The Binary Interacting Network of the Conserved Oligomeric Golgi Tethering Complex*

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Several recent studies have revealed the existence of a conserved oligomeric Golgi (COG) complex consisting of several novel proteins as well as known Golgi proteins that were identified by independent approaches. The mammalian COG complex contains eight subunits: COG1/LdIBp, COG2/LdICp, COG3/Sec34, COG4/Cod1, COG5/GTC-90/Cod4, COG6/Cod2, COG7, and COG8/Dor1. COG1, COG2, and COG7 seem structurally unique to mammalian cells, whereas the other five subunits are structurally conserved in yeast, which also contains three other unique proteins (COG1/Sec36p/Cod3p, COG2/Sec35p, and COG7/Cod5p). We report here the network of intermolecular interactions of the COG complex, revealed by in vitro translation and co-immunoprecipitation approaches. Our results suggest that COG4 serves as a core component of the complex by interacting directly with COG1, COG2, COG5, and COG7. COG3 is incorporated by its direct interaction with COG1 and COG2, whereas COG6 and COG8 do not interact with any individual subunit. Incorporation of COG6 into the complex depends on the concerted interaction of both COG5 and COG7, whereas optional incorporation of COG8 depends on the concerted interaction of COG5, COG6, and COG7. Because COG4 (together with COG1, COG2, and COG3) is among the four essential genes of the COG complex in yeast, this molecular network highlights the structural basis for a crucial role of COG4 in the assembly/function of the complex. A model for the assembly of the COG complex is presented.

Protein trafficking along the secretory and endocytic pathway is primarily mediated by shuttling vesicles that move in either an anterograde or a retrograde direction. The efficient and precise fusion of vesicles with the target compartment is thought to be achieved by bringing the vesicles to a close proximity with the receiving compartment via a process referred to as tethering. The tethering process is believed to be intimately linked to and followed by direct fusion of the two opposing membranes, mediated by a superfamily of proteins referred to as SNAREs. Interaction of v-SNARE on the vesicle with its cognate t-SNARE on the target compartment is the key event in the fusion process (1–7).

It is becoming clear that tethering is mediated by proteins such as small GTPases, their effectors, and other proteins, most of which exist in distinct protein complexes (8–10) or long rod-like coil-coil proteins (11–12). Several tethering complexes have been identified that are involved in the tethering event at distinct intracellular compartments. The exocyst (Sec6-Sec8) complex is the best studied tethering complex, originally identified in yeast, known to be important for polarized vesicle transport to the growing bud during cell cycle. Exocyst complex is similarly involved in the tethering processes on defined regions of the plasma membrane in mammalian cells and is the effector of several small GTPases including Cdc42, RacA, and Arf6 (13–16). In addition, a protein (VFT for Vps 53 or GARP for Golgi-associated retrograde protein) complex consisting of Vps52, Vps53, and Vps54 participates in the tethering process on the late Golgi for incoming traffic from endosomes (17), and Vps51 acts to link the tethering complex to the SNARe complex (18–19). Mammalian homologues of the VFT-GARP complex are identified and could function similarly as tethering proteins in the trans-Golgi network (20). TRAPP-I and -II are two related tethering complex that function in endoplasmic reticulum-Golgi and intra-Golgi transport and act together with Ypt1/Rab1 (21), whereas the HOPS complex (or Class C VPS complex) consisting of Vps11, Vps16, Vps18, Vps33, Vps39, and Vps41 act together with Ypt7 and SNAREs in mediating tether and fusion at the vacuole in yeast (22). The HOPS complex has been shown to function in multiple transport steps in addition to fusion at the vacuolar membrane (23–24). Proteins homologous to components of the TRAPPs and HOPS complexes are found in mammalian cells and could function in a similar manner in their respective tethering processes.

The COG complex was recently identified in both yeast and mammalian cells and consists of eight subunits (COG1–8). Some components such as COG3, COG4, COG5, COG6, and COG8 from yeast and mammalian cells are structurally homologous, whereas mammalian COG1/LdIBp, COG2/LdICp, and COG7 are not structurally related to the remaining three yeast proteins (COG1/Sec36p/Cod3p, COG2/Sec35p, and COG7/Cod5p), although they could represent functional counterparts (25). Among the eight subunits, COG1, COG2, COG3, and COG4 (but not the other four proteins) are essential for cell growth in yeast and are, therefore, essential components of this complex (26).

