Complex Transcriptional Control Links NikABCDE-Dependent Nickel Transport with Hydrogenase Expression in Escherichia coli

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Escherichia coli requires nickel under anaerobic growth conditions for the synthesis of catalytically active NiFe hydrogenases. Transcription of the NikABCDE nickel transporter, which is required for NiFe hydrogenase synthesis, was previously shown to be upregulated by FNR (fumarate-nitrate regulator) in the absence of oxygen and repressed by the NikR repressor in the presence of high extracellular nickel levels. We present here a detailed analysis of nikABCDE transcriptional regulation and show that it closely correlates with hydrogenase expression levels. We identify a nitrate-dependent mechanism for nikABCDE repression that is linked to the NarLX two-component system. NikR is functional under all nickel conditions tested, but its activity is modulated by the total nickel concentration present as well as by one or more components of the hydrogenase assembly pathway. Unexpectedly, NikR function is independent of NikABCDE function, suggesting that NikABCDE is a hydrogenase-specific nickel transporter, consistent with its original identification as a hydrogenase (hyd) mutant. Further, the results suggest that the hydrogenase assembly pathway is sequenced within the cell. A second nickel import pathway in E. coli is implicated in NikR function.

Several energetically difficult reactions, such as nitrogen or carbon fixation, are catalyzed by enzymes with complex metal cofactors (26). A striking feature of the synthesis of these enzymes is the requirement of intricate assembly pathways that utilize several protein cofactors to ensure the fidelity of catalytic-site assembly (18). Metalloenzyme expression levels can be tightly regulated in response to changes in environmental conditions; for example, the nitrogenase operon is induced by nitrogen availability but repressed in the presence of oxygen (14). This shifting metabolism, combined with the biosynthetic cost of making these enzymes, means that the transcriptional regulation of these pathways is both necessary and complex. Cells are unlikely to synthesize large quantities of apoenzyme in the absence of the required cofactor(s), just as they are unlikely to expend the energy necessary to synthesize the transporter and accessory proteins necessary for cofactor assembly when the apoenzyme is not being expressed.

Escherichia coli exhibits a complex transcriptional response to growth conditions at low oxygen tensions (38). Respiration still occurs, but at lower energetic yield, and it requires the presence of an alternative electron acceptor, such as nitrate, dimethyl sulfoxide (DMSO), trimethylamine oxide (TMAO), or fumarate, and a corresponding terminal reductase (2, 16, 32, 42). E. coli can also ferment carbon sources in the absence of a suitable electron acceptor. E. coli expresses NiFe hydrogenases under anaerobic growth conditions (1, 5, 27, 29, 38) when energetic yields are low, for example, during fermentation or with low-energy-yield electron acceptor enzymes such as fumarate. Hydrogenases 1 and 2 (expressed by hya and hyb, respectively) oxidize H₂ in the presence of fumarate to generate ATP. Hydrogenase 3 (hyd) is part of a complex with formate dehydrogenase that converts formate to CO₂ and H₂. The expression of a fourth hydrogenase (hyf) has been observed only under synthetic conditions (30). These hydrogenases require nickel, iron, and organic ligands for catalytic activity (39), and several accessory proteins control the ordered delivery of these cofactors to the active site (3). Nickel is the last cofactor to be inserted into the active site.

The NikABCDE transporter is synthesized under anaerobic conditions to meet the increased demand for nickel resulting from hydrogenase synthesis (24, 43–45). Regulation of nikABCDE expression is positively controlled by FNR (44) and negatively controlled by NikR (9), in both cases by direct protein binding to the nikABCDE promoter (PnikABCDE or Pnik). This arrangement provides two distinct inputs that control nickel uptake. A decrease in oxygen tension results in activation of FNR and repression of nikABCDE expression, while the presence of excess nickel activates NikR, which overrides the action of FNR and results in repression of nikABCDE transcription. NikR forms two distinct DNA complexes in vitro in response to different nickel concentrations (4, 8, 9), suggesting that two modes of NikR-dependent repression of Pnik expression might be observed in vivo. The pattern of Pnik regulation determined thus far has indicated that transcription of nikABCDE is simple and is not tightly linked to the regulation of hydrogenase expression, raising the possibility that NikABCDE levels could be unnecessarily high under conditions of known low hydrogenase expression (i.e., growth in nitrate) and/or low extracellular nickel concentrations (inactive NikR). Further, NikR exhibits picomolar affinity for nickel ions (4, 8), raising the question of whether hydrogenase assembly must compete for available nickel ions with such a rapacious intracellular competitor.

