Here we report that Yip1p and Yif1p, two members of an integral membrane protein complex that bind to the Rab Ypt1p, are required for membrane fusion with the Golgi in vitro. To block fusion, anti-Yip1p or anti-Yif1p antibodies must be added before vesicles bud from the endoplasmic reticulum (ER). These antibodies do not block the packaging of Yip1p, Yif1p, or the soluble NSF attachment protein receptor (SNAREs) into vesicles. We propose that Yip1p and Yif1p perform a critical role in establishing the fusion competence of ER to Golgi vesicles at the time of budding. Consistent with this proposal, we observe that the Yip1p-Yif1p complex binds to the ER to Golgi SNAREs Bos1p and Sec22p, two components of the membrane fusion machinery.

The specificity of membrane traffic through the secretory pathway is ensured by the machinery that mediates vesicle tethering, docking, and fusion. The molecular components required for these events are the tethers, Rabs and SNAREs (soluble NSF attachment protein receptor).1 The tethers include multisubunit complexes and large coiled-coil proteins, whereas Rabs are key in regulating all tethering events. Tethering factors physically bind the correct carrier vesicle to its appropriate acceptor compartment. This occurs through specific interactions between components on the target membrane and the vesicle (1). Exactly how the SNAREs contribute to membrane fusion is the subject of an ongoing debate (2). It has been demonstrated that purified SNARE proteins inserted into in vitro. To block fusion, anti-Yip1p or anti-Yif1p antibodies retained on the columns were acid-eluted, neutralized, and applied to the appropriate fusion protein coupled to Affi-gel matrix (Bio-Rad). The antibodies retained on the columns were acid-eluted, neutralized, and diaлизed into TBS buffer (115 mM potassium acetate, 2.5 mM magnesium acetate, 150 mM sodium chloride, 5 mM potassium chloride, 1 mM EDTA, 0.5% Triton X-100, and 0.1% sodium azide).

Another unanswered question is how the transition from vesicle tethering to membrane fusion takes place. It is thought that the activation of the Rab is pivotal to the initiation of the downstream docking event, commonly defined as trans-SNARE pairing, which is followed by membrane fusion (5, 6). Rab proteins belong to a family of small GTPases that regulate membrane traffic (7). In the cytosol, the GDP-bound form of the Rab is in a complex with guanine nucleotide dissociation inhibitor (GDI). Rabs are recruited to membranes with the aid of a GDI displacement factor (8) and inserted into the membrane via a prenyl group. Once the prenylated Rab is delivered to the membrane, it is activated by a specific guanine nucleotide exchange factor through the exchange of GDP for GTP (9, 10).

Membrane proteins that bind to prenylated Rab proteins have been identified and may provide a clue to the mechanism of Rab attachment to membranes. These factors, which include Yip1p, Yip2p (also called Yop1p), Yip3p, Yip4p, Yip5p, Yif1p, and their mammalian homologs, comprise a family of proteins defined by certain features (11). These include a common domain topology with a cytosolic N terminus, luminal C terminus, and multiple transmembrane-spanning domains. Furthermore, these proteins have been shown to interact with multiple Rabs and with each other (11–13). Yip1p and Yif1p are the only essential proteins in this family (14). Yip1p was first identified using the two-hybrid system as a protein that interacts preferentially with the GDP-bound form of Ypt1p and Ypt31p (13), two small GTP-binding proteins that act in ER-Golgi and intra-Golgi traffic, respectively (15–18). Yif1p was later identified by its ability to interact with Yip1p in a two-hybrid screen (12). These proteins form a complex and bind to multiple Rabs in yeast (11–13). Temperature-sensitive mutations in Yip1p and Yif1p have been shown to block ER-Golgi transport in vivo and lead to the accumulation of ER membranes and 40- to 50-nm vesicles (12, 13).

Little is known about how the Yip1p family of proteins are involved in membrane traffic. Here we show that the Yip1p-Yif1p complex binds to ER to Golgi SNAREs and is required for the fusion of ER-derived vesicles with the Golgi apparatus. Because the Yip1p family of proteins is highly conserved, it is likely that these family members play a key role in membrane fusion at all stages of vesicle traffic in eukaryotic organisms.

