Intramitochondrial Transfer of Phospholipids in the Yeast, *Saccharomyces cerevisiae*

Translocation of phosphatidylinositol, which is synthesized on the outer aspect of the outer membrane of isolated yeast mitochondria, to the inner membrane is linked to phosphatidylinositol synthesis and is therefore a vectorial process. Phosphatidylinositol once integrated into the inner mitochondrial membrane is not transferred back to the mitochondrial surface. Phosphatidyserine is also translocated from the outer to the inner mitochondrial membrane, where it is decarboxylated to phosphatidylethanolamine. We made use of this metabolic modification to characterize the intramitochondrial transfer of phosphatidyserine and phosphatidylethanolamine. Intramitochondrial phosphatidyserine transfer is insensitive to the uncoupler carbonyl cyanide m-chlorophenylhydrazone and to valinomycin and is thus independent of an electrochemical gradient across the inner membrane. Transfer of phosphatidyserine from the outer to the inner mitochondrial membrane occurs not only in intact mitochondria but also in mitoplasts which are devoid of intermembrane space proteins but have the outer membrane still adherent to the inner membrane. This result suggests that specific contact sites are involved in the intramitochondrial translocation of phospholipids. 

Labeled phosphatidylethanolamine synthesized from \[^3H\]serine in isolated mitochondria is readily exported from the inner to the outer mitochondrial membrane without prior mixing with the pool of phosphatidylethanolamine of the inner membrane.

Mitochondria of the yeast, *Saccharomyces cerevisiae*, have the capacity to synthesize glycerophospholipids (Kuchler *et al.*, 1986). Similar to mitochondria of higher eukaryotes, phosphatidyserine decarboxylase and enzymes of cardiolipin synthesis are located in the inner mitochondrial membrane (for a review see Daum, 1985). On the other hand, the inner mitochondrial membrane is devoid of enzymes involved in the biosynthesis of phosphatic acid, phosphatidyserine, and phosphatidylinositol. The corresponding enzymes, glycerophosphate acyltransferase, phosphatidyserine synthase, and phosphatidylinositol synthase, show highest specific activity in the "heavy" microsomal fraction and co-fractionate also with the outer mitochondrial membrane (Kuchler *et al.*, 1986). Synthesis of phosphatidylethanolamine, the major yeast phospholipid, via stepwise methylation of phosphatidylethanolamine is restricted to the endoplasmic reticulum. As a consequence of the spatial separation of the site of biosynthesis and the final destination of phospholipids in organelle membranes, a continuous flow of phospholipids has to traverse the outer mitochondrial membrane in both directions during cell growth and membrane proliferation.

Little is known about mechanisms of intramitochondrial phospholipid transport. Flippases similar to those identified in the endoplasmic reticulum of rat liver or in the erythrocyte membrane (Backer and Dawidowicz, 1987; Devaux, 1988) might trigger transmembrane movement. Intermembrane translocation might occur via specific contact sites between the outer and the inner mitochondrial membrane (Hackenbrock, 1988; Verkleij, 1984). These contact sites are also involved in the transport of proteins into the inner membrane and the matrix space (Schleyer and Neupert, 1985; Schweiger *et al.*, 1987). Participation of contact sites in phospholipid transport to the inner mitochondrial membrane has been suggested before by Baranska and Wojtczak (1984) and Blok *et al.* (1971), but experimental evidence to support this assumption has not been provided.

The aim of the present study was to investigate the process of phospholipid transfer across and between mitochondrial membranes using isolated mitochondria and submitochondrial preparations. Experimental evidence will be presented for the linkage between biosynthesis and intermembrane translocation of phosphatidylinositol, phosphatidyserine, and phosphatidylethanolamine. Furthermore, the question as to the involvement of specific contact sites in phospholipid translocation between the outer and the inner mitochondrial membrane will be addressed.

**EXPERIMENTAL PROCEDURES**

Yeast Strains and Culture Conditions—The wild type yeast strain *Saccharomyces cerevisiae* D273-10 B was grown aerobically at 30 °C on 2% lactate as described (Daum *et al.*, 1982). The phosphatidyserine synthase deficient strain *S. cerevisiae* cho 1 (Atkinson *et al.*, 1980) was grown on YPLac medium (10 g/liter yeast extract, 20 g/liter peptone and 2% lactate). When yeast cells were prelabeled with \[^2-3H\]inositol overnight, the culture medium was the same as described by Klig *et al.* (1985). The pH was adjusted to 5.5 with concentrated KOH.