Here, we show that NikABCDE levels are under complex transcriptional control, which results in an expression pattern that is closely linked to hydrogenase expression levels. Both
TABLE 1. E. coli strains used in these experiments

<table>
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\* Source: Patricia J. Kiley, University of Wisconsin—Madison.
\* Source: E. coli Genetic Stock Center at Yale University (http://gcds.biology.yale.edu).

NikR-DNA complexes act to repress expression from \( P_{\text{nik}} \) although the activity of the first NikR-DNA complex is observed only under conditions of low hydrogenase expression. Additionally, nitrate represses \( P_{\text{nik}} \) expression via the NarLX two-component system. Surprisingly, NikR function does not depend on nickel transport by NikABCD, suggesting the presence of another nickel transporter in \( E. coli \). A model is presented for hydrogenase assembly, showing its isolation from the rest of the intracellular milieu to the extent that it can be considered to constitute a discrete module or circuit within the cell.

MATERIALS AND METHODS

Strains and plasmids. \( E. coli \) strains used in these experiments are listed in Table 1. Gene deletions for nikR (bases 10 to 399), nikABCDER (bases 16 to 1,560), and corA (bases 61 to 891) were constructed using the method of Dat senko and Wanner (12). Numbering refers to base positions relative to ATG.

A translational \( P_{\text{nik-lacZ}} \) fusion was constructed in three steps. A \( P_{\text{nik}} \) fragment (~400 bp) was amplified by PCR from \( E. coli \) genomic DNA using oligonucleotides PC118 (5′-CATGGGCGGGCGAGCAATCGATTAGA-3′) and PC389-GC (5′-GGCTAGCCATATGCACTGTAATGCTGATGATACC CAAATGAGGAAATTTAAGA-3′). The resulting 3,633-bp fragment was amplified by PCR (5′-TCTATT TAAATCCTGGGTGATCAAGACATGATTTACGGATCTG-3′) and PC639 (5′-ACGGCTATTCTAGATATTTTGACCAAGACCACTGATC-3′). The underlined bases correspond to EcoRI and XbaI restriction sites, respectively. The two resulting fragments were purified and combined in a second PCR reaction, the underlined bases corresponding to the circular PCR product. The resulting 6318-bp fragment was amplified by PCR from RZ4500 genomic DNA using oligonucleotides PC118 and PC639. The resulting 3,633-bp fragment was amplified by PCR (5′-TCTATT TAAATCCTGGGTGATCAAGACATGATTTACGGATCTG-3′) and PC639 (5′-ACGGCTATTCTAGATATTTTGACCAAGACCACTGATC-3′). The underlined bases correspond to EcoRI and XbaI restriction sites, respectively. The two resulting fragments were purified and combined in a second PCR mixture that contained PC118 and PC389. The resulting 3,633-bp fragment was digested with EcoRI and XbaI and ligated into pACYC184, cut with the same enzymes to create pC181. This plasmid is a precise fusion between \( P_{\text{nik}} \) and the ATG codon of lacZ. It differs from the previously described pC163 (9), which contains five codons of the 5′ end of the nikA gene fused to the 5′ end of lacZ. pC181 has at least four-fold-greater expression than pC163 under identical conditions (data not shown), suggesting that the nikA codons negatively affect the production of LacZ.

