Regulation of the Nitric Oxide Reduction Operon (norRVW) in Escherichia coli

ROLE OF NorR AND σ54 IN THE NITRIC OXIDE STRESS RESPONSE

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Nitric oxide (NO) induces NO-detoxifying enzymes in Escherichia coli suggesting sensitive mechanisms for coordinate control of NO defense genes in response to NO stress. Exposure of E. coli to sub-micromolar NO levels under anaerobic conditions rapidly induced transcription of the NO reductase (NOR) structural genes, norV and norW, as monitored by lac fusions. Disruption of riopN (σ54) impaired the NO-mediated induction of norV and norW transcription and NOR expression, whereas disruption of the upstream regulatory gene, norR, completely ablated NOR induction. NOR inducibility was restored to NorR null mutants by expressing NorR in trans. Furthermore, an internal deletion of the N-terminal domain of NorR activated NOR expression independent of NO exposure. Neither NorR nor σ54 was essential for NO-mediated induction of the NO dioxygenase (flavohemoglobin) encoded by hmp. However, elevated NO activity inhibited NO dioxygenase induction, and, in the presence of dioxygen, NO dioxygenase inhibited norV induction by NO. The results demonstrate the role of NorR as a σ54-dependent regulator of norVW expression. A role for the NorR N-terminal domain as a transducer or sensor for NO is suggested.

Nitric oxide (NO) is a free radical with multiple and diverse biological functions (reviewed in Ref. 1). NO serves as an intermediate in microbial denitrification (2), a signal molecule controlling the activation of guanylate cyclases (3), and as a neurotransmitter, anti-viral and anti-tumor agent secreted by host immune cells (4, 5). Sub-micromolar NO can inactivate or inhibit critical enzymes, including [4Fe-4S] (de)hydratases and heme-dependent terminal respiratory oxidases, accounting at least in part for the cytotoxic actions of NO (6–11).

Not surprisingly, organisms have evolved mechanisms for NO detoxification. NO reductases (NORs) reduce NO to N₂O and are widely distributed in denitrifying bacteria, nitrogen-dissimilating fungi, and pathogenic bacteria (2). Microbes also express NO dioxygenases (NODs) that utilize O₂ to convert NO to nitrate (12–18). Escherichia coli employs both of these enzymes. An inducible NOD (flavohemoglobin), encoded by the gene hmp (19), detoxifies NO under aerobic growth conditions (12, 15, 20). An inducible O₂-sensitive NOR activity encoded by the norRVW operon detoxifies NO under anaerobic and microaerobic conditions (8, 20). NorV is a di-iron center-containing flavohemoglobin-type NOR with orthologues in the Archeae, strict anaerobes, and facultative anaerobes (21–24). It is distinct from the bacterial heme/nonheme iron-containing cytochrome bc-type NORDs and the fungal P450-type NOR (2). NorV functions as an NADH:flavohemoglobin oxidoreductase (21) and is required for maximal flavohemoglobin-catalyzed NO reduction in cells (8) and in vitro (25). Together, the O₂-dependent NOD and the O₂-sensitive NOR (NorVW) detoxify NO throughout the physiologcal O₂ range (7, 8, 20).

NORs and NODs are induced by NO or NO-generating agents suggesting fine-tuned mechanisms for the coordination of microbial NO defenses to NO stress levels. In denitrifying Pseudomonas and Rhodobacter, cytochrome bc-type NORDs are up-regulated by the Fnr-like DnrD/NnrR transcription regulators in response to nanomolar NO (26–28). However, unlike Fnr (29), DnrD/NnrR do not bear NO-reactive [4Fe-4S] centers, and the NO sensing mechanism is currently unknown (26–28, 30). In the denitrifier Ralstonia eutropha, the tripartite transcription factor NorR regulates denitrification, norAB1 transcription, and NOR activity expression in a σ54-dependent mechanism in response to exposures to sodium nitroprusside, the NO donor compound NOC18, or during growth with nitrite or nitrate (31). E. coli and related microbes contain norR orthologues suggesting a global regulatory role for NorR in controlling defenses (i.e. norVW, norBC, and hmp) against the incipient toxicity of NO and secondarily derived reactive nitrogen species (8, 31, 32).

