Published in "Proceedings of the National Academy of Sciences of the United States of America 109(42): 16882-16887 , 2012" which should be cited to refer to this work.

Supporting Information

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SI Materials and Methods

Yeast Strains, Growth Conditions, Epitope-Tagging, and Western Blotting. Yeast strains were cultivated in standard media as previously described (1). Double- and triple-mutant strains were generated using PCR deletion cassettes and a marker rescue strategy (2, 3). Human cDNA clones were obtained from ImaGenes GmbH. Epitope tagging was performed by homologous recombination using PCR fusion cassettes and correct integration of the fusion cassette was confirmed by colony PCR (2).

To analyze protein secretion into the culture media, proteins were extracted from 3 OD_{600nm} units of cells and from 10 mL of culture supernatant. Proteins were precipitated by TCA and analyzed by Western blotting. Deglycosylation of O-linked glycans was performed by treatment of TCA-precipitated proteins with trifluoromethane sulfonic acid as described previously (4). For GST-pulldown assays, proteins from the culture supernatant corresponding to ~500 OD_{600nm} of cells were concentrated using a spin column (Thermo Fisher Scientific), the pH was adjusted to 7.3, and GST-tagged proteins were purified on glutathione columns according to the instruction supplied by the manufacturer (GE Healthcare). Copurifying lipids were analyzed after lipid extraction of the eluted proteins followed by TLC separation.

Expression and Purification of CAP Proteins from *E. coli*. DNA encoding Pry1, Pry2, and CRISP2 were PCR amplified and cloned into the *NcoI* and *XhoI* sites of pET22b (Novagen, Merck), which contains a signal sequence to direct secretion of the expressed protein into the periplasmic space. Expression of the poly histidine-tagged fusion protein in *E. coli* BL21 was induced by lactose at 24 °C overnight. The cell lysate was incubated with Ni-NTA beads (Qiagen) according to manufacturer instructions; beads were washed and proteins eluted with imidazole, then proteins were concentrated and quantified. Protein concentration was determined by a Lowry assay using BSA as standard and by UV absorption of the denatured protein, taking the number of aromatic residues into consideration.

In Vitro Lipid Binding and Competition Assay. The radioligand binding assay was performed as described by Im et al. (5). For saturation binding curves, the protein (100 pmol) was incubated with radiolabeled cholesterol for 1 h at 30 °C, or for 12 h at 30 °C to determine binding of radiolabeled cholesteryl acetate, in binding buffer (20 mM Tris-HCl, 30 MM NaCl, 0.05% Triton X-100). For the competitive binding assay, equal amounts of the radiolabeled lipid were mixed with an unlabeled competitor lipid; the mixture was dried, resuspended in binding buffer, and incubated with the purified protein (100 pmol) for 1 h at 30 °C. In all binding assays, the protein was separated from the unbound ligand by adsorption to anion exchange beads, and the bound radioligand was quantified by scintillation counting. To determine nonspecific binding, 500 μM of unlabeled cholesterol was included in the incubation. Data were analyzed using PRISM software (GraphPad).

Circular Dichroism. CD spectra were recorded with 200 μg of protein in a buffer containing 50 mM Tris, 150 mM NaCl, and 0.1 mM EDTA using a 1-cm path length cuvettete in a Jasco J-715 spectropolarimeter. Data were acquired from 200 to 250 nm with a bandwidth of 1 nm and a scanning speed of 100 nm/min at 25 °C. Data shown represent the averaged values from five spectra and is expressed in mean residue ellipticity (mdeg). The fractional percentage of the secondary structure was calculated by using software K2D2 (see ref. 6).

