The conserved oligomeric Golgi (COG) complex is strongly implicated in retrograde vesicular trafficking within the Golgi apparatus. Although its mechanism of action is poorly understood, it has been proposed to function by mediating the initial physical contact between transport vesicles and their membrane targets. An analogous role in tethering vesicles has been suggested for at least six additional large multisubunit complexes, including the exocyst, a complex essential for trafficking to the plasma membrane. Here we report the solution structure of a large portion of yeast Cog2p, one of eight subunits composing the COG complex. The structure reveals a six-helix bundle with few conserved surface features but a general resemblance to recently determined crystal structures of four different exocyst subunits. This finding provides the first structural evidence that COG, like the exocyst and potentially other tethering complexes, is constructed from helical bundles. These structures may represent platforms for interaction with other trafficking proteins including SNAREs (soluble N-ethylmaleimide factor attachment protein receptors) and Rabs.

The conserved oligomeric Golgi (COG) complex is essential for normal Golgi morphology and function, yet its structure and precise role are poorly understood. In the yeast Saccharomyces cerevisiae, two COG subunits were originally discovered as Sec34p (now called Cog3p) and Sec35p (Cog2p); yeast carrying temperature-sensitive mutations in either of these subunits display severe phenotypes in yeast and Chinese hamster ovary cells (1, 26). Second, a pool of soluble Cog2p exists in yeast cytosol, suggesting that free Cog2p is likely well folded in vitro and function intra-Golgi transport assay (5, 6). In vivo, depletion of COG causes defects in glycosylation targeting of small vesicles at non-permissive temperatures (1). In mammalian cells several lines of evidence likewise argue for a trafficking role for COG (for review, see Refs. 2–4). Perhaps most directly, mammalian COG was initially purified based on its ability to stimulate an in vitro intra-Golgi transport assay (5, 6). In vivo, deletion of COG causes defects in glycosylation that appear to arise from defects in retrograde trafficking within the Golgi (7–10). Consistent with a retrograde role for COG, genetic and physical interactions between COG and components of the coat protein I (COPI) vesicle coat have been reported in several systems (10–13).

The COG complex belongs to a group of multisubunit protein assemblies commonly termed “tethering complexes” (14–17). Tethering complexes are thought to act upstream of SNAREs, mediating the initial attachment of intracellular trafficking vesicles to their membrane targets. Many, if not all, tethering complexes are also Rab effectors. One model for the molecular function of tethering complexes is that they act as protein interaction hubs, orchestrating the sequential actions of Rabs and SNAREs (and potentially other proteins) during the process of vesicle docking and fusion (4).

A hurdle in critically evaluating such models is a dearth of structural information. Recently, structures of several subunits of the exocyst complex have been determined (18–23). These results are of particular relevance to COG because detectable sequence homology has been reported between regions of some exocyst and COG subunits (17, 24), although the possibility that this homology represents convergent evolution has been raised recently (25). To begin to investigate the structure of COG, we have initially focused on the Cog2p (Sec35p) subunit. The choice of Cog2p was based on several considerations. First, cog2 mutants display severe phenotypes in yeast and Chinese hamster ovary cells (1, 26). Second, a pool of soluble Cog2p exists in yeast cytosol, suggesting that free Cog2p is likely well folded (Ref. 27; but see also Ref. 24). Third, Cog2p is relatively modest in size; at 30 kDa, it is the smallest of the yeast subunits (although its mammalian ortholog is much larger at 83 kDa). Fourth, initial attempts to overproduce Cog2p for the production of antibodies (27) revealed that recombinant Cog2p was largely soluble in Escherichia coli, boding well for structural studies.

Here we report the structure of a fragment constituting a major portion of yeast Cog2p (residues 61–262), determined using multidimensional NMR. Residues 61–108, which are important for solubility in vitro and function in vivo, populate helical conformations in this Cog2p fragment but do not appear
to adopt a fixed tertiary structure. The remainder of the fragment (residues 109–262) forms a six-helix bundle. The fold bears a general resemblance to exocyst subunit domains, strengthening the hypothesis that helical bundle domains are a common structural unit from which both COG and exocyst complexes are constructed (22, 28).