Recently, Hutchings et al. (32) reported NorR-dependent activation of norV transcription by the NO⁺ donor and NO-generating compound nitroprusside in support of the proposed regulatory function. Interestingly, nitroprusside-elicited norV transcription was increased >5-fold by normoxic O₂ suggesting mechanisms for NorR activation involving O₂-derived reactive nitrogen intermediates rather than NO per se. The large oxygen enhancement of norV transcription observed with or without nitroprusside exposure has also supported proposals for aerobic functions for the norRVW operon, including O₂ reduction and the detoxification of O₂-derived reactive nitrogen intermediates (25, 32).

We now report the rapid and robust induction of norV and norW transcription and NorVW activity by sub-micromolar NO via a NorR- and σ54-dependent mechanism in E. coli. We also show that a deletion within the conserved NorR N-terminal
domain activates NorVW expression independent of NO exposure, thus demonstrating the role of the N terminus in NO sensing and signaling. Contrary to the results obtained with nitroprusside, O₂ greatly diminished norV and norW induction by NO. The results are discussed in light of the proposed NO reduction and detoxification function of the norRVW operon within the NO defense network.

MATERIALS AND METHODS

Chemicals and Reagents—Bovine liver catalase (280,000 units/ml) was purchased from Roche Molecular Biochemicals. Glucose oxidase (4,000 units/ml) and bovine liver catalase (260,000 units/ml) were obtained from Roche Molecular Biochemicals. Glucose oxidase (7). Aerobic and microaerobic starter cultures were grown over-night in 5 ml of phosphate-buffered LB medium in 15-ml tubes shaking 214 °C with a 2-mm ISO-NOP NO electrode (World Precision Instruments, Sarasota, FL) in addition to the BclI sites (35), respectively. pUC19NorR was digested with BamHI and religating.

Media, Growth Conditions, and Gas Exposures—Aerobic starter cultures were grown static overnight at 37 °C in 15-ml tubes containing 10 ml of phosphate-buffered LB medium supplemented with 20 mM glucose (7). Aerobic and microaerobic starter cultures were grown overnight in 5 ml of phosphate-buffered LB medium in 15-ml tubes shaking at 200 rpm at 37 °C. Chloramphenicol and ampicillin were added as indicated at 30 and 50 μg/ml, respectively. Cultures growth was monitored by following the turbidity at 550 nm (A550) and by plating and counting. An A550 value of 1.0 in a 1-cm cuvette was equivalent to 3 × 10⁸ bacteria per milliliter for cultures grown in phosphate-buffered LB media. Gases were mixed and delivered to sealed 50-ml growth flasks as previously described (20).

NO Consumption Assays—Whole cell NO consumption rates were measured at 37 °C with a 2-mm ISO-NOP NO electrode (World Precision Instruments, Sarasota, FL) in the presence or absence of O₂ as previously described (12, 20).

β-Galactosidase Assays—Cells were harvested by centrifugation and washed in 100 mM sodium phosphate buffer, pH 7.0. β-Galactosidase activity was measured according to the method of Miller (37) with the following modifications. Frozen cell pellets were suspended at ~ 1 × 10⁶ cells per milliliter in 100 mM sodium phosphate buffer, pH 7.0, and sonicated on ice. Cell-free extracts were prepared by centrifuging ly

Partial NO oxidation within the NO defense network.

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**FIG. 1. Expression of a norV-lacZ fusion in anaerobic cultures exposed to NO.** Strain AG300 was exposed to 960 ppm gaseous NO for various times (A) or to various NO concentrations for 45 min (B), and β-galactosidase activity was measured. Cultures were initiated from static overnight cultures at an A600 of ~0.1 and were grown under an N₂ atmosphere in phosphate-buffered LB medium. At an A550 of ~0.3, cultures were exposed to mixtures of NO in N₂. Cells were harvested, and extracts were prepared and assayed for β-galactosidase activity in triplicate as described under “Materials and Methods.” Error bars represent the S.D. of measurements from three independent exposures.

NO induces transcription of norV and norW but Not norR—norV and norW transcriptional units are arranged in a head-to-tail fashion with the start methionine of NorW located at 12,000 × g for 5 min. Assays were incubated for 15 min at room temperature in a 0.1-ml volume with 1–15 μg of extract protein in a 96-well plate. Extract activities were determined using bovine serum albumin as the standard (38). Statistical Analysis—Statistical significance (p < 0.05) was determined using the Tukey Kramer honestly significantly different method in the JMP program (SAS Institute).

