Flavorubredoxin, an Inducible Catalyst for Nitric Oxide Reduction and Detoxification in *Escherichia coli* *

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**Nitric oxide** (NO) is a poison, and organisms employ diverse systems to protect against its harmful effects. In *Escherichia coli*, *ygaA* encodes a transcription regulator (b2709) controlling anaerobic NO reduction and detoxification. Adjacent to *ygaA* and oppositely transcribed are *ygaK* (encoding a flavorubredoxin (flavoRb) (b2710) with a NO-binding non-heme diiron center) and *ygbD* (encoding a NADH:(flavo)Rb oxidoreductase (b2711)), which function in NO reduction and detoxification. Mutation of either *ygaA* or *ygaK* eliminated inducible anaerobic NO metabolism, whereas *ygbD* disruption partly impaired the activity. NO-sensitive [4Fe-4S] (de)hydratases, including the Krebs cycle aconitase and the Entner-Doudoroff pathway 6-phosphogluconate dehydrogenase, were more susceptible to inactivation in *ygaK* or *ygaA* mutants than in the parental strain, and these metabolic poisonings were associated with conditional growth inhibitions. flavoRb (NO reductase) and flavohemoglobin (NO dioxygenase) maximally metabolized and detoxified NO in anaerobic and aerobic *E. coli*, respectively, whereas both enzymes scavenged NO under microaerobic conditions. We suggest designation of the *ygaA-ygaK-ygbD* gene cluster as the norRVW modulin for NO reduction and detoxification.

**Nitric oxide** (NO) is present throughout the biosphere (1–3). In humans, tightly regulated NO synthases produce sufficient NO to poison pathogens, opportunistic organisms, and neoplastic tissue (4, 5). Nanomolar NO potently inhibits terminal oxidases and aerobic respiration (6, 7) and alters the amphibolic and regulatory reactions of the citric acid cycle enzyme aconitase by destroying its labile [4Fe-4S] center (7–10). In addition, significant secondary toxicity of NO can occur via reactions of NO₂, ONOO⁻, NO⁻, dinitrosyl iron, and nitrosothiols (11–14).

It has become increasingly evident that organisms metabolize and detoxify NO. Enzymes decompose NO in microorganisms (1, 3, 15–18) and humans (7) and prevent the accumulation of toxic NO levels. Nitric-oxide reductases (NORs)¹ metabolize NO to N₂O in anaerobic denitrifying bacteria and fungi and likely serve an additional role in minimizing NO toxicity (1, 3, 19). Nitric-oxygen dioxygenases (NODs) convert NO to NO₃⁻ in organisms as diverse as bacteria and mammals and have been shown to protect aerobic cells from NO damage (7, 20–26). In microorganisms, (flavo)hemoglobins catalyze NO dioxygenation (20–24, 27, 28).

In the accompanying article (17), we provide evidence for an inducible and robust NO-metabolizing and -detoxifying activity in anaerobic *Escherichia coli*. Attempts to biochemically identify the NO reduction system have been complicated by its instability. Moreover, the *E. coli* genome lacks a NOR belonging to either the cytochrome bc complex or cytochrome P450 families (1). The list of proteins displaying a reductase activity for NO in *vitro* with potential for function in *E. coli* is long and includes flavohemoglobin (flavoHb) (27, 28), cytochrome *c* or *c’* (29), multi-heme nitrite reductase (2, 30), copper-nitrite reductase (31), bacterioferritin (32), ribonucleotide reductase (33), Cu, Zn-superoxide dismutase (34), and terminal respiratory oxidases (35). However, none of these candidate systems, including NO-induced flavoHb (17), have demonstrated NO reduction function in cells. Moreover, unlike the inducible NOR in *E. coli* (17), none of these “NOR reductases” are sensitive to inactivation by O₂.

Genomic data and bioinformatics tools (NCBI Protein Database and BLAST) provided a strategy for identifying the *E. coli* system. *E. coli* *ygaA* encodes a protein bearing ~42% identity to the NO-modulated *Ralstonia eutrophus* transcription regulators NorR1 and NorR2 (36). Intriguingly, NorR homologs are also located adjacent to the flavoHb gene (*hmp*) in both *Vibrio cholera* (37) and *Pseudomonas aeruginosa* (38), suggesting a common control for NO detoxification systems in various organisms. Adjacent to *ygaA* and transcribed in the opposite direction with specific promoters are the genes *ygaK* encoding a flavorubredoxin (flavoRb) with a NO-binding diiron center and *ygbD* encoding a flavoRb reductase (39, 40) with potential for a NOR function (see Fig. 1).

