core during solidification. A complete understanding of this, however, will have to wait until we have a better knowledge of those ternary and quaternary diagrams suggested by Poirier. One thing is certain, however, our understanding of these complex questions will depend on observations from many sources. One of the most important of those sources is experiments on the materials present in the core, experiments like the one reported by Fei and Mao. It is the excitement of finding the missing pieces and seeing a picture of some place that is so utterly inaccessible slowly emerge that makes this kind of research so exciting and rewarding.

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Binding Site Revealed of Nature's Most Beautiful Cofactor

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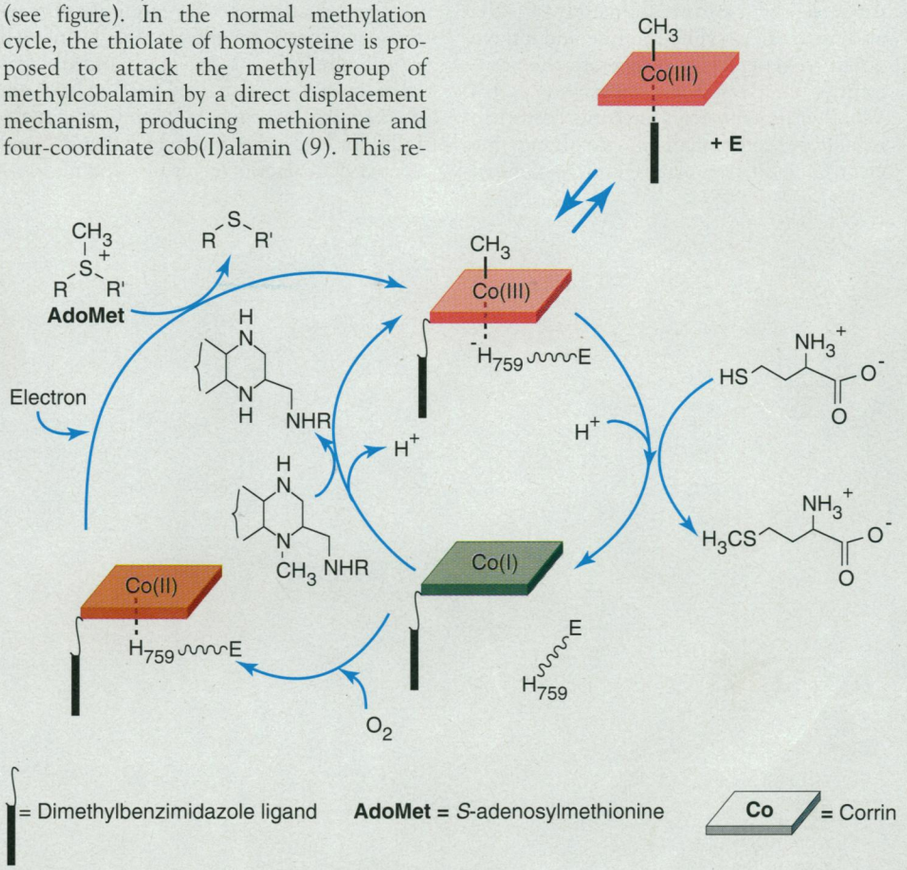
The marvelous structure of vitamin B_{12} was provided by Dorothy Hodgkin and her co-workers in 1956 (*1*). This accomplishment was the prelude to a very exciting period in cofactor biochemistry in which the first carbon-cobalt bond was identified in the two active cofactor forms of the vitamin (*2*)—adenosylcobalamin (AdoCbl) and methylcobalamin (CH_3Cbl). The efforts of chemists, biochemists, and spectroscopists converged to elucidate how these important cofactors and their unusual chemistry participate in enzyme-catalyzed transformations and the biosynthetic pathway of B_{12} (*3*). In addition, the total synthesis of these amazingly complex and beautiful molecules was achieved (*3*). The field then remained dormant until the molecular biological revolution renewed enthusiasm for B_{12}. All the genes of B_{12} biosynthesis have been cloned, sequenced, and expressed, providing an unprecedented—and colorful—view of this pathway and its unanticipated chemistries (*4*, *5*). The crystallization of *Escherichia coli* methionine synthase has now provided the first picture of how methylcobalamin binds to one domain (27 kilodaltons) of this 136-kilodalton (*6*) protein. The unanticipated and mechanistically thought-provoking structure is presented by Drennan, Huang, Drummond, Matthews, and Ludwig in this issue of *Science* (page 1669) (*7*).

