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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 rate 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 sequestered 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 acceptors such as fumarate. Hydrogenases 1 and 2 (expressed by *hya* and *hyb*, respectively) oxidize H$_2$ in the presence of fumarate to generate ATP. Hydrogenase 3 (hyd) is part of a complex with formate dehydrogenase that converts formate to CO$_2$ and H$_2$. 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 (*P*$_{nikABCDE}$ or *P*$_{nik}$). This arrangement provides two distinct inputs that control nickel uptake. A decrease in oxygen tension results in activation of FNR and upregulation 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 *P*$_{nik}$ expression might be observed in vivo. The pattern of *P*$_{nik}$ 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

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NikR-DNA complexes act to repress expression from \( P_{nik} \) although the activity of the first NikR-DNA complex is observed only under conditions of low hydrogenase expression. Additionally, nitrate represses \( P_{nik} \) expression via the NarLX two-component system. Surprisingly, NikR function does not depend on nickel transport by NikABCDE, 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), \( nikABCDE \) (bases 16 to 1,560), and \( coeA \) (bases 61 to 891) were constructed using the method of Danzenko and Wanner (12). Numbering refers to base positions relative to ATG.

A translational \( P_{nis} - lacZ \) fusion was constructed in three steps. A \( P_{nis} \) fragment (~400 bp) was amplified by PCR from \( E. coli \) genomic DNA using oligonucleotides PC118 (5′-CTATGGCGCCCGGCCAAACCTGATTTGCGCGG-3′) and PC38 (5′-CCATGATATGGAATTCATCAGTCTGATGATCAAGC-3′) and PC38-GC (5′-CCATGATATGGAATTCATCAGTCTGATGATCAAGCAAATGATTAAATGAGA-3′). This product was amplified by PCR with the aid of primers PC269 (5′-CTATGATATGGAATTCATCAGTCTGATGATCAAGC-3′) and PC638 (5′-AGGCTATCTAGATTTTATGACACCGACACACTGGG-3′). The underlined bases correspond to Eaqg and XbaI restriction sites, respectively. The two resulting fragments were purified and combined in a second PCR mixture that contained PC118 and PC38. The resulting 3,633-bp fragment was digested with Eagl and XbaI and ligated into pACYC184, cut with the same enzymes to create pPC181. This plasmid is a precise fusion between \( P_{nis} \) and the ATG codon of \( lacZ \). It differs from the previously described pPC163 (9), which contains five codons of the 5′ end of the \( nikA \) gene fused to the 5′ end of \( lacZ \). pPC181 has at least-fourfold-greater expression than pPC163 under identical conditions (data not shown), suggesting that the \( nikA \) codons negatively affect the production of LacZ.

**Growth media.** M63 salts (5×) 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 FeCl₃, 1 μM ZnCl₂, 100 mM (NH₄)₂MoO₄, and 100 mM Na₂SO₄. 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 \( P_{nis} - lacZ \) 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 0.001 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 \( nikhR \) deletion, strains lacking chromosomal \( nikhR \) were transformed with pNIK103 CyoS5Ala (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 \( OD_{600} \) and LacZ activity. \( OD_{600} \) 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 polystyrene 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 \( y = \left(\frac{a}{K_y} + b\right) \left[\frac{1 + (K_y/x)}{1 + (K_y/x)}\right] \) 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), \( K_y \) is the nickel concentration required for half-maximum LacZ activity, and \( n \) 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 NikABCDE levels. \( E. coli \) RZ4500 organisms containing a low-copy-number \( P_{nis} - lacZ \) fusion were grown anaerobically in M63 minimal medium to which glucose, an electron acceptor (nitrate, DMSO, TMAO, or fumarate), and/or formate were added. Changes in LacZ activity were compared to the LacZ activity of cells grown in medium containing glucose alone (Fig. 1A). Nitrate repressed \( P_{nis} \) expression by 70%. TMAO resulted in slight repression of \( P_{nis} - ABCDE \) expression, while fumarate and DMSO resulted in substantial repression of LacZ activity. Formate enhanced \( P_{nis} \) expression by 40%. Thus, NikABCDE expression levels correlate with previously observed changes in hydrogenase activity in different media. The nickel dependence of \( P_{nis} \) 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 \( P_{nis} \) expression levels (Fig. 1A) and nickel-dependent repression of expression has not been examined. \( P_{nis} - lacZ \) 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 \( K_{Ni} \) 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
nickel-dependent regulation of P_nik is strongly correlated with the synthesis of active hydrogenase at limiting nickel concentrations and raise the question of whether NikR activity is altered under these conditions as a result of competition for available nickel ions. The absence of a biphasic repression curve suggested that in vivo roles for the distinct Ni-NikR DNA complexes observed in vitro as a function of nickel (4, 8, 9) are not observable under these conditions.

