The conserved oligomeric Golgi (COG) complex is thought to function in intra-Golgi retrograde trafficking mediated by coat protein I vesicles, a pathway essential for the proper structure and function of the Golgi apparatus. Previous work suggested that COG might act as a tethering factor to mediate the initial attachment between coat protein I vesicles and Golgi membranes. Here, we present extensive in vitro co-translation and immunoprecipitation experiments leading to a new model for the overall architecture of the mammalian COG complex. The eight COG subunits (Cog1–8) are found to form two heterotrimeric subassemblies (Cog2/3/4 and Cog5/6/7) linked by a heterodimer composed of the remaining subunits (Cog1/8). This model is in excellent agreement with in vivo data presented in an accompanying paper (Oka, T., Vasile, E., Pemman, M., Novina, C. D., Dykxhoorn, D. M., Ungar, D., Hughson, F. M., and Krieger, M. (2005) J. Biol. Chem. 280, 32736–32745).

The correct sorting of proteins into different compartments is crucial for every cell. In the eukaryotic secretory pathway, sorting is accomplished by a combination of vesicular transport and organelle maturation (1). The Golgi apparatus plays a particularly central role in the secretory pathway, serving as a hub from which transport vesicles carrying different cargos depart toward different destinations (2). Many proteins are glycosylated as they pass through the successive cisternae of the Golgi stack (3, 4). Within the Golgi apparatus, the glycosylation enzymes that carry out these reactions maintain a nonuniform distribution through a balancing of anterograde and retrograde movement (5, 6). In particular, retrograde movement is crucial for the retrieval of cis-Golgi components from later Golgi cisternae and utilizes coat protein I (COPI)1-coated transport vesicles (7–9). The conserved oligomeric Golgi (COG) complex is essential for proper Golgi structure and plays a major role in glycosylation enzyme homeostasis (3, 10, 11). Mutations in human COG can cause congenital defects in glycosylation (3, 10, 11). COG lacks discernible transmembrane domains and is peripheral associated with the Golgi (17, 27–29). Genetic and biochemical evidence supports dividing the complex into two groups of four subunits, Cog1–4 and Cog5–8 (11, 25). Cog5–8 have been proposed to form a stable subcomplex in mammalian cytosol (11, 30, and 31). Further structural and functional information about tethering proteins. In addition to their putative role in physically linking membranes, tethering factors may also facilitate the engagement of membrane-bridging complexes between soluble NSF-attachment protein receptors (SNAREs). All of the proposed tethering factors display genetic and/or physical interactions with SNARE proteins (21). For example, subunits of the yeast COG complex interact with at least five Golgi SNAREs (14–16, 26, 27). Both yeast and mammalian COG have also been shown to interact with the COPI coat protein, which is present on retrograde vesicles (13–16). Given this network of protein interactions, it is conceivable that COG, or even tethering proteins generally, orchestrate sequential processes triggered by activated Rab proteins that begin with vesicle attachment and conclude with the assembly of SNARE complexes. Such models remain speculative, however, in the absence of further structural and functional information about tethering proteins. Mammalian COG is a large complex containing eight different polypeptides (Cog1-Cog8) with molecular masses ranging from 70 to 110 kDa (11). COG lacks discernible transmembrane domains and is peripherally associated with the Golgi (17, 27–29). Genetic and biochemical evidence supports dividing the complex into two groups of four subunits, Cog1–4 and Cog5–8 (11, 25). Cog5–8 have been proposed to form a stable subcomplex in mammalian cytosol (11, 30, and this study), and deleting any of these subunits in yeast or drastically reducing their expression in mammalian cells (12, 25, 30) has very mild effects. By contrast, deleting any of the mammalian or yeast subunits Cog1–4 is deleterious or even lethal (3, 11, 25, 28, 31). Furthermore, quick freeze/deep etch/rotary shadow electron microscopy of gluteraldehyde-
dehydrate-fixed bovine COG revealed two lobes of similar size (11). Taken together, these observations led to the proposal that COG consists of two lobes, lobe A (Cog1–4) and lobe B (Cog5–8) (11). This hypothesis received further support from a recent analysis of binary subunit interactions within the COG complex (32).

