StarD10, a START Domain Protein Overexpressed in Breast Cancer, Functions as a Phospholipid Transfer Protein

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We originally identified StarD10 as a protein overexpressed in breast cancer that cooperates with the ErbB family of receptor tyrosine kinases in cellular transformation. StarD10 contains a steroidal acute regulatory protein (STAR/StarD1)-related lipid transfer (START) domain that is thought to mediate binding of lipids. We now provide evidence that StarD10 interacts with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) by electron spin resonance measurement. Interaction with these phospholipids was verified in a fluorescence resonance energy transfer-based assay with 7-nitro-2,1,3-benzoazadiazol-4-yl-labeled lipids. Binding was not restricted to lipid analogs since StarD10 selectively extracted PC and PE from small unilamellar vesicles prepared with endogenous radiolabeled lipids from Vero monkey kidney cells. Mass spectrometry revealed that StarD10 preferentially selects lipid species containing a palmitoyl or stearoyl chain on the sn-1 and an unsaturated fatty acyl chain (18:1 or 18:2) on the sn-2 position. StarD10 was further shown to bind lipids in vivo by cross-linking of protein expressed in transfected HEK-293T cells with photoactivatable phosphatidycholine. In addition to a lipid binding function, StarD10 transferred PC and PE between membranes. Interestingly, these lipid binding and transfer specificities distinguish StarD10 from the related START domain proteins Pctp and CERT, suggesting a distinct biological function.

Selective transport of lipids and proteins between organelles is an essential process in the organization of membrane compartments in eukaryotic cells. This process is mainly mediated by budding of transport vesicles from a donor compartment followed by vector trafficking to and fusion with an acceptor compartment (1, 2). Lipids can also be delivered via monomeric exchange between the cytosolic membrane surfaces of different organelles. Monomeric exchange requires desorption of the lipid from the donor membrane, passage through the aqueous phase, and subsequent insertion into the acceptor membrane. Because spontaneous release from the membrane into the aqueous phase is a rare event for most membrane lipids, it has been suggested that specific proteins stimulate lipid exchange between cellular membranes (1, 3).

Several cytosolic proteins with specific lipid binding domains capable of accelerating lipid exchange in vitro have been identified. These proteins include phosphatidylinositol and glycolipid transfer proteins, sterol carrier protein 2, and members of the steroidal acute regulatory protein (STAR/StarD1)-related lipid transfer (START) domain family (4–8). Although phosphatidylinositol transfer proteins have been studied extensively and their role in vesicular transport and signaling has been demonstrated, the cellular function of most START domain proteins still remains elusive.

START domains are ~210 amino acids in length and form a hydrophobic tunnel that accommodates a monomeric lipid (9–11). START domains have been found in 15 mammalian proteins (12). The best characterized family members include STAR/StarD1, which binds cholesterol and is required for cholesterol transport from the outer to the inner mitochondrial membrane (13), and phosphatidylcholine transfer protein (Pctp/StarD2), which is known to exclusively bind and transfer PC between membranes (4, 14). The Pctp subfamily of START domain proteins further comprises Goodpasture antigen-binding protein, StarD7, and StarD10 (12). Recently, a splice variant of the Goodpasture antigen-binding protein/StarD11, termed CERT, was identified to be defective in the Chinese hamster ovary mutant cell line LY-A (15). These cells demonstrate impaired sphingomyelin synthesis due to lack of CERT-mediated transfer of its precursor ceramide from endoplasmic reticulum to Golgi membranes. StarD7 was reported to interact with phospholipid monolayers, indicating a function related to lipid binding (16). Apart from a function in intracellular lipid transfer, START domains are hypothesized to act as lipid sensors with a regulatory role in signal transduction and transcription. For example, the START domain proteins DLC1/2 are candidate tumor suppressors with Rho-GAP activity that...
inhibit Rho-mediated assembly of actin stress fibers (17, 18). In plants, many homeodomain transcription factors contain START domains, whereby lipid/steroid binding is hypothesized to induce conformational changes to control transcriptional activity (19).

