# Comment

## Reversal of Fortune: Do Rab GTPases Act on the Target Membrane?

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The proper targeting and fusion of transport vesicles with the correct membrane is a critical event in the determination of the identity of different compartments within the cell. Work over the last decade has made tremendous progress toward determining a general mechanism by which this occurs. The cornerstones of such a mechanism will have to include two families of proteins: Rab GTPases and SNARE proteins. SNARE proteins are thought to have a central role in catalyzing the fusion of the vesicle with the target membrane (Weber et al., 1998), while Rab GTPases appear to work upstream of this in mediating the initial docking or tethering of the vesicle to the target membrane (Cao et al., 1998; Waters and Pfeffer, 1999). To understand the mechanism by which these two classes of proteins collaborate in this process, it is important to know the arrangements of these proteins with respect to the vesicle and target membrane. Work over the last decade has given us insights as to the membrane surfaces with which these proteins are localized. It is from this localization that the terms vesicle or v-SNARE and target membrane or t-SNARE have their origin. Likewise Rab proteins, such as Sec4, Ypt1, and Rab3 have all been found associated with transport vesicles: Sec4 on post-Golgi vesicles (Goud et al., 1988), Ypt1 on ER-to-Golgi vesicles (Segev, 1991; Lian and Ferro-Novick, 1993), Rab3 on synaptic vesicles (Fischer von Mollard et al., 1990). Other than these clues given by their presence on vesicle or target membrane compartments, no direct evidence of their site of action was known. This is especially important considering that in many cases the SNARE and Rab proteins are present at significant levels on both the target and vesicle membranes. The article by Cao and Barlowe in this issue, provides the first comprehensive test of the site of action of SNAREs and Rab proteins in the fusion of transport vesicles with a target membrane (Cao and Barlowe, 2000). The results are quite surprising.

The authors make use of a two-stage in vitro system for examining the docking and fusion of ER-derived vesicles with the Golgi apparatus. In this assay vesicles are produced by incubation of donor membrane containing $^{35}$S-labeled cargo with purified COPII coat subunits to initiate the production of ER-to-Golgi vesicles. These vesicles, containing radiolabeled cargo, can then be readily separated from the donor membrane by centrifugation. The second stage of the assay is carried out by incubating the isolated vesicles with an acceptor compartment containing the target Golgi membranes. Fusion is measured by the modification of the radiolabeled cargo by an enzyme present in the lumen of the acceptor Golgi membranes. The authors begin to examine the function of specific factors by making use of temperature-sensitive yeast mutants in the v-SNAREs Bet1 and Bos1, the t-SNARE Sed5, the Rab GTPase Ypt1, and the SNARE assembly factor Sly1. Remarkably, the authors are able to reconstitute the temperature sensitivity of each of the mutants in their in vitro system. By then doing mixing experiments with fractions generated from wild-type strains they are then able to examine whether the defect in each mutant resides with the vesicle fraction or the acceptor or target membrane fraction. For example, in experiments with donor or acceptor membranes containing mutant forms of the v-SNAREs Bos1 or Bet1, the authors find a clear temperature-sensitive defect in the ability of the mutant vesicles to fuse with wild-type acceptor membranes but acceptor membranes prepared from these mutants showed no defect. Conversely, when the authors examined the defects associated with a temperature-sensitive mutant in Sed5 or its binding partner Sly1, they saw no defect associated with the mutant vesicles but did see a clear temperature-sensitive defect in the acceptor or target membrane. Therefore, the data so far strongly supports the prevailing model for these proteins: Bet1 and Bos1 serving as v-SNAREs on the surface of ER-to-Golgi vesicles and the t-SNARE Sed5 along with the Sec1 homologue Sly1, functioning on the target Golgi membrane.