Preparation of Subcellular Fractions—Spheroplasts, mitochondria, mitoplasts, and outer and inner mitochondrial membranes were prepared by methods described by Daum *et al.* (1982). Mitochondrial fractions were characterized by the marker enzymes kynurenine hydroxylase (outer membrane) (Schnaitman and Greenawalt, 1968), succinate dehydrogenase (inner membrane) (Ackrell *et al.*, 1978), and cytochrome b6 (intermembrane space) (Labeyrie *et al.*, 1978), or by immunotitration (Haid and Suissa, 1983) using monospecific antibodies against porin (outer membrane), ADP/ATP translocator (inner membrane), and cytochrome b6 (intermembrane space). Fractions were also tested routinely by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and protein bands stained with Coomassie Blue were quantitated on a Shimadzu Dual Wavelength Chromatoscanner CS-350.

Phospholipid Synthesis in Mitochondria and Submitochondrial Preparations—Synthesis of phosphatidylinositol with mitochondria
isolated by differential centrifugation was carried out in the presence of 0.6 M mannitol, 100 mM Tris-HCl, pH 8, 5 mM MnCl₂, 1 mM CTP, 1 mM iso-20-40 μCi/ml [2-3H]inositol (20 Ci/mmol). The reaction was started by the addition of 0.5 mg of mitochondrial protein per ml. The incubation temperature was 30 °C; the reaction was linear within 15 min. Samples of 0.1 ml were taken at time points indicated. Phosphatidylserine synthesis was stopped by the addition of 40 mM EDTA, and lipids were extracted with 3 ml of chloroform/methanol (2:1, v/v) according to the method of Folch et al. (1957).

Phosphatidylserine synthesis with intact mitochondria was carried out in the presence of 0.6 M mannitol, 100 mM Tris-HCl, pH 8, 0.6 mM MnCl₂, 1 mM CTP, 0.6-2.5 mM serine, and 10-20 μCi/ml [3-3H]serine (38 Ci/mmol). For phosphatidylserine synthesis with isolated outer mitochondrial membrane vesicles, mannitol was omitted and CTP was replaced by 0.2 mM CDP-diacylglycerol. The concentration of outer membrane protein in these incubations was 0.1-0.2 mg/ml.

Incubations were carried out at 30 °C; the assay was linear within 15 min. When mitoplasts were used for incubations, the mannitol concentration was 0.1 M. Samples of 0.2 ml were taken at the time points indicated. Phosphatidylserine synthesis was stopped by the addition of 10 mM EDTA; lipids were extracted as described above.

Phospholipid Transfer — Phospholipid transfer was used as a tool to determine the surface exposure of phospholipids in isolated mitochondrial or outer mitochondrial membrane vesicles. A phosphatidylinositol/phosphatidylinositol transfer protein from yeast was isolated by published procedures (Daum and Paltauf, 1984). Acceptor vesicles were prepared as follows. Palmotolylolyl phosphatidylcholine (1.6 mg) and tracer amounts of [14C]triaclyglycerol (approximately 3 × 10⁶ cpm) were dried under a stream of nitrogen. Depending on the incubation (intact mitochondria or isolated outer mitochondrial membrane vesicles) lipids were dispersed in 3 ml of 0.6 M mannitol, 10 mM Tris-HCl, pH 7.4, or in 10 mM Tris-HCl, pH 7.4, respectively. Vesicles were prepared by sonication (70 watts) at 2-4 °C under a stream of nitrogen using a Braun Labsonic 2000 sonicator. Multilamellar vesicles were removed by centrifugation in an Eppendorf centrifuge for 10 min at 12,000 × g. The supernatant was saved and kept at 4 °C.

Phospholipid transfer with whole mitochondria was conducted in the presence of 0.6 M mannitol, 10 mM Tris-HCl, pH 7.4. The incubation mixture contained 40 mM EDTA, 6-10 μl/mg phospholipid transfer protein (10 units/mg; 1 unit is defined as 1% phospholipid transferred in a standard assay (Daum and Paltauf, 1984)), and 0.1 mg/ml acceptor vesicle lipid. Transfer was started by addition of mitochondrial protein (0.1 mg/ml) withdrawn from the respective phospholipid synthesis incubation mixture. Transfer was carried out at 30 °C. Samples of 500 μl were taken between 0 and 30 min; under standard conditions, apparent transfer of radioactively labeled phosphatidylcholine vesicles with the aid of a yeast phosphatidylinositol/phosphatidylcholine transfer protein (Fig. 1). During prolonged incubation of mitochondria with [3H]inositol, [3H]phosphatidylinositol became more and more protected against the transfer protein; after 10 min, only 20-30% of [3H]phosphatidylinositol was transferred.

