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FORUM REVIEW ARTICLE

Redox Eustress: Roles for Redox-Active Metabolites in Bacterial Signaling and Behavior

Chinweike Okegbe,[1,*] Hassan Sakhtah,[1,*] Matthew D. Sekedat,[1,*] Alexa Price-Whelan,[2] and Lars E.P. Dietrich[1]

Abstract

Significance: Plant biologists and microbiologists have long discussed and debated the physiological roles of so-called "redox-active metabolites." These are natural products with unusually high redox activity that are not directly required for active growth. Generally, the biological roles of these compounds have been ascribed to interspecies competition and virulence, and they have been considered important sources of distress. ***Recent Advances:*** In this review, we discuss two examples of redox-active metabolites: nitric oxide and phenazines. Both are known for their toxic effects in some organisms and conditions but have recently been shown to provide benefits for some organisms under other conditions. ***Critical Issues:*** Biologists are identifying new roles for redox-active metabolites that are not directly related to their toxicity. These roles prompt us to suggest a dismissal of the paradigm that all biological stress is negative (*i.e.*, distress). ***Future Directions:*** A more accurate view of redox couples requires characterization of their specific biological effects in a condition-dependent manner. The responses to these compounds can be termed "distress" or "eustress," depending on whether they inhibit survival, provide protection from a compound that would otherwise inhibit survival, or promote survival. *Antioxid. Redox Signal.* 16, 658–667.

Introduction

Redox chemistry is a fundamental feature of every known metabolism that supports life. Strong reductants and oxidants are thought to have enabled the origin of life and the evolution of complex life forms, respectively (Fig. 1). While the energy provided by redox chemistry is required for life, unchecked redox activity is a common source of intracellular damage, and organisms possess well-studied mechanisms that allow them to avoid or combat potentially destructive redox processes. However, biologists are uncovering cases in which organisms utilize unusually redox-active compounds for functions that are not directly part of metabolism during active growth. They are finding that compounds often viewed as toxins due to their high level of redox activity in some cells play roles in communication and/or survival in others, and that their effects may vary qualitatively as a function of concentration. These findings emphasize the context- and organism-dependent effects of compounds that participate in redox chemistry *in vivo*. In this review, we will discuss sensing mechanisms that allow bacteria to respond to the redox states of specific molecules with redox potentials that span a broad range (Fig. 1). We will also provide examples of compounds historically known solely for their toxic effects that have recently been shown to play neutral or beneficial roles in survival and community behavior.

Traditionally, certain redox-active compounds have been categorized as "stressors" because they stimulate protective responses, without which organisms of interest would not be able to survive in the molecule's presence. The term "stressor" originated with the work of Hans Selye, a physiologist who, in the 1930s, devised the concept of the "stress response" while examining recovery from harsh treatments in rats (36). This work established a paradigm increasingly applied by biologists describing responses to environmental stimuli. Although Selye recognized that the response to a stressor can be positive ("eustress") or negative ("distress"), this dual nature of stress is often overlooked (37). The word "stress" typically has a negative connotation, and agents that induce pathology in a particular biological context are often treated categorically as negative stressors. However, mechanisms that have evolved to respond to these signals do not always bear the hallmarks of distress. In these cases, the organism is not negatively affected by exposure to the stressor.

Stress has been defined as a "threat to homeostasis" (31). Originally applied in the context of animal physiology and psychology, this definition can also describe responses to

[1]Department of Biological Sciences, Columbia University, New York, New York.
[2]Mount Sinai School of Medicine, Department of Pharmacology and Systems Therapeutics, New York, New York.
*These authors contributed equally to this work.

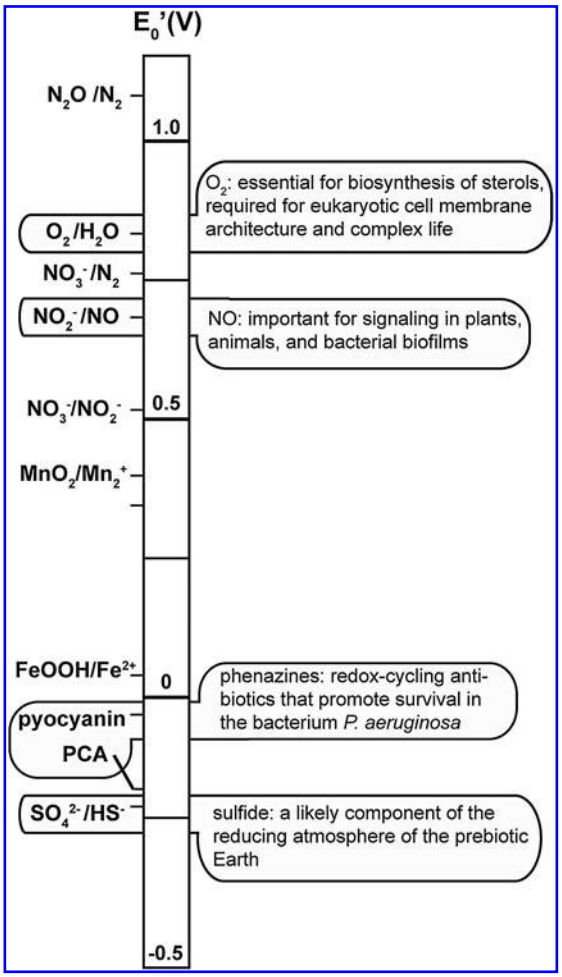

FIG. 1. Redox chemistry is essential for life, but threatening for life in some contexts. Selected redox couples with major roles in biological chemistry are shown organized according to their redox potentials E'_0 at pH 7 (28,33,42). Major events and responses associated with these couples are listed in the figure or described in the main text. FeOOH, ferric oxide-hydroxide; PCA, phenazine-1-carboxylic acid.

