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15. We assume that the surface of the MoO_3 nanocrystal sliding on MoS_2 has the same rectangular lattice as was observed experimentally at the exposed nanocrystal surface, because only integral multiples of the unit cell (along the *b* axis) were observed in thickness measurements of the MoO_3 nanocrystals. We cannot rule out the substitution of some sulfur into the surface oxygen anion layer, although we believe this is unlikely because the magnitude of the observed friction is much larger than was observed for sliding MoS_2 on a MoS_2 substrate (P. E. Sheehan and C. M. Lieber, unpublished results).
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17. The experimental results are only consistent with the atomic interface shown in Fig. 2. No direct method exists to assess the atomic structure of the sliding surface of the MoO_3 nanocrystals.
18. Friction for macroscopic systems is commonly expressed in a load-dependent form, $F = \mu L$, where L is the load and μ is the phenomenological coefficient of friction. In this expression, the friction is apparently independent of the contact area A; in reality, as L is increased between two rough surfaces, the contact area increases. Hence, $F = sA$ represents a more microscopic representation of friction if the contact area is known at the atomic level.
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GS28, a 28-Kilodalton Golgi SNARE That Participates in ER-Golgi Transport

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Little is known about the integral membrane proteins that participate in the early secretory pathway of mammalian cells. The complementary DNA encoding a 28-kilodalton protein (p28) of the cis-Golgi was cloned and sequenced. The protein was predicted to contain a central coiled-coil domain with a carboxyl-terminal membrane anchor. An in vitro assay for endoplasmic reticulum–Golgi transport was used to show that p28 participates in the docking and fusion stage of this transport event. Biochemical studies established that p28 is a core component of the Golgi SNAP receptor (SNARE) complex.

The *N*-ethylmaleimide–sensitive factor (NSF) and the soluble NSF attachment proteins (SNAPs) are required for most vesicle fusion events along the exocytotic and endocytotic pathway (*1*). The cytosolic NSF and SNAPs are recruited to the fusion sites through binding to membrane SNAREs. The specificity of vesicular transport is thought to be determined by correct pairing of vesicle-associated SNAREs (v-SNAREs) with those on the target membrane (t-SNAREs) (*1*). Synaptic vesicle–associated synaptobrevins (v-SNAREs) and presynaptic membrane–associated syntaxin 1A and 1B (complexed with SNAP-25) (t-SNAREs) have been shown to interact with each other to determine the specific docking of synaptic vesicles on the presynaptic membrane as well as to recruit SNAPs and subsequently NSF, resulting in the formation of a 20S fusion (or SNARE) complex (*1*, *2*). Although the 20S SNARE complex was originally formed with Golgi membrane extracts in the presence of NSF and α-SNAP (*3*), the Golgi proteins of this SNARE complex have not been identified.

Syntaxin 2, 3, 4, and 5, Sed5p, Sso1p, Sso2p, Pep12p, cellubrevin, Sec22p (Sly2p), Bet1p (Sly12p), Bos1p, Snc1p, Snc2p, and Sft1p are other possible SNAREs that function in different vesicular transport steps (*1*, *4*). Among these, only syntaxin 5 is implicated in endoplasmic reticulum (ER)–Golgi transport in mammalian cells (*5*).

A cis-Golgi integral membrane protein, p28, has recently been identified by a monoclonal antibody (mAb), HFD9 (*6*). The protein was immunoaffinity-purified from a detergent extract of rat liver Golgi-enriched membranes, and the amino acid sequences of its proteolytic peptides were used to isolate the full-length complementary DNA (cDNA) (*7*) (Fig. 1). The deduced amino acid sequence shows that p28 is a 250–amino acid protein. The major portion of the polypeptide (residues 1 to 230) is predicted to be anchored to the membrane by its 20-residue COOH-terminal hydrophobic tail (residues 231 to 250). Preceding the hydrophobic tail are several regions (residues 67 to 94, 101 to 128, 129 to 152, and 163 to 226) that could potentially form coiled-coil domains. The structure of p28 is similar to that of known SNAREs, although the primary sequence of p28 does not show significant similarity to any of them (*1*, *4*).

