

Phosphatidylserine translocation to the mitochondrion is an ATP-dependent process in permeabilized animal cells

(membranes/phospholipids/organelles/transport)

DENNIS R. VOELKER

Lord and Taylor Laboratory for Lung Biochemistry, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206

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ABSTRACT Chinese hamster ovary (CHO-K1) cells were pulse labeled with [³H]serine, and the synthesis of phosphatidyl[³H]ethanolamine from phosphatidyl[³H]serine during the subsequent chase was used as a measure of lipid translocation to the mitochondria. When the CHO-K1 cells were pulse labeled and subsequently permeabilized with 50 μg of saponin per ml, there was no significant turnover of nascent phosphatidyl[³H]serine to form phosphatidyl[³H]ethanolamine during an ensuing chase. Saponin treatment rendered >99% of the cells permeable as judged by trypan blue exclusion and depleted them of 85% of their complement of cytosolic proteins as determined by residual lactic acid dehydrogenase activity. Supplementation of the permeabilized cells with 2 mM ATP resulted in significant phosphatidyl[³H]ethanolamine synthesis (83% of that found in intact cells) from phosphatidyl[³H]serine during a subsequent 2-hr chase. Phosphatidyl[³H]ethanolamine synthesis essentially ceased after 2 hr in the permeabilized cells. The translocation-dependent synthesis of phosphatidyl[³H]ethanolamine was a saturable process with respect to ATP concentration in permeabilized cells. The conversion of phosphatidyl[³H]serine to phosphatidyl[³H]ethanolamine did not occur in saponin-treated cultures supplemented with 2 mM AMP, 2 mM 5'-adenylyl imidodiphosphate, or apyrase (2.5 units/ml) plus 2 mM ATP. ATP was the most effective nucleotide, but the addition of GTP, CTP, UTP, and ADP also supported the translocation-dependent synthesis of phosphatidyl[³H]ethanolamine albeit to a lesser extent. These data provide evidence that the interorganelle translocation of phosphatidylserine requires ATP and is largely independent of soluble cytosolic proteins.

The biochemical mechanisms by which lipids are translocated among the membranes of eukaryotic cells have not been completely elucidated. Several mechanisms have been proposed, including (i) soluble carrier proteins (1-3), (ii) transport vesicles (4-6), and (iii) contact zones between donor and acceptor membranes. In some studies metabolic inhibitors have been used to infer a role for ATP in the translocation of cholesterol to the plasma membrane (7) and phosphatidylserine to the mitochondrion (8). Metabolic inhibitors have also been used to implicate endocytic pathways as routes for phosphatidylcholine transport from the plasma membrane to the Golgi apparatus (4). The use of metabolic inhibitors with intact cells is compromised by the inability to discover whether the effects are direct or indirect with regard to the process studied. Previous experiments from this laboratory have focused on the conversion of newly synthesized phosphatidylserine to phosphatidylethanolamine as a system to study phospholipid translocation (8, 9). The localization of phosphatidylserine synthase principally in the endoplasmic reticulum and phosphatidylserine decarboxylase in the mi-

tochondrion (10-13) allows one to use the decarboxylation of nascent phosphatidylserine as a measure of the translocation of this phospholipid. Studies from this laboratory using intact cells provided evidence that phosphatidylserine translocation to the mitochondria required ATP (8). More recent studies (14) provided evidence that ATP was not required for the translocation process to occur with isolated organelles (i.e., microsomes and mitochondria). The apparent paradox between these two observations could be a result of the indirect effects of metabolic inhibitors upon intact cells. Alternatively, this paradox may be a consequence of the loss of a critical level of structural organization normally found in the intact cell and missing from the isolated organelles. The purpose of the present study was to more clearly define the putative ATP requirement for phosphatidylserine translocation to the mitochondrion. The permeabilized cell was chosen as a system that could be reconstituted with specific components. The results demonstrate that phosphatidylserine translocation occurs readily in permeabilized cells that are supplemented with ATP.