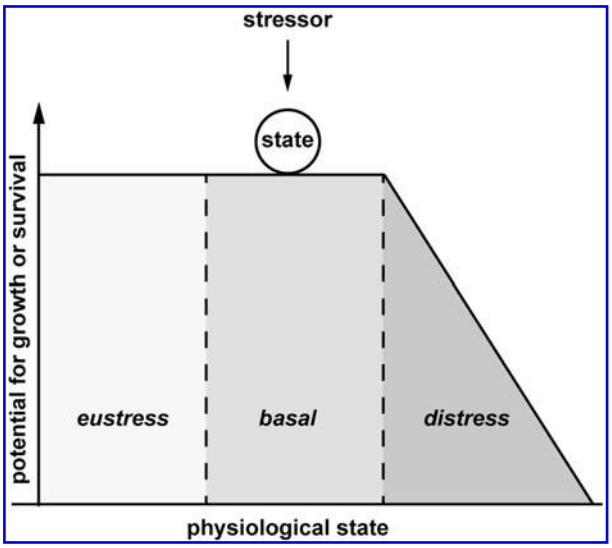

FIG. 2. The influence of stressors on physiology. The normal functional state of a biological system can be likened to a ball on a plateau. Endogenous and exogenous stressors can push the ball to the edge of the precipice, but robust biological systems actively maintain the functional state. This sometimes requires the organism to transition to a new, qualitatively different functional state, as can occur when the stressor is a source of eustress.

stimuli in bacteria. Figure 2 depicts an abstract representation of this concept. An organism maintaining homeostasis, with respect to parameters such as metabolic flux and internal pH, is depicted as a ball sitting on a plateau. When the organism is exposed to a stressor that causes distress, it can no longer maintain a functional physiological state, and this is represented as the ball rolling down the slope. However, when the organism is exposed to a stressor that causes eustress, it enters a qualitatively different physiological state, but still maintains homeostasis. This is represented as a different area atop the same hill.

This review contains examples of specific sensing mechanisms and stimuli involved in eustress affecting bacterial survival and development. These examples concern molecules that are typically referred to as "redox-active metabolites." This is a misleading term because most compounds produced during metabolism are technically "redox-active" in the sense that they readily undergo enzyme-mediated redox transformations within the cell, but the majority of these are unlikely to cause stress. Despite this, biologists use this term for compounds that are unusually reactive and therefore more likely to irreversibly inactivate enzymes or generate additional reactive species. Some of these molecules are also called "redox-cycling compounds" because they enter the cell in a relatively harmless state, but are readily reduced by endogenous enzymes and catalyze downstream reactions producing highly reactive toxic species (Fig. 3A, B). These downstream reactions are oxidizing and regenerate the original compound, which can continuously cycle between reductant and oxidant. We will adhere to convention and refer to unusually redox-active metabolites as simply redox-active or redox-cycling compounds because their high level of redox activity is what distinguishes them from the myriad redox couples present within the cell. However, although these terms carry negative connotations, our use of them does not imply that these compounds are universal sources of distress. In specific contexts, their effects on bacterial physiology are neutral or beneficial. They can transition the organism to a physiological state that is different from the default but still conducive to survival (Fig. 2).

How Do Cells Sense and Respond to Redox Stress?

Redox sensors orchestrate responses to redox-active metabolites and allow for their utilization. Metabolism and redox chemistry are tightly linked as anabolic and catabolic processes involve reductive and oxidative reactions, respectively (12). However, the cellular redox balance is constantly threatened by changes in the availability of electron donors and acceptors and exposure to highly redox-active metabolites. It is monitored by a variety of sensors; some of these directly sense stressors, while others sense their effects on the cell. These take advantage of the redox properties of cofactors such as iron–sulfur clusters, flavins, and hemes (Table 1). Most of these sensors are coupled to outputs that ultimately affect transcription.

