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

Rudolf K. Thauer

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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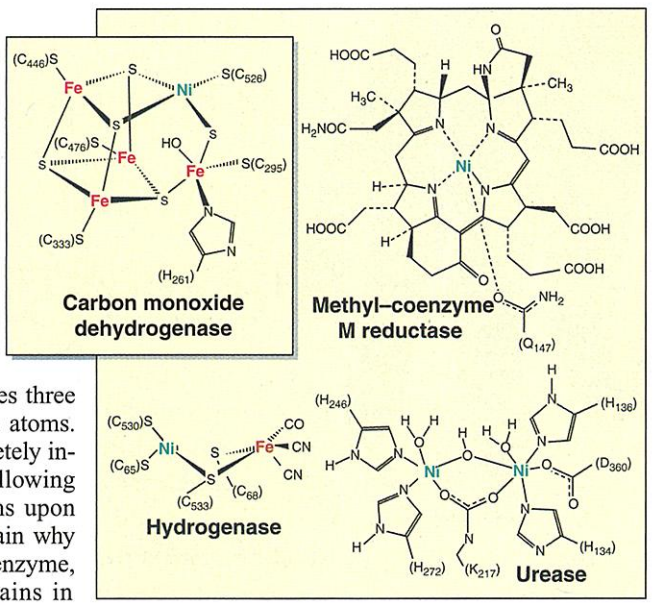
served, as is now evident from the crystal structure. The coordination of nickel in the active site of all nickel-containing CO dehydrogenases should therefore be very similar.

The biggest surprise from the crystal structure is the novel [Ni-4Fe-5S] cluster in the active site (see the figure). The nickel is bound by four S atoms and shares three S atoms with the four Fe atoms. The nickel atom is completely integrated in the cluster, allowing delocalization of electrons upon reduction. This may explain why even in the CO-reduced enzyme, the nickel formally remains in the Ni^{2+} oxidation state (and thus silent in electron paramagnetic resonance spectra) despite the fact that the nickel is the likely site of CO oxidation. But this will have to be shown directly by determining the structure of the enzyme with CO bound; the reported structure is that of the dithionite-reduced enzyme, which probably does not bind CO.



Active site nickel centers. The oxidation state of nickel in active CO dehydrogenase, hydrogenase, and urease is +2, and in active methyl-coenzyme M reductase, it is +1. In the catalytic cycle of CO dehydrogenase, methyl–coenzyme M reductase, and hydrogenase, the nickel center changes its redox state, whereas in that of urease it does not.

Active CO dehydrogenase from *Clostridium thermoaceticum* was obtained by cloning and heterologous expression of its gene in *Escherichia coli*, an organism that does not naturally contain this nickel enzyme (*14*). This indicates that the [Ni-4Fe-5S] cluster can be assembled in *E. coli*.

Another surprise is that nickel CO dehydrogenase is a functional homodimer, in which each monomer harbors one active site [Ni-4Fe-5S] cluster and one [4Fe-4S] cluster; an additional [4Fe-4S] cluster bridges the two subunits. The location of the five metal clusters relative to one another in the dimer indicates that electron transport proceeds from the active site [Ni-4Fe-5S] cluster of one subunit to the [4Fe-4S] cluster of the other subunit and then to the bridging [4Fe-4S] cluster. From the latter, the electrons can be transferred to the iron-sulfur protein electron acceptor (*4*).

The crystal structure was obtained for an enzyme with a specific activity of 14,000 μmol min^{-1} mg^{-1}. Previous analyses with other methods were mostly performed with enzymes with much lower specific activity. This indicates that considerable amounts of inactive enzyme were present and may explain some of the differences in properties reported.

Some aerobic bacteria can also grow on CO, but their CO dehydrogenase contains molybdenum and copper rather than nickel. The Mo-Cu enzyme (*15*) and the nickel enzyme (*4*) are not phylogenetically related, and their crystal structures have completely different topologies. The two enzymes do, however, catalyze essentially the same reaction with different electron acceptors—the same and not the same (*16*).

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PERSPECTIVES: LASER PHYSICS

Getting to Grips with Light

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What is light? This question might at first sight seem an odd one to ask—light is all around us and generally taken for granted. But any attempt to really get to grips with the nature of light takes one on a fascinating journey into the heart of physics. The report by Shelton *et al.* on page 1286 of this issue (*1*) does just that and opens up important areas of research in the generation and synthesis of light fields.

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Light can be thought of as a wave made up from very fast oscillations in an electric field. A typical light wave may have a wavelength of 800×10^{-9} m, which, bearing in mind the speed of light ($\sim 3 \times 10^8$ m/s), gives a frequency for the wave of 3.75×10^{14} Hz. This means that one cycle of the electric field in the light wave takes place in just 2.7×10^{-15} s, or 2.7 femtoseconds (fs). In most situations, this fast variation in the electric field is too rapid to be noticed, and what is observed rather is the envelope function that modulates the underlying fast carrier variation.

Laser systems provide the ideal tool to investigate the properties of light. The light beams produced by a laser are coherent; that is, a fixed phase relationship exists in the output, in contrast to light encountered in every day life. Some modern laser systems are designed to produce light in the form of very short, regularly spaced pulses rather than in the more familiar continuous wave (CW) or "always-on" format (*2*). The pulse periodicity is governed by the physical size of the laser, and the output is a sequence of abrupt short pulses (see the inset of the figure). The pulse duration is short compared with the pulse repetition rate, and the average power from such systems is thus relatively low, but the peak power of the pulses is several orders of magnitude higher.

Recent studies have shown that ultrashort-pulse lasers made from crystals of titanium-doped sapphire can produce pulses of light with durations of less than 5 fs, corresponding to only two cycles of the electric