MATERIALS AND METHODS

Chemicals. Simple salts, buffers, and organic solvents were purchased from Sigma, Mallinckrodt, and Fisher. Saponin, apyrase, ATP, ADP, CTP, UTP, GTP and 5'-adenylyl imidodiphosphate (AMP-P[NH]P) were obtained from Sigma and Boehringer Mannheim. The [G-³H]serine was supplied by DuPont/NEN.

Cells. The Chinese hamster ovary (CHO-K1) cells were obtained from the American Type Culture Collection and maintained in Ham's F12 medium supplemented to 10% fetal bovine serum, 100 units of penicillin per ml, 50 μg of streptomycin per ml, and 2 mM glutamine. Cells were seeded at 1×10^6 per 60-mm dish 22-24 hr prior to labeling and permeabilization experiments. Radiotracer experiments were performed by labeling cells for 1 hr in serine-free Ham's medium supplemented with dialyzed serum and antibiotics and 2 μCi of [G-³H]serine per ml (1 Ci = 37 GBq). After labeling, the cells were washed with Puck's saline G containing 1 mM serine and divided into several groups. The cells that were left intact were either harvested or shifted to normal maintenance medium supplemented to 1 mM serine. The permeabilized cell group was washed with 5 ml of salt solution A, which has an ion composition similar to that found in the intact cell (15): 140 mM KCl, 10 mM NaCl, 2.5 mM MgCl₂, 20 mM Hepes-KOH (pH 6.8), and 1 mM serine. After these washes the cells were treated with 2.5 ml of solution A containing 50 μg of saponin per ml (always prepared immediately before use) for 5 min at 37°C in a humidified atmosphere. Following this permeabilization procedure the medium was aspirated as completely as possible

and the dish was replenished with solution A either with or without nucleotides as specified in the text. The pH of all nucleotide-containing solutions was carefully checked and adjusted in each experiment. The saponin treatment rendered >99% of the cells permeable to trypan blue. Lactic acid dehydrogenase was measured in extracts from the intact and permeabilized cells using a fluorimetric endpoint assay (16). When the permeabilized cells were harvested for lipid analysis they were first cooled on ice and scraped with a rubber policeman without prior removal of the solution A. After collecting the scraped cells the dishes were routinely subjected to a second scraping in the presence of an additional 0.5 ml of solution A, and the lipids were extracted by the method of Bligh and Dyer (17).

Phosphatidylserine Decarboxylase. Intact and saponin-permeabilized cultures of CHO-K1 cells (usually 20 100-mm dishes) were harvested by scraping with a rubber policeman in 0.25 M sucrose/10 mM Tris, pH 7.4/1 mM EDTA (solution B). The cell suspension was sonicated in three 30-sec bursts followed by 1 min of cooling using a Bransonic sonicator at a setting of 4. The sonicated preparation was subsequently centrifuged at $100,000 \times g$ for 1 hr. The pellet containing total cell membranes was recovered and resuspended in 1 ml of solution B. Phosphatidylserine decarboxylase was assayed using a $^{14}\text{CO}_2$ trapping assay as described (18). The reaction mixture in a volume of 0.4 ml contained 50–100 μl of resuspended membranes, 1 mM phosphatidyl[1'- ^{14}C]serine (250 cpm/nmol), 0.5 mg of Triton X-100 per ml, 0.1 M KH_2PO_4 (pH 6.8), 10 mM EDTA, and, where indicated, 2 mM ATP. The reaction was carried out in a gas-tight vessel and terminated by acidification with 0.5 ml of 0.25 M H_2SO_4 . The released $^{14}\text{CO}_2$ was trapped on filter paper saturated with 0.2 M KOH.

