PERSPECTIVES: CELL BIOLOGY

ER-to-Golgi Traffic—This Bud's for You

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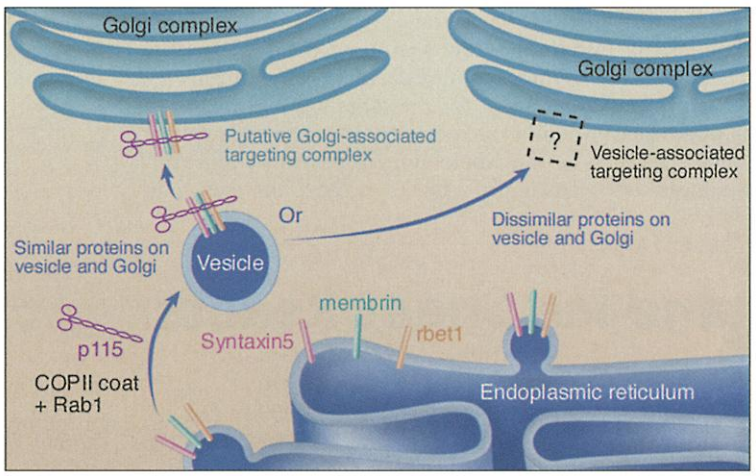
Transport of proteins within the cell depends on membrane-bound carriers, often vesicles, that form under the direction of protein coats (*1*). These coats assemble on membranes and bend them so that eventually they pinch off as vesicles containing protein cargo destined for the next station in the secretory pathway. For example, vesicles bud from the endoplasmic reticulum (ER), the site where proteins destined for secretion are made and the first station in the secretory pathway. These vesicles specifically deliver their contents to the Golgi apparatus, where the secretory proteins are modified en route to their final destinations. This requires that the membranes of ER-derived vesicles and of the Golgi apparatus specifically recognize one another (targeting) and then fuse so that the contents of the vesicles are released into the Golgi. The processes of vesicle formation and targeting/fusion have been considered separate from one another because they are disconnected in time and space and involve unique sets of proteins. However, the study by Allan *et al.* (*2*) on the transport of vesicles from the ER to the Golgi, reported on page 444 of this issue, hints that there is more integration between the formation of vesicles and their targeting and fusion than previously thought.

The protein coat that directs the budding of vesicles from the ER is called COPII. Targeting and fusion of these vesicles with the Golgi membrane depends on a separate group of proteins, collectively called SNAREs, which are integral membrane proteins that project into the cytoplasm. The SNARE family is composed of a large number of related proteins that regulate a variety of different trafficking events in the cell. Most researchers agree that assembly of several SNARE proteins into a complex that forms a bridge between the vesicle and its target membrane is a key event in fusion (see the figure). This SNARE complex is composed of a bundle of four α helices contributed by up to four different SNAREs, with all of the SNARE transmembrane domains clustered at one end of the bundle (*3*). Some argue that assembly of the SNARE complex results directly in membrane fusion (*4*); others suggest that additional downstream events are essential (*5*).



Setting SNAREs to trap Golgi. The interaction of the tethering protein, p115, with SNARE proteins rbet1, membrin, and syntaxin5, during budding of protein transport vesicles from the ER, is dependent on Rab1. The formation of this complex may program the vesicle for its subsequent targeting to and fusion with the Golgi membrane. As many of the molecules necessary for targeting and fusion are carried by the vesicle itself, a question arises about the contribution of the Golgi membrane to the targeting/fusion process. One possibility (**upper left**) is that a similar SNARE-p115 complex exists on the Golgi membrane. Alternatively, the vesicle-associated targeting complex might interact with dissimilar components on the Golgi membrane in an as yet unknown way (**upper right**).

