There are many other proteins containing the predicted metal-binding motif, although none have been reported to be proteases. One is the human protein c6.1A. Chromosomal translocations involving the *c6.1A* gene have been linked to leukemia (*9*). Another example is AMSH (associated molecule with the SH3 domain of STAM) (*10*). STAM proteins are regulators of cell signaling and membrane protein trafficking. STAMs and related endocytosis proteins contain ubiquitin-interaction motifs that are necessary for the monoubiquitination of these proteins and for their normal function. If AMSH were a DUB, its ability to bind to the STAM SH3 domain might enable it to deubiquitinate STAM or an associated ubiquitinated protein. Provocatively, the same SH3 domain in STAM also binds to a known DUB (UBPY), and both UBPY and AMSH use the same noncanonical SH3-binding motif for this interaction (*10*). Together, these data suggest a model wherein both classical and nonclassical DUBs modulate ubiquitin-dependent membrane sorting decisions, possibly at distinct steps in these pathways.

The picture that emerges from these latest studies is of an elaborate interplay between mechanistically diverse proteases that work at multiple stages of the ubiquitin pathway. These intricacies further emphasize the astonishing regulatory capacity of the ubiquitin system and predict that ubiquitin, Ubls, and the enzymes that manipulate them will eventually be found to influence virtually every aspect of eukaryotic cell regulation.

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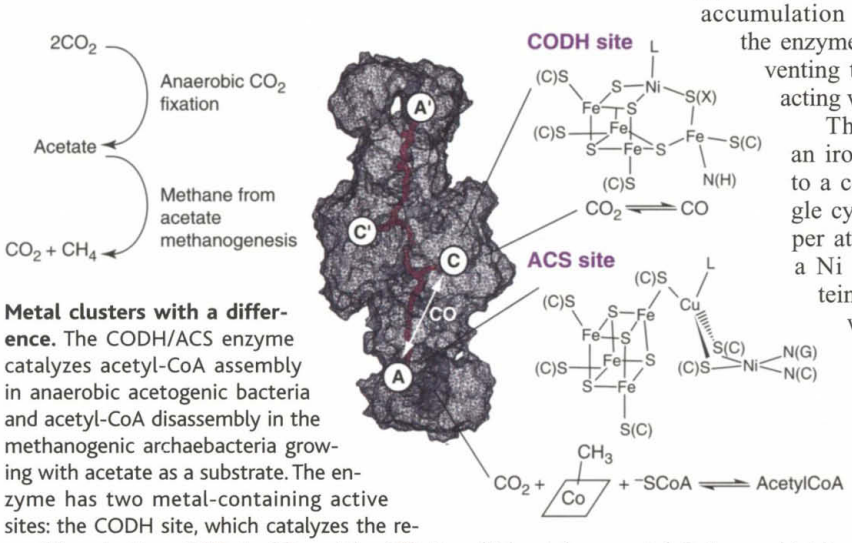
PERSPECTIVES: ENZYMOLOGY

A Trio of Transition Metals in Anaerobic CO_2 Fixation

John W. Peters

Certain acetate-producing anaerobic microorganisms can synthesize biomass via the formation of acetic acid from carbon dioxide. A methyl group and carbon monoxide are derived from carbon dioxide and subsequently condensed with coenzyme A to generate biomass (*1–3*). On page 567 of this issue, Doukov *et al.* (*4*) report the crystal structure of the key enzyme in this process, carbon monoxide dehydrogenase/acetyl–coenzyme A synthase (CODH/ACS).



Metal clusters with a difference. The CODH/ACS enzyme catalyzes acetyl-CoA assembly in anaerobic acetogenic bacteria and acetyl-CoA disassembly in the methanogenic archaebacteria growing with acetate as a substrate. The enzyme has two metal-containing active sites: the CODH site, which catalyzes the reversible reduction of CO_2 to CO, and the ACS site, which catalyzes acetyl-CoA assembly/disassembly. Carbon monoxide travels between these sites via a hydrophobic channel.

The structure reveals, much to the surprise of many investigators, a trio of transition metals at the active site: iron, nickel, and most unexpectedly, copper. Although clusters with two different transition metals are common, no metalloprotein active site with three different transition metal ions has been reported previously. The discovery also points to a new role for copper in biological systems.

In the acetate-production pathway, one CO_2 molecule is reduced to a methyl group in a set of enzymatic reactions using tetrahydrofolate. A second CO_2 molecule is reduced to carbon monoxide at the CODH active site in the CODH/ACS complex. This active site, termed the C-cluster, contains a distorted cubane constructed from one nickel atom, three iron atoms, and four sulfur atoms bridged to an additional iron atom (*5, 6*).

Once formed, the methyl group and the carbon monoxide react with coenzyme A to form the product acetyl–coenzyme A (CoA). This complex reaction of coordinated carbon-carbon bond and carbon-sulfur bond formation occurs at the A-cluster. Methanogenic archaebacteria use analogous reactions involving a similar set of cofactors and coenzymes in the reverse direction to disassemble acetyl-CoA, using acetate as the growth substrate (see the figure) (*7*).

An amazing feature of the CODH/ACS enzyme is the hydrophobic channel that spans nearly the entire complex. This channel is ~13.8 nm long and is reminiscent of the channel in another enzyme, carbamoyl phosphate synthase (*8*). Doukov *et al.* suggest that the channel could allow the accumulation of carbon monoxide in the enzyme for catalysis while preventing the toxic gas from interacting with cellular components.