Transfer of phosphatidylinositol from the outer aspect of mitochondria to the inner membrane as shown in Fig. 1 occurs during active synthesis of phosphatidylinositol. In contrast, translocation of [3H]phosphatidylinositol to the inner mito-
Chondrial membrane did not occur, when \([^{3}H]\)phosphatidylinositol was inserted into the outer mitochondrial membrane by incubating intact mitochondria with \([^{3}H]\)phosphatidylinositol/phosphatidylcholine vesicles in the presence of phosphatidylinositol/phosphatidylcholine transfer protein from yeast. Taken together, these results support the view that phosphatidylinositol translocation between mitochondrial membranes is linked to phosphatidylinositol synthesis and is therefore a vectorial process. Evidence for the linkage of phosphatidylinositol synthesis and intramitochondrial translocation could also be derived from an experiment in which \([^{3}H]\)phosphatidylinositol synthesis in intact mitochondria was stopped by the addition of EDTA (Fig. 1) after 1 min of incubation; subsequent incubation for 19 min did not reduce the percentage (70%) of \([^{3}H]\)phosphatidylinositol accessible to an external phospholipid transfer protein. This result must be interpreted with caution, however, since it cannot be excluded with certainty that EDTA inhibits not only the synthesis, but also the intramitochondrial translocation of phosphatidylinositol. Experiments with exogenously added phosphatidylethanolamine showed that EDTA inhibits the intramitochondrial transfer to approximately 50% of the control (data not shown). The translocation of phosphatidylethanolamine from the inner to the outer mitochondrial membrane (see below, Fig. 5) is not inhibited by EDTA. In analogy, one might therefore conclude that translocation of phosphatidylinositol does also occur in the presence of EDTA.

If phosphatidylinositol transfer was indeed a vectorial process driven by phosphatidylinositol synthesis, then phosphatidylinositol once integrated into the inner mitochondrial membrane (Fig. 2) should be unable to migrate back to the mitochondrial surface. To test this assumption, cellular lipids were prelabeled during 12 h of growth of yeast cells in the presence of \([^{14}C]\)inositol. After the isolation of mitochondria, a pulse-labeling with \([^{3}H]\)inositol was carried out, and the accessibility of differently labeled phosphatidylinositol to a transfer protein was tested as described above. As can be seen from Fig. 3, newly synthesized \([^{14}C]\)phosphatidylinositol was readily accessible to a phosphatidylcholine/phosphatidylinositol transfer protein, whereas the “old” \([^{3}H]\)phosphatidylinositol was protected. This result confirms that intramitochondrial translocation of phosphatidylinositol is a vectorial process directed toward the inner membrane, most likely pushed by the synthesis of the phospholipid.

Translocation of Phosphatidylethanolamine and Phosphatidylethanolamine between the Outer and the Inner Mitochondrial Membrane—Synthesis and decarboxylation of phosphatidylethanolamine are spatially separated: phosphatidylethanolamine synthesis was readily accessible to an external phospholipid transfer protein. Yeast cells were labeled overnight in the presence of \([^{3}H]\)inositol. Prelabeled mitochondria were isolated and incubated for a short period (10 min) with \([^{14}C]\)inositol. Phosphatidylinositol synthesis was stopped by the addition of EDTA, and accessibility of labeled phosphatidylinositol was tested with the aid of a transfer protein as described above. Values are expressed as % “old” \([^{3}H]\)-labeled (—) and “new” \([^{14}C]\)-labeled (——) phosphatidylinositol accessible to transfer.

![Fig. 3. Accessibility of “old” and newly synthesized phosphatidylinositol to an external phospholipid transfer protein.](image)