Methionine synthase in *E. coli* (coded by the *metH* gene) and in mammalian systems is a large, conformationally flexible protein that utilizes all three cofactors with methylating capabilities: *N*,5-methyltetrahydrofolate (CH_3-THF), methylcobalamin, and *S*-adenosylmethionine (AdoMet) (*8*) (see figure). In the normal methylation cycle, the thiolate of homocysteine is proposed to attack the methyl group of methylcobalamin by a direct displacement mechanism, producing methionine and four-coordinate cob(I)alamin (*9*). This reactive species then attacks an "activated" form of *N*,5-methyltetrahydrofolate, again by a direct displacement mechanism, to generate tetrahydrofolate and regenerate methylcobalamin.

The structure of the methylcobalamin domain of methionine synthase has provided unanticipated mechanistic insight into the normal methylation cycle. The dimethylbenzimidazole, providing the sixth ligand of methylcobalamin in solution, is not coordinated to the cobalt on the protein, but is bound instead in a hydrophobic pocket. Furthermore, His^{759} from the protein has now taken its place as an axial cobalt ligand (see figure) (*7*). The structure also has suggested to the authors a mechanism for how the methylcobalamin might cycle, via a protonation and deprotonation shuttle, between six-coordinate cob(III)alamin and four-coordinate cob(I)alamin (see figure).

A second aspect of the structure is also intriguing. The top face of the corrin, which bears the methyl group to be transferred, is almost completely protected by hydrophobic residues from the helix bundle domain. Homocysteine cannot approach this methyl group, when it is in this protected form, to execute a direct displacement reaction. Given the size of intact methionine synthase and the fact that three



Chemistry performed by methionine synthase.

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different substrates must be able to approach the top face of the corrin ring, the domain protecting the top face probably moves out of the way. As with many proteins of this complexity, the static snapshot that a crystal provides is only the starting point for understanding the complex reorganization that may occur during catalysis.

The third cofactor, *S*-adenosylmethionine, required catalytically for methionine biosynthesis, regenerates the active form of methionine synthase when the cob(I)alamin form of the protein is aberrantly oxidized to cob(II)alamin (see figure). In the test tube, this reaction occurs every 100 to 2000 turnovers (*8*). Regeneration of methylcobalamin from the inactive cob(II)alamin requires *S*-adenosylmethionine and an electron source, flavodoxin (*10*). The proposed mechanism of this reactivation reaction is intriguing for several reasons. First, the reduction of cob(II)alamin to cob(I)alamin, if it is on the pathway to methylcobalamin formation, is thermodynamically unfavorable. It has been proposed that this reaction is made favorable by its coupling to the methylation process via the most reactive of the methylating agents, *S*-adenosylmethionine (*11*). Additionally, there are at least two other proteins—the anaerobic *E. coli* ribonucleotide reductase and the anaerobic pyruvate formate lyase—that also use *S*-adenosylmethionine and a flavodoxin to generate or regenerate the active form of their respective proteins (*12, 13*). Whether these systems have mechanistic or regulatory commonalities, involving one-electron reductive cleavage of *S*-adenosylmethionine, remains to be determined.

What is especially noteworthy about methionine synthase is that its cobalamin can shuttle between the Co(II) and Co(III) oxidation states as well as the Co(III) and Co(I) oxidation states (see figure). Its ability to use cob(II)alamin might in fact provide a mechanistic link to the second class of B_{12}-requiring enzymes, those that have a 5′-deoxyadenosyl group in place of the methyl group as an axial ligand. These enzymes use cob(II)alamin as the catalytically active form of the cofactor and are involved in unusual rearrangement reactions in which the cobalamin shuttles between the Co(II) and Co(III) oxidation states (*3*). This linkage is supported by recent sequence alignments of methionine synthase with the two adenosylcobalamin-requiring mutases (methylmalonylCoA mutase and glutamate mutase) which, in conjunction with the methionine synthase structure, suggest a common His^{759} binding motif for the sixth axial ligand (DxHxxG) and a conserved dimethylbenzimidazole binding pocket (SxL, GG) (*7, 8, 14*). One might have thought that the mechanistic problems associated with heterolysis of the carbon-cobalt bond in methionine synthase and homolysis of the carbon-cobalt bond in the mutases would have required different binding strategies for each cofactor.

The structure of a B_{12}-requiring enzyme has long been sought. It has been well worth the wait. New mechanistic ideas have been put forth by Drennan and coworkers that can now be tested experimentally in both the methylcobalamin- and adenosylcobalamin-requiring enzyme systems. Is the sixth axial ligand of the cobalamin really a histidine in some or all adenosylcobalamin-requiring enzymes? Why would nature have chosen to replace the bulky dimethylbenzimidazole ligand, thought to assist carbon-cobalt bond cleavage, with a less bulky imidazole ligand? Does deformation of the corrin ring by mechanical stress accelerate the rate of carbon-cobalt bond cleavage? The ability to manipulate protein function in conjunction with structural information should allow unraveling of the chemical mechanisms at a level not dreamed of 10 years ago.

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