Serinc, an Activity-regulated Protein Family, Incorporates Serine into Membrane Lipid Synthesis*

Madoka Inuzuka, Minako Hayakawa, and Tatsuya Ingi

From the Department of Neurophysiology, Brain Research Institute, Niigata University, 1 Asahi-machi, Niigata, 951-8585, Japan

Cell membranes contain various transporter proteins, some of which are responsible for transferring amino acids across membrane. In this study, we report another class of carrier proteins, termed Serinc1–5, that incorporates a polar amino acid serine into membranes and facilitates the synthesis of two serine-derived lipids, phosphatidylserine and sphingolipids. Serinc is a unique protein family that shows no amino acid homology to other proteins but is highly conserved among eukaryotes. The members contain 11 transmembrane domains, and rat Serinc1 protein co-localizes with lipid biosynthetic enzymes in endoplasmic reticulum membranes. A Serinc protein forms an intracellular complex with key enzymes involved in serine and sphingolipid biosyntheses, and both functions, serine synthesis and membrane incorporation, are linked to each other. In the rat brain, expression of Serinc1 and Serinc2 mRNA was rapidly up-regulated by kainate-induced seizures in neuronal cell layers of the hippocampus. In contrast, myelin throughout the brain is enriched with Serinc5, which was down-regulated in the hippocampus by seizures. These results indicate a novel mechanism linking neural activity and lipid biosynthesis.

Rapid macromolecular synthesis is a necessary component underlying long-term neuronal plasticity (1). In an attempt to identify molecules that are involved in this process, we, as well as others, have used differential cloning strategies to identify genes that are rapidly induced in neurons of the hippocampus by excitatory neuronal activity (2, 3). The set of genes identified includes transcription factors such as c-fos, c-jun, and zf268 (4), as well as effector molecules that may directly modify cellular and synaptic functions. Examples of this latter class of proteins include the inducible form of regulators of G-protein signaling (RGS2) and a key regulatory enzyme of polyamine metabolism (spermidine/spermine N\(^{\text{1}}\)-acyltransferase) (3, 5). These proteins are rapidly induced in neurons and interact with constitutively expressed cellular and synaptic proteins to modify neuronal properties. In the present study, a novel activity-regulated protein family has been identified that serves as an effector molecule that facilitates the biosynthesis of lipids, a major macromolecular component of the nervous system. This effector protein localizes in the endoplasmic reticulum (ER)\(^{2}\) membranes, where most of the membranes including the plasma membrane, are synthesized. Most of lipid biosynthetic enzymes are also embedded in and associated with ER membranes. Because the action of the protein is to incorporate serine into newly forming membrane lipids, it is different from a transporter that transfers amino acids across membranes.

Serine is a nonessential amino acid that can be synthesized by many cells. In the cell, serine serves as a building block for the synthesis of two major classes of membrane lipids, phosphatidylserine and sphingolipids. Although it is well known that cell membranes efficiently incorporate serine into their lipid bilayer despite the hydrophilicity, the molecular mechanism remained unclear. This study has demonstrated for the first time that a protein family having 11 transmembrane domains facilitates the incorporation of serine into both phosphatidylserine and sphingolipids. This is a function novel to proteins and is henceforth referred to as Serinc (serine incorporator). Because the Serinc family showed no amino acid homology to any known proteins, nothing was known about their function, although there have been several studies on this family. First, the predicted membrane topology has 11 transmembrane segments similar to amino acid transporters. Therefore, a recent report classified TMS1, a yeast member of the Serinc family, as an “uncharacterized (orphan) transporter” (6). Secondly, two mouse homolog genes for yeast TMS1, denoted as mouse TMS-1 and TMS-2 (corresponding to Serinc3 and Serinc1, respectively), were identified by another research group, and their neuronal expression and membrane localization were shown experimentally (7). That group also performed experiments to detect amino acid transport activity using TMS1 homolog-expressing cells, but all attempts were unsuccessful (7). Thirdly, some of the family members were reported to be differentially expressed in some environments; TDE1 (corresponding to Serinc3) expression was increased in both human lung tumors and mouse testicular tumors (8, 9); and TPO1 (corresponding to Serinc5) expression was developmentally induced in a terminal differentiation stage of cultured oligodendrocytes (10). These findings implied the important role of this family in multiple aspects of cell physiology, but the function of this family remained unknown.