We demonstrate here the role of the *E. coli* NorR homolog *YgaA* in controlling NOR expression. We also elucidate the role of flavoRb (*YgaK*) and its reductase partner (*YgbD*) in the NO-induced anaerobic NOR activity in *E. coli*. We further demonstrate that NOR and NOD protect NO-sensitive [4Fe-4S]-containing (de)hydratases in critical anabolic and catabolic pathways and thus explain conditional growth defects observed with NO poisoning in *E. coli*. A mechanism for flavoRb-catalyzed NO reduction is envisioned. We suggest renaming the *ygaA-ygaK-ygbD* gene cluster as norRVW and annotating similar genes for a possible NO detoxification function.

**MATERIALS AND METHODS**

*Reagents—DNA restriction and modifying enzymes were obtained from New England Biolabs Inc. Porcine heart isocitrate dehydrogenase, *Aspergillus niger* glucose oxidase, lactate dehydrogenase, casamino acids, HEPES, citrate, 6-phosphogluconate, NAD⁺, lactate, tetrazolium red, and antibiotics were obtained from Sigma. NADH and bovine liver catalase (260,000 units/ml) were purchased from Roche Molecular Bio-

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¹ The abbreviations used are: NORs, nitric-oxide reductases; NODs, nitric-oxygen dioxygenases; flavoHb, flavohemoglobin; flavoRb, flavorubredoxin; ROO, rubredoxin; O₂ oxidoreductase.
Flavobredoxin Detoxifies NO in E. coli

chemicals. Yeast extract, Bacto-agar, and Bacto-Tryptone were purchased from Fisher. Mixtures of 1200 ppm NO balanced with ultrapure N₂, 1.05% O₂ balanced with N₂, and 99.998% N₂ and 99.999% O₂ were from Praxair (Bethlehem, PA). NO (98.5%) and CO (99.999%) were obtained from Aldrich. CO (1 mM) and NO (2 mM) stock solutions were prepared as previously described (22).

**Bacterial Strains, Plasmid, and Plasmid—**E. coli strains AB1157 (F<sup>-</sup> thr-1 ara-C14 leuB6 DEG[prt-proA62]lacY1 tex-33 galR-0 glnV44 galK2 Rac-0 hisG4 rfbD1 mgl-51 spoRS96 rpsL31(strR) kgdK51 xyl-A5 mtl-1 argE3 thi-1) and JTG10 (AB1157gbA::Tn10 kan<sup>®</sup>) were obtained from Dr. Bruce Demple (Harvard University). AG103 (AB1157::Tn5 kan<sup>®</sup>) was prepared as previously described (17). Strain AG501 was created by transducing hnp::Tn5 from AG103 to AG300. Strain JC7623 (F<sup>-</sup> thr-1 ara-C14 leuB6 DEG[prt-proA62] lacY1 sbcC201 tex-33 galR-0 glnV44 galK2 Rac-0 sbcB15 hisG4 rfbD1 spoRS96 recB22 recC22 rpsL31(strR) kgdK51 xyl-A5 mtl-1 argE3 thi-1) was obtained from the E. coli Genetic Stock Center of Yale University. QC719 and QC720 were obtained from Dr. Daniëlle Touati (CNRS, Paris, France) (41). Primers were purchased from Dr. Danie`lle Touati, was randomly transposed to pAlter9G10 by the method of Castillo et al. (43) as modified by Carlho and Touati (41).

Briefly, strain QC720 carrying a Mu prophage and MudIIPR13 was transformed at 30°C with pAlter9G10. The transposition, phage growth, and cell lysis were induced at 44°C in LB medium prepared with 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl prepared in 1 liter of deionized water and supplemented with 20 mM glucose. Phage lysates were used to transduce strain QC719 to ampicillin- and chloramphenicol-resistant colonies and the corresponding open reading frames (b2709, b2710, and b2711, respectively) were chosen from 9610 into pAlter (Promega Corp., Madison, WI) that was modified to express ampicillin resistance.

