PERSPECTIVES: CELL BIOLOGY

A Switch to Release the Motor

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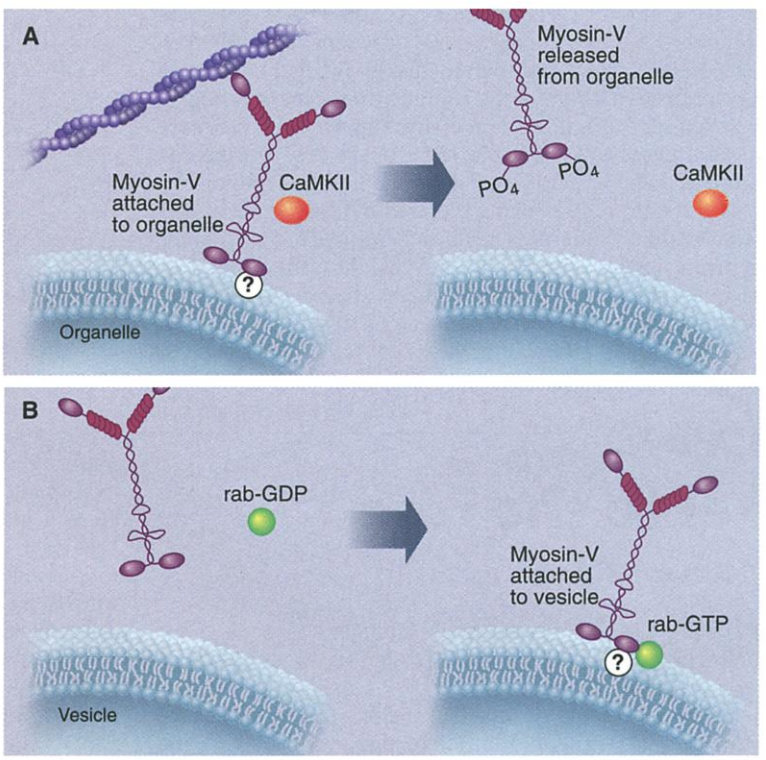
The transport of organelles within eukaryotic cells depends on motor proteins that bind to the organelles (cargo) and move them along tracks composed of microtubules or actin filaments. Although the human genome encodes at least 100 motor proteins, we know surprisingly little about how these motors recognize their cargoes and bind to them. Cells can regulate organelle transport either by regulating the binding of a motor to its cargo or by modulating the activity of a motor after it has bound. On page 1317 of this issue, Karcher *et al.* (*1*) describe a molecular switch that inhibits binding of a motor called myosin-V to its cargo. The molecular switch turns out to be the cell cycle–dependent phosphorylation of the tail region of myosin-V. This work constitutes an important step toward answering the long-standing question of how motors dock with their cargoes.

The class V myosins are perhaps the most ancient and widely distributed group of the myosin superfamily and are leading contenders for the task of driving movement of organelles along actin filaments. They have been implicated in the transport of many organelles, including secretory vesicles and vacuoles in yeast, smooth endoplasmic reticulum in the dendritic spines of neurons, and pigment organelles (melanosomes) in the melanocytes of mammals and the melanophores of amphibians (*2*). The class V myosins have an amino-terminal head (motor) domain that binds to actin filaments, followed by an elongated neck domain consisting of six IQ motifs that bind to light chains such as calmodulin. The tail domain consists of several segments that enable myosin-V to form dimers with itself, and a ~400–amino acid globular region required for organelle binding (see the figure). Although vertebrates express three myosin-V genes, the most widely studied is myosin-Va (*MYO5A*, brain myosin-V, *dilute*), which is particularly abundant in neurons and melanocytes. Mice lacking myosin-Va die 2 to 3 weeks after birth, with from severe seizures and a "diluted" coat color caused by defects in melanosome transport. In humans, defects in myosin-Va are one cause of Griscelli syndrome, a rare hereditary disease characterized by neurological deficits and diluted skin and hair color (*3*).