We identified the START domain protein, StarD10, to be overexpressed in NeuErbB2-induced mammary tumors in transgenic mice, in several human breast carcinoma cell lines, and in 35% of primary human breast cancers (20). Although StarD10 expression alone was not sufficient to transform cells, it potentiated cellular transformation when coexpressed with ErbB1/epidermal growth factor receptor by a yet unknown mechanism. Using intrinsic fluorescence measurements, we showed that StarD10 was capable of interacting with membranes (20), but the identity of lipid ligands was not explored. Here we characterize the lipid binding properties of StarD10 both in vitro and in vivo and demonstrate a dual specificity lipid transfer function for phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

**EXPERIMENTAL PROCEDURES**

**Materials—**Spin-labeled (SL) phospholipids 1-palmitoyl-2-(4-doxyl
pentanoyl-sn-glycero-3-phosphocholine (SL-PC), SL-PE, and -phosphati
diyserine were prepared as described previously (21). Porcine brain lipids (total extract) and the fluorescent lipids 1-palmitoyl-2-[12-(7-nitro-2,1,3-
benzoxadiazol-4-yl)amino-decaneoyl]-phosphocholine (C12-NBD-PC), -phosphoethanolamine (C12-NBD-PE), -phospho-rac-1-glycerol) (C12-
NBD-PC), and N-lissamine rhodamine B sulfonyl)-dioleoylphosphatidyl-
ethanolamine were purchased from Avanti Polar Lipids (Alabaster, AL); pyrene-labeled phospholipids 1-hexadecanoyl-(1-pyrenedecanoyl)-sn-glycero-
3-phosphocholine (pyrene-PC) and -phosphoethanolamine (pyrene-PE) were from Molecular Probes (Leiden, The Netherlands). Pyrene-glycocsylceramide and pyrene-sphingomyelin were synthesized and purified according to the protocol of Kishimoto (22) using pyrene-decanoic acid; 2,4,6-trinitrophenyl-PE (TNP-PE), kindly provided by P. Somerharju, was
prepared as described (23). The MALDI matrix (2,5-dihydroxybenzoic acid; water (65:25:4, v/v) or subjected to MALDI-TOF mass spectrometry. The TLC plates were dipped in 0.4% 2,5-diphenyloxazol dissolved in chloroform/methanol (9:1, v/v) to visualize phospholipids. PE and PC species, brain lipid extracts were subjected to TLC before analysis. The TLC plates were sprayed with the best signal-to-noise ratio. To enhance the spectral resolution, all spectra were baseline corrected before and after the addition of 7.5 μg of StarD10 or Pctp at 37 °C. Fluorescence intensities were normalized to the maximum intensity observed after the addition of Triton X-100 (0.5% final concentration).

**Binding of Endogenous Lipids—**Vero cells were grown in Dulbecco’s modified Eagle’s medium (150 μg/300 nmol of total phospholipid for 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin and were radiolabeled with [3H]acetic acid (37 kBq/ml; 17 nm; Amersham Biosciences) for 48 h. After washing the cells with phosphate-buffered saline containing 0.5 mg/ml bovine serum albumin, total cellular lipids were extracted by the method of Bligh and Dyer (26), and SUVs were prepared by sonication in 20 mM Heps, pH 7.2, 100 mM NaCl, 5 mM EDTA. Lipid vesicles were determined to contain 55% PE, 35% neutral lipids/cholesterol, 5% glycolipids, 3% PG/phosphatidic acid, and 3% sphingomyelin by measuring the amount of phospholipid phosphorus (27). The vesicle suspension was incubated with purified StarD10 (230 μg/300 nmol of total phospholipid) or Pctp (115 μg/150 nmol of total phospholipid) for 60 min at 25 °C. For MALDI-TOF mass spectrometry, vesicles composed of porcine brain were prepared and incubated with purified StarD10 (150 μg/300 nmol of total phospholipid) for 60 min at 25 °C. The protein was separated from the lipid vesicles by centrifuga-
tion at 1000 × g for 30 min using a Centricon 100-kDa cut-off filter (Millipore, Bedford, MA). Lipid vesicles were recovered for analysis by centrifugation of the inverted filter at 1,000 × g for 2 min. Control experiments without protein showed that the vesicles were completely retained by the filter since no phospholipid was detected in the filtrate. Cosylceramide and pyrene-sphingomyelin were synthesized and purified before and after the addition of 7.5 μg of StarD10 or Pctp at 37 °C. Fluorescence intensity was recorded at 535 nm (excitation, 479 nm; slit widths, 250 (Danbury, CT; 10 min; duty cycle, 20%; output control, 2). The fluorescence intensity was recorded at 395 nm (excitation, 340 nm; slit widths, 4 nm) before and after the addition of 7.5 μg of StarD10 or Pctp at 37 °C. Fluorescence intensities were normalized to the maximum intensity observed after the addition of Triton X-100 (0.5% final concentration).