The structure of p28 and its cis-Golgi localization (*6*) prompted us to examine whether p28 functions in ER to cis-Golgi

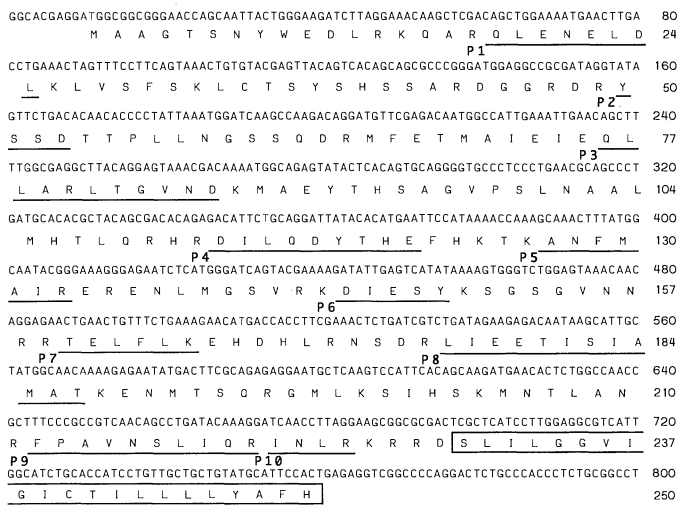

Fig. 1. The cDNA and deduced amino acid sequence of p28. Peptide sequences obtained by microsequencing of proteolytic fragments of purified p28 are underlined. The COOH-terminal membrane anchor is boxed. This sequence has been deposited with GenBank (accession no. U49099). Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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transport. We used a well-established in vitro system (8) that reconstitutes vesicular transport of the envelope glycoprotein of vesicular stomatitis virus (VSV-G) ts045 from the ER to the cis/medial-Golgi in NRK cells or from the ER to the cis-Golgi in CHO15B cells. In NRK cells, this assay monitors the conversion of VSV-G from endoglycosidase H (endo H)–sensitive to endo H–resistant forms. In CHO15B cells, conversion of VSV-G from endo D–resistant to endo D–sensitive forms is measured. p28-specific mAb HFD9, its Fab fragment, and recombinant cytoplasmic domain (residues 1 to 230) of p28 (p28cyto) (9) each inhibited the transport (Fig. 2A). Neither the recombinant cytoplasmic domain of cellubrevin (9, 10), which functions in the endocytotic pathway (10), nor a mAb against the cytoplasmic tail of the cis-Golgi–enriched KDEL receptor (anti-p23) (11) (or its Fab fragment) affected the transport (Fig. 2A). Preincubation of p28 mAb with stoichiometric amounts of p28cyto resulted in a mutual abolition of the inhibitory effect of the other (Fig. 2A), suggesting that mAb binding to the endogenous p28 is responsible for the inhibition of the mAb and that the epitope recognized by the mAb must be exposed for p28cyto to carry out its inhibitory effect. Titration studies showed a half-maximal inhibition concentration at about 0.5 μg for p28cyto and less than 2 μg for the mAb (Fig. 2B). To determine whether intra-Golgi transport is affected by p28cyto or the mAb, we used an assay that measures transport of VSV-G protein from the ER to the trans-Golgi network (TGN), where it is sialylated (8). Kinetic studies, in which p28cyto was added after incubation of the assay for various times, suggested that transport from the cis/medial-Golgi to the TGN was not affected (Fig. 2C). p28 mAb also did not inhibit transport from the cis/medial-Golgi to the TGN (not shown). Kinetic and morphological studies (not shown) indicate that p28 participates in the docking and fusion stage of transport. To clearly establish this role, we used a two-stage transport assay (12) in which transport is first performed in the presence of EGTA, which inhibits the ER-Golgi transport at the docking and fusion stage by chelating Ca^{2+} (12). The docking and fusion stage can subsequently be resumed by incubation of the cells in fresh complete transport mixture, enabling this step to be analyzed independently. As shown in Fig. 3, p28cyto inhibited this docking and fusion stage of ER-Golgi transport, as did the mAb. In contrast, mAb against β-COP or control mAb [anti–hemagglutinin A (anti-HA)] did not inhibit this docking and fusion event.