Lipid Analysis. Total lipid extracts were prepared from cell preparations using the procedure of Bligh and Dyer (17). During the extraction, carrier lipid (100 μg each of phosphatidylserine and phosphatidylethanolamine) was added. The methanol water phase was adjusted to 0.05 M HCl during the initial extraction and the resultant lower phase was washed with an upper phase containing phosphate-buffered saline (137 mM NaCl/8 mM Na_2HPO_4 /2.7 mM KCl/1.5 mM KH_2PO_4 , pH 7.4). The lipid extracts were dried under a stream of N_2 and resuspended in chloroform/methanol (2:1) prior to chromatography. Thin-layer chromatography was performed on activated (100°C for 1 hr) silica gel H plates (Analtech) using the solvent system chloroform/methanol/2-propanol/triethylamine/0.25% KCl in water, 90:27:75:54:18, vol/vol. The lipids were visualized by spraying the chromatograms with 0.1% aqueous 8-anilino-1-naphthalene sulfonic acid and exposure to ultraviolet light. The appropriate areas of the chromatogram were scraped into an emulsion-based liquid scintillation fluor (National Diagnostics, Manville, NJ), and the radioactivity was measured by liquid scintillation spectrometry.

RESULTS

ATP Stimulates the Translocation-Dependent Decarboxylation of Nascent Phosphatidylserine. Phosphatidylserine has previously been shown to be an important precursor of phosphatidylethanolamine in tissue culture cells (8, 9, 18–21). The topology of the enzymes involved in phosphatidylserine metabolism requires interorganelle translocation before decarboxylation can occur. The data presented in Fig. 1 show the metabolism of newly synthesized phosphatidylserine in intact and permeabilized CHO-K1 cells. In the intact cell phosphatidylserine and lesser amounts of phosphatidylethanolamine were readily labeled during a pulse with [^3H]serine. In the ensuing chase phase the phosphatidylserine was metabolized to give rise to additional phosphatidylethanol-

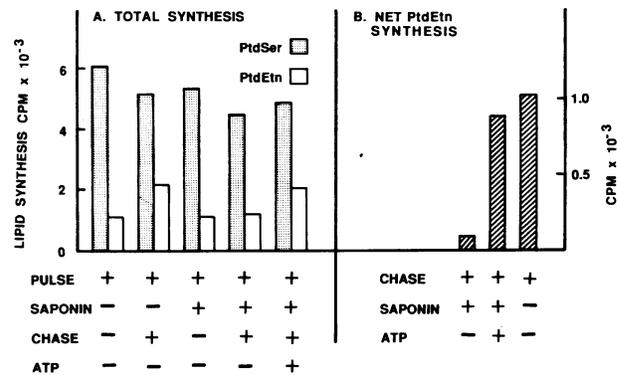


FIG. 1. Decarboxylation of nascent phosphatidylserine by intact and permeabilized CHO-K1 cells. (A) Monolayers of CHO-K1 cells were pulse labeled with 6 μCi of [^3H]serine (2 $\mu\text{Ci}/\text{ml}$, 8.0 Ci/mmol) for 1 hr. Following this pulse label the cells were washed with Puck's saline G and divided into five groups as indicated. Cells that were not treated with saponin after the pulse were either harvested immediately after washing or chased in medium supplemented to 1 mM serine for 2 hr. Cells that were permeabilized with saponin after the pulse were either harvested after permeabilization or chased for 2 hr in a medium of ionic composition similar to that found within the cell that was either unsupplemented or contained 2 mM ATP. The lipids were extracted from each group and analyzed by thin-layer chromatography. The radioactivity present in phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) was measured. (B) The net incorporation of radiolabel into PtdEtn was determined by subtracting the radioactivity found at the end of the pulse labeling period from that found at the end of the chase period. Data shown are from a representative 1 of 12 experiments with duplicate determinations in each experiment.