**Table I.** Transfer of phosphatidylethanolamine from the outer to the inner mitochondrial membrane

<table>
<thead>
<tr>
<th>Condition</th>
<th>Radioactivity in phosphatidy-lethanolamine (cpm)</th>
<th>% control*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20,490</td>
<td>100</td>
</tr>
<tr>
<td>-EDTA</td>
<td>42,190</td>
<td>63</td>
</tr>
<tr>
<td>+CCC (20 or 100 µM)</td>
<td>20,610</td>
<td>95</td>
</tr>
<tr>
<td>+Valinomycin (2.5 µM)</td>
<td>20,610</td>
<td>85</td>
</tr>
<tr>
<td>+Sucinate (3%)</td>
<td>24,680</td>
<td>82</td>
</tr>
<tr>
<td>+Ethanol (7%)</td>
<td>19,390</td>
<td>90</td>
</tr>
<tr>
<td>+CN- (5 mM)</td>
<td>27,690</td>
<td>12</td>
</tr>
<tr>
<td>+N2 (5 or 50 mM)</td>
<td>12,490</td>
<td>97</td>
</tr>
</tbody>
</table>

*In the control experiment, 16-20% of total phospholipid radioactivity was associated with phosphatidylethanolamine.

**% control** is compared to zero incubation (100%)

Co-fractionates with microsomes and the outer mitochondrial membrane, whereas phosphatidylethanolamine decarboxylase is an enzyme of the inner mitochondrial membrane. Conversion of phosphatidylethanolamine to phosphatidylethanolamine can therefore serve as the basis to assess the minimum requirement of phosphatidylethanolamine containing the minimum required per se is present in the mitochondrial membranes. The relative low concentration of \([^{3}H]\)phosphatidylethanolamine remaining in the inner membrane after its import (see below, Fig. 4) might serve as an indication, that decarboxylation is not the limiting step on the way of phosphatidylethanolamine from its site of synthesis to its metabolic conversion. Therefore, the appearance of \([^{3}H]\) phosphatidylethanolamine due to incubation of mitochondria with \([^{3}H]\)serine and CTP can serve as an estimate for the rate of transfer of newly synthesized phosphatidylethanolamine to the inner mitochondrial membrane. Some characteristics of this transfer process are summarized in Table I. Phosphatidylethanolamine transfer is insensitive to the uncoupler cyanide cyanide m-chlorophenylhydrazone and to the ionophore valinomycin, which destroy the electrochemical gradient across the inner mitochondrial membrane. Metabolic stimulation of mitochondria by the addition of succinate or ethanol had no effect on phosphatidylethanolamine transfer. The possibility of an ATP requirement of phosphatidylethanolamine translocation from the outer to the inner membrane could not be assessed in these experiments, because the co-substrates for phosphatidylethanolamine synthesis, CTP or CDP-diacylglycerol, were present during incubation. All inhibitors used in this experiment except CN- did not affect the enzyme activities of phosphatidylethanolamine synthase and phosphatidylethanolamine decarboxylase. CN- inhibits the conversion of phosphatidylethanolamine to phosphatidylethanolamine, but this effect has nothing to do with an involvement of the respiratory chain, because N2 was completely ineffective. Independent measurement of phosphatidylethanolamine decarboxylase activity, using \([^{3}H]\)phosphatidylethanolamine as a substrate, showed that CN- partially inhibited the enzyme (data not shown). An additional effect on phosphatidylethanolamine translocation cannot be excluded.

When yeast mitochondria are subject to a mild hypotonic treatment (0.1 M mannitol), the outer membrane is disrupted and the intermembrane space is released (Daum et al., 1982).
In contrast to mammalian mitoplasts, yeast "mitoplasts" have the outer membrane still adherent to the inner membrane, very likely via original membrane contact sites. Yeast mitoplasts have a similar capacity to synthesize phosphatidylserine as mitochondria. Using the decarboxylation of in situ-synthesized [3H]phosphatidylserine to phosphatidylethanolamine as a measure for phosphatidylserine transfer to the inner mitochondrial membrane, we tested the following hypotheses: if contact sites were the sites of lipid translocation between the outer and the inner mitochondrial membrane, [3H]phosphatidylethanolamine should be formed at the same rate in intact mitochondria and in mitoplasts; if proteins of the intermembrane space catalyzed phospholipid transfer, the appearance of [3H]phosphatidylethanolamine in mitoplasts should be dramatically reduced. Data summarized in Table II show that the conversion of phosphatidylserine to phosphatidylethanolamine is even enhanced in mitoplasts as compared to intact mitochondria supporting the hypothesis that specific contact sites mediate phosphatidylserine translocation between the two membranes. To rule out the possibility that intermembrane phosphatidylserine transfer had occurred by unspecific fusion of membranes during the incubation procedure, the following control experiment was carried out. When mitoplasts isolated from the phosphatidylserine synthase deficient mutant strain S. cerevisiae cho 1 (Klig et al., 1985) were incubated with outer mitochondrial membrane vesicles from a wild type yeast strain, decarboxylation of [3H]phosphatidylserine (synthesized from [3H]serine exclusively in preparations of outer membrane vesicles) was marginal. This result shows that collision contact of isolated outer and inner mitochondrial membranes during incubation is not sufficient for phosphatidylserine transfer. The fact that the rate of conversion of phosphatidylserine to phosphatidylethanolamine is always higher in mitoplasts than in intact mitochondria (see Table II) is not completely understood. We explain this observation by the sensitivity of yeast phosphatidylserine decarboxylase to polyvalent cations (Carson et al., 1984) and the facilitated access of EDTA to phosphatidylserine decarboxylase in mitoplasts as compared to intact mitochondria.

