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MINIREVIEW

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Metallocenter assembly in nickel-containing enzymes

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Abstract The five known nickel-dependent enzymes include urease, hydrogenase, carbon monoxide dehydrogenase (and CO dehydrogenase/acetyl-coenzyme A synthase), methyl-*S*–coenzyme M reductase, and one class of superoxide dismutase. Consistent with their disparate functions, these Ni enzymes have distinct metallocenter structures that vary in Ni coordination geometry, number and types of metals, and the presence of additional components. Sophisticated cellular Ni processing systems have been devised to allow for specific and functional incorporation of Ni into these proteins. This review highlights several themes that are common to the enzyme activation processes and summarizes current concepts related to the enzyme-specific Ni assembly pathways.

Key words Nickel enzymes · Metal Transport · Metallochaperone · Metal shuttle · Accessory protein

Introduction to nickel-containing enzymes

Ni-containing enzymes [1] catalyze five distinct biological activities including urea hydrolysis, reversible hydrogen oxidation, interconversion of carbon monoxide and carbon dioxide (often associated with acetate metabolism), methane generation, and dismutation of superoxide. The following paragraphs provide brief overviews of each of the Ni proteins associated with these activities. Subsequent sections of this review examine common themes and specific pathways related to metallocenter assembly in these Ni enzymes.

Urease (see Eq. 1) plays a central role in the nitrogen metabolism of many plants, fungi, and bacteria, and serves as a virulence factor for numerous pathogens [2]. The protein isolated from jack bean seeds was the first enzyme crystallized [3] and the first demonstrated to possess Ni [4]. The three-dimensional structure of *Klebsiella aerogenes* urease [5] reveals the presence of a dinuclear Ni-active site in which the two metals are bridged by a carbamylated lysine residue (Fig. 1A). Ni-1, acting as a Lewis acid, is proposed to coordinate and polarize the carbonyl oxygen of urea, and Ni-2 is thought to activate a bound solvent molecule that carries out the hydrolysis reaction releasing ammonia. The carbamate product spontaneously degrades in solution to form a second molecule of ammonia and carbonic acid.

$$H_2N\text{-}CO\text{-}NH_2 + 2\ H_2O \rightarrow 2\ NH_3 + H_2CO_3 \quad (1)$$

Hydrogen gas is formed or consumed in microorganisms as part of their energy metabolism by using hydrogenase enzymes (see Eq. 2), many of which possess Ni. The crystal structure of *Desulfovibrio gigas* hydrogenase highlights a set of three Fe-S clusters that function in electron transfer reactions and a dinuclear center containing Ni and Fe bridged by two cysteinyl residues (and an oxygen atom in at least one state of the enzyme) [6]. Three additional Fe ligands were originally modelled as water molecules; however, recent FTIR spectroscopy and chemical analysis studies of the *Chromatium vinosum* enzyme [7, 8] as well as additional structural and FTIR studies with the *D. gigas* enzyme [9] have provided evidence for one CO and two cyanide ligands in the metallocenter structure (Fig. 1B). The detailed mechanism by which hydrogenase catalyzes the deceptively simple reaction remains controversial [9].

$$H_2 \rightleftarrows 2\ H^+ + 2\ e^- \quad (2)$$

Carbon monoxide dehydrogenase (CODH) activity (Eq. 3) is affiliated with two distinct classes of Ni- and Fe-S cluster-containing enzymes [10]. *Rhodospirillum rubrum* CODH is prototypical of the most simple class that solely catalyzes the illustrated reaction to provide