**EXPERIMENTAL PROCEDURES**

*Antibody Preparation and Purification—Polyclonal antibodies were raised in rabbits using purified recombinant proteins. Recombinant Yip1p was expressed in bacteria as an N-terminal His tag fusion to amino acids 1–106 (pET-15, Novagen) and purified on nickel-nitrilotriacetic acid-agarose resin (Qiagen) according to the manufacturer’s instructions. To obtain purified recombinant Yif1p, an N-terminal GST fusion to amino acids 16–206 was expressed in bacteria using the vector pGEX-5x-3 and purified using glutathione-Sepharose 4B (Amersham Biosciences). Antibodies were affinity-purified on columns containing the appropriate fusion protein coupled to Affi-gel matrix (Bio-Rad). The antibodies retained on the columns were acid-eluted, neutralized, and dialyzed into TBS buffer (115 mM potassium acetate, 2.5 mM magnesium acetate, 150 mM sodium chloride, 5 mM potassium chloride, 1 mM EDTA, 0.5% Triton X-100, and 0.1% sodium azide).*
sium acetate, 25 mM HEPES, pH 7.2, 250 mM sorbitol). For pre-absorption of anti-Yif1p antibody, 1 mg of purified GST or GST-Yif1p was transferred to nitrocellulose and incubated with affinity-purified antibody. Fab fragments were generated by digesting affinity-purified antibodies with papain using the ImmunoPure Fab preparation kit (Pierce) according to the manufacturer’s protocol.

Construction of Gal1-YIP1 and Generation of a yip1 Temperature-sensitive Mutant—To place YIP1 under the control of the inducible GAL1 promoter, the open reading frame of YIP1 was cloned into pNB529 (LEU2 integrating vector), linearized, and transformed into a diploid strain in which the genomic copy of YIP1 had been disrupted with the HIS3 gene. Sporulation, dissection, and tetrad analysis were performed to obtain the haploid strain SFNY1159 (Gal’ ura3–52 his3 aro2–3 leu2–3, 112 his32300 yip1Δ::HIS3 ura3–52 pSFN905 (YIP1 URA3 CEN)). The plasmid containing YIP1 was subsequently lost by purifying the transformants on minimal media containing uracil and 1g/liter 5-fluoroorotic acid (Sigma). Surviving colonies were then tested for temperature sensitivity on YPD (1% yeast extract, 2% peptone, 2% dextrose) plates. One mutant allele named yip1–3 was obtained. DNA sequence analysis revealed the presence of five mutations (E70G, T88A, K130E, F142L, and S146P).

In Vitro Transport Assay—Standard transport assays, as well as the preparation of fractions and reagents, were performed as described previously (20, 21). In all assays where antibody was included, a 20-min preincubation was performed on ice prior to the 20 °C incubation. Vesicles used for immunoprecipitation were produced during an in vitro budding reaction as described previously (20). Reactions were performed in the presence or absence of antibody (8–12 μg of anti-Yif1p or anti-Yip1p) as indicated with the exception that the protein A-Sepharose beads were washed four times with TBS buffer before boiling in sample buffer. The presence or absence of antibody, vesicles were allowed to bud from donor cells during a 20-min incubation at 20 °C before the donor cells were pelleted during a 1-min spin in a microcentrifuge. Golgi membranes with or without antibody were then added to the reactions. Following a preincubation on ice to allow the antibody to bind, reactions were incubated at 20 °C for 1 h. Reactions were processed to obtain ConA-precipitable counts. Anti-outER chain-precipitable counts were normalized to the ConA-precipitable counts released from donor cells in the absence of antibody. The vesicle tethering assay was performed as described before (22).