**Phospholipid Transfer by StarD10**

In an Aminco Bowman Series 2 spectrofluorometer (SLM Instruments, Rochester, NY) before and after the addition of 23 μg of StarD10 (L/P = 20). Maximal dequenching (Fmax) was obtained by adding 0.5% Triton X-100. The percentage change of fluorescence dequenching (FDQ) was calculated as FDQ = (F - F Fmax / Fmax ) × 100, where F and F are the fluorescence intensities before and after the addition of the protein.
FIG. 1. StarD10 interacts with phospholipids in solution. SL-PC (A) and SL-PE (B) were dissolved in buffer, and ESR spectra were recorded at 25 °C in the absence (−) and in the presence of StarD10 or Pctp (L/P = 3). The arrows denote a signal arising in the presence of the proteins, which was extracted by spectral subtraction, yielding the spectra of the immobilized component (i.e.) shown for SL-PC. The distance between the arrows represents the outer hyperfine splitting in care of StarD10. C, SL-PC was dissolved in buffer, and the ESR spectrum was recorded at 25 °C in the presence of GST (L/P = 3).

with 200 μl of [3H]choline and 10-azi-stearic acid for 16 h in delipidated medium (32). Cells were washed with phosphate-buffered saline containing 0.5 mg/ml bovine serum albumin and irradiated for 2 min with UV light at 4 °C. Cells were scraped into hypotonic 20 mM Hepes buffer, pH 7.2, supplemented with Complete protease inhibitors and sheared using a 25-gauge needle, and lysates were then clarified by centrifugation at 12,000 × g for 15 min. Immunoprecipitation was performed by incubating lysates after the addition of NaCl to a final concentration of 120 mM with anti-FLAG monoclonal antibody (Sigma). Immune complexes were captured using protein G-Sepharose and washed 4 times with buffer containing 120 mM NaCl, 20 mM Hepes, 1% Triton X-100, protease inhibitors, pH 7.2. Immunoprecipitates were separated by SDS-PAGE. Before drying and autoradiography, the gel was incubated in 1 M sodium salicylate and 1% glycerol. An aliquot of the immunoprecipitates was separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane for analysis by Western blotting. Membranes were blocked in phosphate-buffered saline containing 5% milk and 0.1% Tween 20 before incubation with anti-FLAG antibody followed by incubation with horseradish peroxidase-conjugated secondary antibody (Bio-Rad) in the same buffer. For detection of secondary antibodies, the ECL plus kit (Amersham Biosciences) was used, and fluorescence was scanned on a FLA-3000 Fuji Imaging System (Raytest, Straubenhardt, Germany) equipped with a 488-nm laser and a 515-nm long pass emission filter. Image analysis was performed using Aida Image Analyser 3.24 software (Raytest, Straubenhardt, Germany).