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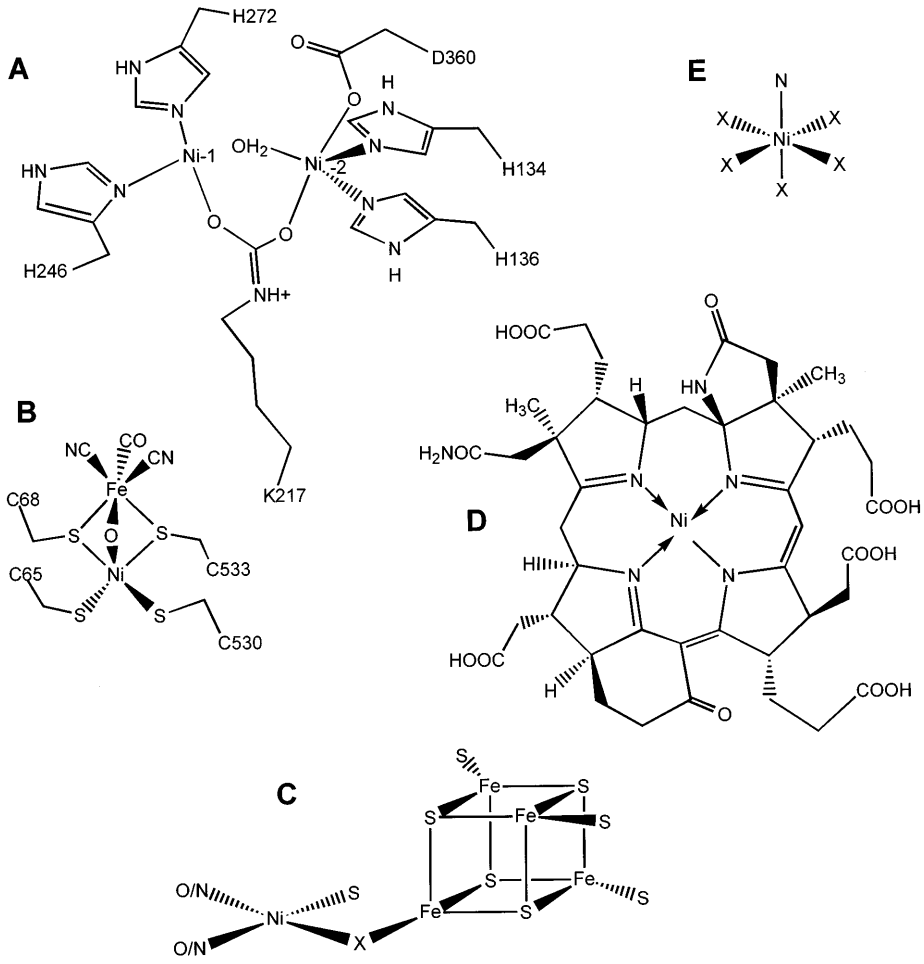


Fig. 1A-E Schematic illustration of the Ni metallocenters found in Ni-containing enzymes. **A** Dinuclear Ni site of *Klebsiella aerogenes* urease (PDB 1KAU); **B** dinuclear metallocenter of *Desulfovibrio gigas* hydrogenase (the oxygen bridging the two metal atoms is likely to be absent in other states of the enzyme); **C** spectroscopically characterized Ni/[4Fe4S] center of CO dehydrogenases (the bridging ligand labeled *X* has not been identified); **D** coenzyme F430 of methyl-*S*–CoM reductase; **E** tetragonal site with an axial N ligand of Ni-SOD

energy for cellular growth. A second paradigm is found in proteins with CODH activity isolated from acetotrophic methanogens and acetogenic microorganisms. These enzymes are thought to possess two Ni/[4Fe-4S] metallocenters, each of which is related to the single metallocenter in *R. rubrum* CODH. One center in each protein supports CODH activity, whereas the second site either splits the C-C bond of acetate to form CO_2 and methane (in the methanogen enzyme) or catalyzes acetyl-coenzyme A synthesis (in the acetogen enzyme). No crystal structure is available for any CODH; however, a spectroscopically based model of the Ni metallocenter has been proposed [10], as illustrated in Fig. 1C. A possible mechanism for how this metallocenter functions in CODH and acetate cleavage/formation has been described [10].

$$CO + H_2O \rightleftarrows CO_2 + 2\,H^+ + 2\,e^- \quad (3)$$

Methyl-*S*–coenzyme M reductase serves an essential role in methanogenic bacteria where it catalyzes release of methane and formation of an energy-yielding heterodisulfide (Eq. 4; HS-CoM, mercaptoethanesulfonate; HS-HTP, 7-mercaptoheptanoylthreonine phosphate). The enzyme has not been crystallized; however, the bright yellow Ni tetrapyrrole in this protein was extracted and structurally characterized [11]. This compound, referred to as F430 and illustrated in Fig. 1D, appears to undergo reduction and methylation at Ni during the catalytic cycle [12].

$$CH_3\text{–S-CoM} + \text{HS-HTP} \rightarrow CH_4 + \text{CoM-S-S-HTP} \quad (4)$$

A novel class of superoxide dismutase (SOD, Eq. 5) is the most recent addition to the family of Ni enzymes. Ni-SOD, examined in several species of *Streptomyces* [13–15], is distinct from the Cu/Zn-, Fe-, or Mn-forms of SOD based on subunit size, N-terminal sequence, antigenic cross reactivity, and other properties. The structure of the Ni metallocenter is unknown; however, EPR spectra are consistent with the presence of Ni(III) in a tetragonal environment with a single nitrogen axial ligand [13], as illustrated in Fig. 1E.

$$2\,H^+ + 2\,O^-_2 \rightarrow H_2O_2 + O_2 \quad (5)$$

Common themes in Ni metallocenter assembly

Although the five described Ni enzymes possess widely different metallocenters (Fig. 1), their biosynthetic pathways are very likely to share several unifying themes. Below, I describe how comparable demands on the cells (i.e., the need to synthesize one or more en-

zymes containing Ni) lead to similar metal processing steps to facilitate activation of these unique catalysts.