**EXPERIMENTAL PROCEDURES**

**Protein Overexpression and Purification—** Recombinant Cog2 constructs were generated by PCR and expressed in *E. coli* BL21 as glutathione S-transferase fusion proteins using the expression plasmid pGEX-4T1 (GE Healthcare). Cultures were grown in Luria Bertani media at 37 °C to an *A*₅₅₀ of 0.6–0.8 before adding isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.5 mM. Cells were grown an additional 12–16 h at 23 °C and then harvested by centrifugation and resuspended in buffer 300 (300 mM NaCl, 20 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol, 4 mM EDTA) supplemented with 1 mM phenylmethylsulfonyl fluoride. The resuspended cells were lysed using an EmulsiFlex homogenizer (Avestin); the resulting lysates were cleared by centrifugation at 24,000 × *g* and applied to glutathione-agarose resin (Sigma). After washing the immobilized fusion proteins sequentially with buffer 300, buffer 500 (containing 500 mM NaCl), and buffer 150 (containing 150 mM NaCl), the Cog2p moiety was released by thrombin cleavage. Thrombin was removed from the eluate using benzamidine-agarose affinity chromatography (GE Healthcare). Final purification was accomplished using size exclusion chromatography (Superdex 75 or Superose 12; GE Healthcare) in buffer 300. For isotopic labeling, cells were instead grown in M9 minimal media with [15N]HCl and/or uniformly [13C]-labeled glucose (Cambridge Isotope Laboratories) as the sole source of nitrogen and/or carbon (29). Cultures in M9 media were grown as above but were harvested 4 h after induction.

**Circular Dichroism—** Circular dichroism (CD) experiments were carried out using 7–15 μM protein in 1 mM potassium phosphate (pH 7.0), 100 mM KCl, 250 mM β-mercaptoethanol. Protein concentration was determined using a ninhydrin assay (30). Spectra were collected in a 0.1-cm path length quartz cuvette using Aviv 62DS or Jasco 810 CD spectropolarimeters. All wavelength scans were collected at 4 °C with 1-s averaging times and represent the average of three scans.

**Generation of Yeast Expression Constructs—** Plasmids were based on pSV15 (27), which contains the entire Cog2 gene along with ~500 bp of genomic flanking sequence at both the 5’ and 3’ ends, in a pRS415 background (31, 32). Each deletion was created by PCR, using 5’ primers designed to loop out a region corresponding to residues 2–60 or 2–96. The 5’ primers included a HindIII site upstream of the start codon, whereas the 3’ primer included a BamHI site in the 3’-flanking region. Yeast cells were co-transformed with an excess of the resulting PCR product together with pSV15 that had been digested with BsgI and AvrII to remove sequences corresponding to Cog2p residues 57–262 and a portion of the 3’-flanking region. Transformants capable of growing on SC–Leu were screened by PCR for the desired deletions, the presence of which was subsequently confirmed by DNA sequencing.

**Structure of Cog2p**

**Haploid Growth Curves—** Colonies from single germinated spores were grown in 5 ml of rich media (yeast extract/peptone/dextrose) overnight at 30 °C. From these cultures, 2.5 *A*₅₅₀ units were transferred to 25 ml of fresh media in a 250-ml Erlenmeyer flask, for an initial *A*₅₅₀ of ~0.1, and the optical density was monitored during a further 12 h of growth at 30 °C.

**NMR Sample Preparation—** Purified Cog2p fragments were exchanged into 3 mM Tris-HCl (pH 7.0), 10 mM NaCl, 1 mM dithiothreitol, 0.5 mM EDTA, 5% D₂O, and 0.02% sodium azide using a NAP5 column (GE Healthcare). The exchanged proteins were concentrated to ~1 mg using pre-rinsed UltraFree 4 centrifugal concentrators (Millipore).