RESULTS

**Table I**

| Strain or plasmid | Characteristic or description | Reference |
|-------------------|------------------------------|-----------|
| Strains AB1157    | F-, thr-1, leu-6, proA2, his-4, thi-1, argE3, lacY1, galK2, ara14, xyl-5, mtl-1, tdx-35, strA21, sup-37, λ | (33) |
| YMC18             | YMC10 ropNglnF208::Tn10; Te⁺ | (34) |
| AG500             | ropN::Tn10 P1 transduction from YMC18 into AB1157 | This work |
| AG200             | AB1157 φnorR-lacZ266; Cm⁺ | (5) |
| AG300             | AB1157 φnorV-lacZ222; Cm⁺ | (8) |
| AG400             | AB1157 φnorW-lacZ211; Cm⁺ | (8) |
| AG305             | AG500 ropN::Tn10 φnorV-lacZ232; Cm²Te⁺ | This work |
| AG301             | AG300 hmp::Tn5 Kn⁺ | (20) |

- Plasmids
  - pUC19 Vector: Ap⁺
  - pUC19NorR The norR regulatory region and the norR structural gene on a 1.9-kb SalI fragment cloned in pUC19
  - pUC19NorRΔ30–164 NorR gene with internal deletion of amino acids 30–164 in pUC19

- Table I: E. coli strains and plasmids used in this study

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Regulation of the E. coli norRVW Operon

Expression of norV, norW, and norR-lacZ fusions

Cultures were grown under anaerobic N₂, 0.5% O₂, or 21% O₂ to A500 = −0.3. Anaerobic and fully aerated (21% O₂) cultures were grown with or without exposure to 960 ppm NO for 45 min. Low O₂ cultures were grown with or without exposure to 600 ppm NO for 45 min. Cells were assayed for β-galactosidase activity as described under “Materials and Methods.” Anaerobic and (micro)aerobic cultures were initiated at an A500 = 0.1 from static and aerated overnight cultures, respectively. Activities were determined in triplicate for three to five independent exposures.

Table II

| Strain | Genotype | with N₂ only | Control | NO |  | with 0.5% O₂ | Control | NO | with 21% O₂ | Control | NO |
|--------|----------|--------------|---------|----|---|-------------|---------|----|--------------|---------|----|
| AG300  | ΦnorV-lacZ232 | 1 ± 1 | 2591 ± 314 | 1 ± 1 | 282 ± 273 | 1 ± 1 | 120 ± 7 |
| AG400  | ΦnorW-lacZ111 | 3 ± 1 | 538 ± 91 | 3 ± 1 | 294 ± 99 | 4 ± 1 | 38 ± 15 |
| AG200  | ΦnorR-lacZ286 | 19 ± 2 | 13 ± 3 | 24 ± 1 | 22 ± 4 | 26 ± 1 | 35 ± 1 |

Fig. 2. Effect of σ⁴⁴ on constitutive and induced norV transcription and NO metabolism. β-Galactosidase activity was measured in strain AG300 and σ⁴⁴-deficient strain AG305 grown under an N₂ atmosphere containing 0.5% O₂ balanced with N₂ in the absence (white bars) or presence of 600 ppm gaseous NO (black bars). Anaerobic (B) and aerobic (C) NO consumption activities were measured for the parental strain AB1157 and the σ⁴⁴-deficient strain AG500 grown under an N₂ atmosphere containing 0.5% O₂ (white bars) or 0.5% O₂ and 600 ppm NO (black bars). Cultures were initiated at A500 = −0.1 from aerated overnight cultures grown in phosphate-buffered LB medium. Cultures were grown to A500 = −0.3 and were either exposed to NO or maintained under an atmosphere containing 0.5% O₂ in N₂. After a 45-min exposure, cultures were shifted to a N₂ atmosphere and immediately harvested for the assay of β-galactosidase activity, or anaerobic NOR activity and aerobic NOD activity as described under “Materials and Methods.” Error bars represent the S.D. of three independent experiments. Asterisks indicate p < 0.05 relative to the corresponding value for AG300 (A) or AB1157 (B).