To reveal the structural and molecular basis underlying the assembly/function of the COG complex, we have systematically investigated the inter-molecular interactions of these subunits, and our results suggest that COG4 is a core component that links up the three other essential components (COG1, COG2, and COG3) with the other four subunits. Based on our findings, we present a model for the assembly of the COG complex.

EXPERIMENTAL PROCEDURES

Materials—Anti-Myc and anti-HA antibodies (rabbit polyclonal IgG) were obtained from Upstate Biotechnology. Anti-FLAG antibodies were
Molecular Network of the COG Complex

Table I
Human cDNA clones used for the study

| Species               | GenBank™ accession no. | Source of clone          | Reference |
|-----------------------|------------------------|--------------------------|-----------|
| COG1 H. sapiens       | AB037802 (Kiaa1381)    | Kazusa DNA Research Institute | 28        |
| COG2 H. sapiens       | AI492237               | IMAGE consortium         | 28        |
| COG3 H. sapiens       | AK26305                | NEDO human cDNA sequencing project | 28        |
| COG4 H. sapiens       | AA28818                | Wash-U (EST) Project     | 28        |
| COG5 H. sapiens       | AA163412 (SCOD1M4)     | Sean Munro               | 27        |
| COG6 H. sapiens       | NP_859422              | Human Universal QUICK-Clone II (cDNA template) | 28        |
| COG7 H. sapiens       | AB032960 (Kiaa1134)    | Kazusa DNA Research Institute | 25        |
| COG8 H. sapiens       | BE261220               | Human Universal QUICK-Clone II (cDNA template) | 25        |

Results

Because the COG complex is one of the few major tethering complexes in all eukaryotic cells, understanding its inter-subunit interactions and structure will be of great importance to gain insight into its assembly, function, and regulation. Because little is known about the structural and molecular mechanisms fundamental to the assembly of the complex, we have thus initiated this current systematic study. In our hands after trying several different approaches, it turned out that *in vitro* translation of subunits of interest using rabbit reticulocyte lysate followed by co-immunoprecipitation provided the most optimal system to assess the protein interaction network of COG complex. Although the reticulocyte lysate-based *in vitro* translation system may contain some endogenous COG proteins and other cytosolic proteins, our results showing specific interactions among selective COG subunits indicate strongly that this system could be used for investigating protein-protein interactions. The high specificity obtained in our comprehensive study suggests that the reticulocyte proteins did not contribute significantly to interactions detected among the tagged COG subunits. The interactions detected are, thus, an indication of direct interactions that are being examined, despite the caveat that conclusive demonstration of direct interaction will have to await a more stringent assay using purified proteins. Keeping this point in mind, we have used the term “direct interaction” in a relative sense.

Direct Interaction of COG4 with COG1, COG2, COG5, and COG7—We initially tested all possible combinations of interaction among any two subunits. Each subunit was tested for possible interaction with the other seven subunits by co-immu-

from Sigma. The TNT T7 Quick Master Mix was obtained from Promega. Protein A-horseradish peroxidase was used as secondary antibody detection for Western blotting (BD Transduction Laboratories). Restriction enzymes were all purchased from Roche Applied Science. Human Universal QUICK-Clone II (cDNA template) was purchased from BD Biosciences Clontech. cDNA clone BE261220 was purchased from Invitrogen. Synthetic oligonucleotides were ordered from ProLogio Singapore Pty Ltd.

Expression Constructs for Myc, HA, or FLAG Epitope-tagged COG1, COG2, COG3, COG4, COG5, COG6, COG7, and COG8—The cDNA clones for COG1, COG3, COG5, COG6, COG7, and COG8 are all of human origin, and the detailed information about the clones is listed in Table I.

For Myc-COG4, primer 1 (5′-GAG-CTG-CAG-GCG-GAC-GCTT-GAT-CAG-TCG) and primer 2 (5′-CAG-TGG-GAAT-CTT-GAT-GAA-GA-G) were used to amplify an ~700-bp fragment from plasmid SCOD1M4 (generously provided by Dr. Sean Munro) (27), and the resulting PCR fragment was digested with Xhol and BglII. This fragment was ligated into a fragment retrieved from SCOD1M4 by digestion with BglII and XbaI together with pDMyc-neo vector pre-cut with Xhol and XbaI.

