Figure S1. Reversible OPA1 aggregation under low salt.

(**A**) OPA1 was dialyzed into buffer containing no salt and examined by uranyl acetate staining and electron microscopy. Protein aggregates are visible. (**B**) OPA1 was dialyzed into buffer containing no salt and then incubated for 1 hour with cardiolipin-containing liposomes. The sample was stained with uranyl acetate and viewed by electron microscopy. Scale bars indicate 200 nm.

Figure S2. Effect of guanine nucleotides on sedimentation and liposome binding.

In the top three panels, the sedimentation of OPA1 was analyzed as in Fig. 1A, except that the indicated guanine nucleotide was present. In the bottom panel, the effect of guanine nucleotides on binding of OPA1 to cardiolipin-containing liposomes was analyzed by sedimentation. The assay was done using 300 mM NaCl. S, supernatant; P, pellet.

Figure S3. Categories of OPA1/liposome morphologies

OPA1 was incubated with cardiolipin-containing liposomes and examined by negative staining. The labels indicate representative liposomes of the "deformed" and "tubular" categories.

Figure S4. Sedimentation of OPA1 mutants under varying salt concentrations.

Sedimentation of the indicated mutants was performed as in Figure 1A.

Video S1. Movie of membrane tubule extension by OPA1-S1.

OPA1-S1 was added to immobilized cardiolipin-containing liposomes labeled with DiO. The elapsed time is 30 minutes.

Figure S1

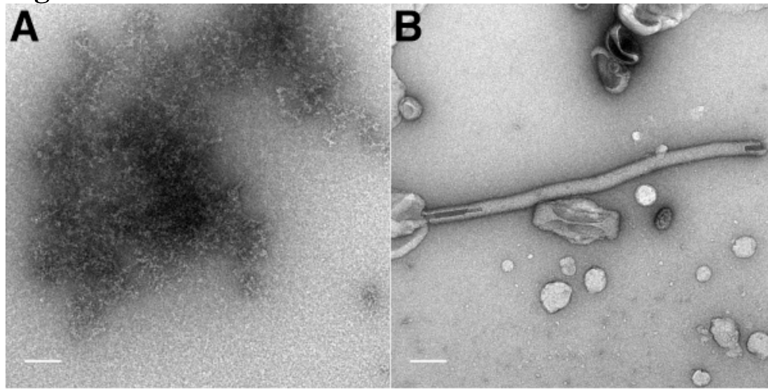


Figure S2

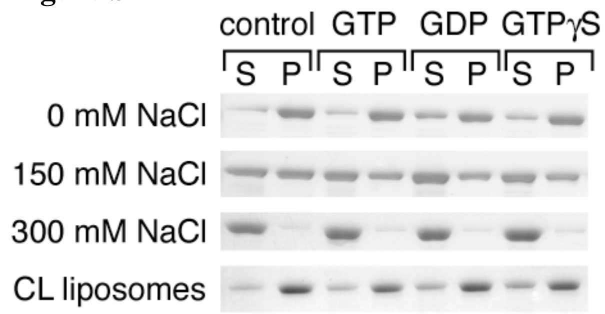


Figure S3

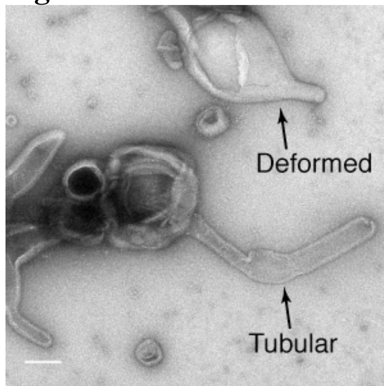
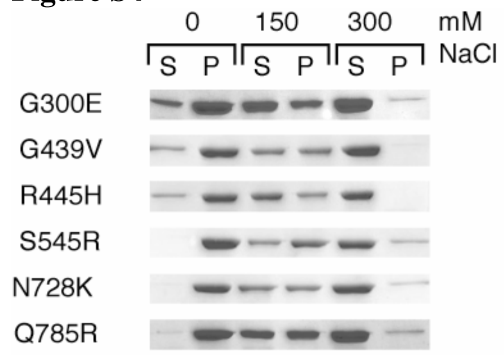