Fig. 3. Effects of NorR and a NorR N-terminal domain deletion mutant on the expression of NOR and NOD activities. Anaerobic NOR (A) and aerobic NOD (B) activities were measured in the norR-lac deletion strain AG200 containing either pUC19 (control), pUC19NorR (NorR), or pUC19NorRΔ30–164 (Δ30–164). Cultures were either maintained under an atmosphere containing 0.5% O₂ balanced with N₂ (white bars) or exposed for 60 min to 600 ppm gaseous NO in 0.5% O₂ balanced with N₂ (black bars). Cultures were initiated, grown, and harvested as described in the legend to Fig. 2, except that ampicillin was added at 50 µg/ml. Error bars represent the S.D. for three to five independent trials. Asterisks indicate p < 0.05 relative to the value of the corresponding control.

within the coding region of norV suggesting coordinate transcription and translation in response to NO stress (8). In contrast, norR is divergently transcribed from norVW (8) and is autogenously regulated (32).

Strain AG300 carrying a norV-lacZ fusion within the norV genomic locus and lacking inducible anaerobic NOR activity (8) was used to measure the responsiveness of norV transcription to authentic NO. Exposure of anaerobic AG300 to 960 ppm gaseous NO (≤2 µM in solution) induced β-galactosidase activity by ~50-fold within 5 min. β-Galactosidase expression peaked after 30–45 min of exposure resulting in ≥1000-fold induction (Fig. 1A). A 30-fold increase in norV transcription was observed with 120 ppm NO (≈0.25 µM in solution) (Fig. 1B). Maximal induction of β-galactosidase activity was observed with 480 ppm NO (≈1 µM in solution). Expression was blunted with 960 ppm NO exposure suggesting toxicity of NO under these conditions.

NO similarly induced norW-lacZ in strain AG400 under anaerobic conditions (Table II). However, the norW-lac fusion was induced to a 10-fold lower extent than that observed for the norV-lac fusion following a similar NO exposure.

The dampened response of norW-lac to NO may be explained by the production of significant NOR activity from norV expression within strain AG400 (8), thus resulting in a lower steady-state NO level. It is also possible that higher steady-state NO levels are required to activate maximal norW transcription. Nevertheless, the results clearly demonstrate a rapid, robust, and coordinate up-regulation of norV and norW transcription in response to low levels of NO. The results in Table II also demonstrate a low non-inducible level of transcription from norR consistent with previous results obtained using nitroprusside as the potential inducer (32).

O₂ Decreases norV and norW Transcription in Response to NO—NO-mediated induction of norV-lac and norW-lac fusions was significantly inhibited by O₂. Under fully aerated conditions (~200 µM O₂), induction ratios for norV-lacZ and norW-lacZ fusions were reduced 200- and 20-fold, respectively, with no change in the basal expression levels (Table II). At a lower O₂ concentration (~5 µM), norV-lac and norW-lac induction ratios were reduced 3.1- and 1.8-fold, respectively. Lower norV and norW induction in the presence of O₂ can be explained by the decrease in cellular NO levels achieved by the inducible NOD. Indeed, NOD expression decreased norV-lac expression by ~96% in cells exposed to an atmosphere containing 960 ppm NO in 21% O₂ for 45 min. NOD-deficient strain AG301 and control strain AG300 produced 3453 ± 493 and 149 ± 23
Fig. 4. Conservation within the N-terminal domain of NorR orthologues. An alignment of NorR orthologues was performed using the ClustalW program (MacVector 7.0). Dark shading, identical amino acids; light shading, similar amino acids; double dots, close similarities with a threshold comparison value of \( \leq 0.50 \). GenBank accession numbers are as follows: E. coli, NP_417189 using the second start methionine for a 504-amino acid protein; S. typhimurium, NP_461760; K. pneumoniae, contig 661 with three base pairs added to maintain reading frame (available at genome.wustl.edu); R. eutropha NorR2, CAC00712; R. eutropha NorR2, CAC00712; P. aeruginosa, NP_251355; V. cholera, NP_232582.

Fig. 5. NO defense network in E. coli. NO induces expression of NOR (NorVW) and NOD activities that scavenge NO and prevent damage to critical cellular targets, stasis, and death throughout the physiological O2 concentration range. NorR controls norVW transcription in response to NO stress via an \( \sigma^{54} \) (\( \sigma^{54} \))-dependent mechanism. A putative histidine-aspartate kinase (\( \sigma^{54} \)) senses NO levels and phosphorylates and activates NorR and norVW transcription. Alternatively, NO activates NorR directly. NO reacts with Fnr and de-represses \( \sigma^{54} \)-dependent promoters. Unknown regulator (X) controls \( \sigma^{54} \) transcription under anaerobic conditions (29). Unknown regulator (X) controls \( \sigma^{54} \) transcription under aerobic conditions (29). Additional genes activated by NO-sensing regulators constitute a NO defense network.