amine. When the cells were permeabilized with saponin at the end of the pulse label there was some diminution in the recovery of phosphatidylserine, and the levels of phosphatidylethanolamine were equivalent to those found in the intact cells. If the permeabilized cells were subjected to a chase period without any supplementation, insignificant amounts of new phosphatidylethanolamine were formed. However, supplementation of the permeabilized cells with 2 mM ATP led to levels of phosphatidylethanolamine synthesis that closely approximated those found in intact cells. This result is more apparent from the data shown in Fig. 1B, in which the net synthesis of phosphatidylethanolamine (phosphatidyl[^3H]ethanolamine present at the end of the chase minus that present at the end of the pulse) is shown. In 12 independent experiments the level of phosphatidylethanolamine synthesis in ATP-supplemented, permeabilized cells was $83.3\% \pm 6.5\%$ (mean \pm SEM, $n = 12$) of the value found in the intact cell. ATP itself has no effect upon the activity of phosphatidylserine decarboxylase activity measured in intact liver mitochondria (14) and detergent-solubilized mitochondria from control and saponin-treated cells (Table 1). The results provide direct evidence that ATP is required for the translocation of phosphatidylserine from its site of synthesis to the mitochondrion. The permeabilization procedure used not only permitted the repletion of cells with ATP but also depleted the cells of their cytosol. The release of the enzyme lactic acid dehydrogenase was monitored to determine the extent to which the permeabilized cells were depleted of cytosolic macromolecules. The saponin treatment rendered >99% of the cells permeable to trypan blue and released $85\% \pm 6.0\%$ (mean \pm SEM, $n = 4$) of the lactic acid dehydrogenase. The residual activity of the dehydrogenase was not readily removed by additional washings, suggesting that this remaining enzyme was tightly bound to other subcellular structures. In contrast to the loss of lactic acid dehydrogenase, the mitochondrial enzyme cytochrome *c* oxidase was completely retained in the permeabilized cells

Table 1. Phosphatidylserine decarboxylase activity is unaffected by ATP

| Enzyme | Saponin treatment* | Decarboxylase activity, [†] nmol/30 min per mg of protein |
|-----------|--------------------|-----------------------------------------------------------------------|
| Membranes | - | 12.3 ± 1.5 (6) |
| + ATP | - | 11.4 ± 1.8 (6) |
| Membranes | + | 12.3 ± 2.4 (5) |
| + ATP | + | 12.0 ± 2.1 (5) |

CHO-K1 total cell membranes were prepared and assayed for phosphatidylserine decarboxylase. Where indicated, 2 mM ATP was added to the reaction.

*CHO-K1 cell membranes were also prepared from cultures that had been rendered permeable by treatment with 50 μ g of saponin per ml.

[†]Values are the mean \pm SEM. The number of independent experiments (*n*) for each condition is shown in parentheses.

(recovery = 126% \pm 6.7%; mean \pm SEM, *n* = 4) compared with nonpermeabilized cells. These results provide evidence that phosphatidylserine translocation can occur in permeabilized animal cells. The process requires ATP and is largely independent of cytosolic proteins.

Phosphatidylserine Translocation Is a Time- and ATP Concentration-Dependent Process in Permeabilized Cells. The decarboxylation of nascent phosphatidylserine was examined as a function of time of incubation and the results are shown in Fig. 2. For reference, the turnover of phosphatidylserine and synthesis of phosphatidylethanolamine by intact cells are also shown in Fig. 2. In these experiments CHO-K1 cells were first pulse labeled for 1 hr with [³H]serine. After the labeling period the cells were washed and "chased" with unlabeled serine. During the "chase" phase the cells were either left intact or permeabilized with 50 μ g of saponin per ml. The permeabilized cells were incubated with or without a 2 mM ATP supplement as indicated. When permeabilized cells were supplemented with