The most surprising results came when the authors examined the function of Ypt1, the Rab GTPase implicated in ER-to-Golgi transport. Vesicles prepared from a temperature-sensitive mutant in Ypt1, *ypt1-3*, had no defect on their ability to fuse with wild-type acceptor membranes. However, Ypt1 mutant acceptor membranes showed a dramatic defect in their ability to allow fusion with wild-type vesicles. Consistent with this the authors also find that very little Ypt1 is recruited onto vesicles when they are produced with purified COPII components, suggesting that Ypt1 normally associates with these vesicles after their initial COPII-dependent budding. However, unlike the membrane-embedded SNAREs, Ypt1 and Rabs are known to cycle on and off membranes with the assistance of a protein known as GDI. This protein has the ability to solvate the hydrophobic geranylgeranyl group at the COOH terminus of Rab proteins and extract them off the membrane which then allows them to attach to an-

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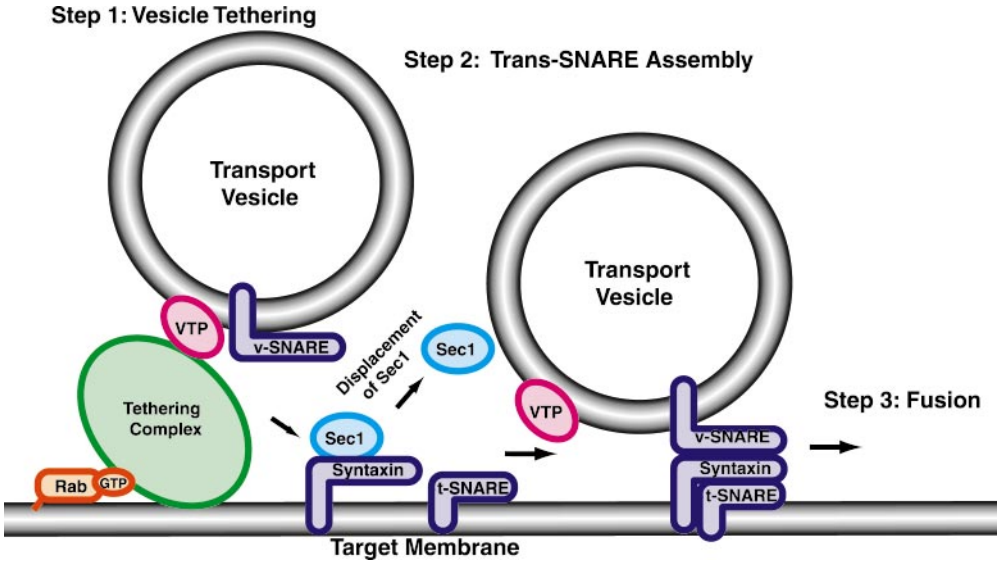


*Figure 1.* A Model for Rab GTPase function on the target membrane. Heterotypic vesicle docking and fusion is thought to proceed in 3 sequential steps. (Step 1) The tethering of vesicles to the target membrane. This would involve interaction of the tethering complex, such as the exocyst, TRAPP, Uso1 or Sec34/35 with GTP-bound Rab on the target membrane as well as the interaction of the tethering complex with the vesicle through a vesicle-bound tethering protein, or VTP. This ternary interaction may involve a kinetic proofreading function by the Rab GTPase analogous to the role of EFTu during translation (Bourne, 1988). (Step 2) The initial assembly of t-SNARE and trans-SNARE complexes. The full engagement of the tethering machinery would be coupled to SNARE assembly by regulating the displacement of a Sec1 family member (called Sly1 in ER-to-Golgi transport) from a Syntaxin family member (Sed5 or Syntaxin 5 in ER-to-Golgi transport) which is a prerequisite for assembly of Syntaxin family members with other t-SNAREs (Sec9 or SNAP-25 in post-Golgi transport) as well as trans-v/t-SNARE complexes. (Step 3) Finally, the association of the SNAREs would lead either indirectly (Ungermann et al., 1998) or directly (Weber et al., 1998) to the fusion of the vesicle and target membranes.

