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Cofactor biosynthesis through protein post-translational modification

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Post-translational modifications of amino acids can be used to generate novel cofactors capable of chemistries inaccessible to conventional amino acid side chains. The biosynthesis of these sites often requires one or more enzyme or protein accessory factors, the functions of which are quite diverse and often difficult to isolate in cases where multiple enzymes are involved. Herein is described the current knowledge of the biosynthesis of urease and nitrile hydratase metal centers, pyrroloquinoline quinone, hypusine, and tryptophan tryptophylquinone cofactors along with the most recent work elucidating the functions of individual accessory factors in these systems. These examples showcase the breadth and diversity of this continually expanding field.

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Introduction

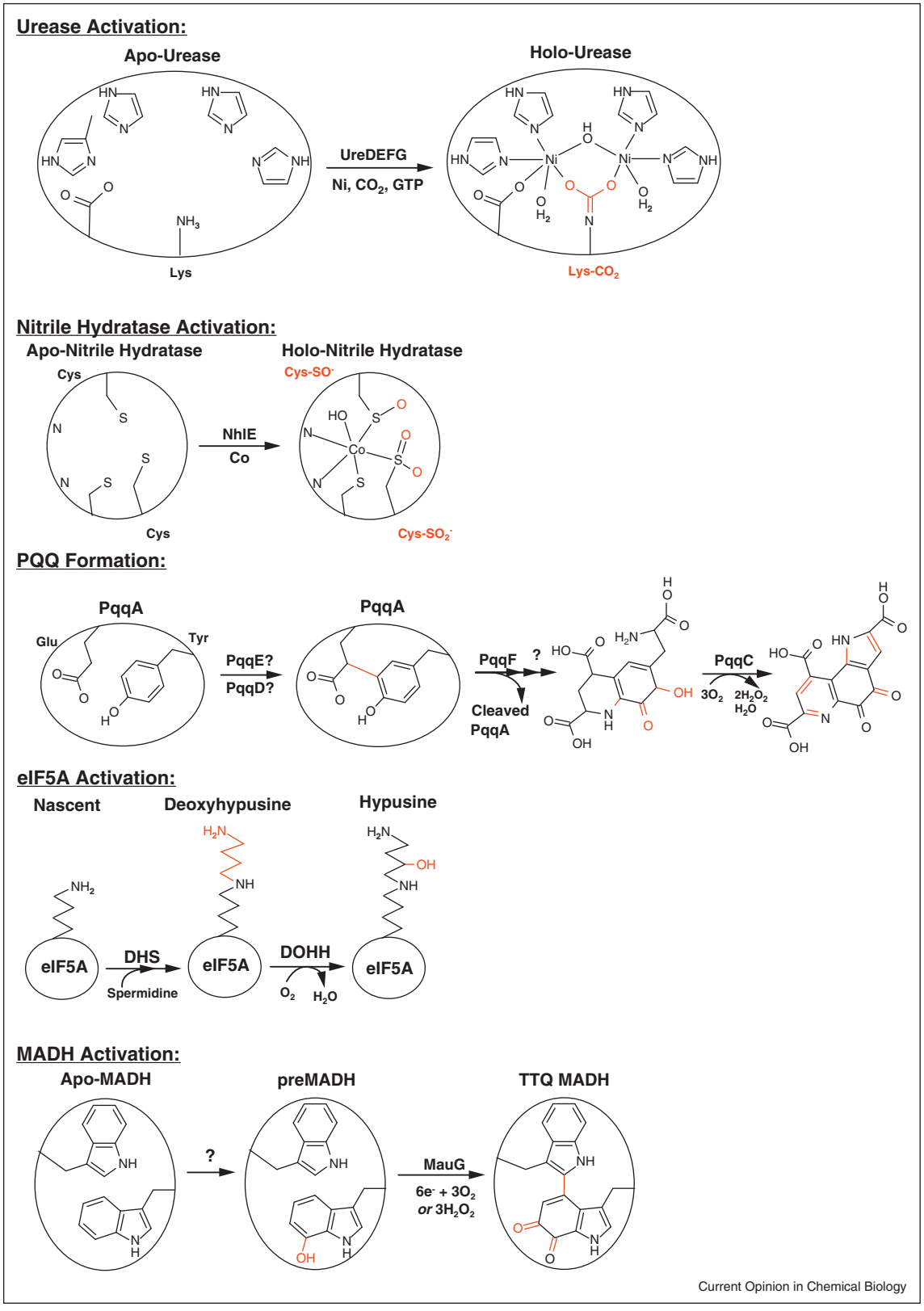
The role of post-translational modifications as regulatory or cell localization strategies has long been recognized. However, the observation that amino acids can be modified to generate cofactors with novel functions is relatively recent [1••]. In some cases, the biosynthesis of these cofactors is autocatalytic, requiring only the proper protein fold and perhaps a second cofactor such as a heme or metal ion to initiate amino acid modification and complete cofactor formation. A well-known example of such autocatalytic synthesis comes from the copper amine oxidases, where generation of the topaquinone (TPQ) cofactor from a Tyr residue requires only copper and oxygen [2]. In other cases, one or more accessory proteins are required for cofactor maturation. This review discusses some interesting examples of such systems and the recent advances in understanding the enzymes which generate these protein-derived cofactors.