Here, we have used systematic biochemical approaches to better define the overall architecture of the COG complex. Our data suggest that the basic bilobed model is oversimplified; instead, we propose a new model in which two heterotrimeric subcomplexes are connected by a heterodimer.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The GenBank accession numbers for the COG subunit cDNAs used were as follows: COG1, NM_018714; COG2, NM_007357; COG3, NM_031431; COG4, NM_015386; COG5, NM_006348; COG6, NM_020751; COG7, NM_153603; COG8, NM_032382. The vectors used are indicated below. It should be noted that the Kazusa COG1 cDNA clone KIAA1381, used in other studies (32), encodes an incompletely processed COG1 cDNA. Nonetheless, we did not observe functional differences between the Kazusa cDNA and the correct cDNA used in this study.

**Antibodies**—Antibodies and antiserum used in this work included rabbit anti-Cog4 serum (CRP Inc., Berkeley, CA).

**Yeast Two-hybrid Assays**—All COG subunit genes were cloned as both Gal4 activation domain and binding domain fusion constructs using the pGAD/pGBDU vectors (33). The different fusion constructs were transformed into yeast of opposite mating type (genotype: trp1–901 leu2–3,112 ura3–52 his3–200 gal4::GAL7-lacZ::LYS2::GAL1-HIS3 GAL2-AD2 met2::GAL7-lacI2). Diploid yeast containing all pairwise combinations of COG subunits plus empty vector controls were created by mass mating. The diploids were subsequently scored for growth on media lacking histidine or adenine and for the level of β-galactosidase expression, as measures of subunit interactions. Only the Cog2/4, Cog5/7, and Cog4/5 pairs showed strong bidirectional interactions (e.g. both AD-Cog2 with BD-Cog4 and AD-Cog4 with BD-Cog2). Strong unidirectional interactions were observed between Cog1/8, as well as between Cog6 and itself (Fig. S1). Additional weaker or unidirectional interactions, the importance of which is unclear, are also summarized in supplemental Fig. S1.

Because the yeast two-hybrid screen did not yield a comprehensive map of subunit interactions and because this method is prone to false negative as well as false positive results (34), we used a second approach to verify and extend the list of interactions. We chose in vitro co-translation of subunits followed by immunoprecipitation; a similar pairwise approach was used to map out subunit interactions within the yeast exocyst complex (35). In subsequent experiments, co-translation and immunoprecipitation of 3–6 subunits was used to build and test structural models.

In briefly, we constructed 16 plasmids in which the cDNA encoding each subunit is under the control of the T7 promoter and is either untagged or tagged with a C-terminal HA epitope for immunoprecipitation with an anti-HA antibody. These plasmids were linearized and transcribed in vitro to generate mRNA. The quantity of mRNA required to obtain efficient translation in a reticulocyte lysate was individually determined for each of the 16 constructs to allow all experiments to be performed under conditions in which the co-translated subunits were present in similar amounts. Furthermore, to rule out the possibility that endogenous COG might cause spurious interactions between co-translated COG subunits, we used immunoprecipitation with several anti-COG subunit antibodies to verify that detectable endogenous COG was absent from the lysates (Fig. 2C, lane R, and data not shown).