RESULTS

StarD10 Binds Phospholipids—To identify potential phospholipid binding partners of StarD10, we employed ESR measurement of spin-labeled lipid analogs bearing a long fatty acid chain on the sn-1 position and a short fatty acid chain (penta-noic acid, C5) with a doxyl moiety on the sn-2 position. In aqueous solution at concentrations above their critical micellar concentration, the ESR spectra of spin-labeled lipid analogs are composed of two components, which reflect the equilibrium between micelles and lipid monomers in aqueous solution; they are (i) a broad component caused by strong spin-spin interaction of the analogs within the micelles and (ii) an isotropic signal with three defined peaks arising from freely tumbling analog monomers in the bulk solution (33). Due to the homology of StarD10 with Pctp, we first analyzed potential binding of SL-PC to the protein. ESR spectra of SL-PC were recorded in the absence and presence of recombinant human StarD10 protein purified from Escherichia coli (Fig. 1A, top panels). The addition of StarD10 to SL-PC (lipid to protein ratio, L/P = 3) gave rise to a new component in the ESR spectra that was extracted by spectral subtraction (Fig. 1A, i.e. StarD10, see arrows). This component can be explained by lipid binding of StarD10. The large outer hyperfine splitting of this component (distance between the arrows) reflects a high immobilization of bound SL-PC, implicating a tight association of the analog with the protein (33). In agreement with the binding of SL-PC to StarD10, micelle formation was strongly reduced, as deduced from the disappearance of the broad component. By ESR measurement, StarD10 was also found to interact with SL-PE in a similar manner as with SL-PC (Fig. 1B, top and middle panels), whereas interaction with SL-phosphatidylserine was less evident (data not shown). We further investigated the interaction of Pctp with these lipid analogs. Similar to StarD10, Pctp was found to interact with SL-PC (Fig. 1A, bottom panels, see arrows). Notably, we also found binding of SL-PE to Pctp (Fig. 1B, bottom panel), confirming previous observations that Pctp binds PE in the absence of PC (14). However, the outer hyperfine splitting and, thus, the immobilization of protein-bound lipid analogs were much lower in comparison to that of StarD10. This suggests a comparatively loose association of lipids with Pctp. GST was used as a control protein and had no influence on the ESR spectrum of SL-PC (Fig. 1C).

Because ESR utilizes lipids in solution, we next employed an assay based on the principle of fluorescence resonance energy transfer to analyze the interaction of StarD10 with PC and PE in membranes. Here, lipid analogs labeled with an NBD fluorescent energy donor at their fatty acid tail were incorporated into SUVs in the presence of head group-labeled rhodamine-PE, which functioned as the energy acceptor. Due to the close proximity of the fluorescent donor/acceptor pair, energy transfer to rhodamine resulted in significant quenching when NBD fluorescence was excited. Upon the addition of StarD10 to these vesicles (L/P = 20), a rapid increase in the NBD fluorescence was observed, which was most likely due to protein-mediated extraction of NBD-labeled lipids from vesicles, allowing fluorescence emission to occur. NBD fluorescence intensity was highest in the case of NBD-PC and was lower for NBD-PE, whereas only a small increase in NBD fluorescence was observed for NBD-PG. In comparison, Pctp displayed the reported specificity for PC in membranes containing other phospholipid species. For both proteins, quantification of lipid binding relative to that of PC is shown in Fig. 2. The domain proteins such as STAR and MLN64 have been shown to bind cholesterol. Using dehydroergosterol, a natural cholesterol analog with intrinsic fluorescence, and TNP-PE as a fluorescence quencher, we did not observe binding of StarD10 to sterols (data not shown). Taken together, these results indicate that StarD10 is capable of interacting with phospholipids in solution and in...
membranes, with a preference for PC and PE.

**StarD10 Binds Endogenous PC and PE**—To test whether StarD10 interacted with endogenous lipids, we prepared SUVs with lipids extracted from [14C]acetic acid-labeled Vero monkey kidney cells. Recombinant StarD10 was incubated with these vesicles, and after separation of the protein from the lipid vesicles by membrane filtration through a 100-kDa cut-off filter, lipids bound to StarD10 were extracted and separated by thin layer chromatography. This approach revealed extraction of both PC and PE by StarD10 (Fig. 3A). Recombinant Pctp, which was previously shown to bind PC in a similar assay, was loaded as a control (14). Scintillation counting of lipids bound by StarD10 revealed a 10:1 ratio of PC and PE (Fig. 3A). Because vesicles contained 55% PC and 10% PE (see “Experimental Procedures”), the affinity of StarD10 for PC is ~2-fold higher than for PE, which is in accordance with the binding preferences observed using NBD lipids.