Fractionation, Immunoprecipitation, and Western Blot Analysis—Lysates were prepared from yeast strains that had been shifted to 37 °C for 1 h (Fig. 4A) or from strains that had been grown in YPD for 14 h to deplete cells of Yip1p (Fig. 4B). Cells were centrifuged to spheroforms and lysed in 20 mM HEPES, pH 7.2. Unbroken pellets were collected during a brief spin at 450 × g for 5 min. Lysates were centrifuged at 42,000 rpm in an SW50.1 rotor (170,000 × g) for 1 h. The pellets were resuspended in a volume of buffer equal to the supernatant fractions, and all fractions were boiled in sample buffer for analysis by SDS-PAGE and Western blot analysis.

Glass bead lysates were prepared for immunoprecipitation of Yip1p. Briefly, 2 mg of lysis was diluted to a volume of 1 ml with IP buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitors). Lysates were incubated for 1 h with 12 μg of affinity purified anti-Yif1p antibodies. The anti-Yip1p antibodies were bound to Protein A-Sepharose beads during a 1-h incubation. Beads were pelleted and washed five times with 1 ml of IP buffer, followed by boiling in 50 μl of SDS sample buffer.

In Vitro Binding Assays—Full-length Yip1p fused at its N terminus to MBP (maltose-binding protein) was expressed in bacteria and purified on amylose resin (New England BioLabs) as described by the manufacturer. His6-tagged fusion proteins were expressed in bacteria and purified on nickel-nitrotriacetic acid-agarose (Qiagen). Prepurified Yip1p was expressed in yeast as a His-tagged fusion protein and purified from the membrane fraction as described before (23). Sso1p (amino acids 1–265) and Sec9p (amino acids 416–651) were purified as described before (24). HisYip1p was pre-loaded with nucleotide as described previously (25). In vitro binding assays were performed by incubating varying amounts of tagged proteins, Sso1p, or Sec9p with 100 nm MBP-Yip1p (immobilized on beads) in a 100-μl reaction containing binding buffer (25 mM HEPES, pH 7.5, 140 mM potassium acetate, 0.1% gelatin, 0.2% CHAPS, 10% glycerol, 10 mM MgCl2, 1 mM diithiothreitol). Binding buffer contained 40 μg GTP in binding assays using pre-loaded Yip1p. Also, binding experiments with non-preyylated Yip1p were done in binding buffer containing 0.01% Triton X-100. Samples were incubated overnight at 4 °C or at room temperature for 1 h, and the beads were washed five times with 1 ml of binding buffer. The bound protein was eluted in SDS-sample buffer and detected by Western blot analysis using the ECL method (Amersham Biosciences).
regenerating mix at 20 °C for 20 min in the absence of antibody. A parallel set of assays was prepared in which antibody was added at the beginning of the budding reaction. Donor cells were then pelleted in all samples, and the supernatant that contained the vesicles was removed. Vesicles formed in the absence of antibody were mixed with Golgi membranes and buffer, anti-Yif1p antibody, or anti-Yip1p antibody. Vesicles formed in the presence of antibodies were mixed with Golgi membranes. Following a 20-min incubation on ice to allow the vesicles to form in the absence of anti-Yif1p antibody, does not correlate with the block in fusion. Assays were performed in the presence of the indicated amount of antibody. Maximum budding is the vesicle release observed in the absence of antibody. Similarly, maximum fusion is the outer-chain precipitable counts obtained in a reaction without antibody.

Fig. 1. Anti-Yif1p antibodies block ER to Golgi transport. A standard transport reactions were performed in the absence (lane 1) or presence of 8 μg of anti-Yif1p antibody (lane 2), pre-absorbed to GST (lane 3) or GST-Yif1p (lane 4). Pre-absorption of anti-Yif1p antibody to GST-Yif1p, but not to GST, restored transport activity. Anti-Yif1p Fab fragments (15 μg) also blocked transport (lane 5). B, the decrease in budding efficiency, observed in the presence of anti-Yif1p antibody, does not correlate with the block in fusion. Assays were performed in the absence of antibody. Maximum budding is the vesicle release observed in the absence of antibody. Similarly, maximum fusion is the outer-chain precipitable counts obtained in a reaction without antibody.

