The Metal-Selective Response of the *Escherichia coli* Nickel Metalloregulator NikR

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Nickel is required in many microorganisms as a metalloenzyme cofactor (1). However, nickel can also produce potent cellular damage (2), so intracellular exposure to unprotected nickel ions is tightly controlled. In *Escherichia coli* (*E. coli*), expression of the nickel uptake transporter is regulated at the transcriptional level by the nickel-responsive repressor NikR (3, 4). In the presence of nickel, NikR binds tightly to the promoter region of the transporter operon and is thought to block transcription. Although the metal selectivity of the DNA-binding response of NikR must be a critical aspect of nickel ion homeostasis, little is known about the metal-selective properties of NikR or how they are achieved.

NikR binds stoichiometric nickel with picomolar affinity, but it also binds several other divalent metals such as Cu(II), Zn(II), and Cd(II) with similar affinities (5). One equivalent of these metal ions induces DNA binding with moderate affinity, indicating that this metal-responsive activity is not selective for nickel (6). However, nickel binding provides more resistance to denaturation and affords a greater degree of protection from protease digestion than any of the other metals tested, suggesting that nickel induces a different conformational change in the protein. DNA-binding experiments support the hypothesis that there are in fact two metal-binding sites that control two different levels of regulation. Furthermore, the binding of a second metal ion produces a nickel-selective response, suggesting that it is this signal that is physiologically relevant. Experiments are underway to locate and characterize the putative second metal-binding site. These results form the basis of a working model for the in vivo activity of this nickel metalloregulator.

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