For Myc-COG5, primer 3 (5′-GAG-CTG-AGG-CGC-CTA-AGG-GATG-GGC-TGG-GTG-GGC-GAC-ATG-ATG-CCT-CTT-GGC-GCC-GGA-GCA-GCT-GCT-ACACCC-A) and primer 4 (5′-ATC-TGAT-TTGA-GCT-GG-AAG-ACC-ACC-ATC-GG-ACG-CTT-GTG-TGA-GG) were used to amplify an ~1200-bp fragment from Universal QUICK-Clone II cDNA template, and the resulting PCR fragment was digested with XbaI and ClaI. This fragment was ligated to another PCR fragment that was generated using the same template with primer 9 (5′-GAG-CTG-CGC-CTA-AGG-GAAT-CTT-GAT-GAA-GA-G) and primer 10 (5′-GAG-CTG-CGC-CTA-AGG-GAAT-CTT-GTG-TGAT-GAA-GA-G) used to amplify the entire coding sequence of COG7 from IMAGE clone AB032960 by PCR, and the resulting product was digested with EcoRI and XbaI and ligated into the corresponding sites of pDMyc-neo vector.

For Myc-COG8, primer 5 (5′-GAG-ATA-CTG-GCC-GAA-AAG-ATC-GT-TGC-AGT-GAA-GA-G) and primer 6 (5′-GATG-CTT-GGA-GGA-GAG-ATC-GT-GAT-GAA-GA-G) used to amplify the entire coding sequence of COG8 from Human Universal QUICK-Clone II cDNA template by PCR, and the resulting product was digested with ClaI and NotI, together with pDMyc-neo vector pre-cut with XbaI and NotI.

For Myc-COG7, primer 5 (5′-GAG-ATA-CTG-GCC-GAA-AAG-ATC-GT-TGC-AGT-GAA-GA-G) and primer 6 (5′-GATG-CTT-GGA-GGA-GAG-ATC-GT-GAT-GAA-GA-G) used to amplify the entire coding sequence of COG7 from IMAGE clone AB032960 by PCR, and the resulting PCR product was digested with EcoRI and XbaI and ligated into the corresponding sites of pDMyc-neo vector.

Myc-tagged constructs of COG1, COG2, COG3 and COG6 were previously described (28). All the above eight subunits of the COG complex, which were constructed in pDMyc-neo vector (28), were also digested using its corresponding restriction enzyme sites out of the vector and ligated to the corresponding sites of pDHIA-neo and pFLAG-neo vector to construct HA-tagged and FLAG-tagged versions of COG1–8.
noprecipitation assay of proteins produced by in vitro translation. COG4 was found to be a core interacting protein, and it forms binary interactions directly with four other subunits, namely COG1, COG2, COG5, and COG7. The results are presented in a logical way, highlighting the interactions detected. When HA-COG4 was co-translated with Myc-COG1, Myc-COG2, Myc-COG5, Myc-COG7, GFP-COG3, FLAG-COG6, and FLAG-COG8 (as indicated) by in vitro translation reactions with nonradioactive methionine. The translated products were divided and immunoprecipitated (IP) with the antibodies (Ab) shown with each set of reactions. The immunoprecipitates and 10% of the respective translation reactions (lanes 1 and 4) were analyzed by SDS-PAGE followed by immunoblot analysis with the respective antibodies (as stated for each reaction).

**A**

- HA-CO4G + myc-COG1
  - probed with α-HA Ab

- HA-CO4G + myc-COG2
  - probed with α-HA Ab

- HA-CO4G + myc-COG5
  - probed with α-HA Ab

- HA-CO4G + myc-COG7
  - probed with α-HA Ab

**B**

- HA-CO4G + GFP-COG3
  - probed with α-HA Ab

- HA-CO4G + Flag-COG6
  - probed with α-Flag Ab

- HA-CO4G + Flag-COG8
  - probed with α-Flag Ab

**Fig. 1. Direct interaction of COG4 with COG1, COG2, COG5, and COG7, but not with COG3, COG6, or COG8.** HA-COG4 was co-translated with Myc-COG1, Myc-COG2, Myc-COG5, Myc-COG7, GFP-COG3, FLAG-COG6, and FLAG-COG8 (as indicated) by in vitro translation reactions with nonradioactive methionine. The translated products were divided and immunoprecipitated (IP) with the antibodies (Ab) shown with each set of reactions. The immunoprecipitates and 10% of the respective translation reactions (lanes 1 and 4) were analyzed by SDS-PAGE followed by immunoblot analysis with the respective antibodies (as stated for each reaction).