Growth media. M63 salts (5x) were treated overnight with (2 g/liter) Chelex-100 resin (Sigma) to remove trace metals, including nickel. Trace metal nutrients, excluding nickel, were then added back at the following concentrations: 1 mM MgCl2, 100 mM MnCl2, 2 μM FeCl2, 1 μM ZnCl2, 100 mM (NH4)2MoO4, and 100 mM NaSeO3. Nutrient concentrations were individually optimized for maximal growth by measuring optical density at 600 nm (OD600) values after overnight growth (14 to 16 h) at 37°C under anaerobic conditions in capped microcentrifuge tubes with no headspace. Glucose (0.25%) was used as a carbon source and potassium nitrate (KNO3), sodium formate, sodium fumarate, DMSO, or TMAO was added when required, each at a final concentration of 10 mM, except when noted otherwise. Nickel was added by making serial 10-fold dilutions into minimal growth media from a 1 mM stock. Concentrations higher than 10 μM NiCl2 were toxic, as judged by a 20 to 30% decrease in the OD600 after 14 to 16 h of growth at 37°C.

β-Galactosidase assays. Strains containing pPC181, the \( P_{\text{nik-lacZ}} \) fusion, and pNIK103 (9), which provides a low level of NikR expression in the absence of any inducer, were inoculated in the defined media to a starting OD600 of 0.0001 and grown 14 to 16 h at 37°C in capped microcentrifuge tubes with no headspace. For experiments examining the nickel-dependent effects of a nikR deletion, strains lacking chromosomal nikR were transformed with pNIK103 Cys95Ala (7), which produces a stable variant of NikR with a mutation in the high-affinity nickel binding site. For every experiment, two separate aliquots (100 μl) of cells were extracted to measure OD600 and LacZ activity. OD600 values ranged from 0.3 to 0.8, and LacZ activity was constant over this range for a given growth condition. The LacZ activities of cultures grown in microcentrifuge tubes with no headspace were similar to the activities measured in 2-ml cultures grown in 15-ml polyethylene tubes in an anaerobic chamber (data not shown). Data were collected in duplicate or triplicate from separate overnight cultures started from the same inoculum. Error bars indicate standard errors between these measurements. In all experiments, the relative LacZ activity was normalized to the level measured for \( E. coli \) RZ4500 grown in media containing glucose alone (3,500 to 4,000 Miller units). For example, a relative LacZ activity of 0.25 is equivalent to ~1,000 Miller units. Nickel titration data for each growth condition and/or strain were fit to the equation \( y = [(a - b)/1 + (K_{\text{m}}/r^2)] \) where \( a \) is the fraction of LacZ activity at a low nickel concentration (i.e., upper baseline), \( b \) is the fraction of LacZ activity at a high nickel concentration (i.e., lower baseline), \( K_{\text{m}} \) is the nickel concentration required for half-maximum LacZ activity, and \( r \) is a cooperativity term required to fit the data set from growth in media containing formate (Fig. 1B).

RESULTS

Hydrogenase expression in \( E. coli \) is regulated by the particular electron acceptors present in the growth medium under anaerobic growth conditions, as well as by formate, which is a product of pyruvate-formate lyase. Hydrogenase activity is low in the presence of nitrate and high in the presence of fumarate and/or formate (1, 5, 29). Changes in hydrogenase expression levels should correlate with a changing requirement for nickel and a corresponding change in NikABCD levels. \( E. coli \) RZ4500 organisms containing a low-copy-number \( P_{\text{nik-lacZ}} \) fusion were grown anaerobically in M63 minimal medium to which glucose, an electron acceptor (nitrate, DMSO, TMAO, or fumarate), and/or formate was added. Changes in LacZ activity were compared to the LacZ activity of cells grown in minimal medium containing glucose alone (Fig. 1A). Nitrate repressed \( P_{\text{nik}} \) expression by 70%. TMAO resulted in slight repression of \( P_{\text{nikABCDE}} \) expression, while fumarate and DMSO resulted in slight repression. Formate enhanced \( P_{\text{nik}} \) expression by 40%. Thus, NikABCD expression levels correlate with previously observed changes in hydrogenase activity in different media.