Representative autoradiograms are shown in Fig. 1, A and B. Each includes, as controls, translation reactions of the individual subunits. Similar controls were included in every experiment but are omitted from subsequent figures for clarity. Cog8 and HA-tagged Cog1 interact (Fig. 1A); both subunits when co-translated (IHA + 8, input lane) were recovered in the immunoprecipitate (IHA + 8, IP lane). Untagged Cog1

**RESULTS**

**Pairwise COG Subunit Interactions**—To establish the subunit connectivity within the mammalian COG complex, we first used a directed yeast two-hybrid approach. Every possible pairwise human COG subunit interaction was tested using Gal4 activation (AD) and binding (BD) domain fusion constructs (33) introduced pairwise into diploid yeast. The diploid strains were monitored for growth on media lacking histidine or adenine and for the level of β-galactosidase expression, as measures of subunit interactions. Only the Cog2/4, Cog5/7, and Cog4/5 pairs showed strong bidirectional interactions (e.g. both AD-Cog2 with BD-Cog4 and AD-Cog4 with BD-Cog2). Strong unidirectional interactions were observed between Cog1/8, as well as between Cog6 and itself (Fig. S1). Additional weaker or unidirectional interactions, the importance of which is unclear, are also summarized in supplemental Fig. S1.

For immunoprecipitation experiments, 20 μl of a translation reaction was centrifuged for 5 min and added to a final volume of 200 μl of IP buffer (125 mM KCl, 30 mM Tris, pH 8.0, 0.5% Tween 20) containing 2 μl of HA.11 antibody (raw ascites) and 0.5 mM phenylmethanesulfonyl fluoride. These buffer conditions are known to maintain the stability of the intact COG complex during biochemical purification (11, 17). After 1 h of incubation and another 5 min of centrifugation, the mixture was added to 20 μl of a 50% slurry of protein G-Sepharose, which was blocked with 2% bovine serum albumin and equilibrated with IP buffer. The resulting slurry was incubated for 25 min and transferred into a macro spin column (Nest Group, Southborough, MA) with an additional 300 μl of IP buffer. The beads were washed with 3× 500 μl of IP buffer by repeated centrifugation and then incubated at 75 °C for 15 min in SDS-PAGE sample buffer to elute the precipitated material. The obtained samples were analyzed by SDS-PAGE on 20-cm-long slab gels followed by autoradiography. For the Cog4-Cog7 binary interaction, which was detected by immunoblotting, translation and immunoprecipitation were performed identically but in the presence of cold amino acids. The samples were analyzed by immunoblotting as described (11).
Subunit Architecture of the Mammalian COG Complex

FIGURE 1. Binary and ternary interactions among COG complex subunits. A, co-translation/immunoprecipitation demonstrates that Cog1 and Cog8 interact. HA-tagged Cog1 (Cog1HA) and Cog8, or Cog1 and Cog8HA, were co-translated in the presence of [35S]methionine and immunoprecipitated using the HA.11 anti-HA antibody. The immunoprecipitates (IP) and input samples (Input), as well as the singly translated proteins (Inp), were separated by SDS-PAGE and visualized using autoradiography. The band labeled with an asterisk is an anomalous product of the Cog8 translation reaction. B, Cog5 and Cog8 do not interact. Cog5HA and Cog8, or Cog5 and Cog8HA, were co-translated and precipitated as in panel A. Note that translation of Cog5HA produces a doublet. C, additional pairwise interactions are detected between Cog2/3, Cog2/4, and Cog5/7. Only input and immunoprecipitate samples are shown for clarity. D, Cog6 interacts with the Cog5/7 dimer. Cog5, Cog6HA, and Cog7 were co-translated pairwise or all together and precipitated. A 3-fold longer exposure of the IP samples is also shown. E, schematic representation of all observable binary and ternary COG subunit interactions. Subunits are represented by numbered ovals whose size is proportional to molecular mass.

and tagged Cog8 likewise coimmunoprecipitated (Fig. 1A, 1+8HA lanes). This interaction was also observed in the yeast two-hybrid assay although, because the binding domain-Cog8 construct was self-activating, a bidirectional interaction could not be verified. Cog5 and Cog8, on the other hand, represent one of many examples of subunit pairs for which no interaction was detected; only the HA-tagged subunits could be immunoprecipitated (Fig. 1B, IP lanes).