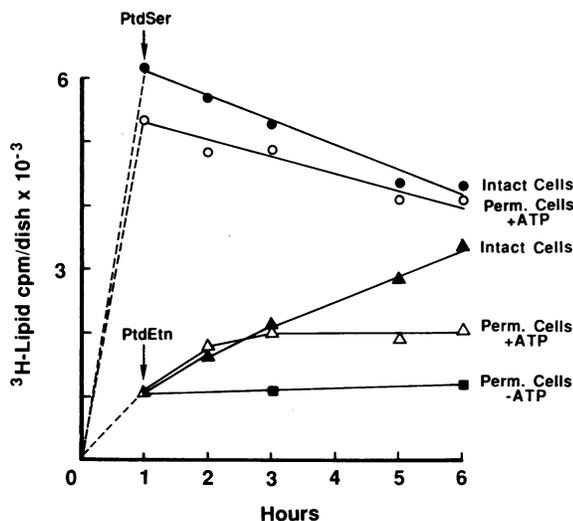


FIG. 2. Time-dependent labeling of phosphatidylethanolamine from phosphatidylserine in intact and permeabilized CHO-K1 cells. Monolayers of CHO-K1 cells were pulse labeled with 6 μ Ci of [³H]serine (2 μ Ci/ml, 8.0 Ci/mmol) for 1 hr. Following the pulse label the cells were washed with Puck's saline G and divided into three groups: (i) Intact cells were replenished with Ham's F12 medium containing 1 mM serine and harvested at the indicated time points. (ii) Perm. cells were permeabilized with 50 μ g of saponin per ml and maintained in medium of ionic composition similar to that found within the cell. (iii) Perm. cells + ATP were permeabilized cells supplemented with 2 mM ATP as indicated. The incorporation of radiolabel into phosphatidylserine (PtdSer) (○, ●) and phosphatidylethanolamine (PtdEtn) (△, ▲, ■) was determined by analyzing thin-layer chromatograms. Data shown are the average of three experiments with duplicate determinations in each experiment.

2 mM ATP, phosphatidylethanolamine synthesis initially proceeded at a rate very comparable to that observed in the intact cell. After 2 hr of chase the synthesis of phosphatidylethanolamine in the permeabilized cells essentially ceased. This latter result was not due to depletion of exogenous ATP, as readdition of ATP failed to support further synthesis of phosphatidylethanolamine. In the intact cell the synthesis of phosphatidylethanolamine from a serine precursor is obligately coupled to the turnover of phosphatidylserine. In the permeabilized cell system this same phosphatidylserine turnover was also observed to occur. The overall rate of phosphatidylserine turnover in the permeabilized cells was slightly reduced relative to intact cells. When ATP was omitted from incubations with permeabilized cells there was no significant synthesis of phosphatidylethanolamine. The translocation-dependent decarboxylation of pulse-labeled phosphatidylserine was also studied as a function of the ATP concentration, and the results are presented in Fig. 3. The synthesis of phosphatidylethanolamine was found to be stimulated by ATP in a saturable manner.

The results presented above provide evidence that the rate of decarboxylation of nascent phosphatidylserine in the saponin-treated cells that are supplemented with ATP is similar to the rate found in the intact cell. The ATP levels required to support this rate of decarboxylation at near maximal levels are within the physiological ranges found in cultured cells (18, 22).

Nucleotide Specificity of the Translocation Process. The nucleotide specificity of the translocation process was examined and the results are presented in Table 2. ATP at concentrations of 2 mM consistently yielded the highest levels of phosphatidylserine translocation. Inclusion of apyrase in the incubation mixture with ATP prevented phosphatidylserine translocation by converting the ATP to AMP. The nucleotide AMP failed to support the translocation process. Likewise the nonhydrolyzable analog of ATP, AMP-P[NH]P, failed to support translocation. ADP at a concentration of 2 mM did yield significant translocation rates. A significant difference was consistently observed for ADP obtained from Sigma and Boehringer Mannheim as indicated. The basis for this difference is not yet known. Other nucleoside triphosphates, UTP, CTP, and GTP, at concentrations of 2 mM [\approx 10 times the normal intracellular concentration (22)] partially supported phosphatidylserine translocation but none were as effective as ATP. GTP was the

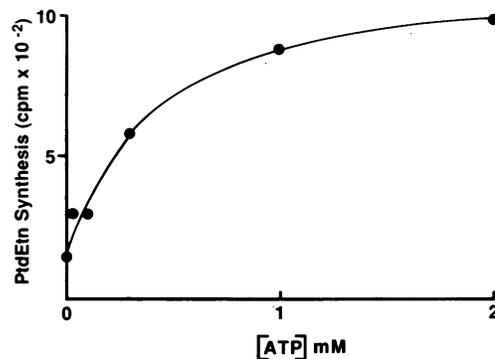


FIG. 3. ATP-dependent synthesis of [³H]phosphatidylethanolamine from [³H]phosphatidylserine. Monolayers of CHO-K1 cells were pulse labeled with 6 μ Ci of [³H]serine (2 μ Ci/ml, 8.0 Ci/mmol) for 1 hr. Following this pulse label the cells were washed with Puck's saline G and permeabilized. Following permeabilization the cells were supplemented with medium of ionic composition similar to that found within the cell and varying concentrations of ATP as indicated. Lipids were extracted from the cells and analyzed by thin-layer chromatography. Data shown are the average of three experiments with duplicate determinations.