**Insertion of Mu Transposons in the ygaA, ygaK, and ygbD Genes—**Disruptions in the ygaA, ygaK, and ygbD genes were created by Mu transposon insertion. MudIIPR13 (cam<sup>®</sup>), a generous gift of Dr. Bruce Demple (Harvard University), was transformed at 30°C with pAlter9G10. The transposition, phage growth, and cell lysis were induced at 44°C in LB medium prepared with 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl prepared in 1 liter of deionized water and supplemented with 20 mM glucose. Phage lysates were used to transduce strain QC719 to ampicillin- and chloramphenicol-resistant colonies and the corresponding open reading frames (b2709, b2710, and b2711, respectively) were chosen from 9610 into pAlter (Promega Corp., Madison, WI) that was modified to express ampicillin resistance.

**Construction of ygaA, ygaK, and ygbD Chromosomal Mutations—**Plasmids were linearized with XbaI, and the DNA was used to transform strain JC7623 to chloramphenicol resistance (45). Several chromosomal-resistant, ampicillin-sensitive colonies were selected for analysis. Site-specific insertions in the ygaA, ygaK, and ygbD genes were confirmed by linkage to gshA using strain JTG10 as the recipient in P1 transduction analysis. Mutations were subsequently transduced to strain AB1157.

**Bacterial Growth and Exposures—**Cultures were grown in phosphate-buffered LB medium (8) unless otherwise indicated. Minimal salts medium (ph 7.0) contained 60 mM K<sub>2</sub>HPO<sub>4</sub>, 33 mM KH<sub>2</sub>PO<sub>4</sub>, 7.6 mM (NH<sub>4</sub>)<sub>2</sub>S<sub>4</sub>, 1.7 mM sodium citrate, 1 mM MgSO<sub>4</sub>, 10 μM MnCl<sub>2</sub>, and 10 μg/ml thiamin HCl and was supplemented with either 20 mM glucose or 2% potassium gluconate. Minimal medium was supplemented with 40 μg/ml 1-arginine, 1-histidine, 1-leucine, 1-proline, and 1-threonine or with 0.25% casamino acids as indicated. Overnight 5-ml aerobic cultures were grown at 37°C in 15-ml culture tubes with vigorous shaking. Overnight 10-ml anaerobic cultures were grown at 37°C in 150-ml Erlenmeyer flasks. For anaerobic cultures, cultures were grown at 37°C in rubber stopper-sealed 50-ml Erlenmeyer flasks continuously flushed with gas mixtures at 30 ml/min at a culture/flask volume ratio of at most 1:5 with vigorous shaking at 275 rpm. To minimize the disturbance of gases, culture aliquots were removed from flasks using a 1-ml tuberculin syringe connected via narrow tubing to the culture medium. Cell density was determined from culture absorbance at 550 nm by and plotting and counting. An absorbance of 1.0 was taken to equal 3 × 10<sup>8</sup> and 7 × 10<sup>7</sup> bacteria/ml for cells grown in phosphate-buffered LB medium and minimal salts medium, respectively.

**NO Consumption Assays—**Aerobic and anaerobic NO consumption activities were measured amperometrically using a 2-mm ISO-NOP NO electrode (World Precision Instruments, Sarasota, FL) as previously described (16, 17). Aerobic and anaerobic activities were measured at 37°C and at 1.0 and 1.5 μM NO, respectively, unless otherwise indicated.

Acetate, 6-Phosphogluconate Dehydrogenase, and Protein Assays—Cells were harvested; extracts were prepared; and acetate, 6-phosphogluconate dehydrogenase, and protein were assayed as previously described (16, 46–48).

**Statistical Analysis—**Significance of differences between data (p < 0.05) were determined using Student's t test.

**RESULTS**

Inducible Anaerobic NO Consumption Activity Is Dependent upon ygaA, ygaK, and ygbD—To test the individual roles of the ygaA, ygaK, and ygbD gene products in the anaerobic NO consumption activity, we constructed strains carrying insertion mutations using random Mudlac transposition (Fig. 1). The anaerobic and aerobic NO consumption activities of strains AG200, AG300, and AG400, with Mudlac insertions in ygaA, ygaK, and ygbD, respectively, were measured and compared with those of the parental strain, AB1157. Strains bearing mutations in ygaA or ygaK showed no anaerobic NO consumption activity following exposure to 960 ppm NO under anaerobic growth conditions, whereas the ygbD mutant produced a rate ~40% lower than that of its parental strain (Fig. 2A). None of the strains showed significant anaerobic NO consumption activity in the absence of NO exposure. We also measured the aerobic NO consumption activity of anaerobically induced cells. Each strain showed the normal basal level of aerobic NO consumption activity (Fig. 2B, compare white bars), and this activity was induced to high levels by NO (black bars). The absence of ygaA, ygaK, or ygbD resulted in small increases in the induction of the aerobic NO consumption activity (compare black bars). This aerobic NO consumption activity is fully attributable to flavoHb (20).