In this study, yeast proteomic data and serine-incorporating experiments using *Escherichia coli* offered the key to an understanding of the function of Serinc in the central nervous system. The background is that serine is universally used for the synthesis of membrane lipids in a broad range of organisms. Phosphatidylserine is present in both prokaryotic and eukaryotic cells, but it is formed in different manners among diverse species. In *E. coli* and yeast, phosphatidylserine syntheses catalyze the formation of phosphatidylserine from serine and CDP-diacylglycerol (11, 12). In mammalian cells, phosphatidylserine is synthesized by a calcium-dependent base exchange reaction in which the head group of a preexisting phospholipid is replaced by serine (13). This serine base exchange enzyme (recently called mammalian phosphatidylserine synthase) is the sole mechanism available for phosphatidylserine formation by mammalian cells. On the other hand, sphingolipids are not found in most prokaryotes but are distributed in all eukaryotic cells. In various eukaryotic species, the first and committed step in *de novo* sphingolipid synthesis is catalyzed by serine palmitoyltransferase, which condenses...
serine and palmitoyl-CoA to form 3-ketodihydrophosphinosine in a pyridoxal 5'-phosphate-dependent reaction (14).

Sphingolipid molecules (particularly sphingomyelin and glycolipids) are found predominantly in the noncytoplasmic half of membranes, where they self-assemble into multimolecular clusters known as lipid rafts, which function as platforms for the attachment of proteins when selected membranes are transported by some routes of intracellular membrane trafficking (15). An increasing number of recent studies indicates that lipid rafts are important mediators in cell biology (16–18). Here we demonstrate that three Serinc proteins specifically enhance the synthesis of phosphatidylethanolamine and sphingolipids. Because sphingolipids are major components of lipid rafts, Serinc is likely to function as a lipid raft maker in activity-dependent neural plasticity and oncogenic transformation.

MATERIALS AND METHODS

Animals and Differential Screening of cDNA Library—Adult male rats (Sprague-Dawley) were used in studies of Serinc regulation. Kainate-stimulated rats as described previously (3). The reaction mixture contained 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM KH2PO4, 1.8 mM CaCl2, 0.4 mM MgCl2, and 10 mM glucose. After washing three times with ice-cold phosphate-buffered saline, cells were lysed by adding 0.1 ml of 10% SDS. Radioactivity was analyzed by liquid scintillation counting using ReadyProtein®.

Base Exchange Enzyme Activity and E. coli Serine-incorporating Activity—The base exchange reaction mixture contained 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 15 mM CaCl2, 0.5 mM [3H]serine (60 μCi), and the indicated amount of cell lysates in a total volume of 0.35 ml. After incubation at 37 °C for 20 min, the reaction was terminated by adding 1 ml of chloroform/methanol (1/2, v/v), and phospholipids were extracted by the method of Bligh and Dyer (21). In screening assays of serine-incorporating activity using Serinc1-expressing E. coli, 1.4 mg of E. coli total lysates was incubated for 20 min at 37 °C with 30 μCi of [3H]serine in the same solution or with 8 μCi of [14C]glucose (0.3 Ci/mmol) omitting serine.

Phosphatidylethanolamine Synthase Activity of E. coli Membranes—A 0.25-ml reaction mixture contained 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM MgCl2, 0.5 mM [3H]serine, 0.2 mM CDP-diacylglycerol, and 3 mM ATP. The reaction was initiated by adding 0.45 mg of membrane protein, and after 20 min at 37 °C, it was terminated by the addition of 1.5 ml of chloroform/methanol (1/2).

Serine Palmitoyltransferase Activity—The reaction mixture contained 0.1 mM HEPEs, pH 8.3, 5 mM dithiothreitol, 2.5 mM EDTA, 50 μM pyridoxal 5'-phosphate, 0.2 mM palmitoyl-CoA, 1 mM [3H]serine (60 μCi), and the indicated amount of membrane proteins or cell lysates in a total volume of 0.3 ml. After incubation at 37 °C for 10 min, the reaction was terminated by the addition of 1.5 ml of chloroform/methanol (1/2). After 25 μg of sphinganine was added as carrier, the lipid products were extracted by the method described previously (22).
A Novel Family of Serine Incorporators

**FIGURE 1. Regulation of Serinc1, Serinc2, and Serinc5 mRNA in the rat brain by seizure.** In situ hybridization of the three Serinc subtypes in rat brain coronal sections is shown. The brains are composites of half-brains from a naive control rat (C-side) and a rat that received the kainate-induced seizure stimulation 3 h before being killed (S-side). The three Serinc mRNAs individually show unique neural localization throughout the brain. Identical hybridization patterns were detected in independent experiments (n = 5–6). arrow, arbor vitae of the cerebellum; cc, corpus callosum; CA, hippocampal CA fields; dg, dentate gyrus; ec, external capsule; gcl, cerebellar granule cell layer; hip, hippocampus; neo, neocortex; pcl, cerebellar Purkinje cell layer.