Table 2. Nucleotide specificity of phosphatidylserine translocation in permeabilized CHO-K1 cells

| Nucleotide | % translocation |
|-----------------------------|-----------------|
| ATP | 100 |
| + apyrase* | 3.0 ± 3.1 |
| AMP | 0 |
| AMP- <i>P</i> [NH] <i>P</i> | 2.3 ± 4.0 |
| ADP (Sigma) | 36.3 ± 7.7 |
| ADP (Boehringer Mannheim) | 73.5 ± 23.3 |
| UTP | 47.8 ± 18.9 |
| CTP | 66.2 ± 16.2 |
| GTP | 77.6 ± 23.4 |
| GTP (0.2 mM) | 13.0 ± 2.0 |

All nucleotide concentrations were 2 mM unless otherwise indicated. Values are the mean ± SD of three to nine determinations. *2.5 units/ml.

most effective nucleoside triphosphate after ATP. Physiological levels of GTP yielded only 13% of the translocation of phosphatidylserine as seen for ATP.

DISCUSSION

A fundamental problem of membrane biogenesis in eukaryotic cells is the elucidation of the mechanisms by which the constituents of a given organelle membrane are translocated and assembled into the mature structure. Information regarding the movement of proteins among membrane domains is substantial and there are clear examples of general principles and mechanisms (23, 24). In contrast, understanding of the mechanisms of phospholipid movement among membrane domains is quite limited. A large body of data identifies phospholipid exchange proteins as macromolecules capable of effecting the intermembrane translocation of phospholipids *in vitro* (1–3). The role of these proteins in the intact cell, however, remains obscure. The activity of these proteins is elevated in neoplastic tissue and normal tissues, with elevated lipid translocation requirements suggesting that their activity changes with changing growth state and the need for new membrane synthesis (25–28). Other investigators have, however, observed discrepancies between the activities observed for the proteins *in vitro* and events that occur in the intact cell (8, 29). Previous work from this laboratory demonstrated that phosphatidylserine translocation to the mitochondrion of BHK-21 cells could be disrupted by depleting cellular ATP levels (8). The alterations in phosphatidylserine transport in the BHK-21 cells were not paralleled by changes in phosphatidylserine exchange protein activity measured in cell extracts. This line of investigation has been further pursued in the present study. In CHO-K1 cells that have been permeabilized with saponin no significant phosphatidylserine translocation to the mitochondria (assessed by the decarboxylation reaction) occurs. Supplementation of the permeabilized cells with ATP promotes phosphatidylserine translocation. The translocation of phosphatidylserine is dependent upon time and ATP concentration. This ATP requirement appears to mechanistically precede the import of phosphatidylserine into the mitochondria (*per se*) as determined from reconstitution studies using isolated organelles from rat liver (14). Experiments with isolated microsomes and mitochondria provide strong evidence that in a system where the two organelles can collide freely, phosphatidylserine can be readily imported into the mitochondria in an ATP-independent manner. These results suggest that the ATP is required prior to the formation of collision complexes between mitochondria and some donor compartment. The donor compartment (for phosphatidylserine) that is proximal to the mitochondria may be the endoplasmic reticulum or some transitional membrane structure such as small vesi-

cles. If the immediate donor organelle is the endoplasmic reticulum, then ATP may be expended to mechanically move this membrane system into a position that permits collision with the mitochondria. Alternatively, if structures such as small vesicles are utilized, then ATP may be utilized as an energy source in the budding of the vesicles from the endoplasmic reticulum or other organelles. It is further plausible that in either of these mechanisms (or others) mechanical force generated by cytoskeletal elements may account for the ATP requirement. Although involvement of cytoskeletal elements seems an attractive hypothesis, experiments with the inhibitors colcemid, nocodazole, and cytochalasin D have consistently failed to alter the translocation-dependent decarboxylation of phosphatidylserine in intact CHO-K1 cells (unpublished observations).