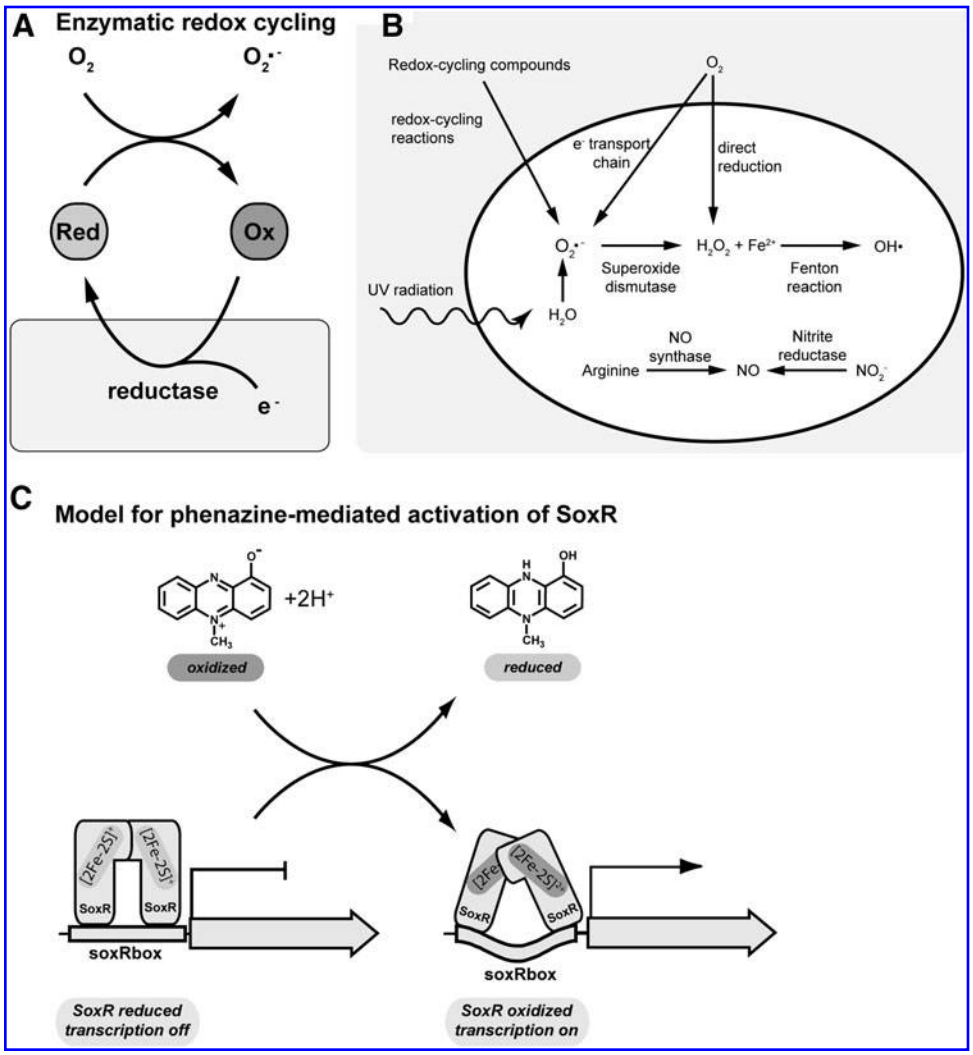

FIG. 3. Phenazines are redox-cycling compounds that activate SoxR. (A, B) The distinction between "redox-active" and "redox-cycling" metabolites. The term "redox-active metabolite" typically refers to compounds that do not participate constructively in primary metabolism during growth. These compounds react with metabolites, nucleic acids, proteins, and/or lipids without the aid of enzymes. The term "redox-cycling metabolite" is used to describe compounds that enter the cell in an oxidized state, participate in enzyme-mediated redox transformations to become reduced, then react with other substrates without the aid of enzymes. **(C)** The transcription factor SoxR is a homodimer that binds upstream of its target gene. In its reduced form it prevents transcription. In *P. aeruginosa*, SoxR is thought to react directly with pyocyanin, such that the iron–sulfur cluster becomes oxidized, inducing a conformational change that activates transcription.

Redox sensors have been best studied as mechanisms for protecting from distress, but recently, some have been implicated in coordinating neutral or positive responses to redox-active metabolites. These alternate responses occur because different functions for identical redox-sensing systems have evolved to meet the needs of specific organisms. Thus, two homologous redox-sensing systems that detect the same redox signal are linked with different outputs with opposing functions, such as coping mechanisms for distress (also known as "stress responses") in one organism versus developmental signaling in another. Two examples that illustrate this concept are (i) the nitric oxide (NO) sensor NsrR in *Escherichia coli* and *Neisseria meningitidis* and (ii) SoxR, a sensor for redox-cycling compounds in *E. coli* and *Pseudomonas aeruginosa*. Both NsrR and SoxR are transcriptional regulators that sense redox-active signals through iron–sulfur clusters (15,43).

NsrR: stress response to NO vs. utilization of NO in primary metabolism

In *E. coli*, NsrR induces approximately 20 genes that mediate a response to NO toxicity including *hmp*, *nrfA*, and *ytfE*. Hmp converts NO to nitrate in the presence of oxygen (26), NrfA converts nitrite to ammonium through an NO intermediate (44), and YtfE is involved in repairing iron–sulfur clusters that have been damaged by NO (25). In this scenario, the regulon encodes mechanisms that enable the bacterium to cope with exposure to the redox-active compound, suggesting that NO causes distress in *E. coli*. A fully functional stress response ensures that redox homeostasis is maintained.

In a contrasting scenario, *N. meningitidis* NsrR regulates a smaller gene set (19), including *aniA* and *norB*. AniA reduces nitrite to NO, which is further reduced by NorB to nitrous oxide. This was originally interpreted as a way for the bacterium to clear NO released from macrophages during infection (41). However, these reactions can also enable the maintenance of redox homeostasis by allowing *N. meningitidis* to utilize NO as an electron acceptor. This function may support survival in the oxygen-deprived environments that occur during infections, due to accumulation of mucus in the nasal cavity and throat (44).

SoxR: a sensor of exogenous and endogenous redox-cycling compounds

SoxR is a well-established stress response regulator in *E. coli* that senses redox-cycling xenobiotics such as methyl viologen, phenazine methosulfate, and 4-nitroquinoline (10–12,18). In *E. coli*, SoxR activates expression of the transcription factor

Table 1. Examples of Redox-Sensing Proteins Organized According to Their Redox-Active Cofactor and Stimulus