Intracellular protein trafficking requires repeated vesicle transport events to relay the protein cargo through a number of membrane-bound compartments en route to the plasma membrane. The core components for most of these trafficking steps include a protein coat for generating vesicles, and sets of specific SNAREs for targeting and fusion of the vesicles with their target membranes. The cell has supplemented this core machinery with a number of other important molecules (*6*) including the Rab proteins. Members of this family of Ras-like guanosine triphosphatases (GTPases) act at distinct steps in the secretory pathway. Also involved are the so-called "tethers," a set of evolutionarily nonconserved proteins, such as p115, that collaborate with the Rabs to facilitate the initial physical interaction of a vesicle with its target membrane.

One current model of vesicle transport is as follows: Vesicles bud from the donor membrane (containing a number of SNAREs) under the direction of a protein coat. The vesicles travel to the target membrane and lose their protein coats as they do so. They then become attached to the target membrane through the coordinated action of Rab and tethering proteins. Finally, the vesicle-associated SNAREs together with the SNAREs in the target membrane assemble into a four–α-helix bundle, which either directly or indirectly results in membrane fusion (see the figure). In this model, budding of vesicles is isolated from the targeting/fusion process. Yet, incorporation of SNAREs into vesicles as they form is crucial for the successful interaction of vesicles with the SNAREs of their target membranes. The Allan *et al.* findings show that vesicle formation is a much more proactive process than previously thought, involving Rab-dependent assembly of a large protein complex containing SNAREs and the tethering proteins necessary for subsequent vesicle targeting and fusion.

The new work shows that the tethering protein p115 is loaded onto vesicles budding from the ER through a direct interaction with the Rab protein, Rab1. The interaction between p115 and Rab1 only occurs when Rab1 is bound to GTP. This observation is important because those proteins that bind exclusively to the GTP-bound form of Rab are known from previous studies to be critical for membrane targeting (*7, 8*). Perhaps even more interesting is the observation that p115 is found in a complex with a number of SNAREs (membrin, rbet1, and syntaxin5) on vesicles traveling from the ER to the Golgi. These three SNAREs must be associated with the vesicle to ensure its subsequent targeting to and fusion with the Golgi membrane. Rab1 is not part of this complex, which suggests that it interacts with p115 transiently. Thus, during budding, the Rab protein directs the assembly of a tethering protein (p115) into a complex of SNAREs that will be subse-

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quently involved in the targeting fusion of the vesicle (see the figure). As Allan *et al.* put it, budding "programs" the vesicle for subsequent targeting/fusion.

The discovery of a Rab-dependent step in the assembly of a targeting complex during budding raises a number of interesting questions. For example, does assembly of analogous complexes occur during other budding events in the cell? How does this p115-SNARE complex relate to the well-studied role of p115 in tethering mitotic Golgi fragments together during reassembly of the Golgi apparatus after mitosis (*9*)? Does the COPII coat participate in Rab-dependent formation of this p115-SNARE complex (see the figure)? In this regard, it is notable that COPII interacts with some SNAREs in yeast (*10*), and depletion of Rab1 inhibits budding from the ER in mammalian cells (*11*).

New questions about the events after vesicle budding are also raised by the Allan study. If most of the components required for targeting/fusion are already on the vesicle, what does the target membrane contribute to the reaction (see the figure)? One possibility is provided by recent work that analyzes ER-to-Golgi trafficking of proteins in yeast. In this model system, targeting requires two additional protein complexes: TRAPP and the Sec34/35p complex (*12*). TRAPP is particularly notable because it is statically localized to the Golgi, whereas SNAREs are mobile, moving back and forth between the ER and Golgi. Thus, TRAPP may be the marker protein that dictates vesicle targeting, a job originally postulated for the SNAREs attached to target membranes. Nevertheless, this still leaves open the question of whether the Golgi contributes SNAREs to the fusion process. The vesicle complex described by Allan *et al.* contains three SNAREs, each of which could contribute one α helix to the four–α-helix SNARE bundle. Thus, a SNARE contributing the fourth helix may reside on the Golgi membrane, providing targeting specificity and acting in the fusion process.

