Nitric oxide-sensing mechanisms in
Escherichia coli

S. Spiro
School of Biology, Georgia Institute of Technology, 310 Ferst Drive, Atlanta, GA 30332-0230, U.S.A.

Abstract
Exposure of Escherichia coli to nitric oxide (NO) or nitrosating agents causes significant changes in patterns of gene expression. Three recent studies have used microarrays to analyse the response of the E. coli transcriptome to NO and nitrosative stress. Drawing on the array data, I review our current understanding of the E. coli regulatory systems that are involved.

Introduction
Nitric oxide (NO) is a water-soluble free radical gas which is potentially toxic in biological systems because of its ability to react with a variety of cellular targets, especially thiol groups and transition metal centres in proteins. NO reacts with superoxide to form the potently toxic peroxynitrite, a chemistry that is exploited by eukaryotic phagocytes in efforts to kill invading pathogens [1]. Bacteria may also be exposed to endogenously generated NO, especially if they are able to use nitrite as an electron acceptor for anaerobic respiration (Scheme 1). The enteric bacteria such as Escherichia coli reduce nitrite to ammonia under anaerobic growth conditions, but traces of NO are apparently formed as a by-product of this metabolism [2]. Thus the responses of bacteria to NO have attracted considerable interest in recent years. In the present paper, I briefly review our understanding of the regulatory systems in E. coli that respond to NO, drawing especially on results from recent microarray studies. In considering the data obtained from microarray (and other) studies, the following points should be borne in mind. In addition to aqueous NO, investigators have also used a range of compounds to mimic NO effects and/or to induce ‘nitrosative stress’. These compounds typically release NO in solution (as in the case of the NONOates, for example, and acidified nitrite) or they release NO+ as an electron acceptor for anaerobic respiration. Each study uniquely identified numerous genes not found in the other two (here I focus on genes up-regulated by NO). Six genes (ytfE, soxS, nrdI, sufA and ilvC) were identified in two studies [4,5], but not in the third [6]. Note that nrdI and nrdH are almost certainly co-transcribed, so the identification of nrdH but not nrdI in a microarray study [6] may reflect a bias in the data towards promoter-proximal genes. Differences in experimental design are likely to account for the different outcomes of the array analyses (and, of course, the reporting of a gene as up-regulated relies on an arbitrary choice of a cut-off value for the induction ratio). For example, as mentioned above, GSNO has a complex mode of action that may involve both NO and NO+. Furthermore, GSNO can be taken up through a dipeptide permease and can act intracellularly by heterolytic transfer of NO+ [7]. It is likely that the permease is expressed at a higher level during growth in tryptone-based rich media, which may, in part, account for the smaller effect of GSNO on the transcriptome of cells grown in defined medium, as compared with rich medium [4,6]. Indeed, we have found that GSNO has a more potent effect on a reporter fusion to an NO-inducible promoter in cells grown in rich medium than in cells grown in a defined medium.

Below, I describe the properties of some of the regulatory systems that have been implicated in the response of E. coli to NO and/or nitrosating agents. Key issues are the mechanisms of action of the regulatory proteins, and the roles (if any) of the genes they regulate in the response to NO.

Ferric uptake regulator
The ferric uptake regulator, Fur, can be inactivated in vitro by nitrosylation of the protein-bound iron, and exposure to

Key words: Escherichia coli, gene regulation, microarray, nitric oxide, NO, regulatory system.
Abbreviations used: FNR, fumarate and nitrate reductase regulatory protein; GSNO, S-nitrosoglutathione.
*email stephen.spiro@biology.gatech.edu

©2006 Biochemical Society
NO can cause de-repression of Fur-repressed genes in vivo [8]. Comparison of the first two microarray studies suggested that GSNO effects on the Fur regulon are only significant in cells grown in rich medium [4,6], and it was argued that this is because rich medium is (paradoxically) partially limited for iron, such that Fur can more easily be inactivated by NO under these conditions [6]. However, in a later study, effects of aqueous NO on the Fur regulon were evident in cells grown anaerobically in a minimal medium [5]. The suf operon is regulated by Fur, and its products are required for the biogenesis of [Fe-S] clusters, especially under conditions of oxidative stress [9]. Enzymes containing [Fe-S] clusters can be damaged by NO [10]; thus at least one representative of the Fur regulon has a function that may be rationalized in terms of a physiological response to NO.