Thin-layer Chromatography and Fluorography—Lipid extracts from assays were dried under \( \text{N}_2 \), redissolved in chloroform/methanol (2/1), and spotted on a silica gel 60 HPTLC plate (Merck, Darmstadt, Germany). In assays of phosphatidylserine synthesis, the plates were developed with chloroform/methanol/acetic acid/formic acid (50/30/4.5/6.5 or 65/25/10/0). In assays of serine palmitoyltransferase, the plates were developed radioactive bands, the plates were treated with 2-methylnaphthalene containing 0.4% 2,5-diphenyloxazole and exposed to X-O-MAT film at \(-80^\circ\text{C}\) for 1–4 days. To quantitate the radioactivity in each band, the silica gel was scraped off and analyzed by liquid scintillation counting using Econofluor.

**RESULTS**

Expression and Activity-dependent Induction of Serinc1 mRNA in the Hippocampus—Adult rats were injected subcutaneously with the glutamate analogue kainate (8 mg/kg of body weight), which induce seizures and long-term synaptic enhancement in the brain (2, 5), and then sacrificed 3 h after kainate-induced seizure, Serinc1 mRNA is significantly induced in neuronal cell layers of the hippocampal CA1–3 fields (Fig. 1, left panels, arrow). Thus, following the kainate-induced seizures, Serinc1 mRNA levels increase rapidly in discrete brain regions.

The Serinc Family of Various Eukaryotic Species Exhibit Transmembrane Domains—Serinc1 mRNA encodes a protein of 453 amino acids (Fig. 2A) with 98% identity to mouse TMS-2 protein. This result, together with the identical neuronal localization, suggests that they are counterparts. Two other reported proteins, human TDE1 and rat TPO1, show homology to Serinc1 with amino acid identities of 58 and 38%, henceforth referred to as Serinc3 and Serinc5, respectively (Fig. 2A). Moreover, four novel mammalian family proteins and five Arabidopsis thaliana family proteins were identified by an NCBI data base search and were termed Serinc2, Serinc3B, Serinc4, Serinc4B, and A. thaliana Serinc 1–V, respectively (Fig. 2). Thus, the Serinc family is highly conserved among eukaryotes, whereas no homologs were found in prokaryotic data bases. The mammalian family members share 58–31% amino acid identity (Fig. 2C) and have similar molecular masses (Fig. 2A, 432–473 amino acids). Furthermore, hydrophathy analysis revealed that these protein members contain 58–53% hydrophobic amino acids, clustered into 11 regions of up to 30 amino acids long, suggesting membrane-spanning domains (Fig. 2A, I–XI regions). However, none of the protein functions were known.

Comparative Localization and Regulation of Serinc2 and Serinc5 mRNA in Brain—We further examined the mRNA distribution of Serinc2 and Serinc5 in control and seizure-treated rat brains (Fig. 1). Overall levels of Serinc2 hybridization are less than either Serinc1 or Serinc5, although intense staining was apparent in the dentate gyrus of the hippocampus and the cerebellar Purkinje cell layer. Seizures strongly induced Serinc2 mRNA, which is restricted to the dentate gyrus (Fig. 1, center panels, arrow). Serinc5 is distinct from Serinc1 and Serinc2 in the dense staining pattern of the white matter such as the external capsule, corpus callosum, and arbor vitae of the cerebellum. Moreover, in contrast to the up-regulation pattern of Serinc1 and Serinc2, expression of Serinc5 is down-regulated in the hippocampal CA fields and the dentate gyrus by seizures (Fig. 1, right panels, arrow). Thus, the divergent spatial and cellular expression and their different transcriptional controls indicate that the three subtype proteins play multiple distinct roles in the activity-dependent plasticity of the central nervous system.