We tested all of the 64 – 8 = 56 possible pairwise combinations of tagged and untagged COG subunits. Compared with the yeast two-hybrid assay, fewer pairwise interactions were detected, and all of these were detected regardless of which subunit was HA-tagged. These results underscore the stringency of the co-translation/immunoprecipitation assay. The results confirm the strong Cog2/4 and Cog5/7 interactions observed in the yeast two-hybrid assay (Fig. 1C). In addition, a Cog2/3 interaction was observed in the co-translation/immunoprecipitation experiments (Fig. 1C) but very weakly or not at all in the yeast two-hybrid assay. On the other hand, neither the Cog4/5 (see below) nor the Cog6/6 interaction observed using yeast two-hybrid assays was detectable using in vitro co-translation and immunoprecipitation.

Larger COG Subcomplexes—The set of pairwise interactions detected using co-translation/immunoprecipitation (Cog1/8, Cog2/3, Cog2/4, and Cog5/7) did not allow construction of a global model because no subunit, with the exception of Cog2, interacted with more than one partner. In addition, Cog6 was not found to participate in any binary interaction. To identify broader connectivities within the COG complex, we therefore expanded the analysis to translation reactions containing more than two subunits. In each reaction, only one subunit was HA-tagged, with the exception of Cog2, which interacted with more than one partner. In addition, Cog6 was not found to participate in any binary interaction.
these isCog2/3/4, as deduced from the ability of Cog2 to bind both Cog3 and Cog4 (and confirmed in experiments described below). The other is Cog5/6/7; Cog5 and Cog7 coimmunoprecipitate with HA-tagged Cog6 in a reaction that requires all three partners (Fig. 1D). A weak pairwise interaction was also observed between Cog5 and Cog6. As before, identical results were obtained regardless of which subunit was HA-tagged (data not shown).

Cog2/3/4 and Cog5/6/7 Do Not Interact Directly—The interactions obtained so far identify three COG subassemblies, two containing three COG subunits (Cog2/3/4 and Cog5/6/7) and one containing two COG subunits (Cog1/8) (Fig. 1E). To clarify the connectivity among these subassemblies, co-translation and immunoprecipitation experiments were conducted using up to six subunits at a time. We first tested whether the Cog2/3/4 and Cog5/6/7 subassemblies interact with one another by translating all six subunits, including one subunit carrying an HA tag. These experiments were in general somewhat challenging to interpret, because the efficiency of the individual translation reactions decreases, not necessarily in a linear manner, in a mixture. Furthermore, the mobilities of several of the COG subunits on SDS-PAGE are very similar. Nonetheless, we were able to rule out a significant interaction between the Cog2/3/4 and Cog5/6/7 subassemblies. For example, HA-tagged Cog2, when co-translated with the other five subunits (Cog3–7), pulls down only Cog3 and Cog4 (Fig. 2A, left). Similarly, when HA-tagged Cog7 was co-translated with the remaining five subunits, only Cog5 and Cog6 were pulled down (Fig. 2A, right).

Previously published results, however, are at odds with our conclusion that Cog2/3/4 and Cog5/6/7 do not interact directly. Based on in vitro translation and co-immunoprecipitation of HA- and Myc-tagged COG subunits, Loh and Hong (32) reported direct pairwise interactions between Cog4 and four other COG subunits: Cog1, Cog2, Cog5, and Cog7. Two of these interactions, Cog4/5 and Cog4/7, would force direct links between the Cog2/3/4 and Cog5/6/7 subassemblies. Prompted by this discrepancy, we re-examined our evidence regarding potential pairwise interactions between Cog4 and either Cog5 or Cog7. Although yeast two-hybrid experiments support an interaction between Cog4 and Cog5 (Fig. S1), no Cog4/5 interaction was observed by co-translation/immunoprecipitation (Fig. 2B and data not shown). For Cog4 and Cog7, no interaction was found by the yeast two-hybrid method (Fig. S1). Interpretation of the corresponding co-translation/immunoprecipitation experiment was, however, complicated by the similar mobilities of radiolabeled Cog4 and Cog7. Therefore, we used immunoblotting to confirm that Cog4 and HA-tagged Cog7 do not interact significantly in our system (Fig. 2C, compare IP lanes); the same result was obtained for HA-tagged Cog4 and Cog7 (data not shown). Notably, our in vitro translation/immunoprecipitation experiments are internally consistent; in experiments containing six subunits, we did not observe an interaction between Cog2/3/4 and Cog5/6/7, whereas in experiments containing two subunits, we did not observe any pairwise interaction that could link the two subassemblies.