Levels of LacZ expression in LB medium were qualitatively similar (data not shown). However, the maximum LacZ activity was at least twofold lower and the repression curve was shifted to \( 10^2 \)-fold higher \( (K_{Ni} \approx 10 \mu M) \). Components of rich medium likely influence hydrogenase expression in anaerobically growing E. coli as well as restrict nickel availability (44). The addition of 0.1% peptone to M63 minimal medium resulted in a 2.5-fold decrease in \( P_{nic-lacZ} \) expression but did not affect \( K_{Ni} \) compared to that after growth in M63 (data not shown). We also observed a twofold pH-dependent difference in \( P_{nic-lacZ} \) 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 \( P_{nic-lacZ} \) 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 \( P_{nikABCDE} \) expression. The significant decrease in the basal levels of \( P_{nik-lacZ} \) 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 \( P_{nik-lacZ} \) expression was observed at nitrate concentrations \( \geq 1 \) mM (Fig. 2A), which is inversely correlated with the NarLX-dependent positive regulation of nitrate reductase (narG) expression (40). To identify whether \( P_{nik} \) regulation was NarLX dependent, \( P_{nic-lacZ} \) expression was assayed in the absence or presence of 15 mM nitrate in mutant strains deleted of the narLX, narP, or narQ gene. Only narLX-deficient E. coli showed a partial loss of repression of \( P_{nic-lacZ} \), compared to the expression of the narP-deficient, narQ- and narP-deficient, narQ- and narQ-deficient, or parent strain at low nickel concentrations when strains were grown in nitrate-containing media (Fig. 2B). Repression of \( P_{nik} \) was unaffected at high nickel concentrations. Little effect on LacZ activity was seen for the 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 \( P_{nik} \) expression.

Nitrate (NarLX) and nickel (NikR) independently regulate \( P_{nikABCDE} \) expression. Deletion of the narLX genes did not result in complete restoration of \( P_{nik} \) 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 \( P_{nik} \). NarL is a response regulator that, when phosphorylated, has increased affinity for DNA (22), suggesting a direct mechanism for nitrate-dependent regulation of \( P_{nik} \). To determine whether NarL- and NikR-dependent regulation of \( P_{nik} \) 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 NarLX and functional NikR showed constant high levels of \( P_{nic-lacZ} \), suggesting that NarL and NikR are sufficient to account for the \( P_{nic} \) repression observed under the conditions tested here (Fig. 3). The difference in \( P_{nik-lacZ} \) 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 \( P_{\text{nik}} \) promoter (data not shown). Recent bioinformatics approaches to identify transcription factor binding sites in \( E. coli \) have not predicted a NarL-binding site in the region of the \( P_{\text{nik}} \) promoter (6, 19, 23); however, these studies have also not predicted the FNR-binding site (TTGAT-N4-AACAG versus consensus TTGAC-N4-ATCAA) in the \( P_{\text{nik}} \) 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 \( \text{nikABCDE} \) operon should dramatically restrict the amount of intracellular nickel available for nickel-binding proteins, including NikR. The effect of the \( \text{nikABCDE} \) deletion should mimic the effect of the Cys95Ala high-affinity nickel-binding mutant of NikR on \( P_{\text{nik}}-\text{lacZ} \) activity by reducing the amount of functional NikR in the cell. Deletion of \( \text{nikABCDE} \) (Fig. 4) or \( \text{nikA} \) alone (data not shown) had no effect on the nickel-dependent repression of \( P_{\text{nik}}-\text{lacZ} \) 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 \( \text{corA} \) 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). This 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, which 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, 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.
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 O$_2$, NarL (negative) is phosphorylated by NarX in the presence of NO$_3^-$, NikR (negative) is activated by Ni$^{2+}$, 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 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, $P_{\text{nic}}$-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 and likely 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 Zn$^{2+}$ (21), suggesting a tight link between nickel transport and hydrogenase assembly. The original identification and designation of nikABCDE as hydC (43) may have presaged 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**