Although ATP is required for phosphatidylserine translocation, it appears that the normal complement of cytosolic proteins is not necessary. The permeabilization procedure removes ≈85% of the cellular lactic acid dehydrogenase activity. Even in the virtual absence of cytosolic proteins, the translocation of phosphatidylserine proceeds at a rate comparable to that of the intact cell for ≈2 hr. Furthermore, the addition of cytosol from CHO cells back to the permeabilized cells fails to stimulate the rate of decarboxylation of phosphatidylserine (data not shown). These results suggest that some of the steps involved in phosphatidylserine translocation to the mitochondria are independent of freely soluble proteins within the cell. However, in the present study, phosphatidylserine transfer protein activity was not detectable in extracts of control or saponin-permeabilized cells. The inability to detect phosphatidylserine transfer activity prevents an unequivocal assessment of exactly how much of this protein is lost from the cell as a consequence of permeabilization. Based upon the results with lactate dehydrogenase, a minimum of 85% of this protein in freely soluble form would be depleted from the cells. In addition, the ionic strength of the permeabilization medium exceeds the levels sufficient to displace the nonspecific lipid transfer protein from mitochondria (30). Collectively the conditions under which phosphatidylserine translocation occurs in the permeabilized cells (2 mM ATP, 160 mM salt concentration, and 85% depletion of cytosol) favor removal of all transfer protein that is not membrane enclosed (31) or very tightly bound to intracellular membranes.

The ATP-dependent translocation of phosphatidylserine in the permeabilized cell terminates abruptly after 2 hr of incubation, and further addition of ATP is without effect. This may be a consequence of inactivation or depletion of some specific factor. Alternatively, there may be only a limited pool of the newly synthesized phosphatidylserine that is competent for translocation to the mitochondria. Such competence may be a consequence of subcellular location or some driving force such as the continued synthesis of phosphatidylserine. The translocation process is saturable with respect to ATP concentration. The maximal rate of translocation occurs at levels of ATP that are within the physiological range for this nucleotide (8, 22). In addition to ATP, other nucleotides can support the translocation of nascent phosphatidylserine to the mitochondria. The nucleotides UTP, GTP, and CTP at concentrations well above their physiological levels (≈200 μM) (22) can support phosphatidylserine translocation but none is as effective as ATP. This may be due to nucleoside diphosphate kinase activity in the permeabilized cell, which generates ATP from NTPs and residual ADP. Alternatively, the process may simply be able to use different NTPs with different efficiencies. In addition to nucleoside triphosphates, ADP will also partially support phosphatidylserine translocation. Much of this activity is likely to be attributable to the conversion of ADP to ATP by adenylate kinase. In contrast to the nucleoside triphosphates

and ADP, AMP is completely without effect upon the lipid translocation process. In addition, the nonhydrolyzable ATP analog AMP-P[NH]P is also without effect. This latter result supports cleavage of ATP as a likely process in the translocation of phosphatidylserine to the mitochondrion.

Collectively these data provide evidence that phosphatidylserine translocation to the mitochondria can occur in cells depleted of cytosol. The translocation process proceeds in permeabilized cells at nearly the same rate found for the intact cell for a period of 2 hr in the presence of physiological concentrations of ATP. These results implicate a mechanism of phosphatidylserine translocation that is largely independent of soluble carrier proteins such as the phospholipid exchange proteins.

From the data obtained with the permeabilized cell system described in this study and isolated organelles reported elsewhere (14), it is proposed that phosphatidylserine translocation to the mitochondria is a two-step process. In the first step, reconstituted in the permeabilized cell, ATP is required to place phosphatidylserine into an environment that is permissive for the collision-mediated transfer of this lipid to mitochondria. The second step [reconstituted with isolated organelles (14)] is the collision-based transfer of phosphatidylserine to the mitochondria.