The nickel dependence of \( P_{\text{nik}} \) expression has previously been examined in LB medium in the absence or presence of 250 μM or higher nickel ion concentrations (9, 43), but a nickel titration has not been carried out under any growth condition. Additionally, the relationship between \( P_{\text{nik}} \) expression levels (Fig. 1A) and nickel-dependent repression of expression has not been examined. \( P_{\text{nik-lacZ}} \) expression was measured over a range of nickel concentrations in a subset of the growth conditions from Fig. 1A. In all cases, a monophasic decrease in LacZ activity was observed with increasing nickel concentration (Fig. 1B). Interestingly, the \( K_{\text{Ni}} \) value increased substantially in the presence of formate, from 10 nM to 158 nM, and nickel-dependent repression in the presence of formate was more cooperative (\( n = 2.3 \)) than growth conditions with lower hydrogenase expression (\( n = 1 \)). These results suggest that the
addition of 0.1% peptone to M63 minimal medium resulted in a 2.5-fold decrease in $P_{\text{nik}}$-lacZ expression but did not affect $K_{\text{Ni}}$ compared to that after growth in M63 (data not shown). We also observed a twofold pH-dependent difference in $P_{\text{nik}}$-lacZ expression when M63 medium was buffered to below pH 6.2 (normal pH is 7.5). Similarly, buffered LB medium (100 mM morpholinepropanesulfonic acid, pH 7.2) had 1.6-fold-higher $P_{\text{nik}}$-lacZ expression than unbuffered LB medium, but this level was still lower than the expression in M63 medium. *E. coli* organisms acidify LB medium under anaerobic conditions as a function of increasing growth, which influences hydrogenase expression (17) and therefore the nickel requirement of the cell.

**Nitrate-dependent regulation of $P_{\text{nikABCDE}}$ expression.** The significant decrease in the basal levels of $P_{\text{nik}}$-lacZ expression in the presence of nitrate (Fig. 1) suggested a previously unidentified mechanism for the transcriptional regulation of NikABCDE. *E. coli* responds to a range of extracellular nitrate concentrations via the NarLX and NarPQ two-component systems (33). Nitrate-dependent repression of $P_{\text{nik}}$-lacZ expression was observed at nitrate concentrations $\geq 1$ mM (Fig. 2A), which is inversely correlated with the NarLX-dependent positive regulation of nitrate reductase ($\text{narG}$) expression (40). To identify whether $P_{\text{nik}}$ regulation was NarLX dependent, $P_{\text{nik}}$-lacZ expression was assayed in the absence or presence of 15 mM nitrate in mutant strains deleted of the $\text{narLX}$, $\text{narP}$, or $\text{narQ}$ gene. Only $\text{narLX}$-deficient *E. coli* showed a partial loss of repression of $P_{\text{nik}}$-lacZ, compared to the expression of the $\text{narP}$-deficient, $\text{narQ}$- and $\text{narP}$-deficient, $\text{narQ}$- and $\text{narQ}$-deficient, or parent strain at low nickel concentrations when strains were grown in nitrate-containing media (Fig. 2B). Repression of $P_{\text{nik}}$ was unaffected at high nickel concentrations. Little effect on LacZ activity was seen for the $\text{nar}$ mutant strains in the absence of nitrate (Fig. 2B). Thus, the NarLX system, which negatively regulates hydrogenase expression (27, 34), has the same effect on $P_{\text{nik}}$ expression.