When phosphatidylserine translocation between the outer and the inner mitochondrial membrane was followed by sub-mitochondrial fractionation (Fig. 4), the total amount of radioactivity associated with phosphatidylserine was markedly higher in the outer than in the inner membrane. The major part of phosphatidylserine transferred to the inner membrane was converted to phosphatidylethanolamine. The total transfer rate of [3H]phosphatidylserine can therefore be calculated as the amounts of radioactivity associated with [3H]phosphatidylserine in the inner membrane and with [3H]phosphatidylethanolamine in both mitochondrial membranes at a certain time point. Radioactivity present in the outer as well as in the inner mitochondrial membrane was corrected for recovery and cross-contamination according to marker enzymes.

In the same experiment, the amount of [3H]phosphatidylethanolamine was found to be significantly higher in the outer than in the inner mitochondrial membrane at all time points tested (Fig. 5), indicating that newly synthesized phosphatidylethanolamine was preferentially exported from the inner membrane to the mitochondrial surface without mixing with the pool of "old" phosphatidylethanolamine present in the inner membrane.

### Table II

| Intramitochondrial transfer of phosphatidylserine as compared to transport between isolated outer membrane vesicles and mitoplasts |
|---|---|---|
| Mitochondria, mitoplasts, and outer mitochondrial membrane vesicles were isolated by published procedures (Daum et al., 1982). Incubation conditions for the incorporation of [3H]serine into phospholipids were the same as described in Table I. |
| | Radioactivity | cpm/ aliquot |
| | Total lipids | Phosphatidylserine (%) |
| Mitochondria w.t.* | 18,960 | 3,640 (30) |
| Mitoplasts w.t. | 17,450 | 6,000 (34) |
| Mitoplasts cho 1 | ND | ND (0) |
| Outer mitochondrial membrane vesicles, w.t. | 13,800 | 580 (4) |
| Mitoplasts cho 1 + outer mitochondrial membrane vesicles, w.t. | 10,160 | 510 (4) |

* w.t., wild type yeast strain D273-10 B; cho 1, S. cerevisiae cho 1; ND, not detectable.

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**Fig. 4.** Transfer of phosphatidylserine from the outer to the inner mitochondrial membrane and its conversion to phosphatidylethanolamine. Synthesis of [3H]phosphatidylserine using [3H]serine as a precursor was carried out as described under "Experimental Procedures." After stopping the synthesis at time points indicated, mitochondria were subfractionated, and lipids of the outer and inner mitochondrial membrane were extracted and separated by thin layer chromatography. Values corrected for the recovery of the outer and inner membrane (see Fig. 2) are expressed as radioactivity associated with phosphatidylserine of the outer (○—○) and inner (●—●) mitochondrial membrane. The total radioactivity present in phosphatidylserine of the inner membrane and in phosphatidylethanolamine present in both mitochondrial membranes represents the total transfer of phosphatidylserine (×—×) from the outer to the inner mitochondrial membrane.

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**Fig. 5.** Transfer of phosphatidylserine from the inner to the outer mitochondrial membrane. The distribution of [3H]phosphatidylethanolamine formed in the experiment described in Fig. 4 between the inner (●—●) and outer (○—○) mitochondrial membrane is expressed as radioactivity (cpm) associated with [3H]phosphatidylethanolamine in the respective membrane fraction. Values are corrected for the recovery of the outer and inner mitochondrial membrane as indicated in Fig. 2.
DISCUSSION

The intramitochondrial transfer of phospholipids can be dissected into two steps: transport across the outer mitochondrial membrane (in either direction) and translocation between the two mitochondrial membranes. What are the mechanisms underlying these processes? Are phospholipids freely exchangeable between the mitochondrial membranes, or is intermembrane lipid flow regulated? These problems we attempted to solve in the present study.