To obtain additional information on the fatty acid chain composition of phospholipids preferentially extracted by StarD10, we analyzed organic extracts of recombinant StarD10 protein before and after incubation with brain lipid vesicles by MALDI-TOF mass spectrometry. Separation of StarD10 from the vesicles was performed by membrane filtration as described above. Selected positive ion MALDI-TOF spectra are shown in Fig. 3B. It is evident that the organic extract of the control filtrate did not contain any lipids (Fig. 3B, trace a). The only detectable peak at mz = 727 corresponded to a typical matrix oligomerization product, whose chemical origin was described previously (34). This peak was present in all spectra and is marked with an asterisk. Recombinant StarD10 analyzed directly after purification from bacteria was identified to contain mainly PE and PG (Fig. 3B, trace b). This is in accordance with the lipid composition of bacterial membranes, which lack PC but contain ~80% PE and 15% PG. When StarD10 was incubated with porcine brain lipid vesicles before lipid extraction, PG was completely exchanged, and StarD10 was found to contain both PC and PE (Fig. 3B, trace c). The identified phospholipid species are summarized in Table I. The main PC lipid species could be assigned to PC 16:0/18:1 (m/z = 766.6 and 782.6), PC 18:0/18:2 (m/z = 786.6 and 808.6), and PC 18:0/20:4 (m/z = 810.6 and 832.6), reflecting the PC lipid species present in porcine brain (Supplemental Fig. 1A and Supplemental Table 1). Interestingly, saturated PC (16:0/16:0) was not extracted by StarD10. The protein was further identified to bind PE 18:0/18:2 (m/z = 766.5 and 788.5) and PE 16:0/18:1 (m/z = 762.5 and 784.5), whereas PE species containing long unsaturated fatty acyl chains on the sn-2 position were not selected. These abundant brain lipid species include PE 16:0/20:4, 16:0/22:5, 16:0/22:6, 18:0/20:4, and 18:0/22:6. For example, the porcine brain lipids displayed similar peak heights for PE 18:0/18:2 (m/z = 766.5 and 788.5) and PE 16:0/20:4 (m/z = 746.5), whereas the StarD10 lipid spectrum contained peaks at 766.5 and 788.5 but at best a strongly reduced peak at 746.5 (Fig. 3B, Supplementary Fig. 1B and Supplemental Table I). These data demonstrate that StarD10 favors lipids containing a palmitoyl or stearoyl chain on the sn-1 position and an unsaturated fatty acyl chain on the sn-2 position (18:1 and 18:2). Estimation of the contribution of the individual phospholipid classes, however, is difficult since each phospholipid species has a different desorption and ionization tendency (34).

**Cross-linking of StarD10 with PC in Intact Cells**—Using a photolabeling approach, interaction of proteins with specific lipids can be visualized in intact cells (32). To investigate whether StarD10 interacted with endogenous PC in vitro, HEK-293T cells were transiently transfected with a FLAG-tagged StarD10 expression plasmid and cultured for 16 h in the presence of [3H]choline and a photoactivatable fatty acid (10-az Stearic acid), both metabolic precursors of PC (32). As controls, we included mock-transfected cells and those transfected with a construct encoding FLAG-tagged Pctp. Before lysis, cells were irradiated with UV light, whereby proteins bound to photoactivatable PC were cross-linked. Autoradiography of anti-FLAG immunoprecipitates separated by SDS-PAGE revealed photolabeling of both StarD10 and Pctp (Fig. 4, top panel). Expression and immunoprecipitation were monitored by Western blotting and found to be equivalent for both proteins (Fig. 4, bottom panel). The lower degree of PC cross-linking of StarD10 compared with that of Pctp is most likely due to its broader lipid binding specificity.