Figure S4



Recombinant OPA1-S1 expression and purification

Recombinant proteins were expressed in Rosetta (DE3) cells (Novagen). Cultures were grown at 37 °C until OD 600 nm = 0.6 and induced with 0.3 mM IPTG for 15h at room temperature. The cells were collected by centrifugation, washed with cold 10% glycerol, and stored at -20°C. Bacterial pellets were resuspended in lysis buffer (50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 0.5% Triton X-100, 10% glycerol) and disrupted with sonication. Bacterial lysates were incubated with Ni-NTA beads (QIAGEN, Valencia, CA) for 3 h at 4 °C. After extensive washing with a detergent-free buffer (50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 1mM DTT, 10% glycerol), proteins were eluted from Ni-NTA beads with 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 1mM DTT, 10% glycerol, and 250 mM imidazole. The eluted protein fraction was further purified by gel filtration chromatography using a HiLoad 16/60 Superdex 200 column (GE Healthcare, Piscataway, NJ) in 50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 1 mM DTT, and 10% glycerol. Purified proteins were frozen in liquid nitrogen and stored at -80 °C. The experiments shown were done with OPA1-S1 containing an N-terminal His_6 tag, but identical results were obtained with OPA1-S1 after cleavage of the tag with thrombin.

Preparation of liposomes

The following lipids were purchased form Avanti Polar Lipids, Inc (Birmingham, AL): 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate (POPA), L-α-lysophosphatidylinositol (PI), 1',3'-bis[1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (cardiolipin). Lipid vesicles compositions were designed to mimic the lipid composition of mitochondrial inner membranes (1). The lipid compositions of the liposomes, by percent-weight, were as follows:

PC liposomes=100% POPC;

PE liposomes =78% POPC, 22% POPE;

PI liposomes =70% POPC, 22% POPE, 8% PI;

CL liposomes =45% POPC, 22% POPE, 8% PI, 25% cardiolipin;

PA liposomes =45% POPC, 22% POPE, 8% PI, 25% POPA;

PS liposomes =45% POPC, 22% POPE, 8% PI, 25% POPS.

The indicated ratios of lipids were mixed in a chloroform solution, dried under a stream of nitrogen, subsequently evaporated for 3 h in a vacuum desiccator. The dried lipids were hydrated overnight in 50 mM HEPES (pH 7.0) at final concentration of 0.8 mg/ml. The resulting multilamellar liposomes were put through five freeze/thaw cycles. The vesicle suspensions were stored in the dark at 4 °C

Membrane tubulation assay

To visualize liposome tubulation, fluorescently labeled liposomes were immobilized in a flow cell. Flow cells were constructed by placing a coverslip over the microscope slide with double-side tape as spacers. An aliquot of 50 nm extruded PC liposomes doped with 0.1 % (mol/mol) 1,2-dipalmitoyl-*sn*-glycero-3- phosphoethanolamine-N-(biotinyl) (Avanti Polar Lipids, Inc) was injected, incubated for 5 min at room temperature, and then washed with buffer (50mM HEPES, 300 mM NaCl, 1m M DTT, pH 7.0). Under these conditions, the small PC liposomes rupture to form a bilayer coating the glass surface. Streptavidin was then added, incubated for 5 min at room temperature, and washed. Large multilamellar CL liposomes (45% POPC, 22% POPE, 8% PI, 25% cardiolipin) doped with 1 % (mol/mol) 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) (Molecular probes/Invitrogen, Carlsbad, CA) and 0.1 % (mol/mol) 1,2-dipalmitoyl-*sn*-glycero-3- phosphoethanolamine-N-(biotinyl) were then introduced into the chamber. This protocol resulted in immobilization of the biotinylated CL liposomes in the flow cell via a streptavidin bridge. OPA1-S1 was injected into the flow cell, and tubulation was observed by fluorescence microscopy (Axiovert 200, Carl Zeiss MicroImaging, Inc., Göttingen, Germany). Random fields were recorded with a digital camera (ORCA-ER, Hamamatsu Photonics, Shizuoka, Japan), and the number of tubulation events was manually scored.

Liposome aggregation assay

Large multilamellar CL liposomes were doped with 1 mol% DiO or 1 mol% 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) (Molecular Probes). The two distinctly labeled liposomes were mixed at a final concentration of 0.1 mg/ml, and OPA1-S1 was added at

a final concentration of 0.2 mg/ml. After incubation for 30 min at room temperature, images of the liposomes were recorded with fluorescence microscopy.

1. Ardail D, Privat JP, Egret-Charlier M, Levrat C, Lerme F, & Louisot P (1990) Mitochondrial contact sites. Lipid composition and dynamics. *J Biol Chem* **265,** 18797-18802.