Fig. 2. Antibodies to Yif1p or Yip1p do not block fusion when added after vesicles bud from the ER. Vesicles were generated during a 20-min incubation in the presence of buffer (lane 1), anti-Yif1p (lane 2), or anti-Yip1p antibody (lane 3). Donor cells were pelleted, and Golgi membranes added to the supernatant were allowed to incubate with vesicles at 20 °C for 1 h before fusion was assayed. Conversely, in lanes 4–6 buffer (lane 4), anti-Yif1p (lane 5), or anti-Yip1p antibody (lane 6) was added to vesicles immediately following the budding reaction. Reactions were preincubated on ice with Golgi membranes to allow the antibody to bind before a 1-h incubation at 20 °C. All samples were normalized to the amount of pro-a-factor released from donor cells (equal vesicles) in the absence of antibody.

Membrane Attachment of Ypt1p Is Not Affected in the Absence of Yip1p Function—Membrane attachment is required for Ypt1p function (26), and Ypt1p is required for vesicle tethering (27, 28). In the yeast two-hybrid system, Yip1p family members bind preferentially to the prenylated form of Rab proteins (11). It has been postulated that these proteins serve as a membrane receptor, or platform, for Rab membrane recruitment. Our in vitro finding that Yip1p and Yif1p are required for vesicle fusion, but not tethering, implies that Ypt1p function is not affected when the Yip1p-Yif1p complex is inactive and that Ypt1p may still be attached to membranes. To determine if this is indeed the case, we monitored the solubility of Ypt1p when Yip1p was defective. First, we generated a yip1 temperature-sensitive mutant using PCR mutagenesis. lysates (T) prepared from wild type and a yip1-3 mutant that had been shifted to 37 °C for 1 h were centrifuged at 170,000 × g, fractionated into supernatant (S) and pellet (P) fractions and then blotted for the presence of Ypt1p. We observed no difference in the distribution of Ypt1p in yip1-3 lysates (Fig. 4A, compare lanes 1–3 with lanes 4–6). In contrast, Ypt1p was
clearly more soluble in lysates prepared from bet2\(^{-1}\) (lanes 7–9), a mutant that is defective in Ypt1p prenylation (26).

We also found that the membrane attachment of Ypt1p was not affected when cells were effectively depleted of Yip1p. A strain was constructed in which the sole copy of \(YIP1\) was placed under the control of the inducible \(GAL1\) promoter. Prior to lysing the cells and fractionating as described above, the \(GAL1-YIP1\) strain (SFNY1105) was grown for 14 h in YPD medium. The solubility of Ypt1p remained unchanged in SFNY1105. C, vesicles co-migrating with Golgi membranes do not fuse in the presence of anti-Yip1p antibody.

To rule out the possibility that Ypt1p was aggregating during sedimentation or was recruited to the wrong membrane, we performed subcellular fractionation studies. Lysates were prepared from SFNY1105 as described above and fractionated on a gradient previously used to separate the ER and Golgi where Ypt1p is normally found (22, 29). The SNARE Bos1p marks the Golgi (Fig. 4C, fractions 3 and 4) and the ER (fractions 8 and 9) on this gradient. Soluble proteins are largely found at the top of the gradient, in fraction 1, where the sample is loaded. Because members of a complex are frequently unstable in the absence of their partner(s), we also blotted for the presence of Yif1p. As shown in Fig. 4C, the Yip1p-depleted fractions also lacked Yif1p (inset). Furthermore, the localization of Ypt1p was unaffected in the absence of the Yip1p\(\times\)H18528Yif1p complex (graph). The same result was obtained when lysates prepared from the yip1\(^{-3}\) mutant were fractionated on this gradient (data not shown). In light of the fact that Ypt1p is required for vesicle tethering, the finding that Ypt1p membrane association is unaffected by the loss of the Yip1p\(\times\)Yif1p complex \(in\) \(vivo\) is con-
SNARE proteins are known to be selectively recruited into budding vesicles and are required for membrane fusion (30, 31). Therefore, anti-Yif1p and anti-Yip1p antibodies may block fusion because of a failure to incorporate SNAREs into vesicles. To address this possibility, vesicles were generated in the presence or absence of anti-Yif1p antibody, and then donor cells were pelleted. Supernatant fractions containing vesicles were subjected to a 200,000 × g spin, and vesicle pellets were solubilized in sample buffer and blotted for the presence of SNAREs. As shown in Fig. 5B, the ER to Golgi SNAREs Bos1p and Bet1p, as well as Yif1p, were efficiently packaged into budding vesicles in the presence of anti-Yif1p antibody. The membranes containing Bos1p, Bet1p, and Yif1p were clearly vesicles and not fragmented ER, because the release of these proteins from the PYCs was time-dependent (not shown) and required ATP (Fig. 5B). Furthermore, a resident of the ER, Sec61p, was not released from the PYCs during the assay. Sec22p and Sed5p were also efficiently packaged into vesicles (data not shown). Thus, the failure of vesicles to fuse with Golgi membranes is not due to a failure to package ER-Golgi SNAREs or to the exclusion of Yif1p or Yip1p from budding vesicles.

