Isolation of a somatic-cell mutant defective in phosphatidylserine biosynthesis

(base-exchange enzyme mutant/Chinese hamster ovary cells/phospholipid metabolism/membrane biogenesis/somatic cell genetics)

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ABSTRACT Mutant clones of Chinese hamster ovary (CHO) cells defective in the base-exchange reaction of phospholipids with choline were isolated by using an in situ enzymatic assay for the reaction in cell colonies immobilized on polyester cloth. The specific activities of the choline-exchange reaction in extracts of one of the mutants (designated 64) grown at 33°C and 40°C were 13% and 6% of those in parental (CHO-K1) cells, respectively. The choline-exchange activity in the mutant was more thermolabile in cell extracts than that in the parent, suggesting that a mutation in the structural gene for the choline-exchange enzyme might have been induced in this mutant. In culture medium supplemented with lipoprotein-deficient serum, mutant 64 grew almost normally at 33°C but divided only twice at 40°C and then stopped growing. Labeling of intact cells with [32P]P, showed that mutant 64 was also strikingly defective in the biosynthesis of phosphatidylserine at 40°C but was normal at 33°C. Most temperature-resistant revertants of mutant 64 exhibited nearly normal ability to synthesize phosphatidylserine at 40°C and also showed choline-exchange activity similar to that in parental cells. The addition of phosphatidylserine to medium supplemented with newborn calf serum, in which mutant 64 grew more slowly than parental cells at 40°C, restored the growth rate of the mutant to the parental level. Our findings suggest that the choline-exchange enzyme functions as the major route for the formation of phosphatidylserine and that the temperature-sensitive growth of mutant 64 is due to a defect in phosphatidylserine biosynthesis at 40°C.

The isolation and biochemical characterization of cell mutants with specific defects in phospholipid metabolism represents a powerful approach for understanding the biological significance and metabolic control of membrane phospholipids (1). Recently, a rapid screening procedure has been developed for detecting mutant colonies of cultured mammalian cells immobilized on discs of filter paper (2) or polyester cloth (3). This procedure has been utilized successfully to isolate mutant clones of Chinese hamster ovary (CHO) cells defective in phospholipid metabolism (4–6).

Mammalian cells contain base-exchange enzymes which catalyze the exchange of free serine, choline, and ethanolamine with polar head groups of pre-existing phospholipids to produce phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine, respectively (for a review, see refs. 7 and 8). Although many enzymological studies on the base-exchange reactions have been conducted over the last two decades, their physiological roles are not understood. It has been suggested that the serine-exchange enzyme might be responsible for phosphatidylserine biosynthesis in mammalian cells (9); however, no direct evidence is available.

In this paper, we describe the isolation of CHO cell mutants lacking the choline-exchange activity by using an in situ enzymatic assay. We have found that one of the mutants (designated 64) with very low choline-exchange activity is defective in the biosynthesis of phosphatidylserine and also exhibits temperature-sensitive growth, suggesting that the choline-exchange enzyme contributes to the formation of phosphatidylserine and is essential for growth of CHO-K1 cells.

EXPERIMENTAL PROCEDURES

Materials [methyl-14C]Choline, l-[U-14C]serine, and [1,2-14C]ethanolamine were from Amersham; [32P]P, (carrier-free) was from the Japan Atomic Energy Research Institute, Ibaragi, Japan; bovine brain phosphatidylserine was from Sigma; Ham's F-12 medium and newborn calf serum (approximately 40 mg of protein/ml) were from Flow Laboratories; trypsin was from Difco; and strain CHO-K1 was from the American Type Culture Collection (ATCC CCL 61). Lipoprotein-deficient newborn calf serum (density ≥ 1.215 g/ml, approximately 130 mg of protein/ml) was prepared by ultracentrifugation as described (10). All other chemicals used were of analytical grade.

Methods Cells were maintained in plastic tissue culture flasks in Ham's F-12 medium supplemented with 10% (vol/vol) newborn calf serum, penicillin G (100 units/ml), streptomycin sulfate (100 μg/ml), and NaHCO3 (1.176 g/liter) in a 5% CO2 atmosphere at 100% humidity at 33°C or 37°C. The growth medium was replaced every 5–7 days in all cloning experiments. For mutant screening, CHO-K1 cells were treated with 400 μg of ethyl methanesulfonate per ml of growth medium at 37°C for 16 hr (2) and then incubated at 33°C for 3 days. The isolation of mutant 38 and mutant 64 from these cells is described in the legend to Fig. 1. Spontaneous temperature-resistant revertants of mutant 64 were isolated as described under Results.