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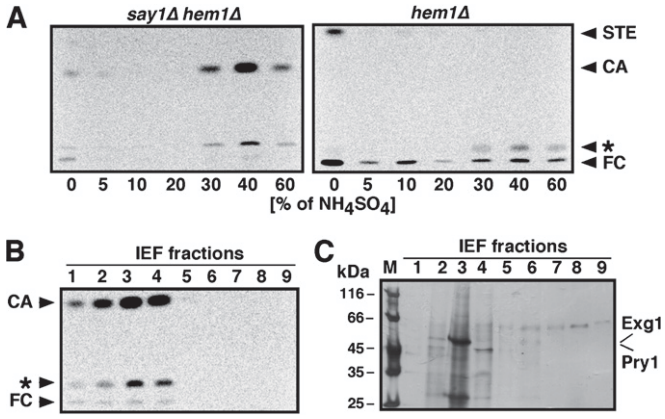

Fig. S1. Identification of candidate cholesteryl acetate–binding proteins. (*A*) Ammonium sulfate fractionation of a cholesteryl acetate-binding activity. Heme-deficient wild-type and *say1*Δ mutant strains were cultivated in the presence of radiolabeled cholesterol. The culture supernatant was fractionated by addition of ammonium sulfate. Lipids were extracted from the salt precipitates, separated by TLC, and radiolabeled lipids were visualized by phosphorimaging. *, position of an unidentified lipid; CA, cholesteryl acetate; FC, free cholesterol; STE, steryl esters. (*B*) Subfractionation of the 40% ammonium sulfate fraction by isoelectric focusing (IEF). Proteins from the 40% ammonium sulfate fraction were separated by liquid-phase IEF and individual fractions, labeled 1–9, were eluted. Lipids were extracted from the IEF fractions, separated by TLC, and visualized by phosphorimaging. (*C*) Separation of proteins from the preparative IEF fractions by SDS/PAGE. Proteins from individual IEF fractions were TCA precipitated, separated by SDS/PAGE, and visualized by silver staining. The band running at ~50 kDa in lane 3 was excised and analyzed by mass spectrometry. The molecular weights of standards are indicated to the left. The data shown are representative of two independent experiments.

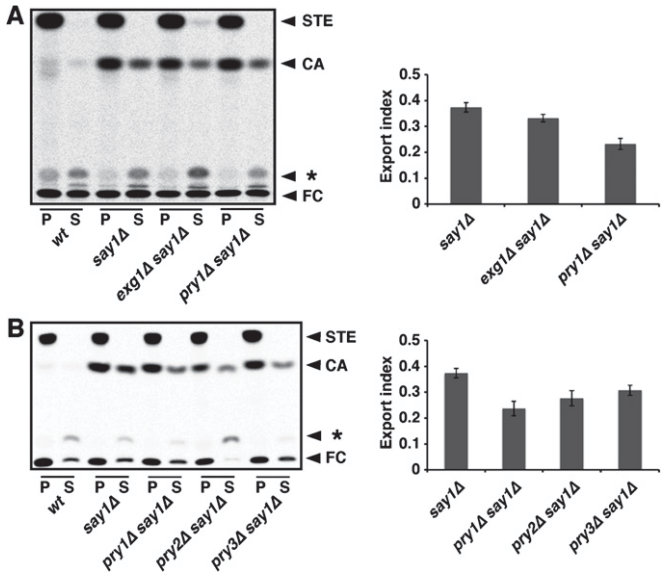

Fig. S2. Efficient export of cholesteryl acetate depends on Pry function. (*A*) Export of cholesteryl acetate is reduced in *pry1*Δ mutant cells. Heme-deficient *say1*Δ, *exg1*Δ *say1*Δ, and *pry1*Δ *say1*Δ mutant cells were cultivated in the presence of radiolabeled cholesterol. Lipids were extracted from the cell pellet (P) and culture supernatant (S), separated by TLC, and visualized by phosphorimaging. The level of cholesteryl acetate was quantified by radioscanning and the ratio between extracellular and intracellular cholesteryl acetate is plotted as an export index. (*B*) Pry proteins have redundant functions in export of cholesteryl acetate. Cells lacking single *PRY* genes, in a *hem1*Δ *say1*Δ mutant background, were tested for export of cholesteryl acetate as described above. Data represent mean ± SD of three independent experiments. *, position of an unidentified lipid; CA, cholesteryl acetate; FC, free cholesterol; STE, steryl esters; wt, wild type.