ER to Golgi SNAREs Bind to Yip1p in Vitro and in Vivo—Our results clearly indicate that Yip1p and Yif1p are required for membrane fusion. To begin to determine if the Yip1p-Yif1p machinery interacts with the SNAREs, we examined the binding affinity of Yip1p for Bos1p in vitro. Bos1p is a stage-specific v-SNARE that is packaged into vesicles and required for anterograde ER-Golgi transport (20, 32, 33). For our studies, full-length Yip1p was fused to maltose-binding protein (MBP-Yip1p) and purified. Because Yip1p has been shown to bind to Ypt1p (13), we tested the ability of MBP-Yip1p to bind to Ypt1p in vitro. Prenylated and non-prenylated forms were tested. Non-prenylated Ypt1p was purified as a His6-tagged fusion protein (His6-Ypt1p) from bacteria, and prenylated Ypt1p was purified from a yeast membrane fraction. Because the binding of GDP-bound Ypt1p to MBP-Yip1p is ~7-fold higher than GTP-bound Ypt1p (data not shown), His6-Ypt1p was pre-loaded with GDP in all binding studies. Incubation of varying amounts of non-prenylated (Fig. 6A, see graph) or prenylated His6-Ypt1p (Fig. 6A, see inset) showed specific saturable binding to MBP-Yip1p immobilized on beads. Prenylated Ypt1p bound to MBP-Yip1p with higher affinity, and saturation was achieved at 0.5 μM. Significant binding of Arf1p, another GTPase, was not seen (Fig. 6C). Thus, binding appears to be specific for members of the Rab family.

Because full-length Bos1p is only soluble in the presence of high concentrations of detergent (1% CHAPS), which elutes MBP-Yip1p from beads, all binding studies were done with a His6-tagged construct of the cytoplasmic domain of Bos1p that was described before (34). Increasing amounts of His6-Bos1p specifically bound to MBP-Yip1p (Fig. 6B, lanes 5–9), but not MBP (lanes 1–4). Quantitation of the data indicated that binding was saturable (not shown). Not all SNAREs bound to Yip1p. Although binding of Sec22p was observed, significant binding of Sso1p and Sec9p, two SNAREs that act in post-Golgi membrane traffic, was not seen (Fig. 6C).

The in vivo relevance of the binding of SNAREs was tested by immunoprecipitation. Cell lysates were prepared by glass bead lysis and immunoprecipitated with anti-Yif1p antibody. The precipitates were then blotted for Bos1p, Sec22p, and Ypt1p. Sec22p was readily found in the precipitate (Fig. 6D, lane 1). A Bos1p band (lane 1), which increased in intensity when Bos1p was overproduced (lane 2), was also observed. The co-precipitation of Ypt1p and Sec22p (lane 3), as well as Bos1p (data not shown), was clearly dependent on the presence of anti-Yif1p

sistent with the observation that vesicle tethering occurs in vitro in the absence of Yip1p or Yif1p function. The Block in Fusion Is Not Due to a Defect in the Packaging of ER-derived Vesicles—The specific recruitment and packaging of cargo and transport machinery into vesicles budding from the ER is critical for successful transport. The failure of vesicles formed in the presence of anti-Yif1p or Yip1p antibody to fuse with Golgi membranes may be the result of a failure to package a protein or proteins required for downstream fusion events, or could reflect a failure of Yif1p or Yip1p themselves to be packaged.