These results demonstrate that YgaA (NorR) and YgaK (NorV, flavoRb) are essential for anaerobic NO consumption. Thus, YgaA and YgaK constitute a novel modulin for NO reduction and detoxification in E. coli; with YgbD (NorW, flavoRb reductase) acting as an accessory for NO reduction. YgaA, YgaK, and YgbD may decrease aerobic NO consumption activities by decreasing steady-state NO levels and flavoHb/NOD expression in anaerobic cells.

**Dependence of the Anaerobic NO Consumption Activity upon NO—**We examined the efficacy of the anaerobic NO consumption activity for NO scavenging. NOR showed an apparent K<sub>M</sub> value of 400 nM (Fig. 3, ○) and was CO-resistant (□) and cyanide-resistant (●). The K<sub>M</sub> value is identical to the values estimated for the flavoHb-type NOD in E. coli (21) and for the cytochrome bc-type NOR in Pseudomonas perfectomarina (49). The results demonstrate the efficiency of the anaerobic system for NO scavenging. The results also indicate low
Affinities of the NO scavenger flavoRb (YgaK) for CO and cyanide.

Role of the ygaA, ygaK, and ygbD Gene Products in Protecting the NO-sensitive Aconitase—To define the NO detoxification function of ygaA, ygaK, and ygbD in cells, it is necessary to understand the protective role(s) of the system for critical target(s) of NO poisoning under physiological conditions. Thus, we tested the role of ygaA, ygaK, and ygbD in the inducible anaerobic protection of the NO-sensitive Krebs cycle enzyme aconitase (16, 17). E. coli (AB1157) exposed to 480 ppm NO gas (<1 μM) lost ~45% of the aconitase activity after 30 min (Fig. 4). Aconitase inactivation increased significantly (p < 0.05) in the presence of chloramphenicol, thus demonstrating the protective role for newly synthesized protein(s). Moreover, loss of aconitase activity in the ygaA (AG200) and ygaK (AG300) mutants was greater than in the parental strain, thus demonstrating critical roles for ygaA and ygaK in the adaptive protection.

In contrast, ygbD (AG400) was not essential for aconitase protection under these conditions.

role of the ygaA, ygaK, and ygbD Gene Products in Protecting E. coli from NO-mediated Growth Inhibition—To further define the functional importance of ygaA, ygaK, and ygbD for E. coli, we investigated the effects of specific insertion mutations in each of these genes on the anaerobic growth of E. coli exposed to a NO stress. Surprisingly, there was little growth inhibition with 240 ppm NO gas (≤0.5 μM) for cells growing on a rich phosphate-buffered LB medium (Fig. 5A). Higher NO exposure levels caused comparable growth inhibition of the mutants and the parental strain (data not shown). These results suggest a limited role for ygaA, ygaK, and ygbD and anaerobic NO metabolism in growth protection. Nevertheless, the exquisite sensitivity of aconitase to NO-mediated inactivation (Fig. 4) (8, 16) strongly suggested that anaerobic growth protection may be better observed under conditions requiring aconitase function, the citric acid cycle, or other NO-sensitive metabolic pathways. Aconitase expression is relatively low under these conditions; and moreover, aconitase function is not expected to be limiting for E. coli growth with glucose supplied as the substrate for energy production (50).

