

P020 DNA binding properties of the *Escherichia coli* nitric oxide sensor NorR: towards an understanding of the regulation of flavorubredoxin.

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Nitric oxide (NO) is an intermediate of denitrification, and is one of the radical species deployed by macrophages against invading pathogens, so bacterial responses to NO are of considerable importance. The *Escherichia coli* flavorubredoxin and its associated oxidoreductase reduce NO to nitrous oxide under anaerobic conditions, and are encoded by the *norVW* transcription unit. Expression of *norVW* requires the NO sensing regulatory protein NorR and RNA polymerase containing sigma-54 (σ^{54} RNAP). We have purified NorR and shown that it binds cooperatively to multiple sites in the *norVW* promoter region. DNaseI footprinting and methylation protection assays have identified three NorR binding sites, located between 54 and 110 bp upstream of the experimentally verified σ^{54} RNAP promoter. As predicted, the *norVW* mRNA initiates 12 bp downstream of the conserved GC motif of the promoter. We have also identified two binding sites for integration host factor (IHF), one between the NorR and σ^{54} RNAP binding sites, and a second downstream of the *norVW* transcription start site. Comparison of the *norVW* promoters of enteric bacteria along with known and putative NorR-regulated promoters from *Ralstonia* and *Pseudomonas* species suggests that NorR binding sites contain an invariant GT-(N7)-AC motif flanking an AT rich central region. This information will help to identify additional members of the NorR regulon.