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Microbial nickel: cellular uptake and delivery to enzyme centers

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Nickel enzymes allow microorganisms to access chemistry that can be vital for survival and virulence. In this review we highlight recent work on several systems that import nickel ions and deliver them to the active sites of these enzymes. Small molecules, in particular L-His and derivatives, may chelate nickel ions before import at TonB-dependent outer-membrane and ABC-type inner-membrane transporters. Inside the cell, nickel ions are used by maturation factors required to produce nickel enzymes such as [NiFe]-hydrogenase, urease and lactate racemase. These accessory proteins often exhibit metal selectivity and frequently include an NTP-hydrolyzing metallochaperone protein. The research described provides a deeper understanding of the processes that allow microorganisms to access nickel ions from the environment and incorporate them into nickel proteins.

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Introduction

Nickel is a micronutrient used by a wide variety of organisms, which incorporate the metal ion into the catalytic centers of enzymes [1,2]. To date, nine nickel enzymes have been identified, and these systems play key roles in global nitrogen, carbon, and hydrogen cycles [1,3,4]. For instance, nickel enzymes catalyze the hydrolysis of urea in the case of urease, methane formation in the case of methyl CoM-reductase, and the reduction of protons to form hydrogen gas in the case of [NiFe]-hydrogenase. The presence of nickel enzymes in an organism requires multiple auxiliary pathways that allow nickel ions to be used as essential cofactors while avoiding toxic side-effects [5,6]. Such systems include transporters that control uptake and efflux of nickel ions across cell membranes, intracellular nickel-binding proteins involved in metal ion distribution, and regulatory factors. These nickel systems present a fascinating display of the diverse bioinorganic chemistry available in biology. Furthermore, due to the fact that several nickel enzymes are virulence factors for human pathogens such as *Helicobacter pylori* and *Escherichia coli* [5,7,8•], nickel enzymes and the support systems required for production are under consideration as possible antibiotic targets.

In this review, we highlight recent insights into some of the nickel-binding factors that sustain nickel enzyme production in microbes (Figure 1). We begin with the metallophores and the import machinery that mediate nickel ion acquisition from the extracellular environment. This is followed by a discussion of the cytosolic proteins that collaborate to deliver the metal ions into the active sites of nickel enzymes. In particular, studies of the maturation of the nickel enzymes [NiFe]-hydrogenase, urease, and lactate racemase are described.