To begin to address these possibilities, we compared the ability of anti-Yif1p or anti-Yip1p antibody to precipitate vesicles when added before or after budding. Anti-Bos1p antibody was used as a control in these studies. Vesicles generated in the presence of antibody were either lysed in 1% Triton X-100 (Fig. 5A, lanes 1, 4, and 7), or precipitated in the absence of detergent (lanes 2, 5, and 8). Vesicles were also formed in the absence of antibody and then precipitated (lanes 3, 6, and 9). The finding that comparable pro-a-factor-precipitable counts were obtained (normalized for differences in vesicle release) when antibody was added before or after budding indicates that the antibody binds to Yif1p or Yip1p on vesicles. Furthermore, these findings also imply that Yif1p and Yip1p are not physically excluded from budding vesicles.

Fig. 5. Anti-Yif1p and anti-Yip1p antibodies do not affect the incorporation of Yif1p, Yip1p, or SNAREs into budding vesicles. A, vesicles containing radiolabeled pro-a-factor, formed in the presence of the indicated antibody (lanes 2, 5, and 8), were precipitated by the addition of protein A-Sepharose. The amount of precipitable counts obtained in the presence of anti-Yif1p or anti-Yip1p antibody was comparable to the amount observed when vesicles were formed in the absence of antibody (compare lanes 5 and 6, and lanes 8 and 9). Anti-Bos1p antibody (4 μg) was included as a control (lanes 1–3). All samples were normalized to the amount of pro-a-factor released from donor cells (equal vesicles) in the absence of antibody. The precipitation of counts required sealed membranes (lanes 1, 4, and 7). In the absence of any antibody, 168 cpm were precipitated with protein A-Sepharose alone. B, vesicles formed in the presence or absence of anti-Yif1p antibody were separated from PYC and pelleted during a 200,000 × g spin. The percent release was normalized to account for the reduction of budding in the presence of anti-Yif1p antibody. As a control, budding reactions were performed with apyrase plus antibody. No Sec61p was released from the PYCs.
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Discussion

A Novel Role for the Yip1p Family in Membrane Fusion—Here we have used an in vitro transport assay that reconstitutes transport between the ER and Golgi to define a role for Yip1p and Yif1p in membrane traffic. Our finding that vesicles are tethered but unable to fuse in the absence of Yip1p-Yif1p function implies that these proteins are required for the transition to, and/or completion of, vesicle fusion. A physical link between the Yip1p-Yif1p complex and components of the membrane fusion machinery is provided by the finding that Bos1p and Sec22p, two ER to Golgi SNAREs, co-purify with Yif1p in vitro. The requirement for Yip1p proteins in membrane fusion may be common to multiple stages of membrane traffic in all eukaryotic cells. Mammalian homologs of two Yip1 family members, Yip1p and Yip2p, have been characterized. The mouse homolog of Yip1p, called Yip1A, is localized to ER exit sites and vesicular-tubular structures, which carry cargo to the Golgi. Evidence for a role in membrane traffic has come from the observation that the overexpression of the cytoplasmic tail of Yip1A inhibits transport of vesicular stomatitis virus envelope glycoprotein to the cell surface in normal rat kidney cells infected with vesicular stomatitis virus (35).