Serinc Protein and Serine Synthetic Enzymes Form an Intracellular Protein Complex—The clue to elucidate the Serinc function came from its interacting proteins. We searched the yeast proteome data base and identified two yeast proteins (SER3 and YGP1) that directly interact with yeast TMS1 (24) (Fig. 3A). SER3 and YGP1 function in the biosynthesis of serine (Fig. 3A). Serine is synthesized from 3-phosphoglycerate, an intermediate in glycolysis. The first and committed step in this pathway is oxidation to 3-phosphohydroxypyruvate, catalyzed by the enzyme 3-phosphoglycerate dehydrogenase (SER3). This \( \alpha \)-ketoacid is transaminated to 3-phosphoserine, which is then hydrolyzed to serine by a phosphatase. YGP1 is a glycoprotein of unknown function (25) but has a highly conserved domain of asparaginase. The putative asparaginase activity accelerates the serine synthetic pathway through the subsequent transamination reaction to glutamate. These findings suggest the involvement of TMS1 in cellular synthesis of serine. To explore whether mammalian Serinc proteins bind to these serine synthetic enzymes, we examined the cellular distribution of Serinc3 mRNA in control and seizure-treated rat brains by performing in situ hybridization. Expression was examined in the half-brains of naive rats (Fig. 1, C-side) and compared with expression in rats that had received kainate-induced seizures and were then sacrificed 3 h later (S-side). Serinc3 mRNA is expressed in the cerebral cortex, hippocampus, and cerebellar granule cell layer (Fig. 1, left panels, C-side), which is closely related to the localization of glutamatergic excitatory neurons (23). This result agrees with a recent report detailing the neuronal localization of mouse TMS-2 mRNA using in situ hybridization (7). Within 3 h after kainate-induced seizure, Serinc1 mRNA is significantly induced in neuronal cell layers of the hippocampal CA1–3 fields (Fig. 1, left panels, arrow). Thus, following the kainate-induced seizures, Serinc1 mRNA levels increase rapidly in discrete brain regions.
enzymes, cDNAs of SER3, YGP1, and their associated proteins were screened by two-hybrid methods using rat Serinc1 as bait (Fig. 3B). Both SER3 and YGP1 were identified as strong interacting partners of rat Serinc1, and PHO12 was identified as a weak interactor. The result indicates that the specific binding to serine synthetic enzymes is conserved among distant members of the Serinc family.

Serinc1 Specifically Enhances the Incorporation of Serine Molecules into the Membrane Lipid, Phosphatidylserine—To investigate the effect of Serinc proteins on cellular serine, we expressed rat Serinc1 in E. coli and mammalian COS cells, first monitoring their cellular and vesicular uptake of [3H]serine. [3H]Serine uptake into Serinc1-expressing E. coli cells was 102% of the base rate of control cells (42 pmol/min/mg of protein), and [3H]serine uptake into Serinc1-expressing COS cells was 99% of the base rate of control cells (530 pmol/min/mg of protein). Thus, the serine transport across these membranes was unaffected by Serinc1, which supports the idea that Serinc is not an amino acid transporter. However, when the E. coli lysate was incubated with [3H]serine, we found that expression of Serinc1 remarkably enhanced the incorporation of [3H]serine into its chloroform-soluble fraction. Examination of the radiolabeled products by thin-layer chromatography revealed that the synthesis of phosphatidylethanolamine increased by more than 10-fold over the base level (Fig. 4B, middle panel, left two lanes). In E. coli cells, serine is incorporated into phosphatidylserine by the catalytic action of phosphatidylserine synthase (Fig. 4A). But phosphatidylserine is found only in trace amounts in the cell because it is rapidly converted to phosphatidylethanolamine, which constitutes about 70% of the phospholipid of this organism (Fig. 4B, left panel), by the presence of phosphatidylserine decarboxylase (26) (Fig. 4A). Therefore, this finding indicates that cellular expression of Serinc1 enhances phosphatidylserine synthesis from [3H]serine. Similarly, when [14C]glucose was incubated with the E. coli lysate, Serinc1 enhanced the [3H]phosphatidylserine synthesis to a detectable level (Fig. 4B, right panel).
A Novel Family of Serine Incorporators