The first required step in Ni processing is to transport Ni across the cytoplasmic membrane [16]. When high levels of Ni are not essential, this is adequately handled by low specificity Mg transport systems; e.g., three distinct Mg transporters in *Salmonella typhimurium* are all capable of Ni uptake [17]. Of more interest, however, are highly specific Ni transport and Ni permease systems. For example, under anaerobic conditions *Escherichia coli* possesses an ATP-dependent, Ni-specific transport system encoded by five genes in the *nikABCDE* operon [18]. NikA is a periplasmic Ni-binding protein, NikB and NikC are integral membrane subunits, and NikD as well as NikE are nucleotide-binding proteins. Mutants defective in this Ni-transport system were originally identified on the basis of hydrogenase deficiency. In contrast to the multisubunit, energy-dependent Nik transporter, Ni permeases appear to be comprised of a single type of integral membrane protein and are not known to require energy. HoxN, the first Ni permease to be described, was initially identified based on the isolation of *Alcaligenes eutrophus* mutants exhibiting a requirement for elevated Ni when growing autotrophically on hydrogen. Heterologous expression of *hoxN* in *E. coli* yields cells with improved Ni uptake capability and increased proficiency to activate the Ni enzyme, urease [19]. A related Ni permease, NixA, has been identified in *Helicobacter pylori*. Heterologous synthesis of this protein in *E. coli* also allows high-affinity Ni transport and enhances urease activity [20]. Site-directed mutagenesis studies of *hoxN* and *nixA* are beginning to identify residues that are essential for Ni transport (T. Eitinger and H. Mobley, personal communications). As recently summarized [16], Ni-permease homologues have been described for several other microorganisms. Of special interest is a related sequence recently revealed in the archaeon *Methanococcus jannaschii* [21]. Because it is a methanogenic organism, *M. jannaschii* has exceptionally high demands for Ni and possibly uses a Ni permease to assist in the synthesis of its Ni-dependent hydrogenase and methyl-*S*–CoM reductase enzymes.

Once inside the cell, the second general step in Ni processing is likely to involve metal ion association with an Ni-binding protein. Ni must be sequestered from metal-sensitive proteins to prevent cellular damage [1], and yet Ni must be made available for incorporation into the target apoprotein. Ni-binding proteins could reasonably assist in Ni enzyme assembly by acting as metal shuttles or metallochaperones. A likely example of a Ni-binding protein serving this role is UreE, such as that encoded in the *K. aerogenes* urease gene cluster. Native UreE (containing a carboxyl terminus in which 10 of 15 residues are histidines) binds approximately 6 Ni per dimer [22]; however, a truncated protein lacking this His-rich region retains cellular function and still binds 2 Ni per dimer [23]. His-rich regions, and associated Ni-binding capabilities, also have been observed in selected hydrogenase-related HypB proteins such as that in *Bradyrhizobium japonicum* [24]. Unpublished evidence has been obtained that the *B. japonicum* protein can store Ni in the cell for later use (R. Maier, personal communication).

A third common theme regarding metal assembly into Ni enzymes is that there must be a mechanism to ensure incorporation of the correct metal ion into the apoprotein at some stage in the metallocenter assembly process. Part of this specificity can be imparted by the transport and metallochaperone systems mentioned above. In addition, the inherent metal-binding properties of the target protein will clearly be important for interacting with the appropriate metal. Nevertheless, additional mechanisms for achieving selectivity must operate in certain cases. For example, Zn is readily available in the cell and can be incorporated into urease apoprotein in vitro [25]; yet, this metal-substituted protein is not formed in cells grown in medium depleted of Ni. It appears likely that some of the metal specificity in Ni proteins is conferred by "accessory proteins" that function in synthesis of the metallocenter, transfer of the metal center to apoprotein, expulsion of incorrectly associated metal, or other role. Accessory proteins, encoded by genes located adjacent to the enzyme structural genes, have been identified for several of the Ni enzymes; however the precise roles of these proteins remain enigmatic.