An alternative possibility is that a complex similar to the one found by Allan *et al.* on ER-derived vesicles is present on the Golgi apparatus (see the figure). Interaction of these complexes as the vesicle and Golgi membranes approach one another might allow fusion of the membranes. Symmetrical biochemical requirements are the norm for the targeting and fusion of similar membranes. However, this cannot be the whole story for ER-to-Golgi trafficking of vesicles because the dissimilar vesicle and Golgi membranes have different biochemical requirements for targeting and fusion (*13, 14*).

Thus, the Allan *et al.* study suggests that vesicle budding from the ER involves Rab-dependent assembly of a protein complex, including a p115 tether and several SNAREs. The important implication of this work is that during budding from the ER the vesicle is given a molecular program that will direct its subsequent targeting and fusion with the Golgi apparatus. Therefore, budding and targeting/fusion, which are separated in time and space, may be more interconnected than we previously thought.

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PERSPECTIVES: CLIMATE CHANGE

The Greenland Ice Sheet Reacts

Dorthe Dahl-Jensen

The Greenland Ice Sheet contains a substantial part of Earth's fresh water. Because it lies on land above sea level, a change in its volume will directly cause a change in sea level: If the whole ice sheet were to melt, sea level would rise by 7 m (*1*). Two reports in this issue assess changes in the Greenland Ice Sheet. On page 428, Krabill *et al.* (*2*) analyze repeated airborne laser altimetry measurements of the surface elevation. They show that the low-elevation areas of the Greenland Ice Sheet are melting but detect no substantial elevation changes in the high-elevation areas. The resulting reduction in the ice sheet's volume corresponds to a sea level rise of 0.13 mm/year, or 7% of the observed rise. On page 426, Thomas *et al.* (*3*) present an independent estimate of the high-elevation balance. They compare the amount of ice that each year passes the 2000-m contour line of the Greenland Ice Sheet with the amount of ice deposited on the surface over the course of a year. They conclude that as a whole, the high-elevation area is showing no net reduction in ice volume, with a substantial thickening in the southwest balanced by thinning in the southeast. Knowledge of the balance of the Greenland Ice Sheet has been sought for decades, and varying estimates have been presented (*1*, *4*). The two reports show that modern techniques now allow sufficiently precise measurements for a reliable estimate to be made.

Enhanced online at www.sciencemag.org/cgi/content/full/289/5478/404

Even small sea level changes can have a severe impact on coastal populations living with the threat of flooding. It is thus important to be able to monitor ice volume change. But it is even more important to be able to predict future changes. The future contribution to sea level change from ice sheets is composed of two terms: A long-term trend determined by the climatic and dynamic history of the ice sheet on centennial to millennial time scales and short-term sea level rise or fall directly related to annual to decadal climate variations (*4*).

The long-term trend arises from several mechanisms. The ice in an ice sheet is in constant flow. If the balance of the mass supplied to the ice sheet by precipitation and the mass lost by melting or production of icebergs shift, the flow will change, thereby influencing the shape of the ice sheet (*5*, *6*). In addition, the flow of ice strongly depends on temperature. During a glacial-interglacial cycle, surface temperatures on the ice sheet will vary by around 20°C (*5–7*). The time scale over which the volume of ice reacts to these variations spans centuries to millennia, because the flow is slow and the temperature changes take thousands of years to penetrate the ice. To complicate matters further, ice from different climatic periods may have different flow properties because impurity concentrations in the ice vary (*8*). Modeling of these long-term changes has shown that the Greenland Ice Sheet is still adjusting to the climate changes reaching back to the last glacial-interglacial transition (*6*, *8*).

The surface elevation changes measured in the laser altimetry surveys between 1993 and 1999 (*2*) are very sensitive to the natural annual to decadal fluctuations in snow accumulation and melt rates over the Greenland Ice Sheet. These fluctuations are believed to be nearly a factor of 10 larger than the long-term elevation changes (*4*, *9*). These annual to decadal

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