Modified amino acid ligands to metal cofactors: urease and nitrile hydratase

Urease and nitrile hydratase are metalloproteins with post-translationally modified amino acid ligands to the metal(s) at the active site (Figure 1). In urease, a dinuclear Ni center is coordinated by a bridging carbamylated Lys residue [3•]. Active site maturation in urease requires typically four accessory proteins (UreDEFG) as well as Ni^{2+}, CO_2 (derived from carbonate), and GTP. Despite intensive study over a number of years, the specific roles of these proteins in urease activation remain elusive. UreE is thought to function as a metallochaperone and UreG as a GTPase. GTP hydrolysis by UreG has been proposed to either cause a conformational change allowing access of Ni and CO_2 to the urease active site, or to generate carboxyphosphate as a CO_2 donor to the active site lysine residue. Less is known about the other accessory proteins. However, successful methods for the soluble expression of UreD (as a maltose binding protein fusion) [4] and a truncated UreF [5] were only recently achieved and may lead to significant advances in this field. The activation of Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) provides some interesting parallels to the urease system in that it also requires carbamylation of a lysine residue [6] in order to bind Mg^{2+} in the active site [7]. Here again an 'activase' protein is involved, although it is not believed to participate directly in Lys carbamylation [8], which appears to occur spontaneously.

Low-molecular-mass nitrile hydratase (L-NHase) utilizes a mononuclear Co site coordinated by two oxidized Cys residues, one Cys-sulfenic acid (–SOH) and one Cys-sulfinic acid ($–SO_2H$) [9]. Expression of the structural genes for the L-NHase $\alpha_2\beta_2$ heterotetramer (*nhlAB*), in the absence of the downstream activator gene *nhlE* resulted in a protein with very little activity, low Co content, and no Cys-sulfinic acid ($–SO_2H$) in the α-subunit [10]. Co-expression of *nhlA* with *nhlE* yields a trimeric complex (holo-αe_2) which contains Co and modified Cys residues in the α-subunit. It was further demonstrated *in vitro* that NhlE was responsible for Co insertion and Cys oxidation [11•]. The holo-αe_2 complex is able to activate apo-$\alpha_2\beta_2$ by a novel mechanism dubbed 'self-subunit swapping' (Figure 2), where two holo-αe_2 complexes exchange α-subunits with apo-$\alpha_2\beta_2$ forming active holo-$\alpha_2\beta_2$ and apo-αe_2 [10,11•]: this mechanism also holds true for the high-molecular-mass nitrile hydratase (H-NHase) [12]. The driving force for the exchange appears to be the formation of a salt bridge between two

Figure 1



Proposed pathways of cofactor biogenesis for Ni-urease, Co-nitrile hydratase, pyrroloquinoline quinone (PQQ), hypusine, and tryptophan tryptophylquinone (TTQ). Post-translational modifications made at each step are shown in red.