Supplemental Methods.

Cloning, expression, and purification of Sec2p and Sec4p. The sequence for *S. cerevisiae* Sec2 (residues 17-167) was cloned into the plasmid pET15b (Novagen). The construct includes an N-terminal hexahistidine tag. *S. cerevisiae* Sec4 (residues 18-187) was cloned into a modified version of the pGEX4T-3 plasmid (Amersham Pharmacia), where a sequence coding for hexahistidine was inserted between the glutathione S-transferase sequence and the SEC4 gene. Both tags can be removed by thrombin cleavage.

Selenomethionine substituted Sec2p and Sec4p were each overexpressed in *Escherichia coli* BL21(DE3) cells at 20˚C according to established protocols (Doublie, 1997). The proteins were each purified by Ni-NTA chromatography (Qiagen) in the presence of 20 mM β-Me. Proteins were eluted from the Ni-NTA resin by cleavage with thrombin. The proteins were further purified by gel filtration on a Superdex-200 column (Amersham Pharmacia) in buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 5 mM $MgCl_2$, 10 mM DTT, and 5% (v/v) glycerol.

Sec2p/Sec4p complex formation and purification. To reconstitute the Sec2p/Sec4p complex, purified Sec2p and Sec4p were mixed together with a molar ratio of about 1:1.5, as judged by SDS PAGE. EDTA was added to a final concentration of 20 mM. The EDTA chelates magnesium ions, stripping both magnesium and bound nucleotides from Sec4p and thereby facilitating complex formation. The mixture was kept at 25˚C

for 10-16 hours; then complex was separated from excess, uncomplexed Sec4p by gel filtration on a Superdex-200 column and concentrated to 15 mg/ml.

Sec2p/Sec4p stoichiometry. To determine the stoichiometry of the Sec2p/Sec4p complex in solution, both the Sec2p GEF domain and the Sec2p/Sec4p complex were analysed in the W.M. Keck Foundation Biotechnology Resources Lab using size-exclusion chromatography coupled with low-angle laser light scattering, SEC-LS (Folta-Stogniew & Williams, 1999). The molecular mass of the Sec2p GEF dimer alone was 38.7 $\pm$ .7 kDa (compared to a theoretical value of 35.8 kDa), and that of the Sec2p/Sec4p complex was 58.7 $\pm$ .8 kDa (theoretical value 55.2 kDa). The difference corresponds to the mass of one Sec4p molecule.

Cloning, expression and purification of Sec4p mutants, other Rab GTPases and Sec4p chimeras. We constructed two Sec4p mutants, Phe45Ala and Ile50Ala, to test the importance of these residues for nucleotide exchange. Mutations were introduced into the pGEX4T3-Sec4p construct described above using the Quickchange site-directed mutagenesis kit (Stratagene).

To confirm Sec2p specificity for Sec4p, we assayed its exchange activity on other rab GTPases. Expression constructs for *Canis familiaris* Rab1a, human Rab2 and Rab6, and *Rattus norvegicus* Rab3a were gifts from the P. De Camilli, G. Warren and I. Mellman labs. Except for Rab3a, which has an N-terminal hexahistidine tag, these Rabs had N-terminal GST tags. Wild type *S. cerevisiae* Ypt1 (residues 1-180) and the same coding

sequence—but with switch I (residues 32-43) substituted by the Sec4p switch I (residues 44-55) --were cloned into the plasmid pET15b between the NdeI and BamHI restriction sites. A Sec4p construct similar to the wild type construct used in crystallization--but with switch I (residues 44-55) replaced by that from Ypt1p (residues 33-43) -- was cloned into the modified pGEX4T-3 plasmid. Sec4p constructs with switch II (residues 75-94), or the P-loop (residues 26-34) replaced by corresponding residues from Rab6 (68-87, 19-27) were constructed from the pGEX4T3-Sec4p construct described above using the Quickchange site-directed mutagenesis kit (Qiagen). A Rab6 construct with the switch II sequence from Sec4p was similarly constructed. All Rab proteins were overexpressed in BL21(DE3) cells at 16 ºC with induction by 0.2 mM isopropyl thiogalactoside (IPTG). Hexahistidine or GST-tags were used in affinity purification and were not removed, except in wild-type Sec4p or Sec4p/Ypt1 chimeras.

References:

Doublie, S. (1997) Preparation of selenomethionyl proteins for phase determination. *Methods Enzymol*. **276**, 523-530.

