accomplish this by linking fully functional cells to a small bead at the end of a cantilevered AFM tip and then measuring the forces exerted on the tip in response to its deflection or attraction to an oriented mineral crystal. The cantilever measurement is directly translated into an interactive force measurement by its alteration of the known spring constant. Using this method, Lower *et al.* measure the approach and retraction forces between an individual cell of *S. oneidensis* and goethite (α–FeOOH) or diaspore (α–AlOOH). Although the minerals have the same crystal structure, goethite is used by *S. oneidensis* as a terminal electron acceptor, whereas diaspore is not. This is because the Fe(III) in goethite can receive an electron but Al(III) in diaspore cannot.

The affinity of *S. oneidensis* for goethite is strongest under those conditions for which electron transfer from the bacterium to the mineral is expected, that is, in the absence of oxygen. Similar affinities are not observed for diaspore. On the basis of specific signatures in the force curves, Lower *et al.* argue that a 150-kD protein in the outer membrane of the cell specifically interacts with the goethite surface to facilitate electron transfer. This protein, along with others in the outer membrane of *S. oneidensis,* was previously identified as a putative electron carrier to iron minerals (*16*). This result is exciting because it opens up the possibility of using nanomechanical measurements to test biochemical and mineralogical hypotheses about what controls mineral respiration.

By combining nanoscale force measurements with molecular genetics and mineralogy, it should soon be possible to find out which components of the electron transfer pathway in the cell are most important for direct electron transfer to minerals. This could be done by knocking out genes thought to encode outer-membrane proteins involved in electron transfer and comparing the interactive forces between the mutant and a mineral to those between the wild type and the same mineral. If substantial differences were measured, this would be compelling evidence for that particular protein's role in direct electron transfer to the mineral surface. As we learn more about how physical force measurements relate to electron transfer, it may be possible to use this technique to quantitate electron transfer reactions directly.

Once we have identified the components of the electron transfer system, the next challenge will be to determine how the relevant proteins work and how they evolved. Are they similar to other electron transfer proteins that participate in different respiratory metabolisms? Which residues in the proteins are critical to electron transfer? Are the proteins used by one species more efficient than those used by another, and can this be correlated with their environmental niche? What structural properties make minerals good electron acceptors? We are far from knowing the answers to these questions, but Lower *et al.*'s work provides us with an exciting new technique with which to approach them.

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PERSPECTIVES: CELL BIOLOGY

A TIP About Rabs

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Eukaryotic cells are filled with membrane-bound compartments, such as the endoplasmic reticulum (ER) and Golgi apparatus, that form a transport network for newly synthesized proteins. In the exocytic pathway, cargo proteins destined for secretion are inserted into the ER and are transported through the various cisternae of the Golgi. They are then sorted into secretory vesicles in the trans-Golgi network (TGN), which fuse with the plasma membrane, releasing their cargo at the cell surface. In the endocytic pathway, proteins in or at the surface of the plasma membrane are internalized into early endosomes, and then are transported in late endosomes to enzyme-filled sacs called lysosomes, where they are degraded.

Like traffic cops at key intersections, a family of small proteins termed the Rab guanosine triphosphatases (GTPases) regulate the sorting and transport of cargo proteins. These molecular switches ensure the specific and efficient targeting of vesicles that move cargo between various cellular compartments (*1, 2*). In addition, Rabs may be required for the formation and movement of transport vesicles, the remodeling of vesicle membranes, the coupling of individual transport steps, and the coordination of protein transport with other cellular processes (*3*). On page 1373 of this issue, Carroll *et al.* shed light on how Rab GTPases carry out their many tasks with help from the effector proteins that they recruit (*4*). First, they show that Rabs may recruit effector proteins for loading protein cargo into budding vesicles. Second, they suggest that Rabs enhance interactions between effectors and effector-binding proteins, implying that Rabs are not only involved in effector recruitment. Finally, they propose that Rabs determine compartment specificity, because each Rab appears to be localized to just one cellular compartment and recruits a unique set of effector proteins only to that compartment.

Transport vesicles—containing both soluble and transmembrane cargo proteins—bud from donor cellular compartments (such as the ER), then fuse with acceptor compartments (such as the early Golgi). After depositing their protein cargo at the plasma membrane, the vesicle membranes are then recycled back to the donor compartment. Sorting of cargo proteins into the correct budding vesicles is important because each donor compartment produces several types of vesicle, and each type has its own specific components. Most compartments are donors for at least two types of vesicle: anterograde vesicles, which carry cargo forward in a pathway, and retrograde vesicles, which return membrane components back to the previous compartment (see the figure). Some compartments, such as the TGN, serve as a donor for more than one type of anterograde vesicle.

Protein cargo is sorted into budding vesicles by cargo receptors that span the compartment membrane (see the figure). These receptors interact with cargo on the inner (lumenal) surface of the budding vesicle and with vesicle-forming