The base-exchange activities in cell extracts were assayed as described in the legend to Table 1 and our previous report (6). Labeling with [32P]P, extraction, and analysis of phospholipids were done as described (6) with the modifications indicated in the legends. Protein was measured according to Lowry et al. (11), with bovine serum albumin as a standard.

RESULTS

Isolation of Mutants Defective in the Choline-Exchange Reaction. To isolate mutants with conditional lesion in the choline-exchange reaction, CHO cells mutagenized with ethyl methanesulfonate were grown and replicated on polyester cloth at 33°C, the permissive temperature. The colonies immobilized on polyester cloth were further incubated for 1 day at 40°C, the restrictive temperature, and then the choline-exchange activities of the individual colonies were determined by an autoradiographic in situ enzyme assay as described in the legend to Fig. 1. Approximately 5000 colonies...
were screened, and two mutant clones (designated mutant 38 and mutant 64, respectively) were found to have markedly decreased levels of the choline-exchange activity. Fig. 1A shows the Coomassie blue-stained polyester cloth on which mutant 64 was found by visual comparison with the corresponding autoradiogram (Fig. 1B); the arrows indicate the position of mutant 64. The two mutant colonies were retrieved from the master dishes and subjected to purification. Each single colony of these two mutants at the third cycle of purification was picked and used for the experiments described below.

Comparison of Base-Exchange Activities in the Mutants and the Parent. Choline-exchange activities of the mutants and the parent (CHO-K1) were quantitatively assayed. The specific activities of the choline-exchange reaction in extracts of both mutant 38 and mutant 64 grown at 33°C were strikingly reduced, as shown in Table 1. When cells grown at 33°C were incubated at 40°C for 24 hr just before harvesting, the specific activities of the two mutants were further reduced, whereas the specific activity of the parent remained unchanged (Table 1). These findings suggested that the choline-exchange activity in these two mutants was more thermostable in situ than that in the parent. The choline-exchange activity of the two mutants was also found to be more thermostable in cell extracts than that of the parent; namely, when the extracts of mutant 38, mutant 64, and the parent grown at 33°C were preincubated for 5 min at various temperatures and the choline-exchange activities then were assayed, the choline-exchange enzyme of both mutants exhibited higher sensitivity to heat than that of the parent (Fig.

**FIG. 1.** Identification of a CHO cell colony defective in the choline-exchange reaction. Mutagenized cells were seeded into 110-mm-diameter dishes treated with poly-L-lysine (3) to yield approximately 200 colonies per dish at 33°C. After 1 day, the cells were overlaid with polyester discs and glass beads (2, 3) and were incubated at 33°C. After 20 days, the polyester disc was removed from the master dish and transferred to another dish containing 5 ml of growth medium. After this dish was incubated in a CO2 incubator for 1 day at 40°C, the polyester disc was washed with phosphate-buffered saline, blotted on a paper towel, placed in a dish containing 2.0 ml of phosphate-buffered saline, and then frozen at −70°C. The frozen polyester disc was subjected to three cycles of freezing and thawing, blotted on a paper towel, and then placed in a dish containing 1.5 ml of the assay mixture for the choline-exchange reaction [0.1 mM {methyl-14C}choline (50 μCi/μmol; 1 Ci = 37 GBq)/5 mM CaCl2/50 mM Hepes, pH 7.5]. After the dish had been incubated at 40°C for 20 min, the reaction was terminated by transferring the polyester disc to another dish containing 2 ml of 10% (wt/vol) trichloroacetic acid. Each polyester disc was washed three times with 50 ml of 5% (wt/vol) trichloroacetic acid, autoradiographed for 14 days at room temperature with Fuji RX medical x-ray film, and then stained with Coomassie blue to visualize the attached colonies (2). The colonies were examined for choline-exchange activity by superimposing the autoradiogram on the stained polyester disc. Mutants identified as having no corresponding spots on the autoradiogram were retrieved from the master dishes, which had been supplied with 7 ml of growth medium plus 10% (vol/vol) dimethyl sulfoxide and stored at −70°C throughout these manipulations. (A) Coomassie blue-stained polyester disc. (B) Autoradiogram of the polyester disc. The arrows mark the position of the mutant colony.