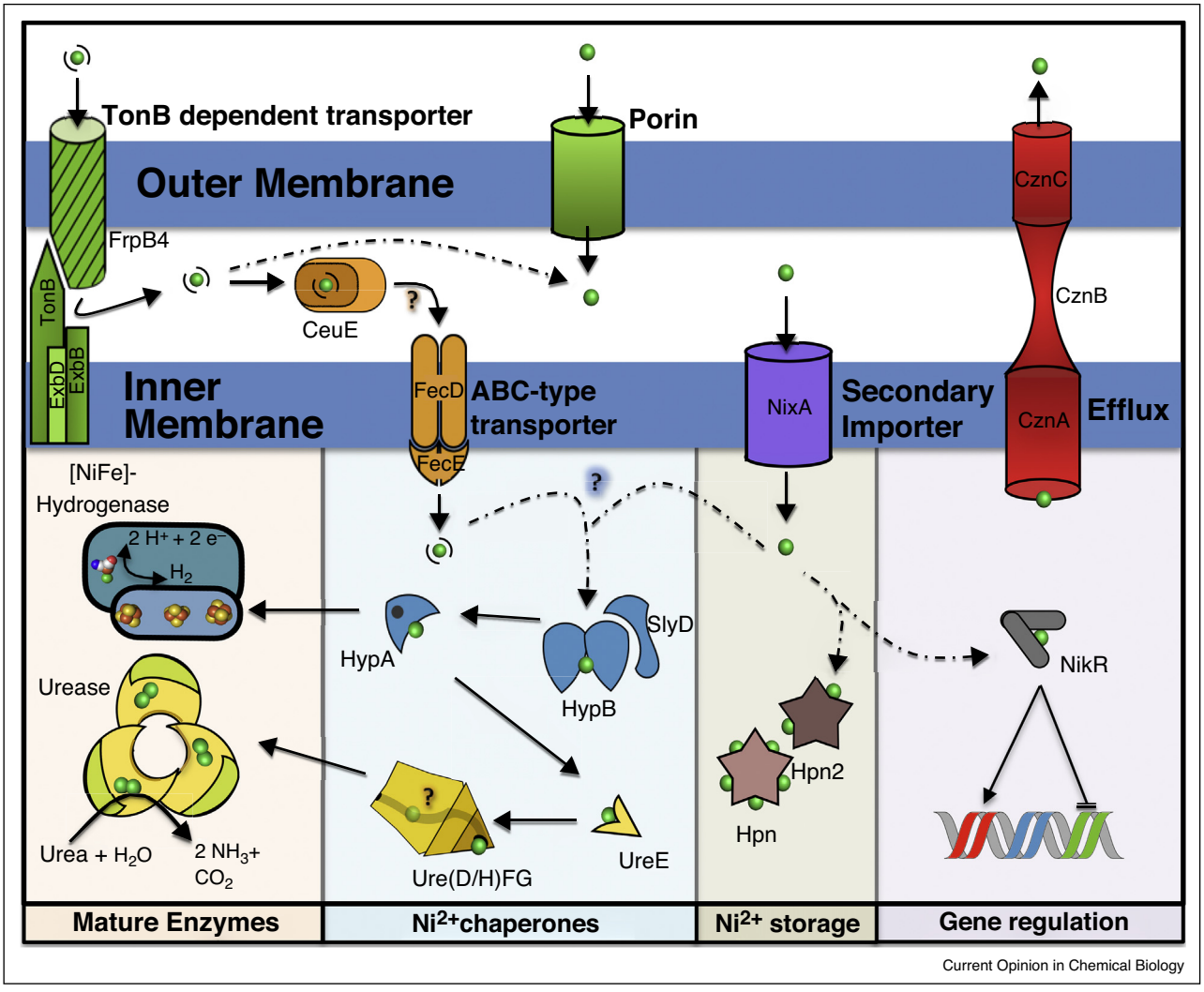
Nickel metallophores

Recent work on nickel uptake indicates that in some cases the nickel import proteins recognize Ni(II) chelates instead of lone nickel ions. Small molecule ligands, either produced by the organism or supplied by the local environment, may help organisms access sufficient nickel in the face of competition for limited resources. After all, while the nickel requirement of some bacteria is substantial [9,10••], environmental nickel is often scarce, typically at low nM concentrations [11,12].

Outer-membrane transporters

The uptake of nickel complexes is consistent with the observation that several Gram-negative bacteria actively import nickel across the outer membrane via TonB-dependent transporters (TBDTs). TonB, in cooperation with ExbB and ExbD, supplies energy to several dedicated transporters, which import molecules that cannot cross the membrane through diffusion or non-specific mechanisms [13]. Many metal-chelates require TBDT for import, including iron siderophores, heme, and cobalamin [13,14]. Nickel import through TBDTs has been observed in *H. pylori* [15,16], and is likely to occur in other species as well [14,17,18]. However, the structures of the nickel complexes transported have not been established.

Figure 1



Components of nickel metabolism in *Helicobacter pylori*. The schematic shows the paths of nickel ions as they are mobilized by *H. pylori* from outside the cell into the active sites of cytosolic nickel enzymes. This organism is used as an example that highlights many of the themes of microbial nickel homeostasis. It is possible that Ni(II) complexes enter the periplasm through TBDTs. Nickel can also reach the periplasm through low affinity porins in the outer membrane. The brackets surrounding the green spheres (nickel ions) indicate possible organic ligands such as histidine. Ni(II)(L-His)$_2$ is a substrate for ABC-type nickel importers in many species, and may be a substrate of the CeuE, FecD/E system in *H. pylori*. Metallochaperone proteins deliberately guide nickel ions through the cytoplasm to the active sites of nickel enzymes such as urease and [NiFe]-hydrogenase. Hpn and Hpn2 contribute to nickel ion storage and urease maturation in this organism. Genetic regulation and nickel export (for instance by NikR and CznABC, respectively) are critical aspects of nickel homeostasis, however they are not discussed in this review.

Ni(II)(L-His)$_2$ complex

After nickel ions reach the periplasm, they are subsequently transported across the inner-membrane in a process that often involves an ATP-binding cassette (ABC) transporter. These transporters include a periplasmic soluble binding protein (SBP) that captures the substrate and delivers it to the trans-membrane protein components. Recent structural studies of several nickel-binding SBPs suggested that L-His is a key piece of this process [19•]. Histidine was initially implicated in *E. coli* nickel uptake because supplementation of the growth media with L-His, but not D-His, enhanced nickel import, and the purified SBP NikA binds Ni(II)(L-His)$_2$ [20]. A crystal structure revealed that NikA braces the Ni(II)(L-His)$_2$ complex through electrostatic and π-stacking interactions, and that His-416 displaces a carboxylate ligand of one L-His to contact the nickel ion [21]. Perhaps as a result of these interactions, the arrangement of the ligands around Ni(II)(L-His)$_2$ in NikA is distinct from Ni(II)(L-His)$_2$ in solution (Figure 2). SBPs from other organisms have also been observed to bind Ni(II)-histidine complexes, with some variations in coordination