**Nitrate (NarLX) and nickel (NikR) independently regulate $P_{\text{nikABCDE}}$ expression.** Deletion of the $\text{narLX}$ genes did not result in complete restoration of $P_{\text{nik}}$ expression in nitrate-containing growth media. Additionally, the nickel-dependent repression at high nickel concentrations was NarLX independent, raising the question of whether NikR and NarLX-dependent repression rely on the same operator site in $P_{\text{nik}}$. NarL is a response regulator that, when phosphorylated, has increased affinity for DNA (22), suggesting a direct mechanism for nitrate-dependent regulation of $P_{\text{nik}}$. To determine whether NarL- and NikR-dependent regulation of $P_{\text{nik}}$ expression were independent, LacZ levels were measured in nikR-deficient and nikR narLX-deficient strains containing wild-type NikR or a mutant protein, the Cys95Ala protein, which lacks high-affinity nickel-binding activity and shows no DNA binding in vitro with up to 1 $\mu$M NiCl$_2$ (7). Cells containing Cys95Ala NikR showed increased LacZ activity with all nickel concentrations (Fig. 3). Cells lacking both NarL and functional NikR showed constant high levels of $P_{\text{nik}}$-lacZ, suggesting that NarL and NikR are sufficient to account for the $P_{\text{nik}}$ repression observed under the conditions tested here (Fig. 3). The difference in $P_{\text{nik}}$-lacZ repression at low nickel concentrations in the narLX-deficient strain indicates that NikR likely binds at a site distinct from that required for NarL-dependent repression. Additionally,
mutations in the NikR operator that diminish NikR binding (9) retained nitrate-dependent repression of the P_{nik} promoter. Recent bioinformatics approaches to identify transcription factor binding sites in *E. coli* have not predicted a NarL-binding site in the region of the nik promoter (6, 19, 23); however, these studies have also not predicted the FNR-binding site (TTGAT-N4-AACAG versus consensus TT-GAC-N4-ATCAA) in the nik promoter (24, 44).

These data reveal a role for the high-affinity nickel-binding site in NikR function at low total nickel concentrations. They also suggest that NikR is active when nitrate is present in the growth media but that its function is somehow inhibited under conditions that favor the expression of hydrogenase isozymes and their corresponding assembly proteins (Fig. 2B), such as the presence of formate in the growth medium.

**NikABCDE is not required for NikR function.** An obvious mechanism for inhibition of NikR function is the exclusion of nickel from inside the cell. Deletion of the nikABCDE operon should dramatically restrict the amount of intracellular nickel available for nickel-binding proteins, including NikR. The effect of the nikABCDE deletion should mimic the effect of the Cys95Ala high-affinity nickel-binding mutant of NikR on P_{nik}-lacZ activity by reducing the amount of functional NikR in the cell. Deletion of nikABCDE (Fig. 4) or nikA alone (data not shown) had no effect on the nickel-dependent repression of P_{nik}-lacZ expression, leading to the surprising conclusion that NikR repression is independent of nickel transport by NikABCD. The nickel- and NikR-dependent repression pattern was not inhibited by the high concentrations of magnesium (4 mM) known to block nickel import by CorA (31), and deletion of corA had no effect on the nickel-dependent repression curves observed here (data not shown). These data suggest the presence of another nickel import pathway in *E. coli*.

**DISCUSSION**

A complex and hierarchical set of inputs controls gene expression in anaerobically growing *E. coli* cells (38). Intricate regulation results in the synthesis of an enzyme complement that produces the highest energy yield under a given growth condition. *E. coli* NiFe hydrogenases are upregulated under fermentative growth conditions or in the presence of a low-energy-yield electron acceptor, such as fumarate. Here, we have shown that transcription of the NikABCD operon is essential for hydrogenase activity (24, 44, 45),
is regulated by several distinct mechanisms in order to match the hydrogenase expression level of the cell.