Another commonality associated with activation of Ni enzymes is that Ni seldom is the only component incorporated into the apoprotein. As is clear from Fig. 1, the better characterized Ni sites additionally include a carbamylated protein side chain, an Fe site with three diatomic coordinating ligands, a [4Fe-4S] cluster and unidentified bridging ligand, or a tetrapyrrole ring. Ni-SOD does not appear to require any additional cofactor, but it is intriguing that the other systems exhibit this extra level of complication. The marked differences in the Ni sites and lack of homology among sequences of the five Ni enzymes imply that most accessory proteins will be functional for a single enzyme system. As an example of this specificity, hydrogenase-defective mutants of *Rhizobium leguminosarum* are uniformly urease positive, and urease-defective mutants are all positive for hydrogenase activity (T. Ruiz-Argüeso, personal communication).

A final general comment about the synthesis of Ni enzymes is that correct folding of the protein must occur prior to, or concomitant with, metallocenter assembly. Especially for the larger Ni enzymes, biosynthesis is likely to be facilitated by the typical cellular protein-folding apparatus. In *E. coli*, clear evidence for the requirement of GroE chaperonins in hydrogenase biosynthesis has been reported, and this involvement appears to extend to the Ni insertion step for at least one isozyme [26].

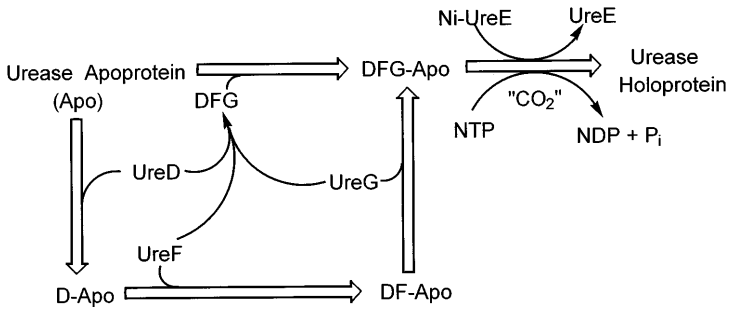


Fig. 2 Urease Ni metallocenter assembly. In vivo activation of urease apoprotein (*Apo*) is thought to occur via a DFG-Apo complex containing Apo and each of the UreD, UreF, and UreG accessory proteins. It is unclear whether DFG-Apo is formed by sequential addition of the auxiliary proteins (forming the D-Apo and DF-Apo intermediate complexes) or by interaction with a DFG species. Activation of the DFG-Apo complex requires a source of Ni (likely donated by the UreE accessory protein), a source of CO_2, and possible nucleotide hydrolysis

Current perspectives on enzyme-specific Ni assembly systems

Urease

Based on studies with proteins encoded by the *K. aerogenes* urease gene cluster (*ureDABCEFG*), a model was developed (Fig. 2) to illustrate how accessory proteins are involved in metallocenter assembly of this enzyme [27, 28]. Expression of the urease structural genes (*ureA*, *ureB*, and *ureC*) in cells containing deletions within any of three flanking accessory genes (*ureD*, *ureF*, and *ureG*) leads to the absence of urease activity and the synthesis of urease apoprotein (Apo)[29]. A fourth accessory gene in this cluster (*ureE*) is not required for synthesis of active enzyme, but it enhances the levels of activation. Overexpression of *ureD* in the presence of the structural genes leads to the formation of a complex (D-Apo) between UreD and Apo [30]. Overexpression of both *ureD* and *ureF* in the presence of *ureABC* leads to the formation of a complex (DF-Apo) containing Apo and both accessory proteins [31]. Finally, evidence has been obtained for the presence of a larger cellular complex (DFG-Apo) comprised of UreD, UreF, UreG, and urease apoprotein [32]. Because each of these accessory proteins is essential for obtaining in vivo urease activity, we proposed that the DFG-Apo complex represents the key urease activation machinery in the cell [27, 28]. Using cells that lack the urease structural genes, but retain the accessory genes, a new protein species (DFG) was identified and shown to contain a 1:1:1 complex of the three required accessory proteins (Moncrief and Hausinger, unpublished observations). As illustrated in Fig. 2, the DFG-Apo complex may arise by direct interaction between the DFG complex and Apo rather than by sequential addition of the accessory proteins to Apo. Finally, the last step in the urease assembly model involves Ni and CO_2 incorporation into Apo accompanied by nucleotide hydrolysis. UreE is likely to shuttle Ni to Apo to yield active urease enzyme, as described earlier, and whole cell studies have suggested that energy is required for Ni assembly into urease [33]. Possibly related to the latter observation, UreG possesses a nucleotide-binding motif, and mutations within this sequence lead to the abolishment of urease activity in cells (unpublished observations).

