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MicroCommentary

A shared mechanism of SoxR activation by redox-cycling compounds

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Summary

In this issue of *Molecular Microbiology,* Gu and Imlay show that a class of compounds known as redox-cycling agents directly activate the transcription factor SoxR of *Escherichia coli* and cause cellular toxicity independent of the production of the reactive oxygen species superoxide. Despite the fact that redox-cycling agents increase formation of superoxide in *E. coli*, the results described in this new publication revise the long-held assumption that superoxide is responsible for the activation of SoxR and for all of the major toxic effects of redox-cycling drugs. This study also suggests that the critical function of the SoxRS regulon in *E. coli* is in protection against redox-cycling agents and not exclusively the defence against superoxide.

Understanding the effects of and responses to redox-cycling agents has been an important problem in biology. Redox-cycling agents are small molecules that are readily reduced and re-oxidized under physiological conditions. They include antibiotics that are produced naturally by a variety of bacteria, fungi and plants, and undergo intracellular redox transformations that can be toxic (Fig. 1). In addition, artificial redox-cycling agents such as paraquat have been used as herbicides since 1962 (Bromilow, 2004). Redox-cycling compounds impair cell function by removing single electrons from carriers and redirecting them to new targets, disrupting normal electron flow and metabolism. For example, oxidation of NADPH-reduced flavin cofactors of enzymes leads to depletion of NADPH (Deller *et al*., 2008), which is critical for many biosynthetic reactions. As shown in this new study by Gu and Imlay (2011), removal of electrons from some metal centres also inactivates key metabolic enzymes. Redox-cycling agents are also well-known for their tendency to reduce molecular oxygen and generate superoxide (Cohen and d'Arcy Doherty, 1987), and it has long been assumed that the deleterious effects of superoxide are largely responsible for the toxicity of redox-cycling agents. However, in one unexpected finding of this paper, the authors demonstrate that redox-cycling agents are toxic to *Escherichia coli* even in the absence of O_2, suggesting that superoxide production is not exclusively responsible for toxicity.

In a second surprising finding, the authors report that, in contrast to the long-held view that redox-cycling agents activate a global superoxide-dependent stress response in *E. coli*, the cognate transcription factor SoxR is in fact directly activated by redox-cycling agents. SoxR (named for superoxide response) was first discovered in *E. coli* in 1990 by two independent groups that were searching for regulators of the global response to redox-cycling agents such as paraquat (Greenberg *et al*., 1990; Tsaneva and Weiss, 1990). Since several different redox-cycling agents had been shown to induce a common global stress response (Hassan and Fridovich, 1978; Greenberg and Demple, 1989), it was generally accepted that activation of SoxR by redox-cycling agents occurred by an indirect mechanism that was common to all of the chemicals. Previous studies had shown that redox-cycling agents also stimulate formation of superoxide. Therefore, it was inferred that these agents activate SoxR through the unifying action of superoxide. Consistent with this model, SoxR-mediated transcription was shown to be activated via oxidation of a bound $[2Fe\text{-}2S]^{1+}$ cluster (Ding *et al*., 1996; Gaudu and Weiss, 1996), which was ascribed to superoxide. However, the work of Gu and Imlay shows that oxidation of the $[2Fe\text{-}2S]^{1+}$ cluster of SoxR can be catalysed directly by redox-cycling agents in the absence of superoxide.

Both *in vitro* and *in vivo* data support this new conclusion. The authors constructed a SoxR overexpression strain to

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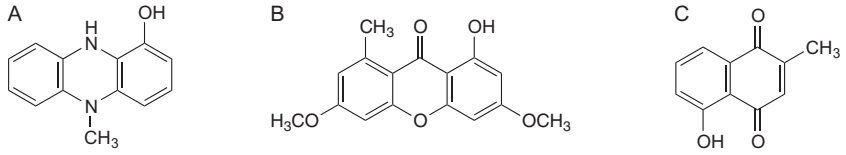

pyocyanin **lichexanthone** **plumbagin**

Fig. 1. Naturally occurring redox-cycling compounds.
A. Pyocyanin, which is bright blue in its oxidized form, is a phenazine produced by the Gram-negative bacterium *Pseudomonas aeruginosa*.
B. The yellow lichexanthone is produced by a variety of lichens.
C. The yellow naphthoquinone plumbagin can be isolated from roots of the plant *Plumbago scandens*.