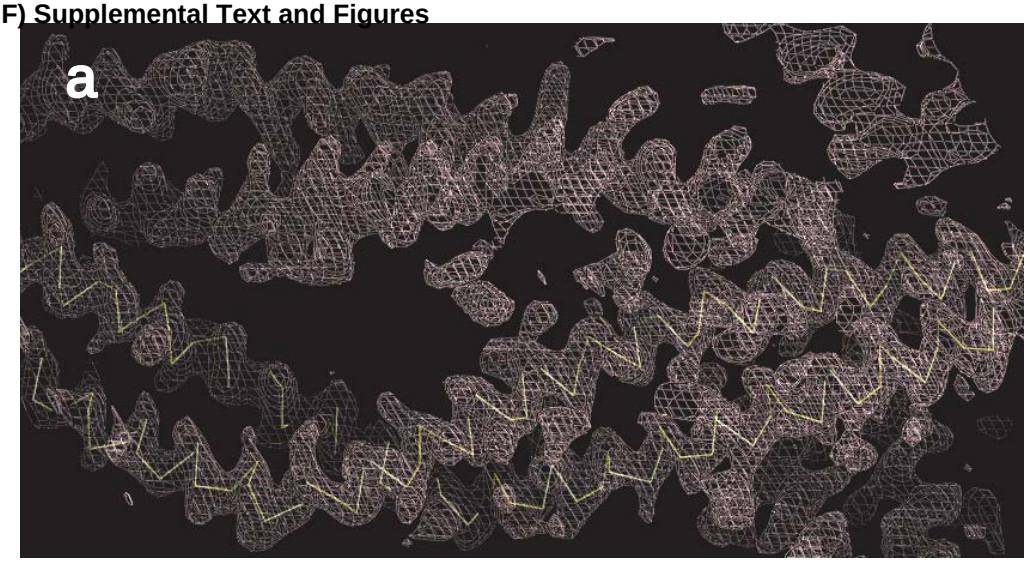
Folta-Stogniew, E. & Williams, K.R. (1999) Determination of molecular masses of protein: implementation of size exclusion chromatography/laser light scattering in a core laboratory. *J. Biomol. Techniques*, **10**: 51-63.

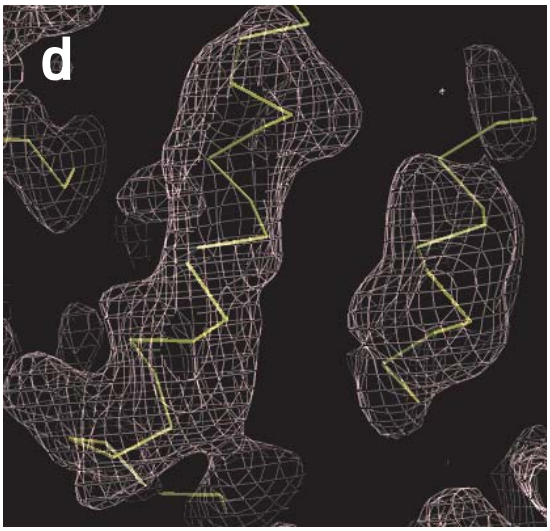
Supplemental Table 1. Data collection and refinement statistics