Redox-sensing moiety	*Redox signal*	*Organism*	*Reference*
Thiol			
OxyR	Hydrogen peroxide	*Escherichia coli*	5
Hsp33	Hydrogen peroxide	*Escherichia coli*	24
OhrR	Organic hydroperoxides	*Bacillus subtilis*	14
ArcB	Redox state of quinone pool	*Escherichia coli*	27
Iron–sulfur cluster			
SoxR	Redox-cycling compounds	*Escherichia coli*	16
		Pseudomonas aeruginosa	10
NsrR	Nitric oxide	*Escherichia coli*	7
		Neisseria spp.	22
Heme			
Dnr	Nitric oxide	*Pseudomonas aeruginosa*	4
Non-heme iron			
NorR	Nitric oxide	*Escherichia coli*	42
Flavin			
NifL	Cellular reductants	*Azotobacter vinelandii*	20
Pyridine nucleotides			
Rex	$NADH/NAD^+$ levels	*Streptomyces coelicolor* A3(2)	3

SoxS, which in turn regulates the expression of >100 genes (32). The products of this regulon include transporters of redox-cycling agents and enzymes that detoxify the products of redox-cycling, including reactive oxygen species. Induction of the SoxRS regulon therefore represents a coping mechanism that protects from the toxicity of exogenous redox-cycling agents.

In nonenteric bacteria such as *P. aeruginosa*, the mechanism of sensing redox-cycling compounds is similar to that of *E. coli* SoxR, but the response is different. *P. aeruginosa* SoxR can be activated by endogenously produced compounds called phenazines (10) in addition to synthetic compounds that have been shown to activate *E. coli* SoxR, such as methyl viologen (Figs. 3A and 4). However, unlike the *E. coli* SoxRS system, *P. aeruginosa* SoxR induces transcription of a small regulon, encoding a resistance–nodulation–cell division efflux pump, a major facilitator superfamily transporter, and a monooxygenase (10). Monooxygenases add hydroxyl groups to organic small molecules, a modification that can facilitate the transport of these molecules across the cell membrane and/or promote their degradation. We have hypothesized that the transporters are needed for the proper shuttling of phenazines (11). Similar observations have been made in *Streptomyces coelicolor*, which produces a redox-active polyketide called actinorhodin. Recent studies suggest that this compound and/or its biosynthetic precursors activate *S. coelicolor* SoxR (9,11,38). Similar to the case for *P. aeruginosa*, its regulon consists of a small set of genes, encoding an ABC transporter, a monooxygenase, and three redox enzymes.

The sensors NsrR and SoxR are two examples of transcription factors that respond to redox-active metabolites in a context-specific fashion. In *E. coli*, they provide effective means to ward off or recover from redox toxicity and allow bacteria to cope with exposure to these compounds. While these mechanisms merely facilitate tolerance to these compounds, there are also cases where these sensors allow the bacteria to exploit the same compounds as signals or metabolic substrates. Examples of these physiological functions are detailed in the following section.

Beneficial Roles of Redox-Active Metabolites

The diversity of redox-active metabolites and their effects on gene expression

Many organisms produce both inorganic and organic redox-active metabolites (Fig. 3B). Intermediates in the full reduction of molecular oxygen and nitrate can be produced both as side products of essential metabolic reactions and by the activities of organic redox-cycling compounds. Despite their high reactivity and potential to damage protein cofactors, DNA, and cellular lipids (21), some of these compounds have been shown to serve signaling functions in a diversity of organisms.

Production of inorganic redox-active metabolites is more likely to occur during active growth. Biosynthesis of redox-active organic compounds, however, is often dependent on the growth phase and conditions, occurring in the stationary phase of the growth curve of a batch culture and during limitation for specific nutrients. In fact, this is one of the properties that have led to their categorization as "secondary metabolites." Secondary metabolism is not directly required for the growth of microbes in laboratory batch cultures. Historically, the major functions ascribed to secondary metabolites have been related to their antibiotic activity and competition between divergent microbes in soil.

Selman Waksman initiated the use of the term "antibiotic" as a noun in the early 1940s when his team isolated streptomycin from the soil actinomycete *Streptomyces griseus* and found it effective against tuberculosis (35). Since then, over a dozen classes of antibiotics have been identified. These compounds are categorized according to their various primary targets within cells, such as DNA gyrase, the subunits of the ribosome, and the cell wall biosynthetic pathway. Interestingly, recent work suggests that despite the diversity in their specific drug target interactions, they share a similar mechanism of killing in that they all alter metabolism such that lethal levels of reactive oxygen species are generated (17).

When Waksman introduced the term antibiotic, it was meant to describe any substance produced by a microbe that is antagonistic to microbial growth (45), but it has evolved to