**Data Collection and Processing—** All data were acquired for Cog2-(61–262) using Varian Inova spectrometers. Preliminary NMR experiments were performed at Princeton University using a 600-MHz instrument. All of the spectra used for structure determination were acquired at the Environmental and Molecular Sciences Laboratory at Pacific Northwest National Laboratory. 13C,H HSQC, HCC-HOCSY, (H)(CO)NH-TOSY, (H)CO(NH)-TOSY, HNCO, and HSQC spectra were collected on a 600-MHz instrument; CBCA(CO)NH, HNCACB, HNCO, and HSQC spectra were collected on a 750-MHz instrument, and 13C- and 15N-edited and 13C,15N-edited NOESY spectra were collected on an 800 MHz instrument. The spectral widths and number of complex points in the F3, F2, and F1 dimensions, with the number of scans per free induction decay, indicated in parenthesis, were: aliphatic 13C,H HSQC, 8000 × 21128.7 Hz, 1024 × 256 (8); aromatic 13C,H HSQC, 8000 × 4800.2 Hz, 1024 × 256 (8); HCH-TOSY, 8000 × 8000 × 12073.6 Hz, 1024 × 124 × 32 (8); (H)(CO)NH-TOSY, 8000 × 4501.3 × 2007.1 Hz, 1024 × 80 × 32 (32); HNCO, 10500.1 × 2262.3 × 2279.2 Hz, 1024 × 100 × 64 (8); CBCA(CO)NH, 10500.1 × 15078.6 × 2279.2 Hz, 1024 × 108 × 64 (32); HNCACB, 10500.1 × 15078.6 × 2279.2 Hz, 1024, 108, 64 (32); HSQC, 10500 × 2279.2, 1024 × 128 (8); 13C,H NOESY-HSQC (with carbon carrier in the aliphatic region), 10999.6 × 9599.2 × 4199.9 Hz, 1024 × 256 × 64 (8); 13C,H NOESY-HSQC (with carbon carrier in the aromatic region), 10999.6 × 9599.2 × 4499.9 Hz, 1024 × 256 × 64 (8); 13C,15N NOESY-HSQC, 10999.6 × 9600 × 2431.5 Hz, 1024 × 256 × 100 (8). Standard Protein-Pack pulse sequences were used for all experiments. Preliminary spectra were acquired at 25 °C, and spectra used for structure determination were acquired at 35 °C. Spectra were processed using NMRPipe (33) and analyzed using NMRView (34).

**Data Analysis and Structure Calculation—** Backbone and most side-chain resonances were assigned using gradient-enhanced HNCO, HNCACB, CBCA(CO)NH, (H)(CO)NH-TOSY, HCC-HOCSY, and 13C,H HSQC-NOESY spectra and standard assignment procedures (35–37). Aromatic side chain resonance assignments required homonuclear two-dimensional TOCSY, NOESY, and double quantum-filtered COSY experiments and a 13C,H NOESY-HSQC experiment acquired with the carbon carrier in the center of the aromatic region. A 13C,H HSQC experiment performed on 10% 13C- and 15N-labeled Cog2-(61–262) was obtained to determine stereospecific assignments of valine and leucine methyl groups. φ and ψ
torsion angle restraints were predicted by the program TALOS (38) based on backbone chemical shifts. Dihedral angle predictions were restrained to 1.5 times the S.D. observed in the TALOS data base, with a minimum of 22.5°. H-bond restraints were assumed for regions of the protein exhibiting strongly helical chemical shift indices, specifically 108–128, 132–150, 159–176, 185–207, 215–242, 249–258. Structures calculated without these restraints were of similar energy and fold. Structures were calculated using CNS (39) and evaluated using CNS, AQUA, and ProcheckNMR (40). A total of 1200 structures was calculated, and the 20 structures with the lowest NOE energy were selected. Structure figures were generated using PyMOL (41).