Table 1. Base-exchange activities in extracts prepared from mutant 38 and 64, temperature-resistant revertant R-32 and R-33, and CHO-K1 cells

<table>
<thead>
<tr>
<th>Strain</th>
<th>Temp., °C</th>
<th>Choline</th>
<th>Serine</th>
<th>Ethanolamine</th>
</tr>
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<tbody>
<tr>
<td>CHO-K1</td>
<td>33</td>
<td>0.79</td>
<td>1.70</td>
<td>2.60</td>
</tr>
<tr>
<td>38</td>
<td>40</td>
<td>0.72</td>
<td>1.74</td>
<td>2.34</td>
</tr>
<tr>
<td>64</td>
<td>33</td>
<td>0.24</td>
<td>1.05</td>
<td>1.59</td>
</tr>
<tr>
<td>R-32</td>
<td>40</td>
<td>0.14</td>
<td>0.96</td>
<td>1.55</td>
</tr>
<tr>
<td>R-33</td>
<td>33</td>
<td>0.10</td>
<td>0.72</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Cells were seeded at 1.5 × 10⁶ cells per 150-mm-diameter dish in medium with 10% (vol/vol) newborn calf serum and incubated at 33°C. After 5 days, some cultures were shifted to 40°C for 24 hr, and then cell extracts were prepared as described (6). Extract containing approximately 100–200 µg of protein was mixed with 50 mM Hepes, pH 7.5/5 mM CaCl2 and either 0.2 mM {methyl-14C}choline (20 µCi/µmol), 0.2 mM L-[U-14C]serine (10 µCi/µmol) or 0.21 mM [1,2-14C]ethanolamine (19 µCi/µmol) in a final volume of 0.2 ml and then incubated 20 min at 33°C. After the reaction was terminated by adding 5 ml of chloroform/methanol (2:1, vol/vol), the lipids were extracted according to the method of Saito et al. (12). A 2-ml portion of the lower chloroform phase was dried, and the radioactivity was measured by liquid scintillation spectrometry in toluene-based scintillation fluid. The properties of the base-exchange reactions in CHO-K1 cells were described previously (6). Specific activities are averages of duplicate assays and varied <10% between duplicates.

24). Mixing experiments in vitro showed that no diffusible (i.e., non-membrane-bound) inhibitor for the choline-exchange reaction was present in extracts of the mutants (data not shown).

Serine- and ethanolamine-exchange activities of the mutants also were assayed and compared with those of the parent. The specific activities of the serine-exchange reaction in both mutant 38 and mutant 64 grown at 33°C were about half that of the parent (Table 1). However, in contrast to the choline-exchange activity, there was no significant difference in

**FIG. 2.** Thermal stability of choline- and serine-exchange activities in cell extracts prepared from mutants and parent (CHO-K1). Cell extracts were prepared as described (6) from cells grown at 33°C in medium containing 10% (vol/vol) newborn calf serum. The extracts were incubated at the indicated temperatures for 5 min, and then the enzyme activities were assayed at 33°C as described in the legend to Table 1. The results are expressed as the percentage of activity relative to the specific activity of each strain without preincubation. The averages of duplicate determinations, which varied by less than 10%, are shown for choline-exchange activity (A) and serine-exchange activity (B) in extracts of CHO-K1 (●), mutant 38 (△), and mutant 64 (○).
the thermal stability of the serine-exchange activity between the mutants and the parent, both in intact cells (Table 1) and in cell extracts (Fig. 2B). With respect to the level of ethanolamine-exchange activity, mutant 38 and mutant 64 exhibited almost the same extent of loss as in the case of the serine-exchange activity when compared with the parent (Table 1) and also showed similar thermal stability to the parent in intact cells (Table 1).