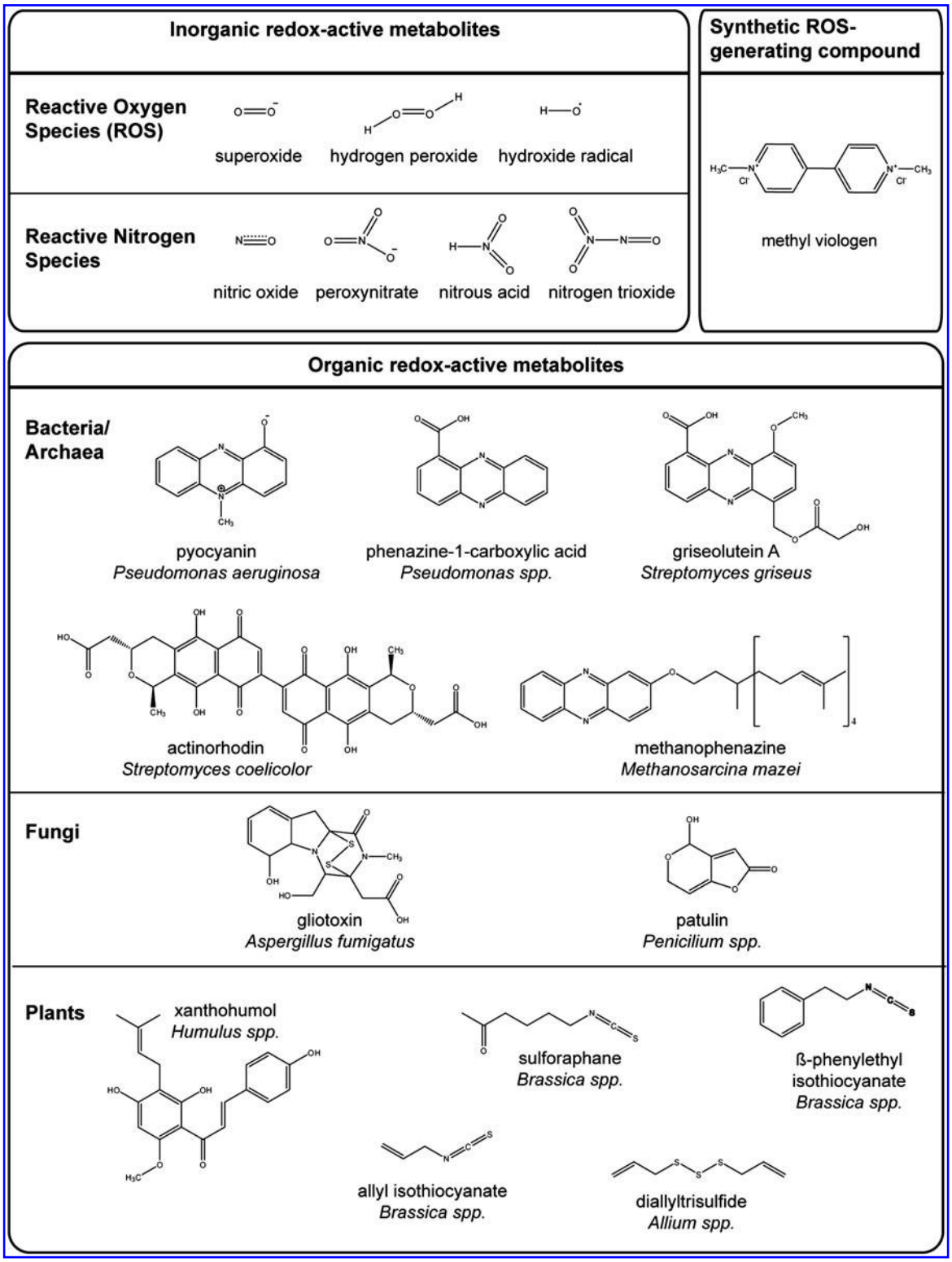

FIG. 4. Examples of inorganic and organic redox-active metabolites.

include all compounds that prevent the growth of microbes. In recent years, levels of canonical antibiotics in soil have been reported that are insufficient for biocidal activity, suggesting that sublethal effects of antibiotics are environmentally relevant. Indeed, many "antibiotics" have been shown to affect gene expression at subinhibitory concentrations (8). It must therefore be considered that the primary roles for these stressors in bacteria are related to the survival of the producing organism rather than the killing of competing organisms.

NO as a signaling molecule in bacteria

NO is a membrane-diffusible free radical gas that can covalently modify thiols and transition metal centers of proteins. When intracellular NO concentrations reach pathological levels, the resulting damage to biological molecules is referred to as nitrosative stress—distinct from oxidative stress. Bacteria and certain fungi reduce NO to N_2O in order to prevent toxicity and maintain homeostasis (18,39). NO-dependent intra- and intercellular signaling (40) has been demonstrated in many types of animal and plant cells (49). Until recently, research on the effects of NO in bacteria has focused on cultures growing planktonically, in which NO is toxic, and the potential for NO to act as a bacterial signaling molecule has been overlooked.

In 2003, Webb and colleagues (48) uncovered new roles for reactive oxygen and nitrogen species in cell lysis and cell dispersal from microcolony biofilms of *P. aeruginosa*. Soon after, the specific role of NO in biofilm development and dispersal was investigated. This work revealed that nontoxic levels of NO indicate to cells in a biofilm that environmental conditions are favorable for planktonic growth, and trigger strategic dispersal (2). NO acts via a signaling pathway to upregulate phosphodiesterase activity, leading to decreased intracellular levels of cyclic di-GMP (2), an intracellular signal that directs the switch from sessile to motile states. As the physiological effects of inorganic redox-active metabolites are investigated in divergent bacteria and under new conditions, additional examples in which such compounds act as signals are expected to emerge.