Although Fig. 2 nicely summarizes our current understanding of the protein species involved in urease maturation, it fails to provide insight into the functions of the accessory proteins. Identification of the roles for these auxiliary proteins became possible with the development of a method for in vitro activation of urease [34]. Incubation of purified Apo with Ni and bicarbonate (provided as a source of CO_2 for carbamylation of the active site lysine residue) leads to the incorporation of 2 Ni per enzyme active site; yet, under optimized conditions only ~15% of the protein present is activated [25, 34]. This low level of activation may arise from competition between productive and non-productive Ni-binding modes. In contrast, purified D-Apo complex is able to be activated in vitro to a level accounting for ~30% of the protein that is present [25, 34]. Similarly, in vitro activation of DF-Apo results in the formation of ~30% active enzyme; however, in this case the presence of UreF reduces the required bicarbonate concentration to more physiological levels [31]. Zn, Cu, and Co inhibit Ni-dependent activation of Apo, D-Apo, and DF-Apo species [25]. We anticipate that fully active urease may be obtained by activating the DFG-Apo complex; however, efforts to purify and characterize this species have thus far proven unsuccessful. Several hypotheses for the roles of UreD, UreF, and UreG in the activation process deserve consideration. These auxiliary proteins may preclude binding by non-Ni metals or may prevent the non-productive binding of Ni. Moreover, the accessory proteins may act to expel from the urease active site any inappropriately bound metal ions. Alternatively, the auxiliary proteins may provide a docking site that allows productive interaction between the Ni donor and Apo. Finally, a role in synthesis of a CO_2 donor is possible, such as the transient generation of carboxyphosphate by a nucleotide-dependent reaction with bicarbonate. Further studies are required to establish the mechanisms of UreD, UreF, and UreG in urease activation.

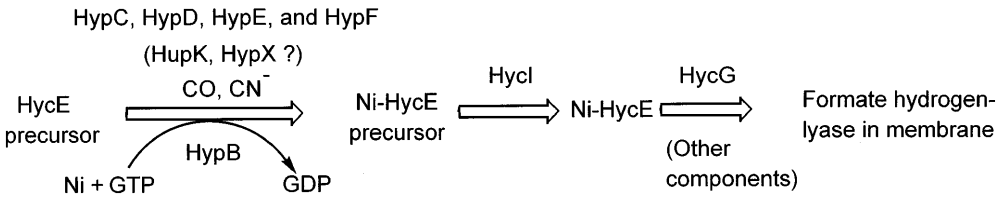
Hydrogenase

As summarized in two recent reviews [16, 35], the biosynthesis of Ni-dependent hydrogenases is a remarkably complex process. For the sake of brevity, this Minireview must focus on the enzyme activation machinery associated with a single hydrogenase; however, especially noteworthy findings in other systems are included. The prototype assembly system for this discussion is that associated with hydrogenase 3 of *E. coli* (Fig. 3). The structural gene for the large subunit of hydrogenase 3 (*hycE*) is located in the nine-gene *hycABCDEFGHI* operon that encodes proteins required to make the formate hydrogenlyase complex [36]. Separated from the *hyc* operon by only 210 bp is a five-gene operon (*hypABCDE*) that is needed for synthesis of all three *E. coli* hydrogenases [37]. More recently, an additional *hy*drogenase *p*leiotropy gene (*hypF*) was identified at a distant location in the chromosome [38]. One gene in the *hyc* operon (*hycI*) and five *hyp* genes (*hypB–hypF*) operate as accessory proteins for hydrogenase 3 activation [39], whereas the remaining *hyc* genes have other roles and *hypA* serves in regulation.