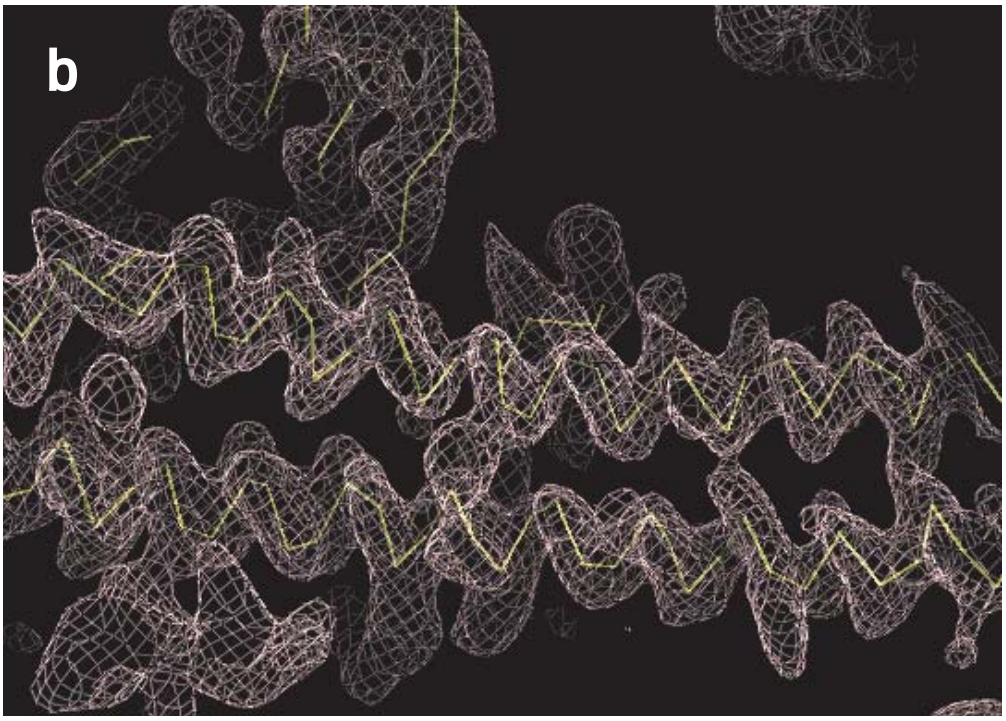
Data collection (anomalous data)	
Space group	$P6_122$
Cell dimensions	a=b=93.00 Å, c=295.91 Å
Wavelength (Å)	0.9794
Resolution (Å)	50-3.3 (3.42)
R_{sym} (%)	7.7 (30.5)
I/σI	14.5 (3.3)
Completeness (%)	97.5 (92.3)
Redundancy	4.2 (3.0)
Refinement	
Resolution (Å)	20.0-3.30
No. reflections	11,817
R_{work}/R_{free} (%)	25.0/32.5
No. protein atoms	3,595
B-factors (average) ($Å^2$)	
all	100.5
Sec2p	84.9
Sec4p	126.6
R.m.s. deviations	
Bond length (°)	0.007
Bond angle (Å)	1.2

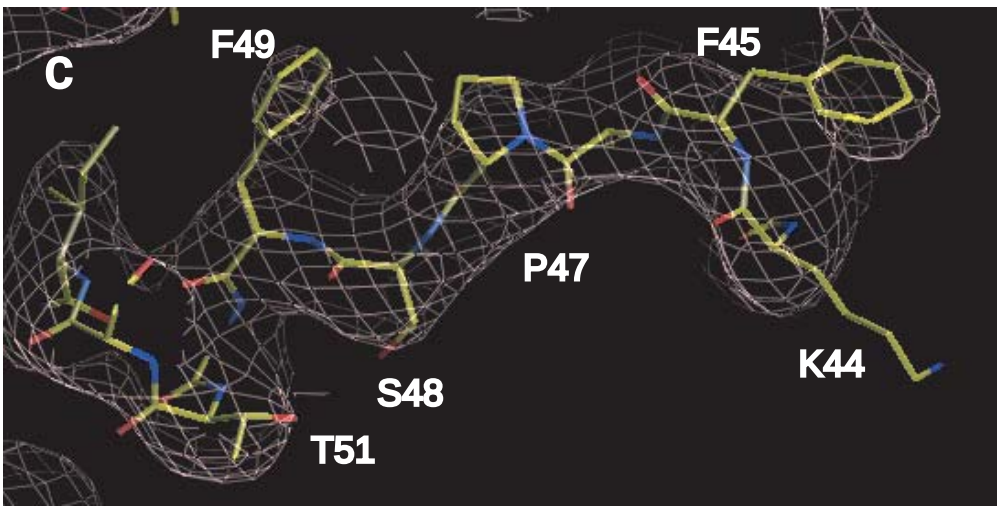
The highest resolution shell is shown in parenthesis.

F) Supplemental Text and Figures









Supplemental Figure 1.
Experimental electron density maps calculated from density modified SAD phases and contoured at 1.0 times the r.m.s. deviation of the map.
a. Density for the Sec2p coiled-coil. A Cα trace for part of one Sec2p dimer is shown.
b. Density for another portion of Sec2p. Parts of the Sec4p core beta sheet are also visible.
c. Density for switch I region of Sec4p, which interacts with Sec2p and is relatively well ordered within the crystal structure.
d. Density for poorly ordered alpha helices in Sec4p.