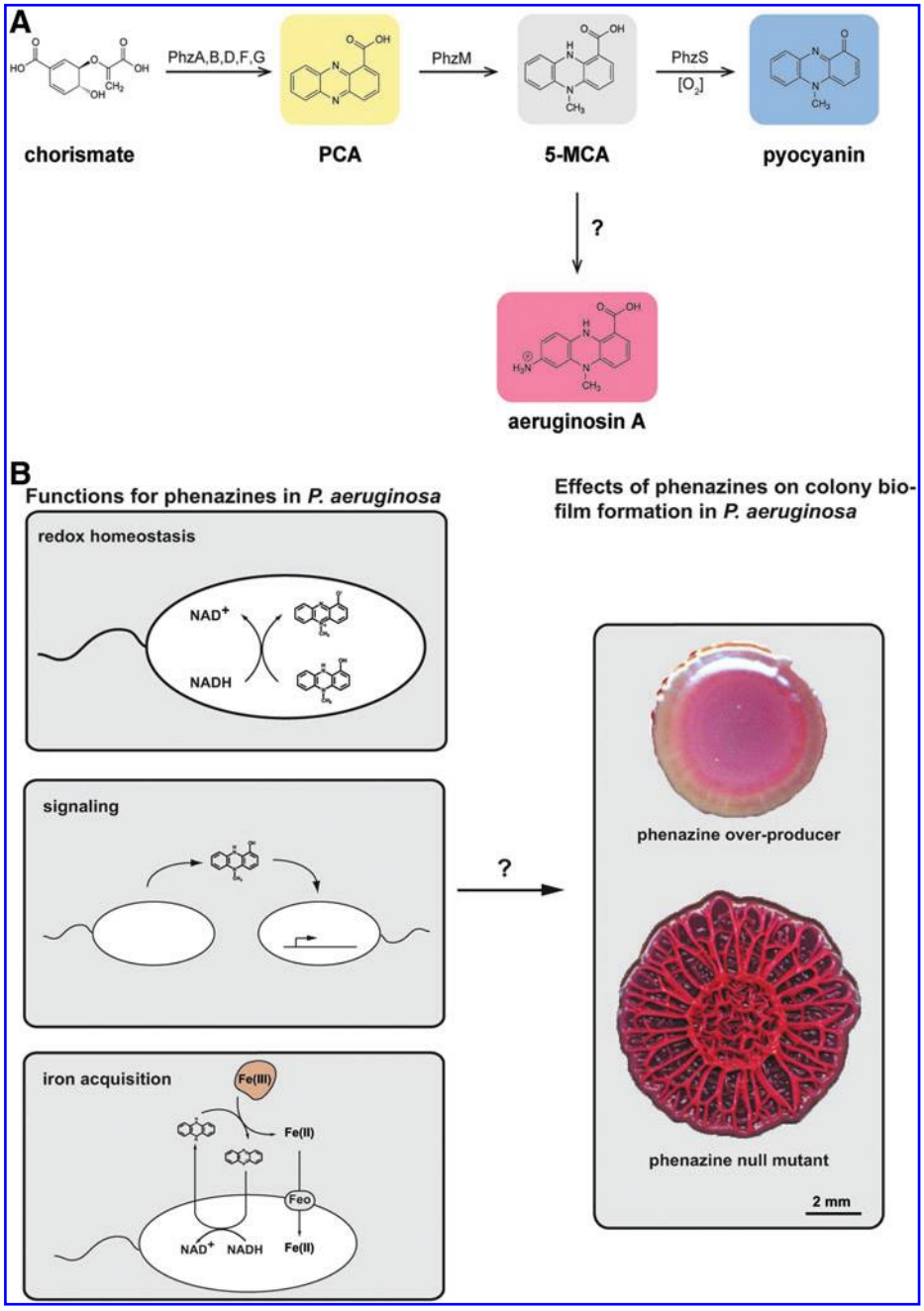

FIG. 5. *P. aeruginosa* phenazine production and physiological roles. (A) The *P. aeruginosa* phenazine biosynthetic pathway. PCA, phenazine-1-carboxylic acid; 5-MCA, 5-methylphenazine-1-carboxylic acid. **(B)** The physiological roles of phenazines in iron acquisition, redox balancing, and signaling may contribute to the drastic morphological switch observed in phenazine-producing versus phenazine-null colony biofilms in *P. aeruginosa* PA14. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Phenazines perform a variety of physiological functions

Phenazines comprise a class of colorful, heterocyclic, redox-cycling compounds. They have long been categorized as antibiotics that generate reactive oxygen species (23), leading to cell death in some organisms. Phenazines are produced by several genera of prokaryotes (1,29), including *Pseudomonas*, *Burkholderia*, *Streptomyces*, and *Methanosarcina*. The bacteria that produce and release high levels of phenazines possess multiple mechanisms that likely contribute to tolerance, including degradation and transport of the compound and increased superoxide dismutase activity (17). Highly diverse naturally occurring phenazine derivatives have been identified, and although most are biosynthetically derived from the yellow compound phenazine-1-carboxylic acid, they display a broad range of chemical structures, physical properties, and biological activities. The best-studied phenazine biosynthetic pathways are those found in pseudomonad species (Fig. 5A).

The toxic effects of bacterial phenazines in non-producing organisms are thought to facilitate competition for resources in the soil in cases of environmental isolates, and contribute to the pathogenicity of producers like *P. aeruginosa*. Beyond this, these compounds also cause eustress in their producers. *P. aeruginosa* is more resistant to ecological levels of phenazines than *E. coli* (Fig. 6, top). Furthermore, such concentrations of phenazines actually fulfill primary physiological roles in *P. aeruginosa*. As discussed above, *E. coli* phenazine sensitivity activates the large SoxRS regulon, including genes to minimize the damaging effects of phenazines (Fig. 6, bottom). At the same concentrations, phenazines activate *P. aeruginosa* SoxR, inducing a small number of genes not involved in protection from superoxide. Phenazines can increase the bioavailability of iron (47), facilitate maintenance of intracellular redox homeostasis (34), and transmit intercellular signals to coordinate gene expression across cell populations (10) (Fig. 5B). Beneficial roles for phenazines have been extensively studied in *P. aeruginosa*. In this section, we will detail the evidence that phenazines play primary roles in biological function, and discuss our ongoing research concerning the significance of these roles in the context of biofilm development in strain PA14, a clinical *P. aeruginosa* isolate.