The regulation of nikABCDE synthesis under different growth conditions is summarized in Fig. 5A and B with curves and diagrams labeled I to IV. FNR upregulates NikABCDE (I and II) in the absence of oxygen. In the presence of nitrate (II), NikABCDE synthesis is repressed by both the NarLX two-component system and NikR. Hydrogenase expression is repressed in the presence of nitrate in favor of the synthesis of nitrate reductase (38), which catalyzes the reduction of nitrate as the terminal electron transfer step in the absence of oxygen. NikR further represses NikABCDE expression at higher nickel concentrations (IV), providing nearly complete repression under these conditions. NarLX-dependent repression is absent when nitrate is not present in the growth medium, while NikR-dependent repression depends on hydrogenase expression level.

FIG. 4. NikABCDE-independent regulation of \( P_{\text{nikABCDE}} \) expression by NikR. (A) Filled squares, \( P_{\text{nik}}-\text{lacZ} \) expression in glucose for the wild type (WT) (RZ4500 plus pNIK103); open squares, \( \Delta \text{nikR} \) (PC269 plus pNIK103 C95A NikR); and open circles, \( \Delta \text{nikABCDE} \) (PC379 plus pNIK103). (B) Filled squares, \( P_{\text{nik}}-\text{lacZ} \) expression in glucose plus 15 mM KNO\(_3\) for the wild type (RZ4500 plus pNIK103); open squares, \( \Delta \text{nikR} \) (PC269 plus pNIK103 C95A NikR); and open circles, \( \Delta \text{nikABCDE} \) (PC379 plus pNIK103). \( K_{\text{Ni}} \) values were 6.6 nM for the wild type (without KNO\(_3\)), 11.3 nM for the \( \Delta \text{nikABCDE} \) strain (without KNO\(_3\)), 10.3 nM for the wild type (with KNO\(_3\)), and 11.7 nM for the \( \Delta \text{nikABCDE} \) strain (with KNO\(_3\)).

FIG. 5. Model for nikABCDE transcriptional regulation. (A and B) Transcriptional regulation of \( P_{\text{nikABCDE}} \) expression as a function of nickel concentration under different anaerobic growth conditions. There are three general states of promoter occupancy. At low nickel concentrations in the absence of nitrate (curves and diagrams I and II), FNR upregulates \( P_{\text{nikABCDE}} \) and some component of the hydrogenase assembly pathway blocks NikR function. The degree of NikR inhibition depends upon the level of hydrogenase expression. In the presence of nitrate at low nickel concentrations (III), NarL and NikR strongly repress transcription. At high nickel concentrations (IV), NikR is the dominant regulator of \( P_{\text{nikABCDE}} \) expression regardless of the presence of nitrate (i.e., NarL). (C) Model of the coordination of hydrogenase assembly and NikABCDE-dependent nickel transport in anaerobically growing \emph{E. coli} based on the data presented here. \( \text{H}_2\text{ase} \), hydrogenase.
els (I and II). The loss of NikR-dependent repression does not appear to be due to a loss of nickel-containing NikR. Rather, NikR function seems to be inhibited by components of the hydrogenase assembly pathway. In particular, the levels of these components are increased in the presence of formate, which induces expression of the hyd-3 operon (28).

There are at least four proteins that either positively or negatively control NikABCDE expression. The activities of these proteins are controlled in some manner by a small molecule: FNR (positive) is inactivated by O2, NarL (negative) is phosphorylated by NarX in the presence of NO3-, NikR (negative) is activated by Ni2+, and NikR function is inhibited by one or more formate-inducible hydrogenase assembly components. This multilayered regulation provides a way for NikABCDE-dependent nickel uptake to be tuned to the hydrogenase requirements of the cell as well as to the external nickel concentration (Fig. 5).