Transmembrane translocation of phospholipids across intracellular membranes is an as yet poorly understood process. Backer and Dawidowicz (1987) demonstrated the existence of a flipase in rat liver microsomes which catalyzes energy-independent flip-flop of phosphatidylcholine when inserted into artificial phospholipid vesicles. Recent experiments in our laboratory (Sperka-Gottlieb et al., 1988) had shown that the outer membrane of yeast mitochondria does not contain flipases that might equilibrate phosphatidylcholine, phosphatidylinositol, or phosphatidylethanolamine between the two leaflets of the membranes. Previous experiments on the localization of phosphatidylinositol synthase (Kuchler et al., 1986) and data reported here (Fig. 1) indicate that phosphatidylinositol synthase is associated with the outer aspect of the outer mitochondrial membrane. Nevertheless, newly synthesized phosphatidylinositol rapidly reaches the inner membrane (Fig. 2). This translocation is unidirectional as was demonstrated by the fact that phosphatidylinositol once integrated into the inner membrane is no longer accessible to an external phospholipid transfer protein, even after prolonged incubation in the presence of phospholipid vesicles (Fig. 3). The vectorial character of intramitochondrial phosphatidylinositol transport can be explained by assuming that trans- and intermembrane movement of phosphatidylinositol is linked to its synthesis. A quantitative estimate shows that, under the experimental conditions employed here, the amount of phosphatidylinositol synthesized per min equals 10% of the phosphatidylinositol present in the outer mitochondrial membrane. At the beginning of the incubation, newly formed phosphatidylinositol obviously enters the pool of outer membrane phosphatidylinositol: 80% of the radioactive phosphatidylinositol is accessible to the external phospholipid transfer protein. As the amount of new phosphatidylinositol in the outer membrane increases, it is preferentially translocated to the inner membrane: only 20% of radioactive phosphatidylinositol remain accessible to the phospholipid transfer protein after 10 min of phosphatidylinositol synthesis. Taken together, these observations suggest that newly synthesized phosphatidylinositol is translocated from the outer aspect of the outer mitochondrial membrane to the inner membrane during or immediately after synthesis. The mechanism of translocation is not yet understood. One might hypothesize that specific contact sites between the two mitochondrial membranes are involved in a “quasi-one-step” transfer of phospholipids from their site of synthesis to the inner membrane. Such a mechanism would be independent of a “classical” flipase and thus be consistent with the absence of such a flipase in the outer mitochondrial membrane (Sperka-Gottlieb et al., 1988).

Phosphatidylserine transfer between mitochondrial membranes occurs probably by a mechanism similar to that discussed above for phosphatidylinositol. Fig. 4 shows that most of the phosphatidylserine synthesized from [3H]serine and CDP-diacylglycerol in isolated mitochondria is converted to phosphatidylserine decarboxylase in the inner membrane. Subfractionation of mitochondria at time intervals between 2 and 10 min revealed that newly synthesized phosphatidylethanolamine was mainly present in the outer mitochondrial membrane without previously entering the pool of total phosphatidylethanolamine of the inner membrane. Such preferential export of newly synthesized phosphatidylethanolamine might occur by physical linkage of phosphatidylserine decarboxylase to the sites of intermembrane phospholipid translocation, which we believe are the specific contact sites between the two mitochondrial membranes. In vivo this mechanism could provide a possibility of transferring phosphatidylethanolamine very rapidly to other cellular membranes, especially to the endoplasmic reticulum, where it is converted to phosphatidylcholine by S-adenosyl methionine-dependent methylation.

As a conclusion, we can summarize that phospholipids do not move freely between and across mitochondrial membranes. Vectorial translocation of phosphatidylinositol and of phosphatidylserine from the outer to the inner mitochondrial membrane, and of phosphatidylethanolamine in the opposite direction, is driven by the biosynthesis of these phospholipids. It is conceivable that migration of phospholipids between the two mitochondrial membranes and across the outer membrane occurs via specific membrane contact sites. There is no evidence for the existence of phospholipid transfer proteins in the mitochondrial intermembrane space (data not shown). Whereas protein import into mitochondria depends on ATP and a membrane potential across the inner membrane (Hartl et al., 1989), phosphatidylserine import into the inner membrane is independent of a membrane potential (Table II). The question as to an ATP (or energy) requirement has still to be answered. An indirect energy dependence is given by the linkage of transport and biosynthesis, the latter requiring CDP-diacylglycerol as a substrate.

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