Alignment and Structure Comparison—BLAST searching revealed three Cog2p homologs with $E < 10^{-17}$, all of them from other fungi. The next best score was $E = 0.06$; iterative searching using Psi-BLAST was needed to detect more distant homologs including human Cog2p (23% sequence identity over 111 residues). ClustalW (42) was used to align S. cerevisiae Cog2p with the three other fungal homologs, Candida glabrata (40% identity over 253 residues), Ashbya gossypii (29% identity over 254 residues), and Kluyveromyces lactis (31% identity over 243 residues). The alignment figure was produced using Alscript (43). Buried residues were defined as those residues with <15% of their side chains exposed to solvent, as calculated using WHAT IF (44).

The previously determined exocyst subunit structures were divided into domains according to the description of each structure in the corresponding original report (18–23). Pairwise Z scores and root mean square deviations for each domain comparison were then calculated using DaliLite (45).

**RESULTS**

The S. cerevisiae Cog2 subunit, originally named Sec35p, contains 262 residues. CD spectroscopy revealed that recombinant Cog2p is highly (∼85%) α-helical (Fig. 1A). Our efforts to produce diffraction quality crystals of the full-length protein were, however, unsuccessful. To identify large fragments that might constitute more favorable targets for structural analysis, we subjected recombinant Cog2p to limited proteolysis using a battery of nonspecific proteases. Two cleavage products appeared, based on their electrophoretic mobility, to be proteolytic fragments with <15% of their side chains exposed to solvent, as calculated using WHAT IF (44).

While scaling up production of Cog2p-(56–262), we observed that it precipitated at concentrations greater than 1 mg/ml. A more soluble variant was produced by eliminating five predominately hydrophobic residues (His-Tyr-Leu-Pro-Leu) to generate Cog2p-(61–262), and this variant was overexpressed and purified to >95% homogeneity. Its CD spectrum was indistinguishable from that of Cog2p-(56–262) (Fig. 1A). Importantly, Cog2p-(61–262) remained soluble and monomeric at concentrations in excess of ∼20 mg/ml (1 mM), as judged by gel filtration and dynamic light scattering (data not shown). Because of its excellent solution properties, Cog2p-(61–262) became the subject of most of the subsequent studies described here. This fragment contains 77% of the full-length Cog2p subunit, including almost all of the “conserved amphipathic helical region” (residues 60–125) identified by Whyte and Munro (17, 24) near the N terminus of several COG, exocyst, and Golgi-associated retrograde protein (GARP) subunits.

To determine whether the N-terminal-truncated versions of Cog2p identified by limited proteolysis were functional in vivo, we analyzed whether they could substitute for the full-length protein in S. cerevisiae. Deletion of the COG2 gene leads to a very severe growth defect at 30 °C and complete inviability at 21 °C (27). CEN-based yeast plasmids were constructed to express a truncated COG2 gene under the control of its own
promoter. These plasmids were transformed into a diploid yeast strain with only one copy of the COG2 gene (Research Genetics); the other copy of COG2 was deleted and replaced by the G418 marker. After sporulation and tetrad dissection, haploid spores were tested for the ability to grow into colonies. Because spores lacking COG2 grow extremely slowly, tetrads from the strain transformed with the parent plasmid (empty vector) never yielded more than two colonies. The same result was observed for a strain transformed with an expression plasmid encoding Cog2-(97–262). Thus, Cog2-(97–262) was unable to functionally replace full-length Cog2p, perhaps because it lacks more than half of the conserved amphipathic helical region. For the strain expressing Cog2-(61–262), however, 16 of 36 dissected tetrads yielded four viable spores. The presence of both the expression plasmid and the chromosomal deletion within the same spores was confirmed by growth on selective media. Dissected tetrads yielding fewer than four viable spores are readily explained by the random segregation of both the plasmid expressing Cog2-(61–262) and the intact chromosomal copy of COG2 into the same spore. These results establish that Cog2-(61–262), but not Cog2-(97–262), can functionally substitute for full-length Cog2p.