Phenazines and iron acquisition

While the facile redox-cycling of phenazines can promote damage inside some cells, where these compounds can produce reactive oxygen species and thiyl radicals (23), the redox-cycling of phenazines outside the cell can be beneficial to the producer and other organisms in the vicinity. This activity can reduce ferric iron to soluble and biologically accessible ferrous iron (47),

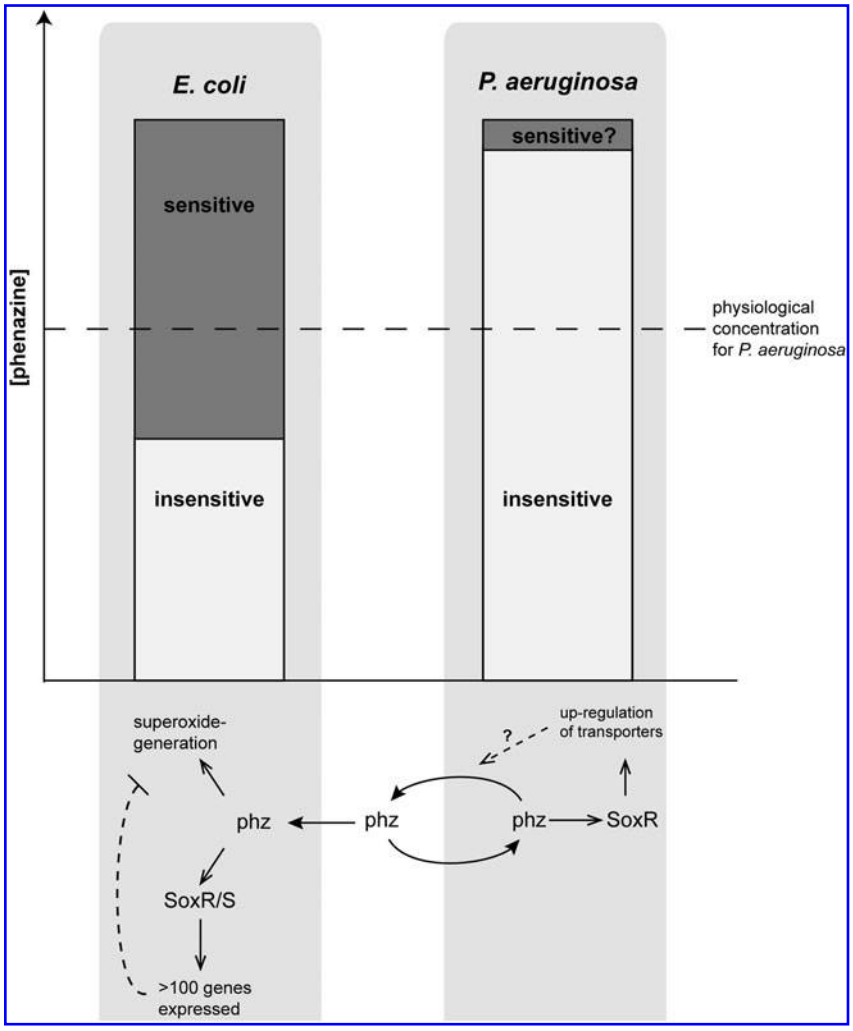

FIG. 6. The transcription factor SoxR is activated by redox-cycling compounds in *E. coli* and *P. aeruginosa*. *E. coli* does not constitutively produce enzymes that provide protection from the toxic effects of these compounds; this protective response is induced by SoxR and its target, SoxS. *P. aeruginosa* is constitutively resistant to higher levels of redox-cycling compounds. In this organism, SoxR induces expression of genes for which the products may facilitate beneficial phenazine redox-cycling (for iron acquisition or intracellular redox balancing) and signaling. In the lower portion of the figure, the gray boxes represent the intracellular space.

and could be important in diverse contexts. Phenazines and their producers are frequently found in the multispecies communities of the rhizosphere, where it is thought that plants and other organisms benefit from their iron-reducing activity (30). Phenazine-mediated iron acquisition may also play a role in pathogenicity. *P. aeruginosa* is infamous for infecting the lungs of cystic fibrosis patients, and phenazine-producing strains are frequently isolated from their sputum. Phenazine-producing strains may be more virulent than non-producers, in part due to their ability to use phenazines to reduce and release iron that is bound to the human protein transferrin (6). It has also been reported that phenazine-1-carboxylic acid promotes the formation of *P. aeruginosa* biofilms in flow cells by facilitating iron acquisition (47). These activities of phenazines may contribute to the survival of *P. aeruginosa* in the host lung.

Phenazines in respiration and redox balancing

Phenazine-producing bacteria catalyze the reduction of phenazines in addition to their biosynthesis. The most striking evidence of bacterial phenazine reduction can be observed in a standing *P. aeruginosa* PA14 culture, which gradually turns from bright blue to bright yellow upon removal from a shaking incubator. This process occurs because the major phenazines produced by *P. aeruginosa* change color depending on their redox state. In the 1930s, Ernst Friedheim demonstrated that pyocyanin production promotes oxygen consumption in *P. aeruginosa* cell suspensions and suggested that phenazines could act as electron carriers in respiratory or redox-balancing reactions (13). He would have been excited to learn that methanophenazine, produced by *Methanosarcina mazei* Gö1, provides unequivocal proof of his hypothesis. This phenazine is not released from the cell but rather resides attached to the cell membrane where it is required for the electron transport that ultimately powers ATP synthesis (1).