We then examined whether p28 is a component of the 20S Golgi SNARE complex. Golgi detergent extracts were analyzed in a sedimentation gradient, and p28 was shown to have a sedimentation coefficient of ~4S [Fig. 4A (a)]. Under conditions that favored assembly of the 20S SNARE complex (2, 3, 13), p28 was shifted to the 20S fraction in a manner that is dependent on both NSF and α-SNAP (b). In contrast, p28 sedimentation was not shifted by equal amounts of NSF and α-SNAP under conditions that promoted disassembly of the SNARE complex (c). To establish that p28 existed in an authentic SNARE complex in the 20S fractions, we immunoprecipitated the p28-containing 20S fractions using mAb HFD9 (14). As shown in Fig. 4B, both NSF and α-SNAP were coprecipitated by p28 mAb (a) (lane 1), suggesting that the p28-containing 20S complex contained both NSF and α-SNAP. Furthermore, NSF and α-SNAP could be released from the precipitated complex under conditions that trigger adenosine 5′-triphosphate (ATP) hydrolysis by NSF and disassembly of SNARE complex (lanes 2 and 4). A control mAb of the same isotype did not precipitate either α-SNAP or NSF (b). We estimated the stoichiometry (in molar ratio) of p28, α-SNAP, and NSF in this

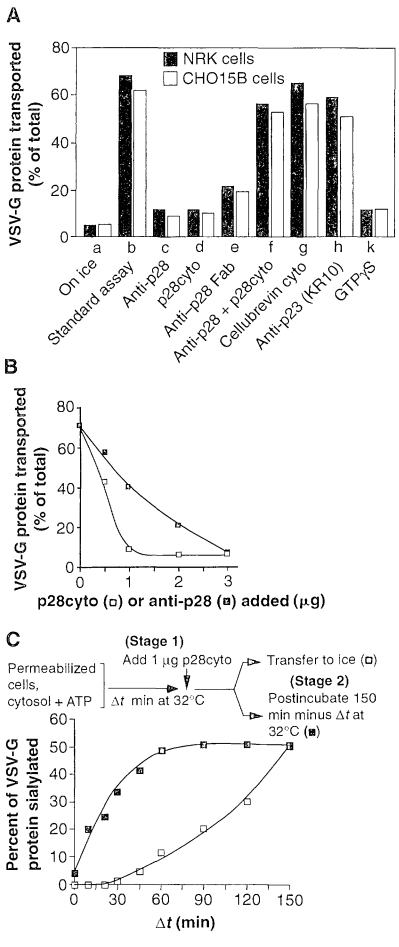

Fig. 2. p28 participates in ER to cis-Golgi transport. (**A**) Permeabilized NRK cells (black bars) or CHO15B cells (open bars), cytosol, and ATP were preincubated in the absence (a and b) or presence of the indicated additional reagents (c to k) for 45 min on ice before incubation at 32°C for 90 min (b to k). (c) mAb HFD9 (3 μg); (d) recombinant p28cyto (1 μg); (e) HFD9 Fab fragments (2.5 μg); (f) p28cyto (1 μg) and HFD9 (3 μg) (preincubated on ice for 60 min); (g) recombinant cytoplasmic domain of cellubrevin (2 μg); (h) mAb against the KDEL receptor (KR10) (11) (3 μg); (k) GTPγS (0.1 μM final concentration). Transport was measured by determining the fraction of VSV-G protein processed to the endo H–resistant form in NRK cells or endo D–sensitive form in CHO15B cells. (**B**) Assays containing permeabilized NRK cells, cytosol, and ATP were supplemented with increasing amounts of HFD9 (■) or p28cyto (□). (**C**) Permeabilized NRK cells were incubated at 32°C in a transport mixture containing cytosol and ATP, supplemented with 100 μM uridine 5′-diphosphate (UDP)–*N*-acetylglucosamine, 250 μM UDP-galactose, and 100 μM cytidine 5′-phosphate–sialic acid to measure transport to the TGN. (□) Standard time course of the incubation. At each time point, a sample of the transport reaction was terminated (stage 1 transport), and another sample was supplemented with 1 μg of p28cyto and incubated on ice for 45 min. Subsequently, the supplemented sample was shifted back to 32°C, and the transport reaction continued for a total of 150 min (stage 1 and 2 transport). Because p28cyto was added at increasing time points after the initial stage 1 incubation at 32°C, it would not inhibit transport of the fraction of VSV-G protein that had reached the cis/medial Golgi for subsequent delivery to the TGN if p28 were involved only in ER to cis-Golgi transport. In this instance, the percentage of VSV-G protein that is sialylated in the stage 1 and 2 transport (■) would be much greater than at the stage 1 transport (□) at some time points (such as those at the 30 min). The observed results are consistent with the interpretation that p28 does not participate in transport from the cis/medial Golgi to the TGN.