To verify further that Serinc1 enhances phosphatidylserine biosynthesis, the conversion of phosphatidylserine to phosphatidylethanolamine was blocked by utilizing the decarboxylase inhibitor hydroxylamine \((\text{NH}_4\text{OCl})\) (11) (Fig. 4B, right panel, 3rd and 6th lanes). Although Serinc1-dependent enhancements of phosphatidylethanolamine completely disappeared in the presence of 10 mM hydroxylamine, \([^{3}H]\)phosphatidylethanolamine accumulated further (Fig. 4B, right panel, 3rd lane) through the action of Serinc1. The imbalance between levels of \([^{3}H]\)phosphatidylethanolamine accumulation and \([^{3}H]\)phosphatidylserine disappearance reflected a cellular feedback regulatory mechanism that reduces the rate of \([^{3}H]\)phosphatidylserine synthesis compared with the amount of accumulation in the presence of hydroxylamine. Although other \(E.\ coli\) lipids were faintly radiolabeled by way of other serine metabolic pathways (Fig. 4B, left two panels, asterisk), these activities were unaffected by Serinc1 (right panel, asterisk), indicating the specific action on phosphatidylserine synthesis.

Membrane Localization of Serinc1 Protein and Function—Specific membrane localization of Serinc1 protein was demonstrated by immunoblotting the total, soluble, and membrane preparations from \(E.\ coli\) cells expressing GST-tagged Serinc1 (Fig. 4C). We incubated the membrane fraction of native Serinc1-expressing \(E.\ coli\) with \([^{3}H]\)serine and CDP-diacylglycerol and monitored the activity of phosphatidylserine synthase (Fig. 4D). Consistent with the result of the experiment using lysates, activity enhancement of phosphatidylserine synthase to 3.1-fold of the base level \((190 \pm 10 \text{ pmol/min/mg of protein})\) was detected in the membranes through the action of Serinc1. These results, together with the predicted topology of 11 transmembrane domains, indicate that a Serinc1 protein localizes in cell membranes, resulting in an acceleration of their phosphatidylserine synthesis rates.

Enhancement of the Phosphatidylserine and Sphingolipid Biosynthesis by Expression of Serinc1 in Mammalian and Yeast ER Membranes—Because Serinc family proteins are found in various eukaryotes, the cellular function was re-investigated with experiments using cultured mammalian COS cells. COS cells were transfected with green fluorescent protein (GFP)-tagged and native Serinc1 plasmids to examine the subcellular distribution and function in mammalian cells. Serinc1-GFP, distributed in a reticular pattern localized around the nuclear envelope, was identical to the intracellular distribution of ER membranes labeled with the dye ER-Tracker (Fig. 5A, left two panels). This result indicates the specific localization of Serinc1 in the mammalian ER membranes, where most of the membranes in a eukaryotic cell are synthesized. The activity of the serine base exchange enzyme was measured in lysates of Serinc1-expressing COS cells (Fig. 5B). Consistent with the results of experiments using \(E.\ coli\), cellular expression of Serinc1 enhanced the synthesis of \([^{3}H]\)phosphatidylserine to 2.8-fold of the base level \((2.5 \pm 0.3 \text{ pmol/min/mg of protein})\). Because the structure and catalytic reaction of a serine base exchange enzyme are completely different from those of an \(E.\ coli\) phosphatidylserine synthase, these results suggest that the function of Serinc1 is to provide serine molecules not for a particular enzyme but for some distinct enzymes in the membranes.

**FIGURE 3.** Specific binding of Serinc proteins to intracellular serine synthetic enzymes. A, functional interaction between the two TMS1-binding proteins, SER3 and YGP1, and their associated proteins with rat Serinc1 as a bait protein. The binding strengths of Serinc1 to SER3 and YGP1 are specific and stronger than to PHO12. PHO12 is the secreted acid phosphatase that directly binds to SER3. The DNA-binding domain vector pCBBD2 and the Gal4 activation domain vector pOAD (20) were used. The screening was performed by transforming a plasmid of pCBBD2-rat serinc1 into a yeast reporter strain PJ69-4a expressing the following Saccharomyces cerevisiae proteins as Gal4 activation domain fusions: Control (no protein), SER3, YGP1, and other proteins. In SER3, YGP1, and PHO12, colonies were detected after 3 weeks of growth on medium lacking tryptophan, leucine, and histidine and supplemented with 3 mM 3-amino-1,2,4-triazole, thus allowing growth only of cells that express the his3 two-hybrid reporter gene. No colony was detected in the control well. To demonstrate the growth rates, the same numbers of positive colonies were picked and replated onto the selection medium; the result of their growth is shown here.