The colonies formed by the strain expressing only Cog2-(61–262) were smaller than the colonies formed by strains expressing full-length Cog2p. A significant growth defect was also observed in liquid culture, with the cells expressing only Cog2-(61–262) displaying a 2.7-fold longer doubling time compared with a control strain (Fig. 1B). Nonetheless, it was clear that Cog2-(61–262) provides sufficient Cog2p function to partially rescue the growth of haploid cells lacking full-length Cog2p. Based on the excellent solution properties of this fragment together with the observation that a fragment of similar apparent molecular weight was produced as a limited digest product by all four proteases tested (chymotrypsin, proteinase K, subtilisin, and trypsin), we conclude that Cog2-(61–262) retains much of the core structure as well as the core function of the full-length protein.

Although Cog2-(61–262) formed large crystals, they did not diffract beyond 8 Å resolution, precluding x-ray structure determination. At 23.4 kDa, however, Cog2-(61–262) presented a potential target for structure determination by multidimensional NMR. The dispersion and relatively uniform intensity of cross peaks in H,15N HSQC spectra confirmed that Cog2-(61–262) is folded and stable (Fig. 2). The size and high α-helicity of the protein gave rise to severe spectral overlap. Nonetheless, by using three-dimensional spectra to resolve ambiguities, it proved possible to make backbone resonance assignments for 182 non-proline residues (91% completeness).

Comparison of the Cα chemical shifts observed for Cog2-(61–262) with those of a random coil revealed six unambiguously α-helical regions: amino acids 107–127, 133–153, 158–
FIGURE 4. Solution structure of Cog2-(109–262). A, 1200 structures were calculated for Cog2-(109–262); residues 61–108 were omitted from structure calculations because of an absence of long-range NOEs (see text for details). The 20 structures with the lowest NOE energy are shown as a stereo pair. B, stereo view of the lowest energy structure, with helices colored in rainbow order from N (blue) to C (red) terminus. C, sequence alignment of selected fungal Cog2 proteins (see “Experimental Procedures”) showing invariant (red) and conserved (yellow) residues. Magenta circles mark acidic patch residues (see also Fig. 5B). Black arrowheads mark buried residues. Cylinders represent α-helices, colored as in B; gray shading represents helices predicted by Whyte and Munro (24).
TABLE 1
Structure calculation statistics for Cog2p-(109–262)

Presented is an ensemble of 20 lowest energy structures, all of which have NOE energies below 6.8 kcal/mol. There were no NOE violations larger than 0.2 Å or dihedral angle violations above 2°. r.m.s., root mean square.

| Average r.m.s. deviation from experimental restraints (Å) | | |
|---|---|---|
| NOE distance restraints | All | 2915 | 0.005 ± 0.0002 |
| Intraresidue | 846 | 0.005 ± 0.0005 |
| Sequential ($i$ − $j$ = 1) | 733 | 0.006 ± 0.0004 |
| Short range ($i$ − $j$ ≤ 2–4) | 781 | 0.006 ± 0.0006 |
| Long range ($i$ − $j$ > 4) | 555 | 0.002 ± 0.0003 |
| Hydrogen bonds (Å) | 194 | 0.005 ± 0.0004 |
| Dihedral angles (%) | 253 | 0.029 ± 0.009 |

| Average r.m.s. deviations from idealized covalent geometry | | |
|---|---|---|
| Bonds (Å) | 0.0012 ± 0.00005 |
| Angles (°) | 0.322 ± 0.005 |
| Improvers (°) | 0.158 ± 0.007 |

| Ramachandran plot statistics* | | |
|---|---|---|
| Most favorable regions (%) | 88.9 |
| Additional allowed regions (%) | 9.1 |
| Generously allowed regions (%) | 1.5 |
| Disallowed regions (%) | 0.5 |

| Average r.m.s. deviations of atomic coordinates (Å) | Among 20 structures | To average structure |
|---|---|---|
| Heavy atoms, residues 109–258 | 1.63 | 1.12 |
| Backbone (N, Cα, C, O) | 0.94 | 0.65 |
| Heavy atoms, helices only | 1.31 | 0.90 |
| Backbone (N, Cα, C, O), helices only | 0.68 | 0.47 |

* Calculated using the program PROCHECK NMR (57).

