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Supplemental materials and methods

Crystallization and data collection

The Δ9 truncation of Sar1A (hamster) was generated and expressed in *E. coli* and purified as described (Kuge, O., C. Dascher, L. Orci, T. Rowe, M. Amherdt, H. Plutner, M. Ravazzola, G. Tanigawa, J.E. Rothman, and W.E. Balch. 1994. *J. Cell Biol.* 125:51–65). The Δ9-Sar1-GDP crystals belong to the monoclinic space group $P2_1$ (see Table I in main text) with two molecules per asymmetric unit, a Matthews coefficient of 2.54 $Å^3$/dalton, and solvent content of 48.9%. The x-ray diffraction data were collected at 100°K on beam lines 7-1 and 9-2 at the Stanford Synchrotron Radiation Laboratory (Stanford, CA). The diffraction data were indexed and processed with DENZO and scaled with SCALEPACK.

Expression and mutagenesis of Sar1

All Sar1A mutants were prepared by site-directed mutagenesis as described previously . In brief, mutagenesis of the NH_2 terminus of Sar1A used forward primer containing a 5′ Nde I site, followed by the start codon and the coding sequence containing the mutation. The reverse primer contained a BamHI site, followed by the stop codon and the last six codons of Sar1A. The forward mutant and reverse primers were used in a PCR reaction with Expand polymerase (Roche) according to the manufacturer's directions to generate the full length Sar1A construct containing the NH_2-terminal mutation of interest. The PCR product was subsequently cloned into pCR2.1 TOPO (Invitrogen) according to the manufacturer's directions and sequenced. The D25-truncated form (D25-Sar1) was cloned using PCR amplification from the full-length Sar1A cDNA using the following primers: 5′-gcgaattCCatgGGTAAACTGGTATTCCTTGG-3′ introducing a Nco I site and an initiation codon at residue 26, and 5′- CCGGGCGGATCCCTAATCGATGTACTGTGCCAT-3′ introducing a BamH I site after the stop codon. All truncations and mutants were subsequently subcloned into the bacterial expression vector pET 11d using Nde I and BamHI into pET11Dhis for protein expression and purification as described (Kuge, O., C. Dascher, L. Orci, T. Rowe, M. Amherdt, H. Plutner, M. Ravazzola, G. Tanigawa, J.E. Rothman, and W.E. Balch. 1994. *J. Cell Biol.* 125:51–65).

Preparation of rat liver cytosol and membrane fractions

Rat livers were homogenized and fractionated to obtain rat liver cytosol as described. Rat liver membranes were recovered from the final 186,000 *g* centrifugation step, resuspended in buffer A at 30 mg/ml, snap frozen in liquid N_2, and stored at −80°C for subsequent use.

Preparation of microsomal membranes from NRK cells

NRK cells were infected with tsO45 VSV as described and microsomes were prepared as described.

Generation of yeast-null strains

A HindIII-Sal1 fragment containing the yeast SAR1 gene was excised from pANY2-9 and subcloned into the pRS415 expression vector. A two-step PCR procedure was then used to make overlapping PCR products which deleted codons 2–6 (generating Δ6scSar1) or codons 2–9 (generating Δ9scSar1). The PCR products were then ligated into pRS415. The *scSar1* mutants were then tested for their ability to complement the yeast *SAR1* knockout strain YSY100-1D MAT α Sar::HIS3 ura3 leu2 trp1 lys2 ade2 his3 (pSar1 on a single-copy URA3 marker plasmid) using a plasmid–shuffle assay. Either the wild-type *scsar1*-pRS415, *Δ6-scsar1*-pRS415, *Δ9-scsar1*-pRS415, or an empty pRS415 vector were transformed into YSY100-1D, and positively transformed colonies were selected on ura-/leu-plates. Positive colonies were restreaked on ura-/leu plates, and then individual colonies were restreaked onto 5FOA plates.

Sar1-exchange assay

Typically, Sar1 was loaded with [^{3}H]GDP by incubating 20 pmol purified wild-type or mutant Sar1 in 100 μl of buffer D (50 mM Hepes, pH 8.0, KOH, 0.1 mg/ml BSA, 0.1% TX-100, 1 mM DTT, and 2.5 μM [^{3}H]GDP (∼2,000 cpm/pmol) (NEN Life Science Products) at 32°C for 1 h. To measure the activity of mSec12 in the absence of detergent, the Triton-X 100 was deleted from buffer D and replaced with 10 mM EDTA during the [^{3}H]GDP loading step. After loading, the free Mg^{2+} concentration was adjusted to 20 mM with $MgCl_2$, and the reaction was incubated at 32°C for 10 min at 32°C. Under these conditions, typically ∼40% of total Sar1 was loaded with [^{3}H]GDP as determined by filtration onto nitrocellulose filters and scintillation counting as described. To follow exchange, Sar1 was mixed with a cocktail containing buffer d (in the presence of absence of detergent as indicated in the Results), 1 mM GTP and the indicated amounts of mSec12 or control fractions as indicated in the Results. Detergent-free mSec12 was prepared by incubating with Extracti-Gel D according to the

manufacturer's directions (Pierce Chemical Co.). Under these conditions, residual detergent was determined to be <0.0001% in the exchange assay. When the exchange activity was measured using intact microsomes or detergent-free mSec12, Sar1 (loaded with [^{3}H]GDP in the absence of detergent) was added to buffer D (lacking detergent) containing 100 μM GDP and 100 μM GTP or GTPγS as indicated in Results. Following incubation at 32°C, the amount of [^{3}H]GDP remaining bound to Sar1 was quantitated by scintillation counting as described.

Sec23/24 GAP assay

Sar1p GTPase activation (GAP activity) of GST-Sec23 or the Sec23/24 complex was performed as described using 0.5 μM Sar1 and 1 μM of recombinant GST-Sec23 or 1 μM of the Sec23/24 purified fraction as described (Aridor, M., J. Weissman, S. Bannykh, C. Nouffer, and W.E. Balch. 1998. *J. Cell Biol.* 141:61–70).

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