**StarD10 Mediates Lipid Transfer between Membranes**—Finally, we sought to determine whether StarD10 possessed phospholipid transfer activity. In a fluorescence resonance energy transfer-based assay similar to the one described in Fig. 1B, the transfer rate of fluorescently labeled lipid analogs from donor vesicles to unlabeled acceptor vesicles was measured. Because C12-NBD-labeled lipid analogs displayed a high rate of spontaneous transfer (data not shown), we used donor vesicles that contained pyrene-labeled lipids as the fluorescence donor and head group-labeled TNP-PE as a fluorescence quencher (35). The presence of TNP-PE caused significant reduction of pyrene fluorescence. When these donor liposomes were mixed with an excess of unlabeled acceptor liposomes, the increase in pyrene fluorescence observed over time was negligible, indicating minimal spontaneous transfer (data not shown). Likewise, the addition of albumin, which has nonspecific lipid binding sites, did not mediate intervesicular transfer of labeled lipids (Fig. 5A, trace c). Upon the addition of StarD10, a steady increase in fluorescence intensity was noted in the case of pyrene-PC (Fig. 5A, trace a). StarD10 was also capable of transferring pyrene-PE with an ~3-fold lower rate than that of PC (Fig. 5A, trace b). Pyrene-labeled glycosylceramide did not serve as substrate (data not shown), confirming the specificity of the transfer assay and further proving that the fluorescence increase was not due to StarD10-mediated transfer of TNP-PE or fusion between donor and acceptor vesicles. In *vitro* lipid transfer rates of StarD10 for different phospholipids were quantified and compared with those of Pctp (Fig. 5B). Although both proteins efficiently transferred PC and promoted very low transfer of sphingomyelin, Pctp completely failed to transfer PE. Taken together, our data show that StarD10 displays phospholipid binding and transfer specificities that are distinct from those of Pctp.
DISCUSSION

StarD10 belongs to a distinct subfamily of START domain proteins comprising Pctp, StarD7, and Goodpasture antigen-binding protein/CERT, which are believed to function in intracellular transport of lipids, lipid metabolism, and cell signaling. In this report we provide evidence that StarD10 is a lipid transfer protein with selective lipid binding specificities.

ESR measurements served as a first approach to explore the interaction of StarD10 with lipids. We could demonstrate that StarD10 interacts directly with SL-PC and SL-PE in aqueous solution. Compared with Pctp, lipids appeared to be more highly immobilized by StarD10. Using fluorescent lipid analogs, we show that StarD10 binds different phospholipids with varying affinity. In agreement with its homology to Pctp, StarD10 was found to strongly bind PC. However, in contrast to Pctp, which displayed selectivity for PC when incubated with membranes containing different phospholipids, StarD10 also interacted with PE. StarD10 is further shown to transfer both PC and PE in vitro, phospholipids with distinct head groups, which implies a certain flexibility of its START domain. It will, thus, be interesting to determine which amino acids in StarD10 are involved in lipid binding by crystallography. This should provide insight into different conformations that can be adopted by START domains and explain the altered substrate specificities compared with Pctp and CERT.

We have previously shown that incubation of StarD10 with brain lipid liposomes resulted in a decrease of its intrinsic tryptophan fluorescence at 335 nm (excitation 280 nm), indicating a conformational change upon interaction with lipids (20). This proved that interaction with phospholipids was not restricted to lipid analogs but extended to naturally occurring lipids. Using a photolabeling approach, we could now visualize the interaction of StarD10 with endogenous PC in vivo. In contrast to Pctp, only a proportion of StarD10 appeared to be stably complexed with PC. One interpretation of this difference is a lower affinity of StarD10 for membrane surfaces. In addition, StarD10 was likely to be engaged in interactions with PE that cannot be monitored under these assay conditions.

By incubating StarD10 with donor membranes generated with radiolabeled endogenous lipids from Vero cells, we demonstrated that StarD10 is able to selectively extract PC and PE. Compared with PC, the intensity of the PE signal was 10-fold weaker (Fig. 3A). However, if the lower abundance of PE is taken into account, the affinity of StarD10 for PE is significant. In intact cells, various organelar membranes display striking differences not only in their lipid composition but also in the distribution of lipids between leaflets. For example, membranes of late secretory organelles display an asymmetric lipid distribution with PE concentrated in the cytoplasmic leaflet, where it is readily accessible for protein-mediated exchange (1, 2).

MALDI-TOF mass spectrometry revealed that recombinant StarD10 protein purified from E. coli contained both PE and PG. In lipid binding assays, NBD-labeled PG was identified as...
are the averages of equation and expressed as the percentage of transfer relative to PC; 100% corresponds to 12.4%/min for StarD10 and 35.6%/min for Pctp. Values initial transfer rates of pyrene-labeled lipids were determined for StarD10 and Pctp by fitting the curves shown in addition of protein. The fluorescence intensities were normalized to the maximum intensity obtained after the addition of 0.5% Triton X-100.