**FIGURE 4.** Serinc1-induced enhancement of \(E.\ coli\) phosphatidylserine synthesis and the membrane localization. A, the biosynthetic and metabolic pathways of phosphatidylserine in \(E.\ coli\) cells. PS, phosphatidylserine; PE, phosphatidylethanolamine; CDP-DAG, CDP-diacylglycerol. B, thin-layer chromatography of lipids extracted from Serinc1-expressing \(E.\ coli\) lysates that were labeled with \([^{3}H]\)serine and \([^{14}C]\)glucose. Control, empty vector-transfected \(E.\ coli\); Serinc1, native Serinc1-expressing \(E.\ coli\); +NH4OCl, in the presence of 10 mM hydroxylamine. 1.4 mg of lysate proteins was incubated with 30 \(\mu\text{Ci}\) of \([^{3}H]\)serine or 8 \(\mu\text{Ci}\) of \([^{14}C]\)glucose for 20 min at 37 °C, and the lipid extracts were developed on a silica gel plate with chloroform/methanol/acetic acid/formic acid (50:30:4.5:6.5). Lipid bands and their radioactive signals on the plate were visualized with iodine vapor (left panel) and fluorography (right two panels), respectively. Lipids were identified with authentic standards phosphatidylethanolamine and phosphatidylserine. The middle and right plates were exposed to x-ray film for 4 and 35 days, respectively. The levels of radioactivity in each band were quantitated using a scintillation counter. C, Western blot analysis of total, soluble, and membrane fraction of GST-Serinc1-expressing \(E.\ coli\) probed with anti-GST antibodies. Each lane contained 0.4 \(\mu\text{g}\) of total cell proteins or their fractions. The GST-tagged Serinc1 (78 kDa) was detected exclusively in the membrane fraction and not in the soluble one. D, Serinc1-dependent activity enhancement of phosphatidylserine synthase in \(E.\ coli\) membrane fractions. 0.45 \(\mu\text{g}\) of native Serinc1-expressing \(E.\ coli\) membranes was incubated with 0.5 \(\mu\text{mol}\) \([^{3}H]\)serine and 0.2 \(\mu\text{mol}\) CDP-diacylglycerol at 37 °C for 20 min, and the lipid extracts were analyzed by thin-layer chromatography.
A Novel Family of Serine Incorporators

In mammals, another class of membrane lipid, the sphingolipids, is formed from serine by the action of serine palmitoyltransferase. This enzyme catalyzes the condensation of serine and palmitoyl-CoA to 3-ketodihydrosphingosine and is the most important control element in the sphingolipid biosynthetic pathway. As shown in Fig. 5C, expression of Serinc1 in COS cells enhanced the incorporation of [3H]serine into their respective phosphatidylserine and phosphatidylethanolamine. The wild-type strain of BY4743 (EUROSCARF accession number Y20000) and the second mechanism involves Serinc acting as a scaffold for serine synthetic enzymes on the cytoplasmic face of the ER (Fig. 6C).

Model of the Serinc Complex in Membranes—Serinc protein is likely to function by the following two mechanisms (Fig. 7). The first mechanism involves Serinc to act as a scaffold for serine synthetic enzymes on the cytoplasmic face of the ER (Fig. 6C).

Serine-incorporating Activities of Serinc2 and Serinc5—To investigate the cellular function of other Serinc subtypes, we expressed rat Serinc2 and Serinc5 in both E. coli and COS cells and monitored the incorporation of [3H]serine into their respective phosphatidylserine and mammalian 3-ketodihydrosphingosine. In a manner similar to that of Serinc1, expression of Serinc2 and Serinc5 caused more than a 10-fold increases in E. coli phosphatidylserine synthase activity (Fig. 6A) and mammalian cells, Serinc2 and Serinc5 significantly enhanced the activities of both serine base exchange enzyme (Fig. 6B) and serine palmitoyltransferase (Fig. 6C).
amphipathic molecule in membrane lipids, a polar head group has to bind to two hydrophobic hydrocarbon tails. However, polar materials such as serine neither dissolve well nor diffuse in the layers of hydrophobic hydrocarbon tails. Because the membrane topology of Serinc contains 11 transmembrane segments similar to amino acid transporters, Serinc may function by carrying a serine molecule into the hydrophobic milieu of membrane lipid bilayers, which is responsible for efficient reactions occurring among serine, hydrocarbon tails, and lipid synthetic enzymes.