**SoxRS**

The SoxS and SoxR proteins principally mediate a response to superoxide. SoxR contains a [2Fe-2S] cluster and is activated by oxidation of the cluster by superoxide. The oxidized form of SoxR activates transcription of the soxS gene and the SoxS protein then goes on to activate transcription of genes in the Sox regulon. Besides superoxide, NO can also activate SoxR, by formation of a dinitrosyl–iron–dithiol adduct [11]. Two array datasets identified the soxS gene as being inducible by NO and nitrosative stress [4,5], but SoxS-regulated genes were noticeably absent – just two (fpr and sodA) were found in one study [4], and none in the other. It is not clear why NO can activate expression of soxS but not SoxS-regulated genes in the array experiments. Activation of the Sox regulon might be expected, given that there is some evidence to suggest that the regulon has a role in protecting cells against NO [12].

**OxyR**

The mechanism of OxyR has recently been the subject of considerable controversy, and has been discussed in several reviews (e.g. [13]). For the purpose of this brief discussion, the pertinent points are as follows. OxyR mediates a response to peroxide stress, by a mechanism that involves formation of a disulphide bond [14] or a sulphinic acid [15]. At least some OxyR-regulated promoters can be activated in vivo by exposure to the nitrosating agent S-nitroso cysteine [16], and S-nitrosylation of a cysteine residue activates OxyR in vitro [15]. The implication is that OxyR plays an important role in the physiological response to nitrosative stress, but in all three array datasets, OxyR regulon members do not appear to be significantly induced [4–6]. In one study [4], the OxyR-regulated genes sufA and fnbF were induced, but, since both are also regulated by Fur, the regulator responsible for induction could not be identified with certainty. The OxyR-regulated grxA gene also responded to nitrosative stress in an OxyR-dependent fashion [4]. One possible interpretation of these results is that nitrosative stress produces a different regulatory output from OxyR than that caused by oxidative stress [15].

**FNR (fumarate and nitrate reductase regulatory protein)**

FNR is a transcriptional regulator, which contains an oxygen-sensitive [Fe-S] cluster and regulates the expression of genes whose products are involved in anaerobic respiration and carbon metabolism. The hmpA gene encoding flavohaemoglobin is repressed by FNR under anaerobic conditions. NO reacts with FNR to form a dinitrosyl–iron–cysteine complex, and this modification leads to de-repression of the hmpA promoter in vivo [17]. The prediction that follows from this result is that expression of other members of the FNR regulon should be sensitive to NO. There is some support for this prediction from one microarray study [5]. The respiratory nitrite reductase Nrf can reduce NO to ammonia and so protect against NO toxicity [18]. The genes encoding Nrf are positively regulated by FNR. If FNR is inactivated by NO, then down-regulation of Nrf synthesis would result, which is inconsistent with a role in mediating NO resistance. However, the array data contain no evidence for a significant role for NO in controlling (either positively or negatively) expression of the genes encoding Nrf.

**MetR**

The hmpA promoter is also regulated by the MetR repressor, in complex with homocysteine. A mechanism for up-regulation of the hmpA promoter by NO has been proposed, in which NO reacts with homocysteine to form S-nitrosohomocysteine, leading to de-repression [19]. Other members of the MetR regulon are induced by GSNO in continuous cultures grown in defined medium, suggesting that this mechanism operates at many or all MetR-regulated promoters, and methionine auxotrophs are sensitive to
nitrosative stress [6]. On the other hand, this regulatory mechanism is not evident in other microarray datasets [4,5].

**NorR**

NorR is an NO-responsive transcriptional activator that regulates expression of the norVW genes encoding flavo-rubredoxin and an associated flavoprotein, which reduce NO to nitrous oxide [20,21]. Microarray data suggest that there may be additional targets for NorR regulation [4]. In contrast with the other regulatory proteins discussed here, NorR apparently serves exclusively to respond to NO. We have recently found that NorR contains a mononuclear non-haem iron and is activated by formation of a mononitrosyl complex [22].

**Conclusions**

*E. coli* has multiple regulatory systems that respond to NO and nitrosative stress, and there is some evidence to indicate that more NO-responsive regulators remain to be discovered [4]. It is clear that mechanisms based on reactive cysteine residues and iron centres predominate in the currently understood regulatory systems. Microarray experiments have provided valuable insights into the global response of *E. coli* to NO and nitrosative stress, but have also produced some conflicting results. A key challenge for the future will be to elucidate the relative physiological importance of each of the regulons that responds to NO.

**Note added in proof**

Recently, we have identified the product of the *E. coli* yje B gene as a repressor of the transcription of hmpA, yifE and ygbA, all of which are up-regulated by NO [23]. These regulatory interactions are confirmed by an analysis using comparative genomics, which suggests further that YjeB (new renamed NsrR) is also a repressor of the hcp-bcr operon encoding the hybrid cluster protein and its reductase [24].

Work in my laboratory is supported by the National Science Foundation (grant MCB-0517174). I am grateful to Ray Dixon (John Innes Centre, Norwich, U.K.) for helpful discussions and comments on this paper.

**References**


Received 8 September 2005