StarD10 binds endogenous PC in vivo. HEK-293T cells (mock transfected or transiently transfected with expression constructs encoding FLAG-tagged StarD10 and Pctp) were grown overnight in the presence of 10-azi-stearic acid and [3H]choline. Cells were ultraviolet (UV)-irradiated to induce cross-linking of proteins with PC. Hypotonic cell lysates were then subjected to immunoprecipitation (IP) with anti-FLAG antibody. Immune complexes were resolved by SDS-PAGE and visualized by fluorography (top panel) or immunoblotting (WB) with anti-FLAG antibodies (bottom panel). Arrow, StarD10; arrowhead, Pctp. con, control.

StarD10 stimulates transfer of PC and PE between vesicles. A, transfer of pyrene-labeled PC (trace a and c) and PE (trace b) from donor to acceptor vesicles was determined in the presence (trace a and b) of StarD10 or bovine serum albumin (trace c); the arrow denotes the addition of protein. The fluorescence intensities were normalized to the maximum intensity obtained after the addition of 0.5% Triton X-100. B, initial transfer rates of pyrene-labeled lipids were determined for StarD10 and Pctp by fitting the curves shown in A to a single-exponential equation and expressed as the percentage of transfer relative to PC; 100% corresponds to 12.4%/min for StarD10 and 35.6%/min for Pctp. Values are the averages ± S.D. of at least two independent experiments. SM, sphingomyelin.

Table 1
Overview of the observed m/z values in the positive ion MALDI-TOF mass spectra shown in Fig. 3B and their assignment to individual phospholipids species

<table>
<thead>
<tr>
<th>Peak position (&lt;i&gt;m/z&lt;/i&gt;)</th>
<th>Assignment</th>
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<tr>
<td>727.0</td>
<td>Oligomerization product of 2,5-dihydroxybenzoic acid matrix</td>
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<tr>
<td>738.5</td>
<td>PE (16:0/18:2) + Na&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>740.5</td>
<td>PE (16:0/18:1) + Na&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>760.5</td>
<td>PE (16:0/18:2) - H&lt;sup&gt;+&lt;/sup&gt; + 2 Na&lt;sup&gt;+&lt;/sup&gt;</td>
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<tr>
<td>760.6</td>
<td>PC (16:0/18:1) + H&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>PE (16:0/18:1) - H&lt;sup&gt;+&lt;/sup&gt; + 2 Na&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
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<td>PE (18:0/18:2) + Na&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>782.5</td>
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<tr>
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<td>PC (16:0/18:1) + Na&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>832.6</td>
<td>PC (18:0/20:4) + Na&lt;sup&gt;+&lt;/sup&gt;</td>
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Note: Neutral PG molecules were assumed to contain sodium ions.

The physiological role of StarD10 remains to be established. By binding PC and PE, StarD10 could function in net transfer of these lipids between subcellular compartments and regulation of cellular signaling events. StarD10 expression is abundant in liver where a putative function is the delivery of phospholipids to hepatocyte canicular membranes for secretion into bile. A similar preference for saturated fatty acids on the sn-2 position did not serve as ligands.
cules, mostly released by the direct action of one or more phospholipases (39, 40). Given its function as a lipid transfer protein, StarD10 may be involved in replenishing membranes with PC and PE metabolized by phospholipases. Alternatively, StarD10 may aid in presenting substrate to phospholipases by solubilizing lipids from membranes, a so-called “liftase” mechanism first established for a ganglioside activator protein in β-hexosaminidase A-mediated hydrolysis of sphingolipids (41). For example, overexpression of phosphatidylinositol transfer protein-α in NIH3T3 cells was shown to activate phospholipase A2, resulting in increased formation of lysophosphatidylcholine and accelerated growth of cells (42). In a similar vein, StarD10 may enhance the activation of specific phospholipases in the cell, thereby contributing to aberrant lipid signaling and cellular transformation.

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