In marked contrast, when HA-COG4 was co-translated with GFP-COG3, FLAG-COG6, and FLAG-COG8 (Fig. 1B), anti-HA antibodies failed to co-immunoprecipitate GFP-COG3, FLAG-COG6, or FLAG-COG8 (lane 5), although HA-COG4 was efficiently immunoprecipitated (lane 1). Furthermore, when GFP-COG3, FLAG-COG6 and -COG8 were efficiently immunoprecipitated by antibodies against GFP/FLAG (lane 6), HA-COG4 was not detected in the immunoprecipitates (lane 3). Similar results were obtained when HA-COG4 was co-translated with Myc-COG3, Myc-COG6, or Myc-COG8 (data not shown). These results suggest that COG4 does not interact directly with COG3, COG6, or COG8.

Because our previous study has demonstrated a direct interaction of COG3 with COG1 or COG2 (28), COG3 is likely incorporated into the complex via direct interaction with COG1 and COG2. Similar results were obtained in our comprehensive analysis of all possible binary combinations (Fig. 2B).

**Binary Interaction between COG5 and COG7—Among our systematic binary interaction assays, we have detected a weak interaction between COG5 and COG7 (Fig. 2A). When Myc-
COG5 and HA-COG7 were co-translated, HA-COG7 was efficiently immunoprecipitated by HA antibody (lane 3). However, a small but significant amount of HA-COG7 could be consistently co-immunoprecipitated by Myc antibodies (lane 2). Consistent with this observation, a small but significant amount of Myc-COG5 was co-immunoprecipitated by HA antibodies (lane 6). Again, Myc-COG5 was very efficiently immunoprecipitated by Myc antibodies (lane 5). Because this weaker but significant co-immunoprecipitation was specifically observed when COG5 and COG7 were co-translated, we believe this weaker interaction is specific.

**Summary of All Binary Interactions Depicts a Sub-complex Consisting of COG1, COG2, COG3, COG4, COG5, and COG7**—The results of all binary interaction assays (each subunit was tested for possible interactions with the other seven subunits) were summarized in Fig. 2B, +, +/-, and − symbols indicate strong binary interaction (such as COG4 with COG1, COG2, COG5, or COG7), weaker but specific binary interaction (such as COG5 with COG7), and no interaction, respectively. Most significant is the observation that COG6 and COG8 did not show any binary interaction with any individual subunits in the two-subunit assay. The results of the detected interactions were illustrated in a diagrammatic manner in Fig. 2C, where direct binary interactions were indicated, assuming that none of the binary interactions are mutually exclusive, which is a point that remains to be established.

**Incorporation of COG6 into the COG Complex Depends on Both COG5 and COG7**—Because COG6 and COG8 did not display any direct interactions with any other subunits, we suspect that their incorporation into the complex is mediated by interacting surfaces that arise from several subunits. To identify those subunits that are important for incorporation of COG6 and COG8, we have taken a different strategy in which different subunits (or combination of various subunits) were omitted from an initial co-translation of all eight subunits. When all eight of the COG subunits were co-translated by in