Milliliters/mg \( \beta \)-galactosidase (n = 4, ±S.E.), respectively. Thus, norV and norW are maximally induced under conditions in which the O2-sensitive NOR functions most effectively (8, 20), and NOD indirectly regulates NOR expression.

Induction of norVW Transcription Is Dependent on \( \sigma^{54} \)—The central domain of the tripartite NorR protein is highly homologous with \( \sigma^{54} \)-dependent response regulators (31, 39) thus suggesting an important role for \( \sigma^{54} \) in the NO response. Furthermore, the region upstream of the norVW genes contains the respective −12 and −24 elements TGGCA and TGGCA characteristic of \( \sigma^{54} \)-dependent promoters (40, 41).

We used rpoN mutants to test the role of \( \sigma^{54} \) in NO-induced norVW transcription and NOR activity expression. \( \beta \)-Galactosidase activity was measured in AG300 and \( \sigma^{54} \)-deficient strain AG305 following a 45-min exposure to 600 ppm gaseous NO under microaerobic conditions. In the absence of \( \sigma^{54} \), norV-lacZ expression was substantially impaired (Fig. 2A). The \( \sigma^{54} \)-deficient strain AG500 and parental AB1157 were similarly exposed to NO under low O2 and tested for anaerobic NOR and aerobic NOD activity. NOR activity was significantly reduced in strain AG500 (Fig. 2B). There was no significant effect of \( \sigma^{54} \) on NOD (\( \sigma^{54} \)) expression under these conditions (Fig. 2C). The results clearly demonstrate a role for \( \sigma^{54} \) in norVW transcription. The residual induction of norV transcription and NOR activity in the absence of \( \sigma^{54} \) suggests ancillary roles for other \( \sigma \) factors in norV transcription or mechanisms for post-transcriptional regulation.

NorR Activates NOR Expression in trans—Expression of NorR from a multicopy plasmid rescued the NO inducibility of NOR activity in the norR deletion strain AG200 (Fig. 3A) thus confirming the trans-acting regulatory role of NorR in the activation of norVW transcription and NOR activity expression (8, 32). In the absence of NO, there was no measurable NOR activity expressed (Fig. 3A, open bars) indicating that overexpression of wild-type NorR does not by itself increase NorVW expression. However, internal deletion of NorR, eliminating amino acids 30–164 containing the putative signaling domain (31, 39), but retaining the entire central \( \sigma^{54} \)-interacting ATPase domain and the C-terminal DNA binding domain, induced NOR activity in the absence of NO (Fig. 3A). Further deletion of NorR to amino acid 214, eliminating part of the \( \sigma^{54} \)-interacting ATPase domain, did not induce \( \beta \)-galactosidase activity (data not shown) thus further delineating the requirement for \( \sigma^{54} \) interaction with NorR for transcriptional activation. Interestingly, the NO-mediated induction of NOD activity was significantly (\( p < 0.05 \)) reduced in strains expressing NorR and elevated NOR activity (Fig. 3B) thus suggesting an indirect role for NorR and NOR in regulating NOD expression by reducing NO levels.

These results demonstrate the signaling function of the N-terminal domain of the E. coli norVW transcription regulator NorR similar to that described for other tripartite regulators (31, 42). In addition, the results demonstrate that neither NorR nor \( \sigma^{54} \) is directly involved in the NO-mediated up-regulation of the E. coli NOD (\( \sigma^{54} \)).
Our data demonstrate that the exposure of E. coli to NO induces transcription of the norV and norW genes via a NorR and α²-dependent mechanism. The data extend the results of Hutchings et al. (32) demonstrating activation of norV transcription by the NO⁺ donor nitroprusside, nitrite, or nitrate in a NorR-dependent fashion. Given the relatively low concentration of NO required for anaerobic norV induction (Fig. 1B), NO is the most probable physiological signal modulating NorR and norVW transcription. Our results differ from those of Hutchings et al. (32) who reported that constitutive and induced norV transcription was greater in the presence of O₂. One likely explanation for the discrepancy is that we used NO gas and Hutchings et al. (32) used nitroprusside as a NO⁺ donor and potential NO-generating agent. Nitroprusside may have deleterious effects on transcription or, alternatively, may generate NO at higher levels in aerobic cells. Pure NO gas is readily available and is clearly preferred for investigations of the effects of NO on NO defense gene regulation.