Regulating traffic. (**A**) Myosin-V (maroon) is a motor that drives transport of organelles such as melanosomes along actin filaments. During mitosis, cell cycle–dependent phosphorylation of myosin-V by CaMKII leads to its detachment from melanosomes. The nature of the putative melanosome receptor to which myosin-V binds remains unknown. (**B**) GTP-bound "active" forms of certain rab proteins regulate vesicle transport and membrane trafficking. Certain rab proteins may be important for linking class V myosins to organelles.

Pigment-containing cells such as melanocytes in mammals and melanophores in amphibians provide a valuable system for investigating organelle transport. Hormones trigger the melanosomes in melanophores to undergo rapid outward movements leading to dispersion, or inward movements leading to aggregation. The long-range movements of melanosomes depend on microtubules, with outward movements powered by the motor kinesin II and inward movements powered by the motor dynein. Myosin-V and actin-based transport are required for shorter-range movements, which result in melanosomes being transported along or tethered to actin-rich regions at the cell periphery (*4*). Karcher and colleagues previously demonstrated that myosin-V is attached to *Xenopus* melanosomes and that actin-based motility could be reconstituted by placing the isolated melanosomes on actin bundles obtained from the seaweed freshwater alga *Nitella* (*5*). Most importantly, treating melanosomes with mitotic extracts from *Xenopus* eggs resulted in phosphorylation of myosin-V, its apparent dissociation from melanosomes, and a profound inhibition of melanosome movement.

In their latest work, Karcher and colleagues first first demonstrate that a molecular construct containing only the globular tail region of myosin-V binds to melanosomes. Incubation of these melanosomes with mitotic *Xenopus* egg extracts leads to phosphorylation of the construct and its release from the organelles. Second, they use powerful mass spectrometry techniques to identify the single residue (serine 1650) in the globular tail region that becomes phosphorylated under these conditions. Third, they show that replacing this critical serine with a residue that cannot be phosphorylated prevents release of the myosin-V globular tail from melanosomes. Furthermore, they provide evidence that calcium/calmodulin-dependent kinase II (CaMKII) is the kinase responsible for the phosphorylation step. Moreover, inhibitors of CaMKII prevent the release of the myosin-V construct from melanosomes.

There is a precedent for CaMKII phosphorylation of myosin-V, and the kinase and the motor have been reported to form a complex in vitro (*6*). There is also a precedent for cell cycle–dependent regulation of

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organelle transport: Microtubule-based organelle transport driven by dynein is blocked by mitotic *Xenopus* egg extracts, and dynein (like myosin-V) detaches from organelles under these conditions (*7*). This finding raises the possibility that myosin-V is released from its cargo so that it may engage in mitosis-specific activities (*8, 9*), a possibility also suggested by the discovery that a yeast class V myosin is required for orienting the spindle apparatus prior to cell division (*10*).

The obvious unanswered question is the identity of the putative receptor for myosin-V on melanosomes. Intriguingly, recent evidence indicates that small guanosine triphosphatases (GTPases) of the rab family may be involved in linking class V myosins to their cargoes (see the figure). Each of the ~60 or so rab proteins in vertebrates appears to be associated with a specific membrane compartment, and these proteins are essential for membrane trafficking and transport of vesicles through the exocytic and endocytic pathways of the cell. Thus, it is extremely exciting that mammalian myosin-Va appears to require a specific rab, rab27a, for attachment to melanosomes (*11*). Myosin-Vb binds directly and specifically to a different rab protein, rab11a, and the two proteins colocalize to an endosomal compartment that is important for recycling certain molecules to the plasma membrane (*12*). Genetic analyses suggest that Myo2p, a yeast class V myosin, interacts with the rab protein Sec4p. Both of these proteins are thought to be elements of an organelle transport complex required for polarized secretion in budding yeast (*13*). Although recruitment of motors to organelles by rab proteins would provide an elegant connection between motors and membrane trafficking, the actual sequence of events in docking of motors to their cargoes remains largely unknown.