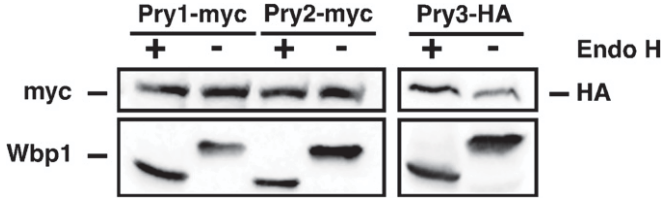

Fig. S3. Pry proteins are resistant to endoglycosidase H treatment. Proteins were extracted from cells expressing epitope-tagged versions of Pry proteins. They were left untreated (–) or were treated with endoglycosidase H to remove *N*-linked glycans. Proteins were separated by SDS/PAGE and detected with antibodies against the respective epitope. The *N*-glycosylated ER protein, Wpb1, serves as control for the efficiency of the treatment.

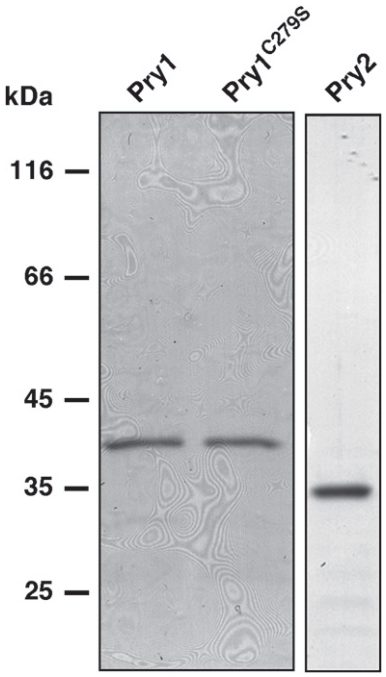

Fig. S4. Purification of His-tagged Pry. 6×His-tagged versions of Pry proteins were expressed in bacteria, affinity purified on nickel-Sepharose beads. A total of 1 μg of the eluted protein was separated by SDS/PAGE and stained with Coomassie blue.

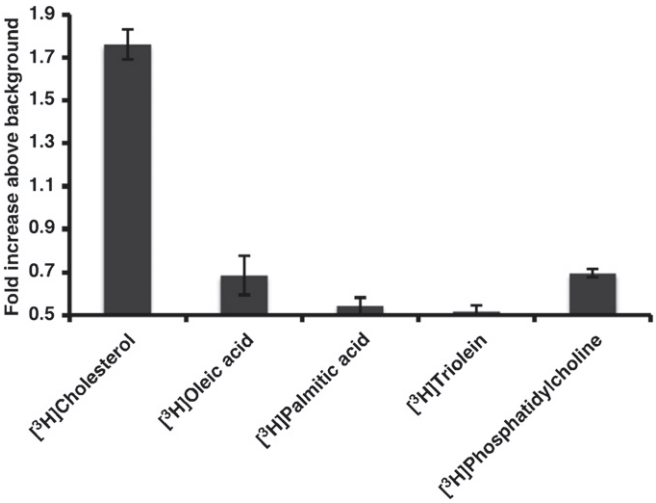

Fig. S5. Lipid-binding specificity of Pry1. The indicated radiolabeled lipids (1 nmol) were incubated with purified Pry1 protein (100 pmol) for 1 h at 30 °C. The protein was separated from the unbound ligand through adsorption to anion exchange beads and the bound radioligand was quantitated by scintillation counting. Data represent mean ± SD of two determinations.