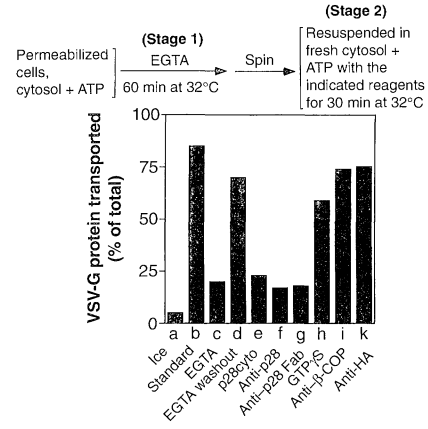

Fig. 3. p28 participates in the docking and fusion stage of ER to cis-Golgi transport. Lanes a and b: permeabilized NRK cells, cytosol, and ATP were incubated for 90 min on ice (lane a) or at 32°C (lane b). Lanes c to k: cells were incubated for 60 min at 32°C in the presence of 5 mM EGTA, which blocks ER to cis-Golgi transport at the docking and fusion stage (12). The cells were then pelleted and resuspended in standard transport buffer containing fresh cytosol, ATP, and the indicated additional reagents: (c) 5 mM EGTA; (d) no additions; (e) p28cyto (1 μg); (f) mAb HFD9 (3 μg); (g) HFD9 Fab fragments (2 μg); (h) GTPγS (0.1 μM final concentration); (i) antibody to β-COP (M3A5) (3 μg); and (k) mAb 12CA5 against hemagglutinin (HA) (3 μg).

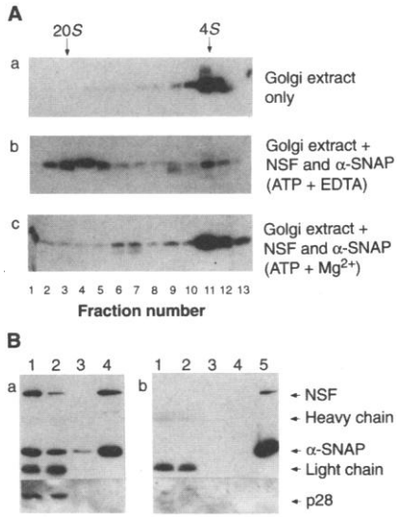

Fig. 4. p28 is part of the 20S Golgi SNARE complex. (**A**) Golgi extracts were incubated without (a) or with (b and c) NSF and α-SNAP in assembly (a and b) or disassembly buffer (c) and then separated on a 15 to 40% glycerol gradient. Gradient fractions were analyzed for the distribution of p28 by protein immunoblotting. BSA (4.6S) and α_2-macroglobulin (20S) were used as sedimentation standards. (**B**) The p28-containing 20S fractions were immunoprecipitated with mAb against p28 (a) or a control mAb (b) under the assembly condition. The immunoprecipitates were incubated in assembly (lanes 1 and 3) or disassembly buffer (lanes 2 and 4). The eluates (lanes 3 and 4) and the beads (lanes 1 and 2) were resolved by SDS-PAGE along with 100 ng of α-SNAP and NSF (lane 5) and then analyzed for the presence of NSF, α-SNAP, and p28 through the use of immunoblotting.

20S complex to be around 1 to 2:1:3 to 4 by comparing the result shown in Fig. 4B with standard curves of each protein in immunoblotting analysis, consistent with the observation that α-SNAP functions as a monomer whereas NSF acts as a homotrimer (*15*). p28 is thus a core component of the Golgi SNARE complex that participates in the docking or fusion stage of ER-Golgi transport (or both). Accordingly, we have renamed this protein GS28 (Golgi SNARE with a size of 28 kD). Because the primary sequence of GS28 is not significantly related to other known SNAREs, GS28 may represent a member of a distinct class of SNAREs that function in vesicle docking or fusion (or both). An unknown protein of 28 kD has been shown to be a component of the Golgi SNARE complex in yeast (*16*). Whether this yeast protein is related to GS28 awaits molecular characterization.