Cog2p primary amino acid sequences are highly divergent across species. Nonetheless, alignment of Cog2p with three other fungal Cog2p sequences (pairwise sequence identity with S. cerevisiae Cog2 29–41%; see “Experimental Procedures”) revealed 16 residues that are strictly conserved across all four sequences and 32 more that are similar (Fig. 4C). The majority of the conserved residues present in the NMR structure are buried (black triangles in Fig. 4C), suggesting that they play a largely structural role. In particular, no region of the protein surface displays a significant clustering of conserved residues. Close examination of the protein surface does, however, reveal two distinctive features, a broad acidic stripe across one end of the bundle (Fig. 5B) and a hydrophobic groove formed by the C-terminal portion of Cog2p (Fig. 5C). These features constitute potential protein-protein interaction surfaces.

**DISCUSSION**

The COG complex is a heterooctamer implicated in retrograde trafficking within the Golgi apparatus. It has been proposed to act as a tethering factor, mediating the initial interaction between trafficking vesicles and their compartmental destinations. COG and other so-called tethering factors may...
act as protein interaction hubs, coordinating the interplay of vesicular transport factors leading ultimately to SNARE complex formation and membrane fusion. A major impediment to further progress in understanding the mechanistic basis for COG function has been the lack of detailed structural information. Indeed, although the network of protein-protein interactions within the COG complex has been the subject of several earlier studies (48–51), there has not previously been high resolution structural information for any COG subunit. In this work we have determined the NMR structure of the stable core (residues 109–262) of *S. cerevisiae* Cog2p.

The Cog2p structure is not unique in lacking N-terminal residues. Indeed, previous structures of exocyst subunits (Sec6p, Sec15p, Exo70p, and Exo84p) all lack at least 62 N-terminal residues (in the case of Exo70p) and in most cases significantly more (18, 20, 22, 23). On the other hand, NMR and crystal structures of the N-terminal Ral binding domain of Sec5p have also been reported (19, 21). This structure adopts an all-β immunoglobulin-like fold bearing no resemblance to the α-helical bundles observed for the other exocyst subunits and Cog2p. In the majority of cases, however, the N-terminal regions appear recalcitrant to structural characterization. It is possible that they are poorly ordered in the absence of the other subunits of the complex. Alternatively, like some SNARE proteins, they might be “natively unfolded.”

The six-helix bundle structure of Cog2-(109–262) does not have striking conserved surface features to guide functional experiments. Indeed, the majority of the amino acid residues conserved among fungal Cog2 subunits are buried, suggesting that they play a role in maintaining structure and stability. A large acidic patch is evident on the surface of the protein (Fig. 5B); however, the residues composing it are not particularly well conserved (Fig.
4C). A second potentially important feature of the Cog2p structure is a groove, formed largely by the fourth and fifth α-helix, where a number of hydrophobic residues are at least partially exposed (Fig. 5C). The majority of the residues contributing to this groove are hydrophobic in all of the aligned sequences. However, genetic evidence suggests that this groove cannot be required for the essential function of Cog2p. A temperature-sensitive mutation in Cog2p (sec35-I) results in the conversion of Tyr-195 to a stop codon (Fig. 4C), removing virtually all of the residues that contribute to the hydrophobic groove. Nonetheless, the sec35-I strain displays no growth defect at temperatures 21–30 °C (27). Overall, therefore, we were unable to identify conserved surface features on Cog2-(109–262) that are essential for its function. Although we cannot rule out that the surface of Cog2p has evolved in conjunction with its functional partners, compromising our ability to detect protein-protein interaction sites through the identification of conserved surfaces, it appears likely that this domain plays a fundamentally structural role in the COG complex.