The use of pure NO gas for the quantitative evaluation of gene expression responses also presents challenges because of the existence of multiple pathways for rapid and inducible NO metabolism and because of the incipient toxicity of NO. Nevertheless, the demonstration that the NO levels required for norV induction correspond with levels shown to exert cellular damage strongly supports the proposed role of the norRVW operon in NO reduction and detoxification. Thus, 240 ppm gaseous NO (≤0.5 μM in solution) inactivated E. coli aconitase and 6-phosphogluconate dehydrogenase and inhibited growth in the absence of the induced NorVW activity (6, 8). Furthermore, the level of NO inducing half-maximal norV transcription (≤0.7 μM) approximates the apparent Kₘ(NO) value of ~0.4 μM determined for NorVW-catalyzed NO reduction (8). These results diminish the likelihood of a significant function of the operon in O₂ detoxification or in the detoxification of unspecified reactive nitrogen intermediates generated from nitroprusside or NO exposure as previously suggested (25, 32).

A search of GenBank™ (NCBI) with the N-terminal 182 amino acids of NorR identifies several NorR orthologues (Fig. 4). As in E. coli, NorR orthologues in Salmonella typhimurium, Klebsiella pneumoniae, Shigella flexneri (AAN44223), and Vibrio vulnificus CMCP6 (NP_763239) are positioned upstream of norVW orthologues. Interestingly, NorR orthologues in the Pseudomonas aeruginosa, Vibrio cholerae, Azotobacter vinelandii (ZP_00091183), Burkholderia sp. strain TH2 (BAC16772), and Burkholderia fungorum (ZP00028693) genomes are found divergently transcribed from flavohemoglobin (hmp) genes suggesting a potential role for NorR in regulating NODs in response to NO. In this regard it is noteworthy that NorR was not required for the induction of NOD activity in response to NO in E. coli (Fig 3B), thus demonstrating the existence of one or more separate NO-responsive regulator(s) of hmp in E. coli.

NorR belongs to the family of two-component response regulators (42). Similar to other tripartite regulators in this family, deletion of the N-terminal signaling or inhibitory domain of NorR activated NorVW expression independent of NO (Fig. 3A). Furthermore, conserved aspartate residues in the NorR N-terminal signaling domain suggest the potential for phosphorylation by a sensor histidine-kinase similar to that described for the NtrB/NtrC pair (43). In particular, aspartates 57 and 62 are in position to accept phosphate, and the conserved acidic residue at position 14 may serve to optimize phosphorylation (Fig. 4) (44). Alternatively, the NorR N-terminal domain could activate transcription by interacting with a signal transducing protein as described for NifL/NifA (45) or by binding NO directly as the formate-sensing transcription regulator FhIA binds formate (46). The NorR N-terminal domain contains potential metal-liganding histidine and cysteine residues that could form the NO sensor module. For example, NorR contains an His₁¹¹-X-Cys₁³⁰ site reminiscent of the Cys²⁷-X-His³⁷ heme iron ligand-switch motif in the carbon monoxide-sensing CooA of Rhodospirillum rubrum (47).

Work summarized herein provides a current view of the NO defense network in E. coli. NO exposure elicits the synthesis of two major NO-metabolizing enzymes, NOD and NorVW by activating transcription of their corresponding genes, hmp and norVW. The respective contribution of each enzyme to NO detoxification depends primarily on the availability of O₂. NOD is effective under aerobic and microaerobic conditions (Kₘ(O₂) = 60–100 μM) (13, 14). The NOR activity of NorVW is unique in that its exquisite sensitivity to O₂ restricts its NO scavenging function to anaerobic or microaerobic conditions (8, 20). The results also support a model in which norVW and hmp transcription are indirectly influenced by O₂ availability, because O₂ levels affect NorVW and NOD activities, which ultimately determine NO steady-state levels and the activity of transcription regulators such as NorR and Fnr (29, 48).

Interestingly, neither SoxRS nor OxyR, which have been persistently proposed to be critical NO stress response sensor-regulators (49, 50) appear to be involved in the regulation of either hmp or norVW in E. coli (Fig. 5) (48). Future investigations will aim to further clarify the diverse roles and mechanisms of NO defense genes, enzymes, and regulators in microbial adaptations to NO in vitro and in various models of infection.

Acknowledgments—We thank Drs. Alex Ninfa and Ken Rudd for supplying strains and phage used in these investigations.

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