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To identify which components in the remaining four subunits (COG1, COG2, COG5, and COG7) are involved in the interaction with COG6, we systematically omitted each of these Myc-COG subunits in the co-translation reactions together with FLAG-COG6 (Fig. 3, C and D). When these five subunits were co-translated, FLAG-COG6 was efficiently co-immunoprecipitated by antibodies against the Myc-tagged-COGs (Fig. 3, C, row 1, and D, panel 1, left lane). Omission of Myc-COG1 did not affect co-immunoprecipitation of FLAG-COG6 by Myc antibodies (Fig. 3, C, row 2, and Fig. D, panel 2), although the efficiency is reduced. Similarly, omission of Myc-COG2 did not affect the interaction of FLAG-COG6 with the remaining Myc-COG subunits as assessed by co-immunoprecipitation, although the efficiency is similarly reduced (Fig. 3, C, row 3, and D, panel 3).
Fig. 3. COG5 and COG7 are necessary for incorporation of COG6 into the complex. A and C, tables showing the various subunits added (shown with symbol +) into the in vitro translation reactions for each experiment, denoted by the numbers on the extreme left column (COG6 is FLAG-tagged whereas all other subunits are Myc-tagged). The extreme right column shows the results of whether FLAG-COG6 was detected in the anti-Myc immunoprecipitates. B and D, immunoblot analysis with anti-FLAG antibodies to detect the presence of FLAG-COG6 in each of the immunoprecipitation experiments, using either Myc (left lane of all panels) or FLAG (right lane of all panels) antibodies. The numbers shown below each set of reactions correspond to the numbers in panels A or C showing which of the subunits was present in each reaction. For each in vitro translation reaction, the translated products were divided and co-immunoprecipitated separately with anti-Myc or anti-FLAG antibodies.

When Myc-COG5 was absent in the translation reaction, FLAG-COG6 was not co-immunoprecipitated with anti-Myc antibodies (Fig. 3, C, row 4, and D, panel 4). Similarly, when Myc-COG7 was omitted, anti-Myc antibodies failed to co-immunoprecipitate FLAG-COG6 (Fig. 3, C, row 5, and D, panel 5). These results suggest that COG5 and COG7 but not COG1 or COG2 are essential for interaction of FLAG-COG6 with the other Myc-COG subunits.

The above results also suggest that COG6 could interact with COG5 and COG7 to form a sub-complex. To further substantiate this point, FLAG-COG6 was co-translated with Myc-COG5 and Myc-COG7. The efficiency of co-immunoprecipitation of FLAG-COG6 by Myc antibodies (Fig. 4A, lane 1) is about 60% that achieved by flag antibodies (lane 2), suggesting that FLAG-COG6 can be efficiently incorporated into a sub-complex with COG5 and COG7. Consistent with this, significant amounts of Myc-COG5 and Myc-COG7 can be co-immunoprecipitated by antibodies against FLAG-tag (lane 4), although the efficiency is somewhat lower. The low efficiency could indicate that COG5 and COG7 exists either as free or COG5-COG7 forms in addition to the ternary complex, consisting of COG5, COG6, and COG7. Alternatively, this low efficiency of co-immunoprecipitation of COG5 and COG7 with COG6 could be due to the possibility that COG5 and -7 are present at much higher levels after in vitro translation. As summarized in Fig. 2B, when FLAG-COG6 was co-translated with either Myc-COG5 or Myc-COG7 independently, FLAG-COG6 was not co-immunoprecipitated with anti-Myc antibodies (Fig. 4B, lane 2). Consistently, anti-FLAG antibodies failed to co-immunoprecipitate Myc-COG5 and Myc-COG7 (lane 6). This illustrates that COG6 interacts with COG5-COG7 binary sub-complex but not COG5 or COG7 alone. This interaction is important for COG6 to be incorporated into the complex. The formation of the sub-complex consisting of COG5, COG6, and COG7 is depicted in Fig. 4C, where the solid line indicates direct binary interaction, whereas the dashed lines indicate interaction via several subunits.