Cog1/8 Bridges Cog2/3/4 and Cog5/6/7—Because we failed to detect any direct interaction between Cog2/3/4 and Cog5/6/7 (except for an unconfirmed yeast two-hybrid interaction between Cog4 and Cog5), we considered the alternative possibility that the Cog1/8 subassembly constitutes a bridge linking Cog2/3/4 to Cog5/6/7. To determine whether the Cog1/8 subcomplex interacts with the Cog2/3/4 subcomplex, we carried out co-translation/immunoprecipitation experiments with all five subunits (Fig. 2D). As expected, Cog1 co-immunoprecipitated efficiently with HA-tagged Cog8. Cog2, 3, and 4 were also observed, in approximately equimolar amounts, in the immunoprecipitate. This experiment demonstrates that Cog1 and Cog8, almost certainly as a Cog1/8 subassembly, bind Cog2/3/4. In an analogous manner, we tested whether Cog1/8 interacts with Cog5/6/7 by co-translating all five subunits (Fig. 2E). Again, Cog1 co-immunoprecipitated with HA-tagged Cog8, as did Cog5, 6, and 7 in approximately equimolar amounts. Thus, Cog1/8 is also able to bind Cog5/6/7. Taken together, these experiments support the model that the Cog1/8 subassembly can bridge Cog2/3/4 and Cog5/6/7.

Characterization of the Cog1/8-Cog5/6/7 Interaction—Having established that the Cog1/8 subcomplex interacts with both the Cog2/3/4 and Cog5/6/7 subcomplexes, we asked which subunits are directly involved in mediating these interactions. As a starting point, we attempted to identify the contributions of the Cog1/8 subassembly components in binding to the other subassemblies. We first asked whether Cog8 might mediate the interaction between Cog1/8 and Cog5/6/7. Evidence consistent with this possibility was already available in the form of a subcomplex, proposed to consist of Cog5, 6, 7, and 8, that can be separated from the intact hetero-octamer by hydroxyapatite chromatography during the course of purifying COG (11). To confirm that the subcomplex contains Cog5, 6, 7, and 8, we subjected it to gel filtration and anion exchange chromatography. Four bands co-elute in
an approximately stoichiometric complex (Fig. 3A). Comparison with the band pattern of the full COG complex reveals that the four bands correspond to Cog5 (or Cog3), Cog6, Cog7 (or Cog4), and Cog8. The ambiguity arises from the similar migration of Cog5 and Cog3 and of Cog7 and Cog4. This ambiguity was resolved by immunoblotting, which demonstrates that neither Cog3 nor Cog4 is present in the Cog5-containing hydroxypatite fractions (30–32) representing the subcomplex (Fig. 3B). These results confirm that the subcomplex contains Cog5, 6, 7, and 8.

The Cog5/6/7 subassembly, therefore, is capable of interacting stably with Cog8. This interaction does not require stoichiometric quantities of any of the remaining COG subunits or of any other protein (Fig. 3, A and B). Nonetheless, our ability to recapitulate the interaction between Cog5/6/7 and Cog8 by co-translating all four subunits was limited (Fig. 3C); when HA-tagged Cog8 was immunoprecipitated, only small amounts of Cog5, 6, and 7 were recovered, presumably because the reticulocyte lysate lacks some feature needed for optimal assembly. The binding observed in vitro is nevertheless specific, in that it requires all four subunits (Fig. 3C, compare lanes 3–6 with lane 2). Taken together, these results show that Cog8 plays an important role in mediating the interaction between Cog1/8 and Cog5/6/7.