allow detection of its [2Fe-2S]$^{1+}$ cluster by EPR and found that the cluster was oxidized by paraquat even under anaerobic conditions. Thus, this reaction occurred in the absence of O_2, which is needed to generate superoxide. This could be explained by two alternative mechanisms: (i) direct oxidation of SoxR by paraquat or (ii) indirect activation by depletion of SoxR's reductant, NADPH. To test the latter possibility, the authors blocked the two primary mechanisms of NADPH regeneration by deleting the genes *zwf* and *pnt*. The NADPH-depleted strain showed slightly elevated expression of the SoxR target gene *soxS*, consistent with results reported in a recent study by Krapp and colleagues (Krapp *et al*., 2010). However, as Gu and Imlay point out, these changes in NADPH concentrations are unlikely to account for the much higher *soxS* expression in the presence of paraquat. The authors went on to compare the activation of SoxR by superoxide and paraquat *in vitro*. Using both EPR and visible absorption spectroscopy, they found that the SoxR [2Fe-2S]$^{1+}$ cluster was more readily oxidized by paraquat than by superoxide. In fact, the rate of oxidation of the [2Fe-2S]$^{1+}$ cluster by superoxide was found to be too slow to be responsible for SoxR activation in cells. Further support that SoxR is not a physiologically relevant sensor of superoxide levels comes from previous studies of Gort and Imlay, demonstrating that SoxR is not activated by the increased superoxide present in strains lacking superoxide dismutases (Gort and Imlay, 1998). These strains produce superoxide at levels sufficient to inactivate some dehydratases and impair growth.

The results of this study provide biochemical proof that SoxR can be activated anaerobically. The fact that *E. coli sodA*, encoding superoxide dismutase, is induced in a SoxR-dependent manner initially fit with the role of *E. coli* SoxR in the defence against superoxide. However, many genes upregulated in response to SoxR activation have no apparent function associated with superoxide detoxification. Rather, proteins encoded by these genes function in export or modification of redox-cycling drugs, suggesting that the major function of the regulon is in the specific defence against redox-cycling agents rather than superoxide. In support of this conclusion, the authors show that growth of a strain that lacks SoxR is impaired under anaerobic conditions in the presence of redox-cycling agents.

Several studies published over the last decade have raised the possibility that the SoxR response is not geared towards superoxide *per se*. Park *et al*. determined that the *sodA* gene is not upregulated in response to paraquat in *Pseudomonas putida* and that a *soxR* deletion strain is not sensitive to paraquat (Park *et al*., 2006). Similar findings have been reported for *Pseudomonas aeruginosa*, where the SoxR regulon is composed of genes encoding the RND efflux pump MexGHI–OpmD, an MFS transporter and a putative monooxygenase (Kobayashi and Tagawa, 2004; Palma *et al*., 2005). Subsequently, it was found that in *P. aeruginosa* SoxR is activated by endogenous, redox-cycling compounds called phenazines. This reaction can occur anaerobically *in vivo*, suggesting superoxide-independent activation (Dietrich *et al*., 2006).

The *P. aeruginosa* SoxR regulon differs dramatically from that of the enterics (Fig. 2). In *E. coli* and *Salmonella enterica* serovar Typhimurium (Pomposiello and Demple, 2000), SoxR directly regulates the expression of just one target gene: *soxS*. SoxS is a transcription factor that controls the expression of more than one hundred genes (Pomposiello *et al*., 2001). To determine which SoxR regulon structure is more typical across the domain Bacteria, a bioinformatic analysis was conducted (Dietrich *et al*., 2008). The results suggested that the regulon architecture found in *P. aeruginosa* – i.e. direct regulation of approximately 1–10 target genes that typically encode predicted mono- and dioxygenases, oxidoreductases and transporters – is representative of that found in most SoxR-containing species, while the *E. coli* SoxR-SoxS arrangement is specific to the enterobacteria.