Temperature-Sensitive Growth of Mutant 64. Mutant 64 grew more slowly than the parent in the growth medium containing 10% newborn calf serum at 40°C, doubling every 22 hr as opposed to 16 hr (Fig. 3B), whereas the growth rates of mutant 64 and parental cells were quite similar at 33°C (Fig. 3A). Furthermore, the growth of mutant 64 was found to be strikingly temperature-sensitive in the medium supplemented with 2% lipoprotein-deficient serum (see the Methods). When cells cultured in this medium were shifted from 33°C to 40°C, mutant 64 divided only two or three times and then stopped growing (Fig. 3C). Mutant 38 could grow normally at both temperatures in the growth medium supplemented with either 10% newborn calf serum or 2% lipoprotein-deficient serum and was not further characterized.

Defective Phosphatidylycerine Synthesis in Mutant 64 at 40°C. To determine the effects of the reduced choline-, serine-, and ethanolamine-exchange activities (Table 1) on phospholipid metabolism in mutant 64, we compared the phospholipid compositions of mutant 64 and the parent. A long-term [32P]Pi-labeling experiment revealed that there was no significant difference in phospholipid composition between the mutant and the parent at 33°C (Table 2). However, the phosphatidylserine content of the mutant was about half that of the parent at 40°C (Table 2). In addition, the mutant contained approximately 30% less phosphatidylethanolamine than the parent did at 40°C (Table 2), suggesting that some part of the phosphatidylethanolamine is derived from phosphatidylycerine (see Discussion). The levels of phosphatidylcholine and other phospholipids, such as phosphatidylinositol and sphingomyelin, in the mutant were not grossly affected at 40°C (Table 2). Similar results were obtained when phospholipid compositions were determined by chemical quantitation of phosphorus (data not shown).

Cells were plated at approximately 5 × 10⁴ cells per 100-mm-diameter dish and incubated for 6 days at 33°C or 3 days at 40°C in medium with 10% (vol/vol) newborn calf serum and [32P]Pi (2 μCi/ml). Cells were harvested with a rubber policeman, washed with phosphate-buffered saline, and extracted by the method of Bligh and Dyer (13). The phospholipids were analyzed by two-dimensional thin-layer chromatography on 250-μm Silica Gel 60 plates (Merck), with chloroform/methanol/acetic acid (65:25:10, vol/vol) as the first solvent and chloroform/methanol/formic acid (65:25:10, vol/vol) as the second solvent (14). After thin-layer chromatography, the spots were visualized by autoradiography. To quantitate the radioactivity in each spot, the silica gel was scraped off and analyzed by liquid scintillation spectrometry. Average data are shown for at least two determinations; deviations from the mean are <10%.

We also investigated the kinetics of [32P] incorporation into individual phospholipids in intact cells. Mutant 64 incorporated 65–80% and 30–50% less [32P]P into phosphatidylethanolamine (Fig. 4B) and phosphatidylethanolamine (data not shown), respectively, relative to the parent at 40°C but was comparable to the parent in incorporation of [32P]P into phosphatidylcholine (Fig. 4E). At 33°C, there was no difference between the mutant and parent in the incorporation of [32P]P into phosphatidylserine (Fig. 4A), phosphatidylethanolamine (data not shown), and phosphatidylcholine (Fig. 4D).

Effect of Exogenous Phosphatidylserine on Growth of Mutant 64. Because CHO cells are able to utilize large amounts of exogenous phosphatidylcholine for membrane biogenesis when endogenous synthesis is impaired (15), we examined the effect of phosphatidylserine added as a dispersion to the medium on the growth of mutant 64 at 40°C. As shown in Fig. 3B, the addition of 50 μM phosphatidylserine liposomes to the growth medium supplemented with 10% newborn calf serum increased the growth rate of mutant 64 to the same level as that of the parent at 40°C, whereas the growth rate of parental cells was not affected by exogenous phosphatidylserine. Similar results were obtained when colony formation was examined at 40°C; namely, colonies of mutant 64 in the growth medium supplemented with phosphatidylserine were bigger than those in the same medium lacking phosphatidylserine. These findings suggested that the defect in the biosynthesis of phosphatidylserine in mutant 64 was responsible for the temperature-sensitive growth of the mutant. In medium supplemented with 2% lipoprotein-deficient serum, exogenous phosphatidylserine at 5–10 μM slightly enhanced the growth of mutant 64 at 40°C; however, phosphatidylserine at >30 μM was very toxic even to parental cells under these conditions (data not shown).