The precise functions of the *hypBCDEF* hydrogenase accessory genes are unknown, but the role of *hycI* is established, and recent progress is beginning to clarify the steps in the overall activation process. For example, an in vitro activation system has been devised and each of these six genes is required for generating active hydrogenase from a Ni-free precursor form [39]. HypB is a GDP- and GTP-binding protein that exhibits a low level of GTPase activity [40]. Examination of cells containing variant HypB proteins with defective nucleotide-binding sites revealed that GTP hydrolysis is essential for Ni insertion into hydrogenase [41]. Unlike the HypB homologs of other microorganisms that possess His-rich motifs and are known to bind Ni (e.g., 24), the *E. coli* protein does not include a corresponding His-rich region. Nevertheless, *E. coli* HypB is likely either to directly bind Ni or to assist some other protein (perhaps HycE) in binding Ni as indicated by genetic studies. Specifically, hydrogenase activity can be restored to mutant *E. coli* cells defective at *hypB* by growth in the presence of elevated Ni [37, 42]. Very little is known about the roles for HypC, HypD, HypE, and HypF, but each is required for incorporation of Ni into hydrogenase. Preliminary experiments have indicated that HypC can bind HycE and perhaps act as a chaperone, HypD contains an Fe-S cluster consistent with a possible role in reductive activation, and HypF contains two Zn-finger-like motifs and does not bind Fe (A. Böck and N. Drapal, personal communication). The best understood hydrogenase auxiliary protein is HycI. This protease cleaves off a carboxyl-terminal fragment of HycE at a site located immediately after the last Ni ligand in the sequence [43]. Ni must be bound to the hydrogenase precursor for processing to occur [44] and Ni is not incorporated into protein that is synthesized without the carboxyl terminal extension [45]. These results are consistent with HycI-dependent proteolysis leading to a substantial conformational change in HycE as one might expect given the observed buried location of the Ni metallocenter in the *D. gigas* enzyme [6, 9]. As the last step in hydrogenase maturation, HycE is incorporated into the membrane as part of the formate hydrogenlyase complex. The latter complex also includes the small subunit of the enzyme, HycG, which binds to both the processed and unprocessed forms of HycE (Böck and Drapal, personal communication).

In microorganisms other than *E. coli*, hydrogenase accessory proteins include homologs to those described above as well as novel components [16, 35]. For example, *A. eutrophus* [46], *R. leguminosarum* [47], and *Rhodobacter capsulatus* [48] each have *hyp* genes that are involved in hydrogenase maturation. *A. eutrophus* also has a HycI-like protein, termed HoxM, that functions as a carboxyl-terminal protease of the membrane-bound hydrogenase [49] and an unrelated protease, HoxW, that is specific for processing of the soluble enzyme in these cells [50]. As found with *E. coli* hydrogenase 3, the presence of the carboxyl-terminal region is required for Ni incorporation into the soluble *A. eutrophus* protein [65]. A distinct auxiliary role in hydrogenase activation has been proposed for the *R. leguminosarum* HupK protein [51]. HupK is related in sequence to the Ni-binding portions of the large subunit of hydrogenases; however, phenylalanine residues align with two ligand cysteines. The authors suggested that HupK may act as a scaffold for metallocenter assembly,

Fig. 3 Hydrogenase Ni metallocenter assembly. The scheme illustrates the biosynthesis of *Escherichia coli* hydrogenase 3 as a general model for hydrogenase activation. HypB-F accessory proteins are required for incorporation of Ni into the precursor form of HycE, the large subunit of this hydrogenase. HypB exhibits GTPase activity and may directly bind Ni or allow Ni binding by HycE. The roles for HypC-F are unknown. By analogy to other systems, a HupK-like protein may act as a scaffold for metallocenter assembly and a HypX-like protein may aid in provision of CO and cyanide that serve as ligands to Fe in the dinuclear site. The Ni-containing protein is subjected to proteolysis at its carboxyl terminus by HycI, possibly leading to burial of the metallocenter within the protein. The processed large subunit incorporates into the membrane as a complex with its small subunit, HycG, and other components of the formate hydrogenlyase complex



but due to the weaker ligation the metal site is readily transferred to the hydrogenase protein. HypX appears to be another novel accessory protein that affects hydrogenase maturation. This protein, encoded within the *R. leguminosarum hyp* operon, exhibits similarities in sequence to N_{10}–formyltetrahydrofolate-dependent enzymes that transfer one-carbon units and to enoyl-CoA hydratase/isomerase enzymes [52]. The authors propose that HypX may utilize formyl groups and unidentified CoA-thioesters for the synthesis of small organic molecules needed for hydrogenase maturation. Given the recent evidence that CO and cyanide are ligands to Fe in the dinuclear active site, this proposal is sure to stimulate further studies. Homologs to both HupK and HypX (often referred to as HoxX) have been identified in several other microorganisms.