Characterization of the Cog1/8–Cog2/3/4 Interaction—We turned next to the interaction between Cog1/8 and Cog2/3/4. We found that binding of Cog1/8 to Cog2/3/4 (Fig. 2D) required Cog1, because without Cog1 there was no co-immunoprecipitation (Fig. 4A). Next, we asked whether Cog1 itself is sufficient for Cog2/3/4 binding. Such an interaction was indeed observed, and co-immunoprecipitation appears to be most efficient when all four proteins are present (Fig. 4B). A small but reproducible amount of Cog2 was also recovered in the precipitate when the Cog2/3 dimer was co-translated with HA-tagged Cog1 (Fig. 4B, lane 3).

The co-immunoprecipitation of Cog3 in many of the lanes seen in Fig. 4B is ambiguous because of a co-migrating Cog1 breakdown product (lane 6). To obtain definitive evidence that Cog1 binds all members of the Cog2/3/4 subassembly, we therefore replaced full-length Cog1 with HA-tagged Cog1 fragments (Fig. 4C). In these experiments, all members of the Cog2/3/4 subassembly are larger than the HA-tagged Cog1 fragment, thus circumventing the complication introduced by the breakdown products of the full-length HA-labeled Cog1. Cog2, Cog3, and Cog4 all co-immunoprecipitated with Cog1a or Cog1ab fragments (Fig. 4D). These experiments indicate that an N-terminal portion of Cog1 mediates the interaction between the Cog1/8 and Cog2/3/4 subassemblies.

Whereas the N-terminal region of Cog1 mediates binding to Cog2/3/4, a more C-terminal region mediates binding to Cog8: both Cog1c and Cog1bc bind Cog8, but not Cog2/3/4 (Fig. 4D). Cog1bc binds more Cog8 than Cog1c; indeed, inclusion of the central “b” region of Cog1 increases the yield of Cog1/8 complex to the nearly stoichiometric levels observed for full-length Cog1 (Fig. 1A). We conclude that both the central and C-terminal regions of Cog1 are needed for efficient binding to Cog8. These results are summarized in Fig. 4E.

**DISCUSSION**

A model for the overall architecture of the COG complex that emerges from our results is shown in Fig. 5. In our model, the two heterotrimeric subcomplexes, Cog2/3/4 and Cog5/6/7, are bridged by the Cog1/8 heterodimer. Cog1 plays a central role, with its N-terminal sequences mediating interactions with the Cog2/3/4 subassembly while its C-terminal sequences mediate binding to Cog8 and, through it, the Cog5/6/7 subassembly. The only other putative tethering complex with a subunit map is the exocyst or Sec6/8 complex, for which pairwise in vitro binding experiments (35, 36) and two hybrid assays (35, 37) were performed. Although there is weak sequence homology between certain exoyect and COG subunits (25), we were unable to draw informative parallels between our COG subunit connectivity map and either the mammalian or the yeast exocyst maps. It is intriguing, though, that Cog8 and Sec5, two subunits reported to share sequence homology (25), are centrally located in their respective complexes (36, and this study).

The co-translation/immunoprecipitation strategies we used allowed us to construct a structural model unbiased by previous hypotheses regarding COG subunit connectivity. We began by assembling the full set of binary subunit interactions and then tested all possible third subunit additions. The co-translation/immunoprecipitation procedure proved stringent; we detected only four two-subunit interactions (of 28 possibilities) and only two three-subunit interactions (of 56 possibilities). Finally, we focused on determining the connectivity among these stable subassemblies and found that only the Cog1/8 bridge was able to interact with both the Cog2/3/4 and Cog5/6/7 subassemblies. A weakness of our approach, and indeed of all hierarchical approaches, is that they undervalue the potential significance of cooperative interactions...
among subunits. For example, we did not exhaustively test all possible ternary combinations, so we would not have found the ternary combination X/Y/Z unless X/Y, X/Z, or Y/Z formed a stable binary interaction. This weakness is compensated, at least in part, by the ability to conduct experiments on up to six subunits at a time. So, for example, we tested Cog2, 3, 4, 5, 6, and 7 with the HA tag in many different locations (Fig. 2A and data not shown) and should have detected any additional stable three-component interactions that occurred within this set of subunits.