Our functional studies suggest that essential regions of Cog2p are located more N-terminally. Whyte and Munro (24) identified a weakly conserved amphipathic helical region within Cog2p (residues 60–125) and several other subunits of the COG, exocyst, and GARP complexes. Our results are consistent with the prediction that residues 60–82 and 92–125 form helices, although the apparent lack of fixed tertiary structure for residues 61–108 means that we are reliant on chemical shift data alone (Fig. 3) to make helix assignments within this region. In vivo, deleting residues 1–60 compromised but did not abolish function, slowing growth ~3-fold (Fig. 1B), whereas deleting residues 1–97 was lethal. The deleted residues may be important for interaction with another COG subunit and, therefore, for the structural integrity of the complex. Alternatively, or in addition, this region may be important for the interaction between COG and another protein with which it functionally collaborates (e.g. a COPI subunit, a Rab protein, or a SNARE). In either case, the intrinsic helicity of the region suggests that it may retain a helical structure in its complexed state. It is interesting to note that both SNAREs and Rab proteins generally recognize helical regions in their functional partners.

Previous reports (18–23) had demonstrated that four different subunits of the exocyst complex all contain helical modules, resulting in extended structures or rods (28). The six-helix bundle of Cog2-(109–262) resembles these helical modules (Fig. 5A), but the presence of only a single module makes the architectural similarity with the exocyst somewhat uncertain. It is worth noting in this regard that the Cog2 subunit is frequently much larger, especially in higher eukaryotes but also in some other fungi, than it is in S. cerevisiae. By analogy with exocyst subunits, it is possible that orthologs of Cog2p contain two (or more) helical bundles. Overall, although further structural investigations are clearly essential, the finding that yeast Cog2p, and presumably its orthologs in higher eukaryotes, contain one or more exocyst-like helical bundles provides an indication of architectural similarity between COG and exocyst complexes that complements indications of functional similarity.

The transport protein particle (TRAPP) complexes I and II are multisubunit tethering complexes essential for trafficking to the Golgi apparatus (52–54). The TRAPP complexes lack any similarity to the COG or exocyst complexes. Several TRAPP subunits do, however, bear a structural resemblance to the “lon- gin” domains found at the N terminus of vesicle (v-)SNAREs including Ykt6p, Sec22b, and Nyv1p (55, 56). This observation led to the suggestion that TRAPP may play a role in SNARE assembly or function. Cog2-(109–262), by contrast, bears a structural resemblance to target membrane (t-)SNAREs with N-terminal helical bundle regulatory domains. Indeed, three of the top eight scores in a Cog2-(109–262) Dali search were achieved by SNAREs (syntaxin 1A (Protein Data Bank (PDB) code 1dn1)) or their isolated N-terminal domains (Tlg1p, PDB code 2c5i) and syntaxin-12 (PDB code 2dnu)). Perhaps during the sequence of events that accompany SNARE assembly and function, helical bundle tethering complexes displace or interact with helical bundle SNARE regulatory domains.

It is intriguing to speculate that exocyst/COG helical bundle subunits are tailored to interact with SNAREs that contain helical bundle domains, whereas TRAPP complex longin domain subunits are tailored to interact with SNAREs that contain longin domains. This hypothesis would explain the observation that tethering protein complexes and SNARE regulatory domains both appear to fall into two main classes. Future efforts to test these and other models will be crucial in understanding the mechanistic basis for tethering complex function.

In conclusion, we have taken an initial step in elucidating the structure, and, ultimately, the molecular function, of the COG complex by determining the NMR structure of the folded core of the subunit, Cog2p. At 23 kDa, this core fragment of a single subunit represents a small part of the entire complex (515 kDa, assuming one copy of each subunit (24)). Nonetheless, its structure reveals an unanticipated similarity with each of the recently determined structures of individual exocyst complex subunits. Our results add new support to the hypothesis that the exocyst and COG complexes share some general structural, and possibly functional, features.

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