Isolation and Characterization of Temperature-Resistant Revertants of Mutant 64. To examine whether the defects of mutant 64 in growth, phosphatidylserine biosynthesis, and the choline-exchange enzyme at 40°C were due to a single

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**Table 2.** Phospholipid compositions of mutant 64, temperature-resistant revertant, and CHO-K1 cells.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Temp. (°C)</th>
<th>% of total phospholipid*</th>
</tr>
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<tbody>
<tr>
<td>CHO-K1</td>
<td>33</td>
<td>6.4 47.9 21.9 7.1 9.8 6.8</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5.8 55.7 17.0 7.7 9.2 4.7</td>
</tr>
<tr>
<td>64</td>
<td>33</td>
<td>6.0 50.6 20.0 7.6 9.0 6.7</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>2.7 62.7 11.4 9.4 8.1 5.6</td>
</tr>
<tr>
<td>R-32</td>
<td>40</td>
<td>5.8 53.3 18.4 8.6 8.7 5.4</td>
</tr>
<tr>
<td>R-33</td>
<td>40</td>
<td>6.0 51.7 18.9 9.2 9.2 5.1</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Growth of mutant 64 (c, d) and CHO-K1 (e, i) cells. Approximately 5 × 10⁴ cells were seeded per 60-mm-diameter dish and then incubated under the various conditions described below. At the times indicated, the cells were dispersed with trypsin and counted with a Coulter Model ZBI Counter. (A) Growth at 33°C in medium supplemented with 10% (vol/vol) newborn calf serum. (B) Growth at 40°C in the same medium in the absence (c, d) or presence (c, e) of 50 μM phosphatidylserine, added as liposomes prepared in the following way: Phosphatidylserine in chloroform/methanol (95:5) was dried in a glass tube under a stream of nitrogen, resuspended to 5 mM in phosphate-buffered saline (pH 7.5), sonicated for 2 min at room temperature and 30 min at 4°C in a 100-W ultrasonic disrupter (Heat System/Ultrasonics, Plainview, NY) equipped with a No. 419 microtip, and then sterilized with a 0.22-μm-pore Millipore filter. To measure the concentration of phosphatidylserine, phosphate was determined as described previously (6). The recovery of phosphatidylserine after the filtration was typically ≥90%. (C) Growth in medium supplemented with 2% (vol/vol) lipoprotein-deficient newborn calf serum. Cells were incubated at 33°C (c, d), and after 40 hr, one set of cultures was shifted to 40°C (c, i).

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mutation, we isolated spontaneous temperature-resistant revertants. Mutant 64 cells (2 × 10^6), repurified just before the experiment, were plated at 10^5 cells per 100-mm-diameter dish containing medium supplemented with lipoprotein-deficient newborn calf serum. After incubation at 40°C for 10 days, six colonies as large as parental cell colonies were found. Mutagen-treatment of mutant 64 increased the reversion frequency 10- to 20-fold (data not shown). Four of the spontaneous revertants were repurified and employed for the following biochemical analyses.

All of the purified revertants grew normally at 40°C (data not shown). The rates of synthesis of phosphatidylserine in the four revertants were examined in a pulse-labeling study with [32P]Pi, as described above, and it was found that all of them exhibited the normal level of phosphatidylserine synthesis at 40°C; the results of the experiment with the two representative strains designated R-32 and R-33 are shown in Fig. 4C. The phospholipid compositions of strains R-32 and R-33 were also normal (Table 2) at 40°C. Furthermore, enzymatic analyses revealed that the choline-exchange activities of the two revertants (R-32 and R-33) were almost the same as that of parental cells (Table 1). The serine- and ethanolamine-exchange activities of the two strains were also at nearly normal levels (Table 1). The other two revertants showed choline-exchange reaction activity intermediate between those of mutant 64 and strain R-33 (data not shown).