**DISCUSSION**

The present study suggests that Serinc proteins play a pivotal role in the regulation of lipid biosynthesis. Eukaryotic ER membranes were enriched with Serinc protein, where it bound directly to the key enzyme of sphingolipid biosynthesis, serine palmitoyltransferase. Moreover, Serinc directly interacted with an intracellular serine-synthesizing protein complex that included 3-phosphoglycerate dehydrogenase. Thus, synthetic activities of serine and serine-derived lipids were bridged by an intermediary protein, Serinc, and both activities were linked to each other. Because the 3-ketodihydrosphingosine synthesis is the first committed step in sphingolipid biosynthesis, Serinc is thought to function by increasing the cellular sphingolipid content.

One of the notable features of Serinc localization is that the highest concentration of Serinc5 is found in white matter, and the distribution patterns of Serinc5 and white matter are virtually identical throughout the brain. The predominant element of white matter is the myelin sheath, which is characterized by a high proportion of lipid (80% of the total dry weight). Myelin membranes contain strikingly large amounts of glycolipids such as galactocerebrosides, and in this respect, the lipid composition of white matter (glycolipid is 27% of the total lipid) is significantly different from that of gray matter (7%). Therefore, this observation suggests that a major role of Serinc5 is to provide serine molecules for the formation of myelin glycosphingolipids in oligodendrocytes. Another notable feature is the localization of Serinc1 in glialmateric neurons. This observation, together with the activity-dependent mRNA induction in these neurons, suggests that the action of Serinc1 may be coupled with synaptic glutamate transmission, because a transamination reaction from a glutamate molecule to 3-phosphoserine enhances the biosynthesis of serine (Fig. 3A).

Mammalian Serinc subtypes may be divided into two main groups based on structural similarity; one group consists of subtypes 1, 2, 3, and 3B, which share more than 42% amino acid identity (Fig. 2C, bold numbers), whereas subtypes 4, 4B and 5 belong to the other group. Between the two groups, neural localization and regulation of the mRNAs are different. Although Serinc5 is expressed prominently in oligodendroglial cells throughout the brain, the high concentrations of Serinc1 and Serinc2 are seen in neuronal cell populations of the hippocampus and cerebellar cortex, which is supported by a recent report indicating neuronal localization of mouse Serinc1 (TMS-2) and Serinc3 (TMS-1) (7). Moreover, in the hippocampus, the seizure up-regulated the mRNA levels of Serinc1 and Serinc2, which contrasts with the down-regulated pattern of Serinc5. These changes may be a reflection of the neuronal process extension and myelin sheath degradation, respectively, which occur at the site of neural circuit reconstruction.

The phylogenetic analyses of the Serinc family and its interacting proteins provide a deeper insight into the function of Serinc protein complexes and their role in cell-specific maturation of membranes. First, 3-phosphoglycerate dehydrogenase (SER3) is found in both prokaryotic and eukaryotic cells; the structure and catalytic reaction are highly conserved throughout evolution, which reflects the fact that phosphatidylserine and phosphatidylethanolamine are utilized as major classes of membrane lipids by a broad range of organisms. Secondly, Serinc proteins are evolutionarily conserved among eukaryotes such as animals and fungi, but no counterparts are found in prokaryotes, which is consistent with the prevalence of sphingolipids in eukaryotes. Although sphingolipids are found in the membranes of all eukaryotic cells, the concentration is highest in the cells of the central nervous system. Moreover, some glycosphingolipids included in the brain, such as galactocerebrosides and gangliosides, are not found in as high quantities elsewhere. Furthermore, localization of the highest concentration of Serinc5 mRNA in the brain was identical to the distribution of the white matter, a glycosphingolipid-rich tissue. This line of co-localizations suggests that Serinc proteins had emerged to meet the particular lipid requirements of eukaryotic organisms. Abundant evidence indicates that sphingolipids form a lipid raft and play pivotal roles in the sophisticated communication among mammalian cells, such as neuronal networks, and in their pathologic state (malignant tumors).

Thirdly, YGP1 and PHO12 are yeast-specific proteins because their counterparts are not found in other species, which suggests involvement in the formation of yeast-specific sphingolipids. It is well known that mammals and yeasts produce structurally different types of sphingolipids, although the early steps of their synthetic pathway are similar (27). Although animal cells contain sphingomyelin and a large number of complex glycosphingolipids, yeasts synthesize only three types of complex sphingolipids: inositol-phosphorylceramide, mannose-inositol-P-ceramide, and mannose-(inositol-P)2-ceramide. Recent studies identified YGP1 and its homolog (SPS100) as maturation factors that induce cellular adaptation into stationary phase and facilitate spore wall formation (28). Because YGP1 is a highly glycosylated secreted protein, it may function as the mannose donor for yeast-specific sphingolipids in the spore walls. Further studies of the Serinc complex will be performed to elucidate the elementary mechanism of the neural signaling and plasticity, as well as other complicated intercellular communications.