Surprisingly, NikR function does not depend on nickel import by NikABCDE at any nickel concentration or under any growth condition tested here. The high affinity of NikR for metal ions (8, 41) suggests different possibilities for the activation of NikR in the absence of added nickel. Either nickel ions enter the cell by some previously unidentified pathway or NikR is activated in vivo by a different transition metal. The growth medium used in these experiments was treated to remove nickel, but the addition of other metal supplements after this treatment would have resulted in some very low level of nickel ions entering the cell by some previously unidentified pathway or under any growth condition tested here. The high affinity of NikR for metal ions (8, 41) suggests different possibilities for the activation of NikR in the absence of added nickel. Either nickel ions enter the cell by some previously unidentified pathway or NikR is activated in vivo by a different transition metal. The growth medium used in these experiments was treated to remove nickel, but the addition of other metal supplements after this treatment would have resulted in some very low level of nickel being added back to the medium. It is not possible, based on the data presented here, to differentiate between the two NikR activation mechanisms described above. However, in the complete absence of any added nickel, Pnix-lacZ expression was 10 to 15% higher than with 10 pM nickel, suggesting that E. coli cells can sense even very low extracellular nickel concentrations. At higher nickel concentrations, NikR repression exhibits a consistent dependence on the added nickel concentration. This observation provides strong evidence for a second pathway for nickel import into E. coli that is independent of the previously identified NikABCDE and CorA routes for nickel import.

The tight nickel-binding affinity exhibited by NikR in vitro poses a paradox with regard to intracellular metal trafficking. Because NikR can bind nickel at a concentration (< 5 pM) well below that corresponding to a single nickel ion inside the cell (~1 nM), there might be competition for nickel ions between functional and regulatory pathways. The data presented here suggest that there is no such competition. Instead, the hydrogenase assembly pathway sequesters nickel ions within the cell, beginning with the NikABCDE transporter, and another nickel transporter is required for NikR function, which clearly establishes a second “pool” of nickel ions within the cell. Previously, it was shown that the presence of a functional NikABCDE system is required for the correct insertion of nickel into hydrogenase 3 in the face of competition from high extracellular concentrations of Zn2+ (21), suggesting a tight link between nickel transport and hydrogenase assembly. The original identification and designation of nikABCDE operon hydC (43) has been reassigned its apparently specific function. The model presented here suggests a nickel-independent mechanism for “competition” between functional and regulatory pathways, in which hydrogenase assembly components block NikR function when hydrogenase synthesis is high and nickel concentrations are limiting (Fig. 5). The hydrogenase assembly pathway is complex (3), and it is likely that more than one component affects NikR function (J. L. Rowe and P. T. Chivers, unpublished results).

E. coli likely requires a second nickel uptake pathway not linked to hydrogenase expression. Glyoxalase I (GlxI), has maximal activity in vitro in the presence of nickel ions (11, 13, 15, 35). This enzyme, which detoxifies methylglyoxal produced from dihydroxyacetone phosphate, is expressed under aerobic growth conditions (20) and thus requires an independent source of nickel for its activity. This source of nickel may also be important for NikR function.

A number of microbial genomes carry a nikR ortholog. However, the nickel requirements of these microbes will be different based on the differing complements of nickel enzymes encoded by their genomes. For example, Helicobacter pylori has a large requirement for nickel to support both urease and hydrogenase activity, both of which are essential for the colonization of the stomach (25, 37). Methanogens have an absolute requirement for nickel in three enzymes involved in their central carbon metabolism (36). This diversity of nickel-related physiology raises interesting questions about the biochemical properties of different species of NikR, their corresponding biological roles, and differences in the regulatory inputs controlling intracellular nickel homeostasis in these different microbes. The nitrate-dependent regulation of nickel uptake observed in E. coli will not be a common feature of all microbes, because many NikR-encoding microbes lack both the ability to respire nitrate and a nitrate-responsive two-component system. Further, in microbes that have more than one high-abundance nickel enzyme or nickel enzyme assembly pathway, such as H. pylori or methanogenic archaea, nickel must be trafficked to multiple sites before NikR regulation of uptake can be allowed to occur. Differences in the numbers of nickel transporters and their structures may also influence how each microbe responds to nickel. Thus, the results from E. coli provide a conceptual model of how NikR might function in a microbial cell but illustrate that its function is not solely governed by its in vitro ligand-binding properties.

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REFERENCES