Methanophenazine is the only small organic pigment for which a direct role in respiration has been characterized. However, decades after Friedheim proposed that *P. aeruginosa* phenazines could react with "labile hydrogen" inside the bacterium, new studies have shown that these phenazines facilitate intracellular redox balancing and survival. Planktonic cultures of phenazine-deficient *P. aeruginosa* mutants exhibit a higher $NADH/NAD^+$ ratio in stationary phase, suggesting that an inability to produce phenazines results in a reduced intracellular environment (34). The NADH reoxidation coupled (directly or indirectly) to phenazine reduction may allow primary anabolic reactions to proceed under conditions in which other terminal electron acceptors are unavailable, as is the case in dense, oxygen-limited stationary-phase cultures. Intriguingly, in anaerobic reactors containing electrodes poised at phenazine-reducing potentials, phenazines promote *P. aeruginosa* survival (46). The bacteria catalyze phenazine-mediated extracellular electron shuttling, using the electrodes as the terminal oxidant. While the specific reactions that support bacterial survival in this case remain to be identified, it appears that phenazine secondary metabolites can play primary metabolic roles under some conditions.

Phenazines are signaling molecules

Gene expression in populations of bacterial cells can be coordinated via secretion of specific molecules. In quorum sensing, a mechanism of intercellular signaling common to many divergent bacterial species, these molecules tend to be structurally complex regulators of a discrete set of genetic loci that are not directly involved in primary metabolism during exponential growth. Several types of small molecules have been identified that participate in quorum sensing in gram-negative bacteria, including the N-acylhomoserine lactone and 2-alkyl-4-quinolone signals produced by *P. aeruginosa*. Recent work has shown that phenazines also coordinate gene expression in populations of *P. aeruginosa*, and mutational analysis has demonstrated that pyocyanin is a terminal intercellular signal in the quorum sensing cascade (10). Pyocyanin up-regulates a discrete set of genes, including those induced by SoxR activation. Similarly, the redox-active pigment actinorhodin induces expression of SoxR-dependent genes in *S. coelicolor*. Strong evidence suggests that the redox-active pigments themselves, rather than the reactive species generated during their redox cycling, directly activate SoxR by oxidizing its iron–sulfur cluster (10,16). In both *P. aeruginosa* and *E. coli*, endogenous and exogenous redox-cycling compounds can activate SoxR under anaerobic conditions (Fig. 6). These findings implicate redox-active metabolites in the regulation of gene expression in oxygen-limited bacterial communities.

Phenazines modulate community development

Many bacteria frequently exist in nature not as unicellular organisms but within complex cellular communities known as biofilms. We have hypothesized that phenazines contribute to biofilm development (i) by acting as intercellular signals and (ii) by balancing the intracellular redox state. Indeed, the effects of phenazine production or exposure on colony biofilm development produce dramatic macroscopic effects: wild-type *P. aeruginosa* PA14 colonies are smooth and smaller than those of the phenazine-null mutant, which are highly wrinkled and spread over a larger surface area of the agar plate (Fig. 5B). Although it has been established that bacterial cells in cultures and flow-cell biofilms can coordinate their gene expression, the ability for 10 billion bacterial cells to organize into a structure like the phenazine-null colony suggests that the mechanisms by which they do so are far more complex than previously appreciated, and probably involve both cell–cell communication and redox balancing.

Cells in biofilms often respond to environmental changes together to ensure the survival of the community rather than individual cells. This concerted approach requires sophisticated signaling pathways that transmit information from the environment to discrete subpopulations of the cell community. Phenazine-dependent signaling pathways have the potential to fulfill this type of role. Biofilm communities are heterogenous due to the uneven distribution of oxygen (50) and other substrates. Oxygen is required for the biosynthesis of the phenazine pyocyanin (Fig. 5A) and affects its redox state; therefore, this heterogeneity could lead to localized control of gene expression. We have also hypothesized that phenazines play an important role in balancing the redox state of cells within anoxic zones (33). Our laboratory endeavors to delineate these mechanisms. We are defining the availability of oxygen and other substrates provided in growth media and measuring the redox state of cells collected from biofilms. Furthermore, we are learning how signals are transduced from the environment to a developing *P. aeruginosa* PA14 colony via phenazines and fully characterizing the genes

involved in the developmental program of a PA14 colony. These approaches will converge to provide a full picture of the eustress that gives rise to the morphology of wild-type (phenazine-producing) *P. aeruginosa* biofilms.

Concluding Remarks

Although Hans Selye distinguished between positive and negative responses to stressors in his original conception of the term "stress response," for microbial physiologists the term "stress" harbors a universally negative connotation. We propose a modification of the current paradigm and have adapted Selye's original concept of eustress so that it can be applied to bacterial communication and behavior. While production or exposure to stressors such as redox-active metabolites may cause negative stress, *i.e.*, distress, under some conditions, examples are emerging in which these compounds promote survival, and the response is more accurately described as positive stress, or eustress. Microbiologists now appreciate that the nutrient-limited, densely populated condition akin to the stationary phase of a laboratory culture is often a closer representation of the conditions experienced by bacterial populations in the environment. As we continue to probe the mechanisms promoting survival under these conditions, we will no doubt uncover additional examples in which redox-active metabolites are the causes of eustress.

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Address correspondence to:
Dr. Lars E.P. Dietrich
Department of Biological Sciences
1212 Amsterdam Avenue, MC 2418
New York, NY 10027

E-mail: ldietrich@columbia.edu

Dr. Alexa Price-Whelan
Department of Pharmacology and Systems Therapeutics
Mount Sinai School of Medicine
1 Gustave L. Levy Place, Box 1603
New York, NY 10029

E-mail: alexa.price-whelan@mssm.edu

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Abbreviations Used

GMP = guanosine monophosphate
NO = nitric oxide
N_2O = nitrous oxide

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