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7. Membrane proteins extracted from salt-washed rat liver Golgi membranes (*6*) were incubated with CNBr-Sepharose conjugated with mAb HFD9. p28 eluted from the beads was subjected to trypsin or endoproteinase GluC digestion. The resulting peptides were sequenced with a model 6600 ProSequencer (Milligen, Burlington, MA). One cDNA fragment of about 400 bp was obtained by PCR with a sense oligonucleotide [GA(T/C)AT(T/C/A)(T/C)TICA(A/G)GA(T/C)TA(T/C)ACICA] to peptide P4 (DILDYQTHE) and an antisense oligonucleotide [TG(A/G/T)ATIA(A/G)I(C/G)(A/T)(A/G)TTIACAGC IGG (A/G)AA] to peptide P9 (FPAVNSLIQR). This cDNA fragment was used to isolate a full-length rat cDNA clone (SKT7p28). Database searches were done with the BLAST program.
8. Transport assay was done exactly as described [C. J. M. Beckers, D. S. Keller, W. E. Balch, *Cell* **50**, 523 (1987); H. W. Davidson and W. E. Balch, *J. Biol. Chem.* **268**, 4216 (1993)].
9. The coding regions of p28cyto and cellubrevin minus the COOH-terminal transmembrane regions were amplified and cloned into pET23(d) (Novagen). This vector yields recombinant proteins with a COOH-terminal hexahistidine tag. Proteins were purified as recommended by the manufacturer.
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13. The 20S complex formation was done as described (*2, 3*). Salt-washed Golgi membranes were extracted with assembly buffer [20 mM Hepes-KOH (pH 7.4), 100 mM KCl, 2 mM dithiothreitol, 2 mM EDTA, 0.5 mM ATP, 0.5% (v/v) Triton X-100] or disassembly buffer (as above plus 8 mM $MgCl_2$). The extracts were then centrifuged at 100,000*g* in a TLA 100.2 rotor (Beckman) for 45 min and the supernatants retained. Recombinant His_6-NSF and His_6–α-SNAP were purified as described (*2, 3, 17*). Golgi extract (300 μg) was incubated with 60 μg of His_6-NSF and 15 μg of His_6–α-SNAP in a final volume of 500 μl. After a 30-min incubation at 4°C, the samples were loaded onto a 15 to 40% (w/v) glycerol gradient in the assembly (Fig. 4A) (a and b) or the disassembly buffer (c). Centrifugation was carried out for 18 hours in a SW41 rotor (Beckman). Fractions of about 0.8 ml were collected manually from the bottom at a flow rate of ~1 ml/min. Samples were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), and the distribution of p28 was determined by immunoblotting.
14. Gradient fractions containing the 20S complex were diluted with assembly buffer containing 0.1% bovine serum albumin (BSA) and preincubated with protein G–Sepharose beads for 2 hours at 4°C followed by immunoprecipitation with either mAb HFD9 or mAb DAD5 (immunoglobulin G1 subtype control) bound to protein G–Sepharose. Beads were washed five times with assembly buffer containing 0.1% BSA, split into two equal portions, and incubated for 1 hour at room temperature with assembly buffer or disassembly buffer [assembly buffer containing 4 mM $MgCl_2$, 4 mM $CaCl_2$, and 25 μM guanosine triphosphate (GTP)]. The beads and supernatants were then processed for immunoblotting with antibodies to p28, α-SNAP, and NSF.
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Vertical Flux of Biogenic Carbon in the Ocean: Is There Food Web Control?

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Models of biogenic carbon (BC) flux assume that short herbivorous food chains lead to high export, whereas complex microbial or omnivorous food webs lead to recycling and low export, and that export of BC from the euphotic zone equals new production (NP). In the Gulf of St. Lawrence, particulate organic carbon fluxes were similar during the spring phytoplankton bloom, when herbivory dominated, and during nonbloom conditions, when microbial and omnivorous food webs dominated. In contrast, NP was 1.2 to 161 times greater during the bloom than after it. Thus, neither food web structure nor NP can predict the magnitude or patterns of BC export, particularly on time scales over which the ocean is in nonequilibrium conditions.

Between 30 and 50% of the CO_2 released from fossil fuel is removed from the atmosphere and exported from the ocean surface to depth as dissolved inorganic carbon and BC derived from primary production (PP) (*1–3*). The downward flux of BC from the ocean surface can be estimated directly from particulate organic carbon (POC) accumulation in sediment traps (*2, 4, 5*) or assessed indirectly (*5–7*) from