**DISCUSSION**

In bacteria and yeasts, phosphatidylserine is formed by the reaction of L-serine with CDP-diacylglycerol catalyzed by phosphatidylserine synthase (16–19); however, such an enzyme is not found in mammalian cells, and the molecular mechanism of phosphatidylserine synthesis in these cells is not well understood. In the present study, mutant 64 was isolated as a variant clone with a lesion in the choline-exchange reaction by using an *in situ* enzyme assay (Fig. 1) and shown to be defective in the biosynthesis of phosphatidylserine (Fig. 4B). Our results obtained on biochemical analyses of the mutant and its temperature-resistant revertants suggest that a single mutation can account for the temperature-sensitive growth (Fig. 3), the conditional lesion of phosphatidylserine synthesis (Fig. 4), and the striking reduction in choline-exchange activity (Table 1) of mutant 64. Because the residual choline-exchange activity of mutant 64 is more thermolabile in cell extracts (Fig. 2), it is likely that the mutation is in the structural gene for the choline-exchange enzyme, although other possibilities such as a defect in post-translational modification have not been excluded. These results provide direct genetic evidence that the choline-exchange enzyme is responsible for the formation of phosphatidylserine and essential for cell growth in CHO-K1 cells. These possibilities are also supported by the fact that the temperature-sensitivity of growth of mutant 64 is suppressed by homogenous phosphatidylserine (Fig. 3B).

How does the choline-exchange enzyme contribute to the biosynthesis of phosphatidylserine in CHO cells? As shown in Table 1, in addition to the striking reduction in choline-exchange activity, mutant 38 and mutant 64 are also about 50% defective in both serine- and ethanolamine-exchange activities. Furthermore, the temperature-resistant revertants of mutant 64 all regained choline- and serine- and ethanolamine-exchange activities similar to those of parental cells (Table 1). These results suggest that there are at least two or three kinds of base-exchange enzymes in CHO-K1 cells; one of the enzymes, in which mutants 38 and 64 are defective, can catalyze the base-exchanges of phospholipids with choline, serine, and ethanolamine, and the other enzymes(s), which is normal in the two mutants, uses specifically serine and/or ethanolamine as a substrate. Because mutant 64 lacking the choline-exchange enzyme is defective in the biosynthesis of phosphatidylserine but normal in that of phosphatidylinositol at 40°C (Fig. 4), it is very likely that the actual role of the choline-exchange enzyme in intact cells is to synthesize phosphatidylserine by catalyzing the base-exchange of phospholipids with serine and that the choline-exchange reaction observed in cell extracts is not significant in terms of total phosphatidylcholine biosynthesis in CHO-K1 cells.

We previously isolated a CHO cell mutant (designated 89.1) with altered phosphatidylcholine metabolism (6). This mutant, which had been screened and isolated as a variant with a lesion in the serine-exchange reaction, was shown to be 65–75% and >90% defective in choline kinase and choline-exchange activities, respectively, in addition to having only about half the parental serine-exchange activity (6). Mutant 89.1 has a normal phosphatidylserine content (6), so the residual activity of the choline-exchange reaction in mutant 89.1 is probably sufficient for phosphatidylserine biosynthesis in these cells. We suggested that the partial defect in the serine-exchange activity in mutant 89.1 was due to a decrease in acceptor phospholipid(s) for the reaction. However, it is now clear that the choline-exchange reaction and a part of the serine-exchange reaction, both of which are defective in mutant 64 and probably also in mutant 89.1, are catalyzed by the same enzyme in CHO-K1 cells. Unlike mutant 89.1, mutant 64 has a normal level of choline kinase (unpublished data), suggesting that the site of mutation in mutant 64 is different from that in mutant 89.1.

As described above, more than one enzyme appears to be responsible for the serine-exchange activity in extracts of CHO-K1 cells. Since it has been suggested that phosphatidyl-
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ethanolamine (9, 20) and phosphatidylcholine (6, 10, 20, 21) can serve as precursors for phosphatidylserine biosynthesis, each of the serine-exchange enzymes may have a different specificity for acceptor phospholipid and, therefore, may have different physiological roles. Isolation of mutants defective in the base-exchange enzyme specific for serine would help to clarify these points.

Recently, Voelker (22) has shown that most of the cellular phosphatidylethanolamine is derived from phosphatidylserine in BHK-21 (baby hamster kidney) and CHO-K1 cells. Our observation that phosphatidylethanolamine synthesis is significantly inhibited in mutant 64 (Table 2) is consistent with his findings. Mutant 64 will facilitate studies on the mechanisms of phosphatidylserine and phosphatidylethanolamine biosyntheses and, furthermore, on the physiological functions of phosphatidylserine in mammalian cell membranes.

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