The effect of NO on anaerobic growth of E. coli mutants was investigated under growth conditions demanding the function of putative NO-sensitive enzymes. The [4Fe-4S]-containing 6-phosphogluconate dehydrogenase of the gluconate-metabolizing Entner-Doudoroff pathway and the [4Fe-4S]-containing α,β-dihydroxyacid dehydratase of the branched-chain amino acid biosynthesis pathway are two enzymes that are predicted to be NO-sensitive (8, 51). Anaerobic growth of strains AG200 and AG300 was significantly impaired by exposure to 240 ppm NO gas under growth conditions requiring gluconate metabolism (Fig. 5B) or amino acid biosynthesis (Fig. 5C). These results...
Flavorubredoxin Detoxifies NO in E. coli

The anaerobic NO consumption activity of flavoRb (YgaK) and flavoHb (HMP) is O₂-sensitive, decaying with a half-life of ~5 min in air (17), suggesting that flavoRb may function poorly, if at all, in the presence of O₂. On the other hand, the aerobic NOD activity of flavoHb shows a rather high Km value for O₂ (60–100 μM) and is potently inhibited by NO under hypoxia (21, 22), suggesting that flavoHb would be a poor catalyst for NO detoxification in E. coli cells under anaerobic conditions. In contrast, flavoRb (YgaK) and NO reduction afforded to 6-phosphogluconate dehydratase by flavoRb (YgaK) and flavoHb (HMP) are each required for anaerobic growth protection by flavoRb (YgaK) and NO reduction afforded to 6-phosphogluconate dehydratase (Fig. 6, compare white and black bars). The results establish the sensitivity of this [4Fe-4S] enzyme to NO and further demonstrate the protection NO metabolism affords against metabolic NO poisoning.

Both flavoRb and flavoHb Detoxify NO under Microaerobic Growth Conditions—The anaerobic NO consumption activity in E. coli is O₂-sensitive, decaying with a half-life of ~5 min in air (17), suggesting that flavoRb may function poorly, if at all, in the presence of O₂. On the other hand, the aerobic NOD activity of flavoHb shows a rather high Km value for O₂ (60–100 μM) and is potently inhibited by NO under hypoxia (21, 22), suggesting that flavoHb would be a poor catalyst for NO detoxification under anaerobic conditions. In contrast, flavoRb (YgaK) and NO reduction afforded to 6-phosphogluconate dehydratase (Fig. 6, compare white and black bars). The results establish the sensitivity of this [4Fe-4S] enzyme to NO and further demonstrate the protection NO metabolism affords against metabolic NO poisoning.

During our investigations of NO toxicity and defenses in E. coli, we have observed a novel O₂-sensitive and NO-inducible anaerobic pathway for reductive NO metabolism and detoxification (17). We have now demonstrated that the E. coli NorR homolog (b2709) encoded by ygaA (36) and the adjacent gene ygaK (encoding a flavoRb (b2710)) (Fig. 1) are each required for expression of the efficient and inducible anaerobic NO metabolic activity (Figs. 2 and 3), the protection of NO-sensitive [4Fe-4S]-containing (de)hydratases (Figs. 4 and 6), and the resistance of E. coli to NO-mediated growth inhibition under various metabolic conditions (Fig. 5). Proximal ygbD (encoding
a NADH:(flavo)Rb oxidoreductase (b2711) appears to play an ancillary role in anaerobic NO metabolism and detoxification, suggesting the existence of other flavoRb reductases. We propose the designation of the *E. coli* ygaA-ygaK-ygbD gene cluster (Fig. 1) as the gicr oxide reduction modulon norR/VW. Similar modulons are also found in the *Salmonella typhimurium* and *Klebsiella pneumoniae* genomes.

We can infer in part from the work of others (36, 54) that NorR (YgaA) acts as 1) the receiver of phosphate from a separate unidentifed NO-sensing histidine-aspartate phosphotransferase (kinase) and 2) the regulator of ygaK-ygbD (norR/VW) transcription in a two-component sensor-receiver regulatory system. Future investigations will be aimed at understanding these regulatory systems.

*E. coli* flavoRb (NorV) belongs to a superfamily of proteins encoded in the genomes of anaerobic archaea and facultative euubacteria, including *Methanococcus*, *Desulfovibrio*, *Pyrococcus*, *Dehalococcus*, *Trepomonas*, *Clostridium*, *Salmonella*, *Klebsiella*, and the photosynthetic cyanobacterium *Synechocystis* (39, 55). *E. coli* flavoRb shares 34% amino acid identity with the *Desulfovibrio gigas* rubredoxin:O2 oxidoreductase (ROO) (55) and shares key amino acids in the diiron center of ROO (Fig. 8). ROO differs most notably from flavoRb in that the rubredoxin domain exists as a separate protein (55). Non-heme iron and FMN stoichiometries and electromagnetic properties of *E. coli* flavoRb support the presence of a diiron center, a tightly bound FMN, and mononuclear iron in the rubredoxin domain (40). ROO has been proposed to function in O2 reduction and detoxification or oxidative stress protection in anaerobes (56, 57). However, a role for ROO and flavoRb in anaerobic NO reduction and detoxification now appears more likely because these organisms often lack an identifiable NOR (1), but clearly express O2-reducing respiratory chains (58).

