je primjer suradnje organela slijed biosintetskog puta aminoglicerofosfolipida, koji se događa uzajamnim djelovanjem endoplazmatskog retikuluma, mitohondrija, a vjerojatno i Golgija. Intracelularni protok intermedijera potreban je da bi se sintetizirali lipidi te lipidima opskrbile one membrane koje same nisu sposobne sintetizirati vlastite lipide. Proteinima kataliziran transport lipida, protok vezikula i translokacija lipida membranskim dodirom mogući su mehanizmi migracije lipida unutar stanice.

Na osnovi eksperimentalnih podataka izgleda da je dodir između organela najvažniji za transport lipida između subcelularnih odjeljaka.