The intriguing results of Karcher *et al.* raise many other questions: Does the cell cycle switch regulating binding of myosin-V to melanosomes direct the binding of other class V myosins to their cargoes? What is the relationship between the cell cycle–dependent regulation of motor-cargo docking reported by Karcher *et al.* and rab-dependent docking during membrane trafficking and vesicle transport? Why would cells regulate attachment of a processive motor such as myosin-V by phosphorylating the motor rather than by phosphorylating its receptor on the melanosome membrane? Do cells in interphase (phases of the cell cycle between cell divisions) regulate attachment of motors in the same way, or do they use other signals such as calcium (which is believed to regulate myosin-Va motor activity)? Answers to these questions will require determination of the exact nature of the motor-cargo attachment—does the motor become attached to the cargo through an as yet uncharacterized cargo receptor, a rab protein, membrane phospholipids, or a combination of these elements?

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PERSPECTIVES: ENZYMOLOGY

Nickel to the Fore

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Nickel was long thought not to be a metal of biological importance. This changed in 1975, when Zerner discovered that urease is a nickel enzyme (*1*). Since then, five other important enzymes that depend on nickel for activity have been identified (*2*). The high-resolution crystal structure of one of them, carbon monoxide (CO) dehydrogenase from anaerobic microorganisms (*3*), is reported by Dobbek *et al.* on page 1281 of this issue (*4*). The structure offers surprising insights into the active site and the likely mechanism through which the enzyme harvests electrons from CO.

The six nickel enzymes known to date catalyze different reactions. Comparison of the available crystal structures shows that the nickel centers in their active sites also vary widely (see the figure). As Dobbek *et al.* show, CO dehydrogenase contains a [Ni-4Fe-5S] cluster (*4*). In other nickel enzymes, the metal is coordinated in a tetrapyrrole complex (*5*), a dinuclear NiFe complex (*6*), and a dinuclear NiNi complex (*7*).

Nickel CO dehydrogenase catalyzes the reversible dehydrogenation of CO and H_2O to CO_2. An iron-sulfur protein (FeSP) serves as both electron acceptor and donor in the reaction

$$CO + H_2O + FeSP \rightleftharpoons CO_2 + FeSP^{2-} + 2H^+$$

where both the forward and the backward reactions are of biological importance. The enzyme is found in many anaerobic microorganisms, both bacteria and archaea, but appears to be absent in aerobic microorganisms.

Nickel CO dehydrogenase has many functions in anaerobic microorganisms. In *Carboxydothermus hydrogenoformans* (*8*) and *Rhodospirillum rubrum* (*9*), it is involved in converting CO and H_2O to CO_2 and H_2. In sulfate-reducing bacteria and archaea, it assists the oxidation of acetate to two CO_2 molecules, in methane-producing archaea the disproportionation of acetate to CO_2 and CH_4, and in many acetate-generating bacteria and autotrophic anaerobes the total synthesis of acetate from $2CO_2$. Up to 10% of the organic compounds generated each year by photosynthesis are estimated to be remineralized by anaerobic microorganisms involving CO dehydrogenase (*10*). This enzyme thus plays a quantitatively important role in the global carbon cycle.

Dobbek *et al.* crystallized the enzyme from *C. hydrogenoformans*. In this organism, CO dehydrogenase is loosely attached to the inner aspect of the cytoplasmic membrane. After cell lysis, it can be recovered and purified from the soluble cell fraction (*8*).

In most other anaerobes studied, the enzyme forms a tight complex with a second nickel-containing cytoplasmic enzyme, acetyl–coenzyme A synthase/decarbonylase, which catalyzes the reversible decarbonylation of acetyl–coenzyme A to CO, coenzyme A, and a (bound) methanol (*11–13*). In the complex, the active sites of the two enzymes are connected by a tunnel through which CO can diffuse from one site to the other.

Crystal structures of this complex or of its components are not yet available, but sequence comparisons have shown that all known nickel CO dehydrogenases are phylogenetically related (*4*). Especially the amino acids involved in coordinating the active site [Ni-4Fe-5S] cluster are highly con-

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