Given the homologous structure of ROO (55) and the redox and NO-binding properties of *E. coli* flavoRb (40), we envision a catalytic NO reduction mechanism in which two NO molecules bind to the diferrous (Fe2+–O–Fe2+) center (Fig. 8), and each is univalently reduced to form two nitroxyl anions and a diferrous center. Two nitroxyl molecules would then combine to form N2O and water, as suggested for the cytochrome *bc*-type NOR (1, 59). For turnover, NADH-dependent flavoRb reductase (NorW) or other reductases (Fig. 2) would supply two electrons to the diferrous center via the rubredoxin domain and the proximal FMN in flavoRb. Alternatively, we can suppose a mechanism in which the diferrous center binds NO and reduces NO by two electrons to produce an Fe3+–O–Fe3+–NO2− (H+) intermediate that reacts with the second NO molecule to form N2O. An analogous mechanism has been suggested for cytochrome P450nor (60). The cyanide and CO resistance and high NO affinity of the activity (Fig. 3) may be explained by this novel non-heme iron mechanism for NO reduction.

Reducive activation of O2 to peroxo (Fe3+–O–OH) or oxenoid (Fe3+–O) intermediates by non-heme diiron centers such as those in deoxynemethyl, methane monoxygenase, and steroyl-(acyl carrier protein) Δ9-desaturase is well documented (61, 62). The formation of these reactive O2 intermediates may account for the rapid and irreversible O2-mediated inactivation of the flavoRb-type NOR activity in *E. coli* (17). O2 sensitivity may also explain the low (∼0.2 s−1) and progressively diminishing *in vitro* O2 reductase activity reported for flavoRb (40).

Future studies will be aimed at understanding the mechanism of NO reduction by flavoRb and the mechanism of O2 inactivation. It is important to point out that reaction intermediates and mechanisms of the two-heme cytochrome *bc*-type NOR and cytochrome P450nor remain to be fully elucidated.

Labile [4Fe-4S] (de)hydratases have explained the conditional toxicity of superoxide to various organisms (41, 47, 48, 51), and it is becoming increasingly apparent that these enzymes may also illuminate important mechanisms of NO toxicity and the physiological roles of NODs, NORs, and other NO defenses in various models (7, 16, 17). Our investigations demonstrate that anaerobic NO toxicity is due, at least in part, to the inactivation of [4Fe-4S]-containing (de)hydratases, including aconitase and 6-phosphogluconate dehydrogenase, and that inducible anaerobic NO metabolism via flavoRb is important for their protection (Figs. 4–7). NO sensitivity of the branched-chain amino acid pathway α,β-dihydroxyacid dehydrogenase may contribute to growth defects observed in minimal medium (Fig. 5C), and aconitase inactivation and poisoning of the amphotropic reactions of the citric acid cycle are expected to have complex and pleiotropic effects on cells.

NORs and NODs are likely to be important for the resistance of microbes to the immune system (3–5) and the NO present in various niches (1, 3). It is hoped that a greater understanding of the complex roles of NOs and NORs and their regulators in NO detoxification and microbial pathogenesis may lead to the development of novel therapies. Thus, inhibitors of NORs, NODs, or their regulators may be useful for enhancing the antibiotic action of NO on pathogens or tumor cells (7). Furthermore, greater knowledge of NO metabolism may be particularly important for understanding the pathogenesis and persistence of certain organisms. For example, we have found an internal 68-amino acid deletion in the flavoRb-coding sequence (norV) encompassing the critical FMN-binding flavodoxin domain in the genomes of enterohemorrhagic *E. coli* O157:H7 isolates from the Michigan (63) and Sakai (64) outbreaks. The norV mutation located among wild-type norR and norW genes suggests an important, yet puzzling role for NO in the pathogenesis and virulence of O157:H7. It seems unlikely that the deletion increases flavoRb function. Rather, the truncated flavoRb may, in combination with the NO
produced by intestinal epithelial cells and other immune cells, increase the synthesis of the bacterial toxins that make O157:H7 so devastating.

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