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- Wirtz, K. W. A. & Zilversmit, D. B. (1968) *J. Biol. Chem.* **243**, 3596–3602.
- Kader, J. C., Douady, D. & Mzaliak, P. (1982) in *Phospholipids*, eds. Hawthorne, J. N. & Ansell, G. B. (Elsevier, New York), pp. 279–311.
- Wirtz, K. W. A. (1982) in *Protein Lipid Interactions*, eds. Jost, P. C. & Griffith, O. H. (Wiley, New York), Vol. 1, pp. 151–231.
- Sleight, R. G. & Pagano, R. E. (1984) *J. Cell Biol.* **99**, 742–751.
- De Silva, N. S. & Siu, C. H. (1980) *J. Biol. Chem.* **255**, 5845–5850.
- Chlapowski, F. J. & Band, R. N. (1971) *J. Cell Biol.* **50**, 634–651.
- Kaplan, M. R. & Simoni, R. D. (1985) *J. Cell Biol.* **101**, 446–453.
- Voelker, D. R. (1985) *J. Biol. Chem.* **260**, 14671–14676.
- Voelker, D. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2669–2673.
- Dennis, E. A. & Kennedy, E. P. (1972) *J. Lipid Res.* **13**, 263–267.
- Van Golde, L. M. G., Raben, J., Batenburg, J. J., Fleischer, B., Zambrano, F. & Fleischer, S. (1974) *Biochim. Biophys. Acta* **360**, 179–192.
- Jelsema, C. L. & Morre, D. J. (1978) *J. Biol. Chem.* **253**, 7960–7971.
- Vance, J. E. & Vance, D. E. (1988) *J. Biol. Chem.* **263**, 5898–5909.
- Voelker, D. R. (1989) *J. Biol. Chem.* **264**, 8019–8025.
- Ueda, T., Chueh, S. H., Noel, M. W. & Gill, D. L. (1986) *J. Biol. Chem.* **261**, 3184–3192.
- Fanestil, D. D. & Barrows, C. H. (1965) *J. Gerontol.* **20**, 462–469.
- Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–917.
- Voelker, D. R. & Frazier, J. L. (1986) *J. Biol. Chem.* **261**, 1002–1008.
- Kuge, O., Nishijima, M. & Akamatsu, Y. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1926–1930.
- Kuge, O., Nishijima, M. & Akamatsu, Y. (1986) *J. Biol. Chem.* **261**, 5790–5794.
- Miller, M. A. & Kent, C. (1986) *J. Biol. Chem.* **261**, 9753–9761.
- Vance, D. E., Trip, E. M. & Paddon, H. B. (1980) *J. Biol. Chem.* **255**, 1064–1069.
- Pfeffer, S. R. & Rothman, J. E. (1987) *Annu. Rev. Biochem.* **56**, 829–852.
- Hartl, F. U., Pfanner, N., Nicholson, D. W. & Neupert, W. (1989) *Biochim. Biophys. Acta* **988**, 1–45.
- Dyatlovitskaya, E. V., Timofeeva, N. G. & Bergelson, L. D. (1978) *Eur. J. Biochem.* **82**, 463–471.
- Poorthuis, B. J. H. M., VanderKrift, T. P., Teerlink, T., Akeroyd, R., Hostetler, K. Y. & Wirtz, K. W. A. (1980) *Biochim. Biophys. Acta* **600**, 376–386.
- Post, M., Batenburg, J. J., Schurmans, E. A. J. M. & Van Golde, L. M. G. (1980) *Biochim. Biophys. Acta* **620**, 317–321.
- Wirtz, K. W. A., Jolles, J., Westerman, J. & Neys, F. (1976) *Nature (London)* **260**, 354–355.
- Yaffe, M. P. & Kennedy, E. P. (1983) *Biochemistry* **22**, 1497–1507.
- Megli, M. M., DeLisi, A., Van Amerongen, A., Wirtz, K. W. A. & Quagliariello, E. (1986) *Biochim. Biophys. Acta* **861**, 463–470.
- Van Amerongen, A., Van Noort, M., Van Beckhoven, J. R., Rommerts, F. F., Orly, J. & Wirtz, K. W. A. (1989) *Biochim. Biophys. Acta* **1001**, 243–248.