Optimal Incorporation of COG8 into the COG Complex Depends on COG5, COG6, and COG7—We next proceeded to identify which subunits are involved in the incorporation of COG8 into the COG complex. When all eight of the COG subunits were co-translated by in vitro translation (Fig. 5A, row 1), FLAG-COG8 was efficiently co-immunoprecipitated by antibodies against the Myc tag (Fig. 5B, panel 1, left lane), suggesting that FLAG-COG8 is efficiently incorporated into a complex (or sub-complexes) with the other seven Myc-COGs. The efficiency of co-immunoprecipitation by Myc antibodies (left lane, panel 1 of Fig. 5B) is close to 90% that achieved by FLAG antibody (right lane), suggesting that the majority of FLAG-COG8 has been incorporated into the complex. Omission of Myc-COG1 from the translation reaction had no effect on incorporation of FLAG-COG8 into the complex formed with the other 6 Myc-COG subunits (Fig. 5A, row 2, and B, panel 2). Similarly, omission of Myc-COG2 (Fig. 5A, row 3, and B, panel 3), Myc-COG3 (Fig. 5A, row 4, and B, panel 4) and Myc-COG4 (Fig. 5A, row 5, and B, panel 5) had no effect on the interaction of FLAG-COG8 with the remaining six Myc-COG subunits. Therefore, these results suggest that COG1, COG2, COG3, and COG4 are not involved in the formation of interacting surface for COG8. When COG5 (Fig. 5A, row 6, B, panel 6) and COG6 (Fig. 5A, row 7, B, panel 7) were omitted, the efficiency of co-immunoprecipitation by Myc antibodies was greatly reduced. When COG7 was omitted, the efficiency of co-immunoprecipitation of FLAG-COG8 by Myc antibodies was about 30% that achieved by FLAG antibody (Fig. 5A, row 8, and B, panel 8). These results suggest that COG5, COG6, and COG7 are necessary for the optimal incorporation of COG8 into the COG complex, whereas COG1, COG2, COG3, and COG4 are dispensable.

To further ascertain this observation, we prepared co-translation reactions consisting of FLAG-COG8 with Myc-COG4, Myc-COG5, Myc-COG6, and Myc-COG7 from which we sequentially omitted each of the Myc-COG subunits (Fig. 6, A and B). When these five subunits were co-translated, FLAG-COG8 was
efficiently co-immunoprecipitated by antibody against Myc tag (Fig. 6, A, row 1, and B, panel 1). Omission of Myc-COG4 did not affect co-immunoprecipitation of FLAG-COG8 by the Myc antibodies (Fig. 6, A, row 2, and B, panel 2). When Myc-COG6 was excluded from the translation reaction, the efficiency of co-immunoprecipitation of FLAG-COG8 by the anti-Myc anti-
bodies was greatly diminished (Fig. 6, A, row 3, and B, panel 3). When Myc-COG7 was omitted, anti-Myc antibodies failed to co-immunoprecipitate FLAG-COG8 (Fig. 6, A, row 4, and B, panel 4). To establish the importance of COG5 in the incorporation of COG8 into the complex, FLAG-COG8 was co-translated together with Myc-COG6 and HA-COG7 (by omitting COG5) were co-translated, and the products were divided and co-immunoprecipitated with anti-FLAG, anti-Myc, or anti-HA antibodies. The immunoprecipitates and 10% of the translation reactions were resolved by SDS-PAGE and analyzed by immunoblot with anti-FLAG, anti-Myc, and anti-HA antibodies, respectively. C, a schematic diagram depicting the possible interactions within the COG5, COG6, COG7, and COG8 sub-complex (the solid line indicates a direct interaction between COG5 and COG7, whereas dashed lines indicate interaction of COG6 with COG5-COG7 or COG8 with COG5-COG6-COG7).