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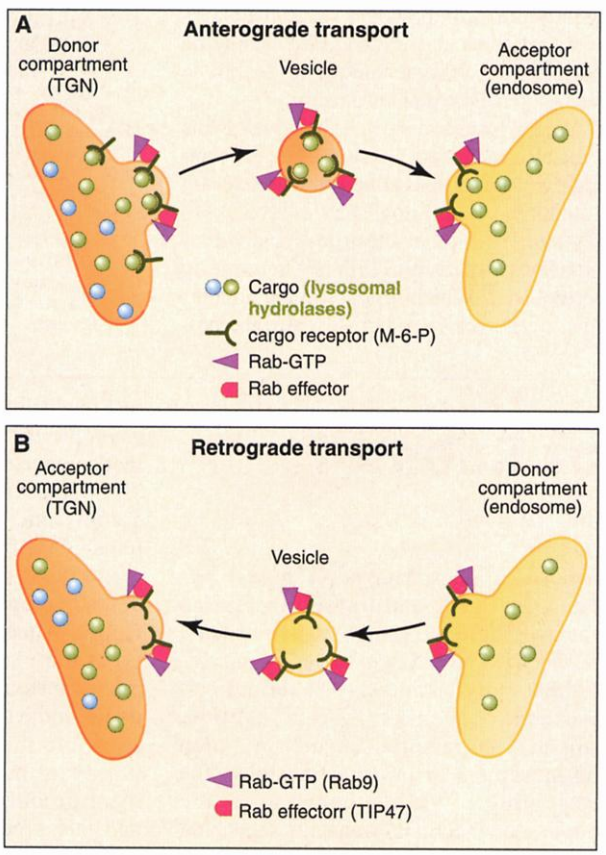
proteins on the outer (cytoplasmic) surface of the budding vesicle. Vesicle-forming proteins include adaptors that interact with cargo receptors, as well as coat complexes such as COPI, COPII, and clathrin (*5*). Adaptors and coat complexes assemble at the compartment membrane, sequester cargo receptors into areas of the compartment membrane where vesicles will form, and trigger formation of a bud that will eventually pinch off as a vesicle.

Members of the Arf/Sar and Rab GTPase families are key regulators of protein transport. GTPases cycle between the GTP-bound "on" state and the guanosine diphosphate (GDP)–bound "off" state. In the "on" state, these GTPases interact with their downstream effectors, which then transmit signals that induce, for example, vesicle formation and targeting. It is well established that Arf/Sar GTPases control vesicle formation, whereas Rabs regulate targeting of vesicles to the next compartment (*6*). New evidence suggests that this distinction is not so clear-cut and that Rabs are also involved in vesicle formation. In yeast, a pair of Rabs, Ypt31 and Ypt32, have been implicated in the production of TGN vesicles, and mammalian Rab5 is thought to sequester cargo proteins into vesicles that form at the plasma membrane (*7, 8*). Alternatively, Rabs have been proposed to program budding vesicles prior to targeting (*9*).

The Carroll *et al.* study (*4*) demonstrates that Rab9 sequesters cargo proteins into budding vesicles, indicating that Rabs are direct players in vesicle formation (*10*). Rab9 is important for shuttling vesicles from endosomes back to the TGN (retrograde transport). In this case, the cargo receptors are mannose-6-phosphate (M-6-P) receptors, and the Rab effector is TIP47, which specifically interacts with the cytoplasmic tails of these receptors. Because TIP47 does not share homology with the components of other coat complexes or adaptors, it may represent a new family of adaptor/coat proteins (*11*). Rab9 stimulates the recruitment of TIP47 to endosomes that bear M-6-P receptors, and promotes the binding of TIP47 to these receptors. In existing models of protein transport, Rabs are proposed to recruit their effectors to the right place at the right time, allowing the effectors to orchestrate various aspects of vesicular transport. But the Carroll *et al.* findings indicate that Rabs may not only recruit effectors, but may also affect their downstream interactions.

Although each Rab can act at multiple steps in a protein transport pathway, they probably do so only within one cellular compartment. For example, the Rabs Ypt1 and Ypt51 are associated with two protein transport steps but in one compartment: the early (cis) Golgi and late endosome, respectively (*12, 13*). Carroll *et al.* suggest a mechanism by which Rabs may determine compartment specificity. M-6-P receptors and TIP47 are not compartment specific: About 50% of TIP47 is in the cytoplasm, and M-6-P receptors are associated with the plasma membrane and the TGN as well as with endosomes. Rabs cycle between compartment membranes and the cytoplasm, but their GTP-bound form is presumably associated only with their specific cellular compartment (*3*). The fact that TIP47 binds to the tails of M-6-P receptors only on endosomal membranes is due to the presence of both M-6-P receptors and GTP-bound Rab9 on these membranes. Both Rab9 and M-6-P receptors enhance the interaction of each other with TIP47 by a factor of about 3. This mutual enhancement of binding might be sufficient for Rab9 to promote interactions between TIP47 and M-6-P receptors only on endosomes. Because one Rab molecule can interact with multiple effectors (*14*), these GTPase molecular switches can stimulate multiple events specific for the compartment membrane on which they reside.

The in vivo requirement for the interaction of Rab9 with TIP47 during cargo sequestration needs to be studied further, and this process should be reconstituted in vitro using purified components. Other open questions include: What triggers the formation of the ternary complex between Rab9, TIP47, and M-6-P receptors, and how does this complex stimulate vesicle formation? Are other Rabs directly involved in vesicle formation? If so, is the same Rab active in both the formation and targeting of a particular type of vesicle? As more effector proteins are discovered, it will become possible to test the notion that Rabs regulate the downstream interactions of the effectors that they recruit.



Directing traffic. The GTP-bound form of Rabs and cargo receptors on the membranes of cellular compartments are cues for the specific recruitment of Rab effectors. A complex among these three players is required for the sequestration of cargo receptors into regions of the compartment membrane where new vesicles will form. (**A**) In the anterograde transport of vesicles from the TGN to endosomes, the M-6-P cargo receptors bind their cargo (lysosomal hydrolases) in the TGN and deliver it to endosomes, where it is released. The identity of the Rab and its effector in this transport step is still unknown. (**B**) In retrograde transport from endosomes to the TGN, cargo-free M-6-P receptors are recycled back to their original TGN donor compartment. During this transport step, Rab9 and its effector TIP47 have been identified as important for sequestering cargo receptors into newly forming vesicles.

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