Our in vitro binding and yeast two-hybrid results are not completely concordant. The main discrepancies involve the Cog2/3 interaction, observed only in vitro, and the Cog4/5 interaction, observed only using the yeast two-hybrid assay. The in vitro binding results proved more useful in generating an internally consistent interaction map, shown in Fig. 5. Multisubunit reactions, difficult or impossible to replicate using yeast assays, provide additional evidence that Cog4 and Cog5, even in collaboration with their subassembly partners, do not stably interact (Fig. 2A and data not shown). Nonetheless, it remains possible that additional interactions, possibly including one between Cog4 and Cog5, contribute to the stability of the intact COG complex. It will be important in future work to explore these and other possibilities more fully.

We note, however, that the in vitro precipitation data, rather than the yeast two-hybrid data, are in better agreement with the complementary in vivo study presented in an accompanying paper (30, see below).
Recently, another study based on in vitro translation/co-immunoprecipitation experiments reached quite different conclusions regarding the subunit connectivity of COG (32). Two particularly significant differences emerge from comparing the two models. On the one hand, Loh and Hong (32) do not report an interaction between Cog1 and Cog8, although in our experiments this interaction was robust (Figs. 1A, 2D and 2E, 4D). On the other hand, Loh and Hong report an interaction between Cog4 and Cog7 that we were unable to reproduce (Fig. 2C; see “Results”). Together, these differences lead to a very different model for the subunit connectivity of the COG complex. Although we do not understand the origin of the discrepancies between our co-translation/immunoprecipitation results and those of Loh and Hong, they might in principle arise from several causes. All COG subunits in the study by Loh and Hong carried green fluorescent protein, FLAG, double-Myc, or double-HA tags, whereas our in vitro co-translation reactions always contained only one single-HA-tagged subunit together with one or more untagged subunits. Also, Loh and Hong used a coupled transcription/translation system that can yield very different quantities of the various co-translated subunits, whereas we titrated individually transcribed mRNAs to generate similar quantities of the different co-translated subunits.

Two contemporaneous studies provide independent support for the model presented in Fig. 5. First, in an accompanying paper (30), we report analyses of COG mutant cells and cell extracts, the results of which are in complete agreement with the structural model we derive here on the basis of in vitro translation/immunoprecipitation experiments, but not with the model proposed by Loh and Hong (32). For example, co-immunoprecipitation of Cog1 and Cog8 from HeLa cells is unaffected by the absence of Cog5 or Cog7 (30). Second, a subunit interaction map of the Saccharomyces cerevisiae COG complex, generated using different methods (38), is in good agreement with our map of the mammalian complex. In terms of the direct interactions discussed above, the yeast data match our in vitro translation/immunoprecipitation data. Specifically, an interaction between the yeast subunits Cog1p and Cog8p was observed; conversely, no interaction between yeast Cog4p and Cog5p, or between Cog4p and Cog7p, could be detected (38). Agreement among the three studies provides cross-validation for all three. Furthermore, it implies that the overall architecture of the COG complex is conserved from yeast to humans.

The COG complex has been shown previously to interact with members of several protein families, including SNAREs, Rab proteins, and coat proteins (13–16, 26, 27), placing it at the nexus of many proteins involved in vesicular trafficking. Deleting different COG subunits has drastically different consequences on cell viability for both Caenorhabditis elegans (25, 31). It therefore seems plausible that COG has more than one function, for example acting in multiple trafficking events, and that these functions might be distributed among the different subassemblies comprising the intact complex. For example, different parts of the complex may interact with different sets of SNAREs, Rab, or coat proteins. Our identification of the underlying structural subassemblies that make up COG should facilitate efforts to determine the molecular bases for these interactions and to elucidate their mechanistic significance.

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