A discussion of hydrogenase activation would not be complete without a few comments regarding HupUV (in *R. capsulatus* or *B. japonicum*) or HoxBC (in *Alcaligenes* species). These proteins are closely related in sequence to the two-component hydrogenases, and appear to have a role in regulating hydrogenase expression by acting as hydrogen and/or Ni sensors [53, 54, 65]. (The *B. japonicum* enzyme is the only hydrogenase shown to be regulated by Ni). Consistent with an ability to bind hydrogen, the *R. capsulatus* protein has been shown to catalyze the H-D exchange reaction [55]. It will be of great interest to learn whether these proteins possess Ni and, if so, to elucidate the mechanism of Ni incorporation. Unlike Ni hydrogenases that are synthesized as precursors and require proteolytic processing (a notable exception is the enzyme from *R. rubrum* [56]), these proteins lack the carboxyl-terminal extensions that have been suggested to be important for Ni incorporation (see above).

CODH

The best characterized CODH assembly system is that from *R. rubrum*. An inactive Ni-deficient form of the enzyme can be activated in vitro by incubation with Ni [57]; however, this process (requiring reduction of a [4Fe4S] cluster) exhibits a K_d for Ni of 0.755 mM [58]. The in vitro requirement for high Ni concentrations suggested the presence of an activation system within the cell, and genetic methods have provided evidence for such a system. Located immediately downstream of the *R. rubrum* CODH structural gene (*cooS*) are three genes (*cooCTJ*), two of which (*cooC* and *cooJ*) are required for Ni incorporation into the enzyme [66]. The predicted sequences of these accessory proteins are unremarkable except that CooC possesses a nucleotide-binding motif, similar to the sequence observed in UreG and HypB, and CooJ possesses a His-rich motif, reminiscent of many UreE and HypB proteins. CODH Ni assembly systems similar to that of *R. rubrum* are likely to be used by the acetotrophs and acetogens. Peptides homologous to CooC are encoded in the genome of *M. jannaschii* [MJ082321; 21] and the CODH (*cdh*) operon of *Methanosarcina thermophila* [59]. In unpublished studies (G. Ferry, personal communication), heterologous synthesis of the *M. thermophila* Ni-containing subunit of CODH (CdhC) in *E. coli* cells was found to require coproduction of methanogen CooC-like protein. The *Clostridium thermoaceticum* CODH possesses a labile Ni associated with one of its Ni/[4Fe4S] sites [60]. Treatment of enzyme with 1,10-phenanthroline eliminated acetyl-CoA synthase activity (without affecting CODH levels), and the acetyl-CoA synthase activity was restored by incubation with 0.5 mM Ni for 2 days. The in vitro activation process was also successfully applied to the isolated peptide that is thought to be associated with acetyl-CoA synthesis [61].

Methyl-*S*-CoM reductase

The biosynthesis of F430 is a major branch of the general pathway for tetrapyrrole synthesis in methanogenic bacteria [62], as illustrated in Fig. 4. The pathway progresses from glutamic acid to 5-aminolevulinic acid, through uroporphyrinogen III, and to dihydrosirohydrochlorin by well established enzymes. After this point and in undefined sequence, Ni is inserted (possibly by a ferrochelatase-like enzyme, but no data have been reported), the ring is extensively reduced, two side chains are amidated, and the five-membered lactam ring is closed to form 15,17^3–seco-F430-17^3–acid [63]. This intermediate undergoes closure of the 6-membered carbocyclic ring to form F430. The enzymes catalyzing the latter steps have not been charcterized. Once the coenzyme is synthesized, it may be able to spontaneously bind to the methyl-*S*–CoM reductase apoprotein according to in vitro reconstitution studies [64]. These results do not rule out the possibility that the cell facilitates this process of coenzyme assembly, but no evidence for additional factors has been described.

Ni-SOD

Knowledge about Ni assembly into Ni-SOD is limited to in vitro reconstitution studies carried out with the enzymes isolated from two *Streptomyces* species [13, 14]. Apoproteins, prepared by dialysis in buffer containing 5 M guanidinium chloride and chelating agents, were reconstituted to a level accounting for 12–12.5% of the starting activity by 24 h dialysis against buffers containing 1 mM Ni salts. Analogous reconstitution efforts with Fe and Mn led to recovery of 2.1% and 4.6% of the starting activity levels; yet, little to no Fe and no Mn was measured in enzyme isolated from the cells. Physiological studies are consistent with some type of Ni-dependent regulation of the Ni-SOD protein level in *S. coelicolor* [15]. Given these findings, one can expect