Evidence that Yip1p family members act at other stages of membrane traffic comes from work on the mammalian homolog of Yip2p, called Pra1p. Pra1p was isolated in several two-hybrid screens as a protein that interacts with multiple Rabs (36–39) in their GTP- or GDP-bound forms. It has been suggested that Pra1p is a GDI displacement factor that recruits RabA to membranes (40). In addition to binding to Rabs, Pra1p binds to the SNARE Vamp2p (39). Although Pra1p localizes predominantly to Golgi membranes (41), it is also present at the presynaptic active zone where it is a component of the cytoskeletal matrix. There it interacts with Piccolo (42), a protein implicated in the regulation of Ca²⁺-mediated exocytosis (43).

An interesting question is how the Yip1p family members, which have been shown to bind to each other, combine to mediate membrane fusion at multiple stages of membrane traffic. One possibility is that their steady-state localizations are distinct and regulated. Evidence that this may be the case comes from work on Pra1p and its isoform, Pra2p. Although Pra1p localizes predominantly to the Golgi, Pra2p has been found to be retained largely on the ER. The sequences that are responsible for this differential localization have been isolated, but the functional significance remains unclear (41). Yip1p and Yif1p largely reside on Golgi membranes at steady state (12, 13), however, it remains to be seen whether other family members exhibit different localization patterns or interact with stage-specific SNARE proteins to mediate fusion throughout the pathway.

The binding of a Rab to the correct intracellular membrane is an important mechanism in the regulation of each stage of vesicle transport. The identification of the Yip1p family of integral membrane proteins as Rab binding proteins led to the proposal that they may function in the recruitment and/or retention of the Rab on membranes (12, 13, 40). Surprisingly, we have observed that the Yip1p-Yif1p complex is not required for the membrane localization of Ypt1p. The loss of Yip1p and Yif1p in vivo does not lead to an increase in the soluble pool of Ypt1p and Ypt1p is recruited to the correct intracellular membrane. Consistent with this result, we find that the tethering of ER-derived vesicles to the Golgi is unaffected in vitro by the loss of Yip1p-Yif1p function.

Our finding that antibodies to Yip1p or Yif1p must be added during vesicle budding to block fusion imply that the Yip1p-Yif1p complex is required early in the transport reaction, specifically at the time a vesicle forms. This may best be explained by proposing that the antibodies may inhibit a function of Yip1p and Yif1p that is critical for establishing the fusion competence of ER-derived vesicles. Once this function has been executed, it cannot be reversed or inhibited by the addition of antibody at a step downstream of the budding process. Perhaps the Yip1p-Yif1p complex is required to coordinate a priming event that would ultimately allow the tethering and fusion machinery to communicate once the vesicle arrives at the Golgi, or it may recruit an unidentified factor to the budding vesicle that is required for fusion. Whether binding of Yip1p-Yif1p directly to the SNARE promotes complex priming, rearrangement, or trans-SNARE pairing is not known. Interestingly, we find that the steady-state level of SNARE complexes, in lysates prepared from yip1-3 cells, is reduced compared with wild type (data not shown). However, this result...
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must be interpreted with some caution due to the observed reduction in budding noted in our in vitro work and its unknown relation to what may happen in vivo.

Implications for Membrane Fusion—Despite significant progress in discovering and detailing the molecular events that constitute vesicle tethering, the transition into the final stages of membrane fusion remain unclear. Since the inception of the SNARE hypothesis (30), the notion that the transmembrane SNAREs play an important role in membrane fusion has remained a basic tenet in the membrane trafficking field. Serving to reinforce this idea, in vitro work with reconstituted liposomes has shown that certain combinations of SNAREs are capable of promoting membrane fusion (44–46). However, an idea is now emerging that SNAREs may play an important role in membrane fusion or by signaling to downstream machinery, remains to be clarified.

Because of the importance of understanding the final events that result in bilayer fusion, it will be of interest to determine the exact nature of the interaction of Yip1p family members with the SNAREs. It is possible that the Yip1p family of proteins may facilitate SNARE-mediated membrane fusion by regulating their activity and, consequently, their ability to form productive trans-SNARE complexes. It is also possible that the Yip1p family may themselves constitute a core fusion machinery that acts in concert with SNAREs. To this end, it will be interesting to see if Yif1p and Yip1p, or other family members, are required for fusion at other stages of membrane traffic.

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