**Acknowledgments**—We thank Stanley Fields for all of the yeast clones used in the two-hybrid assay and Katsuei Shibuki for generous support. We also thank Saeko Maruyama for technical assistance.

**REFERENCES**

1. Nguyen, P. Y., Abel, T., and Kandel, E. R. (1994) Science 265, 1104–1107.
2. Nedivi, E., Hervoni, D., Naot, D., Israeli, D., and Citri, Y. (1993) Nature 363, 718–722.
3. Ingi, T., Krumins, A. M., Chidich, P., Brothers, G. M., Chung, S., Snow, B. E., Barnes, C. A., Lanahan, A. A., Siderovski, D. P., Ross, E. M., Gilman, A. G., and Worley, P. F. (1998) J. Neurosci. 18, 7178–7188.
4. Sheng, M., and Greenberg, M. E. (1990) Neuron 4, 477–485.
5. Ingi, T., Worley, P. F., and Lanahan, A. A. (2001) Eur. J. Neurosci. 13, 1459–1463.
6. De Hertogh, B., Carvajal, E., Tall, E., Dujon, B., Raret, P., and Goffeau, A. (2002) Funct. Integr. Genomics 2, 154–170.
7. Grossman, T. R., Luque, J. M., and Nelson, N. (2000) J. Exp. Biol. 203, 447–457.
A Novel Family of Serine Incorporators

8. Bossolasco, M., Lebel, M., Lemieux, N., and Mes-Masson, A. M. (1999) Mol. Carcinog. 26, 189–200
9. Lebel, M., and Mes-Masson, A. M. (1994) DNA Seq. 5, 31–39
10. Krueger, W. H., Gonye, G. E., Madison, D. L., Murray, K. E., Kumar, M., Sporel, N., and Pfeiffer, S. E. (1997) J. Neurochem. 69, 1343–1355
11. Dowhan, W. (1992) Methods Enzymol. 209, 287–298
12. Carman, G. M., and Bae-Lee, M. (1992) Methods Enzymol. 209, 298–305
13. Kanfer, J. N. (1992) Methods Enzymol. 209, 341–348
14. Merrill, A. H., Jr., and Wang, E. (1992) Methods Enzymol. 209, 427–437
15. Simons, K., and Ikonen, E. (1997) Nature 387, 569–572
16. Tsui-Pierchala, B. A., Encinas, M., Milbrandt, J., and Johnson, E. M., Jr. (2002) Trends Neurosci. 25, 412–417
17. Guirland, C., Suzuki, S., Kojima, M., Lu, B., and Zheng, J. Q. (2004) Neuron 42, 51–62
18. Hakomori, S. I. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 225–232
19. Ingi, T., and Aoki, Y. (2002) Eur. J. Neurosci. 15, 929–936
20. Cagney, G., Uetz, P., and Fields, S. (2000) Methods Enzymol. 328, 3–14
21. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
22. Williams, R. D., Wang, E., and Merrill, A. H., Jr. (1984) Arch. Biochem. Biophys. 228, 282–291
23. Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R., and Nakanishi, S. (1991) Nature 349, 760–765
24. Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M., and Sakaki, Y. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 4569–4574
25. Destrueille, M., Holzer, H., and Klionsky, D. J. (1994) Mol. Cell. Biol. 14, 2740–2754
26. Dowhan, W., Wickner, W. T., and Kennedy, E. P. (1974) J. Biol. Chem. 249, 3079–3084
27. Dickson, R. C. (1998) Annu. Rev. Biochem. 67, 27–48
28. Law, D. T., and Segall, J. (1988) Mol. Cell. Biol. 8, 912–922
Serinc, an Activity-regulated Protein Family, Incorporates Serine into Membrane Lipid Synthesis
Madoka Inuzuka, Minako Hayakawa and Tatsuya Ingi

J. Biol. Chem. 2005, 280:35776-35783.
doi: 10.1074/jbc.M505712200 originally published online August 24, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M505712200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 28 references, 8 of which can be accessed free at
http://www.jbc.org/content/280/42/35776.full.html#ref-list-1