DISCUSSION

We have taken a systematic approach to establish the inter-subunit interactions of the mammalian COG complex. Several different methods were tried, including the yeast two-hybrid system, in vitro co-translation of [35S]Met-labeled proteins revealed by autoradiography, and in vitro co-translation of unradiolabeled but tagged subunits that were detected by immunoblot analysis. After extensive comparisons and many trials, it turned out that the last method was most reliable and consistent. In this regard, each subunit was tagged with a few different tags for our analysis. Two strategies were employed. The first was to identify all possible binary direct interactions via co-translating two different subunits, and this was evaluated by the efficiency of co-immunoprecipitation; the second approach aimed to identify interactions of a particular subunit with a sub-complex consisting of several other subunits via co-translating all eight subunits followed by systematically omitting each subunit. Although the reticulocyte lysate may contain some endogenous COG proteins, our results showing high specificity suggest that the endogenous proteins, if any, did not contribute significantly to the interactions detected. The first strategy has helped to identify seven direct binary interactions: COG4-COG1, COG4-COG2, COG4-COG5, COG4-COG7, COG3-COG1, COG3-COG2, and COG5-COG7. Because COG6 and COG8 did not exhibit any direct binary interaction, the molecular basis for their incorporation into the complex was investigated by the second strategy. By systematic omission of different subunits, it was revealed that COG6 interacts with a binary COG5-COG7 sub-complex. Similarly, it was shown that COG8 interacts with a ternary COG5-COG6-COG7 sub-complex. As more and more protein complexes are being uncovered by proteomic and biochemical studies, in line with the emerging new field of systems biology, we believe our approach in defining the interacting network of COG complex will be applicable to studies aiming to understand the inter-subunit interaction and assembly of other protein complexes. Although multiple sub-complexes could potentially exist, we favor the following model for the assembly of the COG complex.
First, COG4 will drive the formation of COG1-COG2-COG3-COG4 sub-complex (sub-complex I). By the interaction of COG4 with COG5 and COG7, COG1-COG2-COG3-COG4-COG5-COG7 sub-complex (sub-complex II) could be formed. Sub-complexes I and II could be formed at the same time and co-exist in equilibrium. COG5 and COG7, once incorporated into the sub-complex II, will create a high affinity interacting surface for COG6. Incorporation of COG6 leads to the formation of sub-complex III, which has a high affinity COG8 binding surface contributed combinatorially by COG5, COG6, and COG7. Incorporation of COG8 results in the formation of the holo-COG complex.

Our model is not only consistent with our presented results but could also explain several other observations. First, the ultrastructure of purified COG as visualized by quick freeze/deep etch/rotary shadow electron microscopy revealed that the COG complex exists as a 37-nm-long structure comprising two similarly sized globular domains (lobes A and B) connected by smaller extensions (25). This observed bilobed structure is in good agreement with our model. Our results are also consistent with the proposal that COG1, COG2, COG3, and COG4 form one lobe (lobe A), whereas the other lobe consists of COG5, COG6, COG7, and COG8 (lobe B). Most importantly, our results provide a novel insight into the molecular basis that links up the two lobes. Via direct interaction of COG4 on lobe A with both COG5 and COG7 of lobe B, these two lobes are effectively and directly joined together. Secondly, evolutionally and structurally conserved subunits (COG3, COG4, COG5, COG6, and COG8) are located on both lobes of the complex with COG4 serving as the core for the entire complex. COG1, COG2, and COG7 from mammalian and yeast cells do not display structural homologues, and they are similarly distributed in both lobes. The divergences of these subunits on both lobes enable the entire complex to evolve to accommodate the specific needs of different species during evolution. Third, our results also highlight the importance of the COG4 subunit in both serving as a core component of lobe A and also in the assembly of the entire complex by linking up with two subunits (COG5 and COG7) of lobe B. This important structural function revealed here could explain the essential function of COG4 in the yeast
as deletion of COG4 leads to very slow growth (27). We have tried to identify sub-domains of COG4 that are important for each of the four direct binary interactions. Interestingly, a deletion from either the N terminus (200-residue deletion of the 763-residue protein) or the C terminus (163-residue) completely abolished all possible binary interactions (data not shown), suggesting that the entire molecule is necessary and the formation of interaction sites for all binary interactions is coordinated at the level of the entire structure. In addition, among the eight COG subunits, the four essential components (COG1, COG2, COG3, and COG4) are segregated from the other four nonessential components so that lobe A plays a more fundamental role despite the fact that two of the four components are not structurally conserved during evolution. This indicates that the conserved COG3 and COG4 are likely to be involved in a more fundamental role, whereas the more divergent COG1 and COG2 serve to accommodate the needs of different architectures of the Golgi apparatus in different organisms. That three of the four nonessential components in lobe B are structurally conserved between yeast and human indicates lobe B could play a regulatory/integrating role that is well conserved in different species, whereas the divergent COG7 could be linked with more divergent loops of regulation. It is possible that components in lobe B could interact with proteins of other well conserved molecular machineries to modulate the essential function played by lobe A. Consistent with this possibility, COG5 has recently been shown to be particularly important for spermatogenesis in the fly, and it could integrate the general function of lobe A with specialized Golgi architecture that supports rapid and extensive increases in cell surface area during spermatocyte cytokinesis and polarized elongation of differentiating spermatids (29). Future studies along these lines, in the context of our inter-subunit network, will add more insightful understanding about the function and regulation of the COG complex.

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