The emerging paradigms for SoxR activation and SoxR-dependent regulation have been experimentally supported by studies in the soil bacterium *Streptomyces coelicolor*, which produces the redox-active polyketide actinorhodin. Similar to phenazines in *P. aeruginosa*, these endogenous compounds or precursors thereof activate SoxR and induce the expression of several target

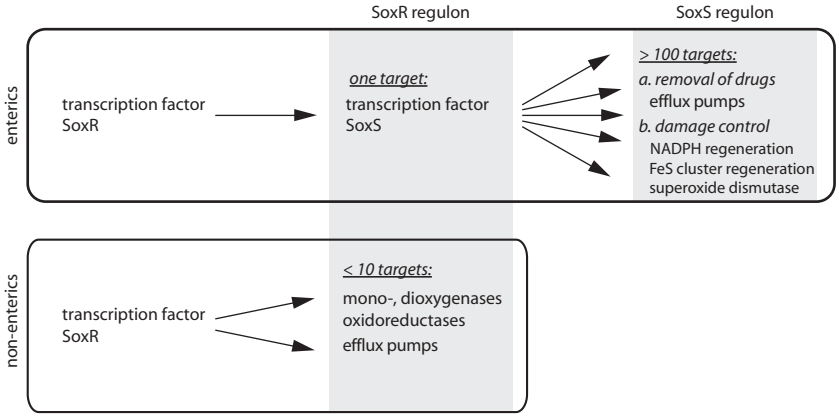

Fig. 2. SoxR response in enterics and non-enterics.

genes, encoding putative reductases, a monooxygenase and an ABC transporter (Dela Cruz *et al*., 2010; Shin *et al*. 2011). The physiological role of SoxR is a particularly intriguing question for organisms such as *P. aeruginosa* and *S. coelicolor* that produce redox-cycling compounds naturally. The small regulons in these bacteria may constitute a specific response geared to the endogenous signals. It is noteworthy that in contrast to the enterics, *soxR* mutants in *P. aeruginosa* and *S. coelicolor* are insensitive to endogenous and exogenous redox-cycling compounds, suggesting that in these organisms SoxR is not part of a detoxification response. *P. aeruginosa* phenazines have been demonstrated to play important roles for the producing organism that go beyond their antibiotic properties, acting as signalling molecules (Dietrich *et al*., 2006) and serving as electron acceptors to balance the intracellular redox state (Price-Whelan *et al*., 2007). The activities of SoxR target gene products may be important for these additional functions. The role of the monooxygenase and its substrates are currently unknown. However, considering that the SoxR-regulated RND efflux pump MexGHI–OpmD appears to be involved in the release of certain phenazines (Dietrich *et al*., 2008), the SoxR response may ensure proper shuttling of these compounds.

The fact that these organisms do not require SoxR to survive in the presence of redox-cycling agents likely reflects a higher constitutive level of expression of the enzymes required for detoxification. In *P. aeruginosa*, two superoxide dismutases, encoded by *sodA* and *sodB*, are regulated by quorum sensing and iron availability respectively (Hassett *et al*., 1999). This suggests that *P. aeruginosa* pre-emptively expresses its superoxide stress response as an adaptation to a specific lifestyle, instead of reacting to the presence of superoxide-generating drugs. This strategy is well-suited to bacteria that make these compounds themselves or that thrive in environments, such as the soil, that contain a variety of bacteria, fungi and plants that produce redox-cycling compounds.

In contrast to the SoxR regulons of *P. aeruginosa* and *S. coelicolor*, the SoxRS regulon in the enterics may represent a more generalized response to a diversity of redox-cycling compounds and their damaging effect on the cell. The observation that the transcription factor SoxS is the only well-established SoxR target in *E. coli* suggests that SoxR likely evolved in an organism that produces redox-active compounds and was then laterally transferred to hitchhike the SoxS regulon. Although the response to SoxR activation differs significantly in the enterics compared with other bacteria, the study by Gu and Imlay suggests that the mechanism of SoxR activation across bacteria may be more similar than previously considered. A system originally dedicated to the sensing, modification and localization of endogenous redox-cycling agents may have been adapted to perform a protective function in non-producing organisms.

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