The A-cluster consists of an iron-sulfur cubane bridged to a copper ion through a single cysteine thiolate. The copper atom is in turn bridged to a Ni atom through two cysteine sulfur atoms. Enzymes with active sites containing complex bridged metal assemblies include nitrogenase, hydrogenase, and sulfite reductase (*9, 10*).

The coordination environments of both the copper and the nickel atoms in the A-cluster are interesting. The copper atom is coordinated by three cysteine sulfur atoms, and the authors have tentatively assigned the fourth ligand to be an acetyl group or perhaps a carbonyl bound in multiple conformations representing an intermediate in catalysis. The nickel atom is in a square planar environment with thiolate and amide nitrogen coordination. The planar environment and amide coordination is reminiscent of the Ni porphinoid in methyl–coenzyme M reductase (*11*).

In their careful structural analysis, Doukov *et al.* used the anomalous scattering properties of the native metal ions in x-ray diffraction data collected near their absorption edges to assign the position

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and composition of the metal ions of the A-cluster. The presence of copper in the CODH/ACS complex eluded investigators previously, although it was reported in the early characterization of the CODH/ACS complex from a methanogenic archaebacteria (*12*).

The presence of copper is surprising given its very low solubility under anaerobic conditions in the presence of sulfide. Although there are a number of examples of copper-containing proteins, the presence of copper in this complex enzyme represents a new role for the metal in biological systems. The occurrence of both copper and nickel at a single active site has not been reported previously and may reflect the complexity of the biochemical reaction of acetyl-CoA condensation and disassembly.

CODH/ACS is believed to be an ancient enzyme. The ACS reaction has been considered as the primordial initiation reaction for a chemoautotrophic origin of life (*13*). Recent experiments aimed at mimicking prebiotic conditions indicate that activated acetate can be generated from CO_2 in the presence of a slurry containing NiS and FeS. A role for copper in these prebiotic scenarios for the synthesis of activated acetate will without doubt be the subject of future studies.

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PERSPECTIVES: NEUROSCIENCE

Does Grammar Start Where Statistics Stop?

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Languages exhibit statistical structure—that is, they show inhomogeneities in the distribution of sounds, words, and phrases. The importance of this type of structure in learning a language is a matter of intense debate, and is tackled by Peña *et al.* (*1*) on page 604 of this issue. The debate originated with Chomsky's 1957 discussion of the sentence "Colorless green ideas sleep furiously" (*2*). This sentence can be immediately recognized as being well formed (compared to "Ideas colorless sleep furiously green") even though in both cases the probability that these words have previously occurred in this order is close to zero. Chomsky concluded that the statistical properties of language are not central to the characterization of linguistic knowledge, an insight that became part of the foundation of modern linguistic theory. Whether statistical properties are important in language acquisition was largely set aside. Instead, research focused on how the child converges on the rules and other components of grammar using a combination of deductive (nonstatistical) reasoning and innate knowledge (*3*).

Recently, there has been a resurgence of interest in statistical learning, with evidence showing that infants and young children incorporate statistical cues when learning about the sounds of a language, vocabulary, and the structures in which words occur (*4–6*). These findings complement evidence from adults demonstrating the use of statistical information in comprehending and producing utterances, suggesting that similar mechanisms may underlie the learning and use of language (*7, 8*).

Although this research establishes that statistical information is used in language acquisition, the extent to which acquisition can be explained in these terms is not yet known. Peña *et al.* (*1*) suggest one possibility: Perhaps there are both statistical processes (based on frequency and distribution of elements in language) and grammatical processes (for example, learning and using rules). Statistical learning may be limited to simpler problems such as learning the sounds of a language and building a lexicon. In contrast, the complexities of grammar may require other nonstatistical procedures. Thus, it seems that learning grammar begins where statistical learning ends.

This reconcilist view is appealing because it preserves the main tenets of the grammar approach while apparently accommodating evidence about statistical learning. In practice, however, it turns out to be difficult to establish a boundary between "grammatical" and "statistical" learning. Any corpus of linguistic stimuli contains a vast array of cues and potential generalizations. Even in carefully designed experiments, conditions intended to isolate grammatical processes may introduce correlated statistical cues that would support performance. For example, in the Peña *et al.* study, adults listened to a continuous stream of nonsense words (see the table). According to the authors, the subjects could extract statistical regularities from the speech stream, but they could formulate rules only when brief pauses were added at word boundaries. Although the language supplied to subjects by Peña *et al.* consisted of only nine words, the corpus derived by concatenating these words afforded a large number of generalizations about the syllable sequences (some of which are shown in the table). Peña *et al.*'s conclusions about grammatical learning concentrated on some properties of the syllable sequences, but other properties could also have cued subjects' responses.

Two previous attempts to isolate a distinct grammatical form of learning (*9, 10*) raised similar concerns. In each case subsequent analyses suggested that the behavior could instead have arisen from statistical regularities that occurred simultaneously with the grammatical patterns (*11–13*). Importantly, these additional findings (like the analysis presented in the table) do not show that grammatical learning does not exist, but rather that statistical learning could also account for the results. Such findings also illustrate the difficulty of working back from observed behavior to the underlying regularities that gave rise to it (*14*). Knowing how many distinct procedures are involved in learning a language is a critical issue; resolving it will require advances on both the "statistical" and "grammatical" fronts.

Discussions of statistical learning need to consider two questions illustrated by the "colorless" sentence. First, what kinds of statistics are people, particularly infants, capable of computing? As in the "colorless" example, most research has investigated the transition probabilities between words or syllables. The Peña *et al.* study is a welcome step forward insofar as it addresses questions concerning nonadjacent elements (*15*). Adult learners can track various types of statistics, including some second-order probabilities and long-distance dependencies (*14, 16*), but the

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