other membrane. This suggested the possibility that in this assay wild-type Ypt1 function on the vesicle may be provided by it "jumping" from the acceptor membrane fraction to the mutant vesicle membranes. This would give the misleading result that any defect associated with a mutant form of Ypt1 was manifested on the target membrane. To get around this problem the authors made use of a chimeric form of Ypt1 in which a membrane-spanning domain is added to the COOH terminus of Ypt1 in place of the geranylgeranyl modification which normally anchors it to the membrane. This form of Ypt1, called Ypt1-TM2, is functional as the sole source of Ypt1 (Ossig et al., 1995) but, the authors show, unlike wild-type Ypt1, is unable to be extracted from the membrane by GDI. When acceptor membranes are made from strains with this as the only source of Ypt1 the authors are able to show that they continue to see high levels of fusion with *ypt1-3* mutant vesicles. Moreover to demonstrate that the chimeric form of Ypt1 is not jumping in the assay they examined the sensitivity of the reaction to inhibition by GDI. When present at high levels GDI will effectively remove Rab proteins from membranes and thus cause significant inhibition of transport. As expected the authors find that fusion assays with wild-type acceptor membranes are sensitive to inhibition by GDI, however the assays with Ypt1-TM2 are completely resistant to inhibition by GDI. Therefore the function of the Rab GTPase during this assay appears to be fulfilled entirely by Ypt1 present on the acceptor or target membrane.

This surprising result suggests the possibility that Rab GTPase function in heterotypic fusion may generally lie on the target rather than vesicle membrane. This would represent a major revision of current models for Rab function upstream of SNARE proteins in vesicle fusion. Recently it has been suggested that Rab GTPases may work in conjunction with other factors to mediate the initial docking or tethering of vesicles to the target membrane. This is thought to be mediated by large hetero-oligomeric complexes such as the exocyst (Guo et al., 1999) in post-Golgi transport or the TRAPP, Sec34/Sec35 or Uso1 complexes in ER-to-Golgi transport (Sacher et al., 1998; VanRheenen et al., 1999; Kim et al., 1999), and p115/giantin/GM130 in intra-Golgi transport (Nakamura et al., 1997). In each case these proteins appear to be stably associated with the target membrane (Bowser et al., 1992; Nakamura et al., 1997; VanRheenen et al., 1999; Barrowman et al., 2000). In previous models it was assumed that the tethering between the target membrane and the vesicle membrane would be mediated by a direct interaction between the complex and the Rab GTPase on the vesicle surface (Pfeffer, 1999). The results by Cao and Barlowe in this issue suggest a different view (seen in Fig. 1) where the tethering complex would be regulated on the target membrane by the Rab GTPase. The absence of a functional Rab on the vesicle suggests a requirement for a new, as yet unidentified, factor which would then mediate the interaction of the vesicle with the tethering complex. Such a factor (called a Vesicle Tethering Protein or VTP for short in Fig. 1) could in principle be provided for by the v-SNARE itself, however previous data has suggested the v-SNAREs are not involved in the tethering reaction (Cao et al., 1998).

In the future it will be important to determine how general the target membrane function of Rab proteins is. For example does the Sec4 GTPase have a similar function on the plasma membrane in Golgi-to-cell surface transport?

Unfortunately, the absence of an in vitro system for this stage of transport in yeast is a major obstacle to obtaining this type of information. Perhaps work in other systems will shed light on the generality of this mechanism. In addition, the delineation of the role of the various complexes (i.e., TRAPP, Sec34/35, Uso1) involved in the ER-to-Golgi tethering reaction will be important. Of greatest importance will be determining which complex mediates the Ypt1 function in tethering and which complex mediates the association of the vesicle with the target membrane during tethering. This finding suggests the existence of a factor on the surface of the vesicle, termed a vesicle tethering protein, which may be recognized by the tethering complex and thus impart some degree of specificity on this reaction. The paper of Cao and Barlowe represents a significant advance in producing a general outline of Rab and SNARE function in vesicle transport, while at the same time making it clear that there is much left to be done before we have a truly have clear model for how these proteins participate in this complex process.

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