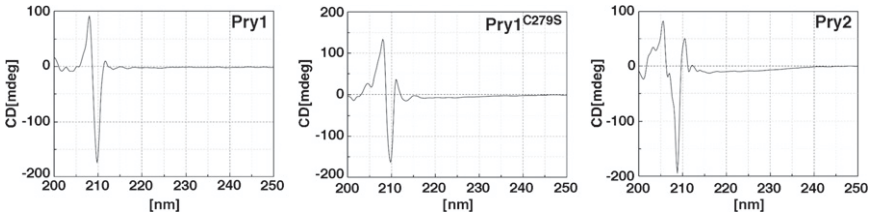

Fig. S6. CD Spectra of Pry proteins. Spectra were recorded using 200 μg of purified protein in a 1-cm path length UV cuvettete at 25 °C.

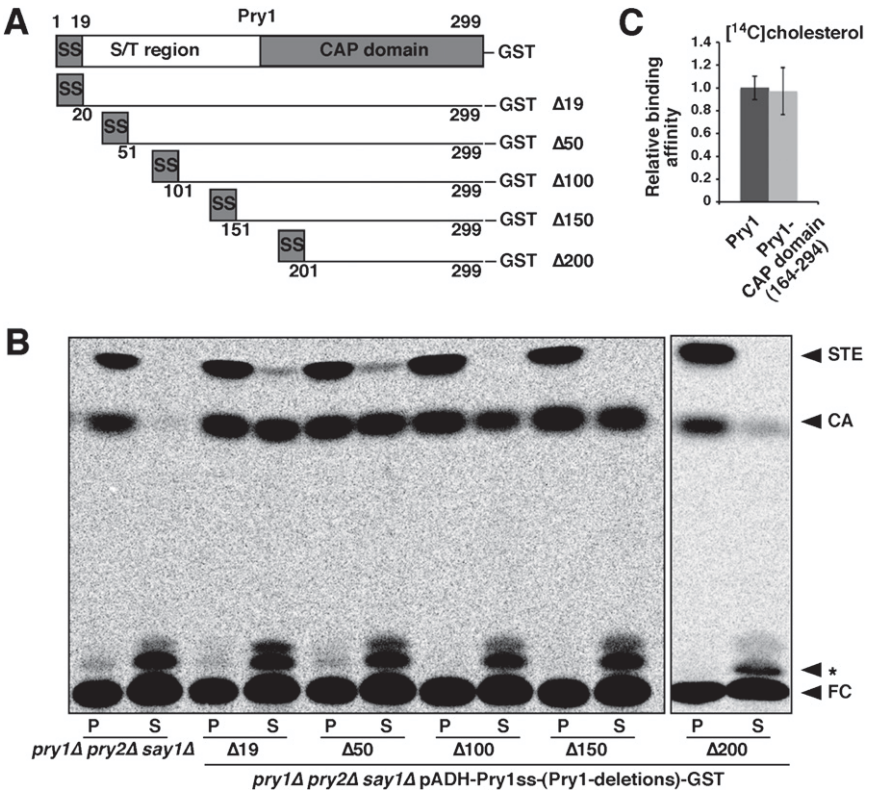

Fig. S7. The CAP domain of Pry1 is required for lipid export and is sufficient for sterol binding. (*A*) Deletion analysis of Pry1. Schematic representation indicating the signal sequence (SS), the Ser/Thr rich N-terminal part, and the CAP domain of Pry1. The N-terminal truncations that were tested for lipid export function are shown. (*B*) The CAP domain is required for lipid export. N-terminal truncations were tested for complementation of the lipid export defect in a *pry1*Δ *pry2*Δ double mutant background. (*C*) The CAP domain is sufficient for sterol binding. The CAP domain of Pry1 (164–294) was expressed in bacteria, purified and tested for sterol binding. Data are mean ± SD of two independent determinations. *, position of an unidentified lipid; CA, cholesteryl acetate; FC, free cholesterol; STE, steryl esters.