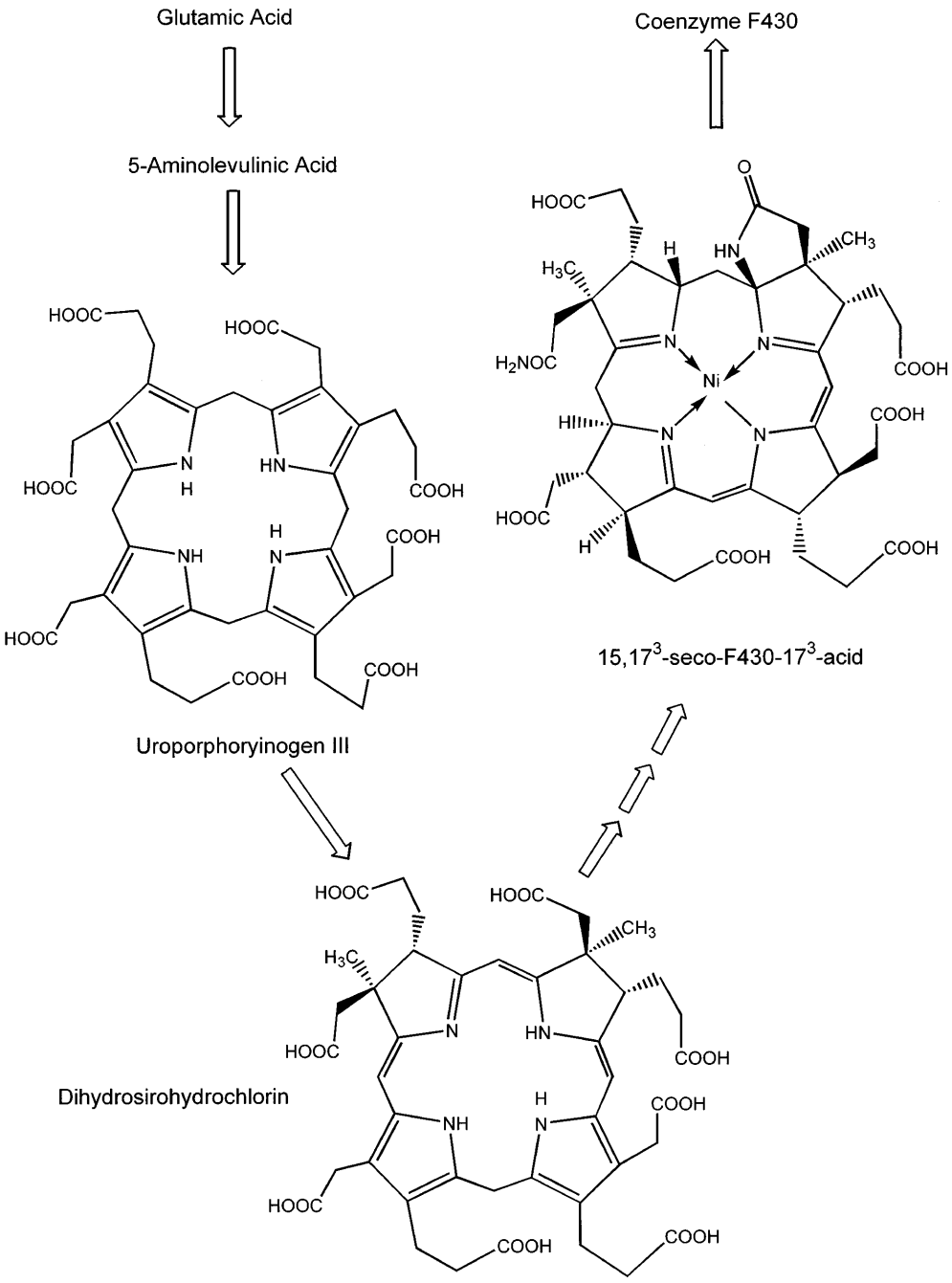


Fig. 4 Biosynthesis of coenzyme F430. Methanogenic bacteria are capable of synthesizing their tetrapyrroles from glutamic acid via 5-aminolevulinic acid and uroporphyrinogen III. F430 is obtained by a series of poorly characterized reactions that include dihydrosirohydrochlorin and 15,17^3–seco-F430-17^3–acid intermediates

that the cell possesses Ni-processing machinery that is able to sense the concentrations of this metal, transport it across the membrane, and specifically incorporate it into the Ni-SOD.

Conclusions

Biosynthesis of Ni-containing enzymes often requires elaborate cellular Ni-processing steps that may include selective metal transport across the membrane, shuttling of the metal within the cell, synthesis of the metal center, transfer of the metallocenter to the apoprotein, proofreading to ensure incorporation of the correct metal, and other actions. These activation steps are likely to be needed for functional biosynthesis of many other metalloenzymes in addition to those containing Ni. I hope that this Minireview will stimulate further studies in the area of metallocenter assembly for Ni-containing and other metalloenzymes.

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