Nature Reviews Microbiology | Published online 11 Jan 2016; doi:10.1038/nrmicro.2015.27

BACTERIAL PHYSIOLOGY

Pushing the envelope on protein repair

> protein repair in the periplasm is induced by … HOCl, and mediated by a wholly novel type of Msr system

Cell envelope proteins of Gram-negative bacteria can be damaged by reactive oxygen species, such as HOCl or H_2O_2, which oxidize Met residues to Met-O (methionine sulfoxide). How this oxidative damage is repaired has been an enigma, as no methionine sulfoxide reductase (Msr) has been identified in the cell envelope. However, a genetic suppressor screen in *Escherichia coli* now identifies a periplasmic Msr that repairs cell envelope proteins, and finds that this Msr uses the respiratory chain as a novel source of electrons.

To identify previously uncharacterized Msrs, Gennaris *et al.* deleted all known Msrs from an *E. coli* strain that is auxotrophic for Met. Initially, the strain was unable to grow using Met-O as the only source of Met, which meant that it lacked the Msr activity necessary to reduce Met-O to Met. However, the authors postulated that uncharacterized Msrs might be transcriptionally silenced. Indeed, a genetic suppressor screen produced a mutant strain that was able to grow using Met-O as the only source of Met. The suppressor mutation was in the YedVW two-component regulatory system, which suggested the presence of an uncharacterized Msr system that is conditionally expressed in response to an external signal relayed by YedVW.

The *yedVW* operon is in close proximity to an operon that encodes a periplasmic molybdopterin-containing oxidoreductase (MsrP; also known as YedY) and its putative redox partner (MsrQ; also known as YedZ), and thus the authors investigated MsrPQ as a candidate for the Msr system that is regulated by YedVW. In agreement with such a role, expression of *msrPQ* was 100-fold higher, and MsrP substantially more abundant, in the suppressor mutant. Furthermore, exposing wild-type cells to either HOCl or H_2O_2 identified HOCl as the external signal relayed by YedVW to induce the expression of *msrPQ*.

To investigate whether MsrPQ constitutes a functional Msr system, the authors generated Δ*msrP* and Δ*msrQ* mutants, and found that both genes are required for Met-O reduction. Furthermore, MsrP was able to reduce Met-O, *N*-acetyl-Met-O (a mimic of the chemistry of methionine within proteins) and oxidized calmodulin (a protein commonly used as a reporter of Msr activity) *in vitro*, confirming that MsrP is a novel Msr. Finally, the authors characterized the functional role of MsrP in the cell envelope by using mass spectrometry to identify 20 periplasmic proteins for which oxidation can be repaired by incubation with MsrP. *In vivo* experiments confirmed the role of MsrPQ in protecting two of these proteins, the chaperone SurA and the lipoprotein Pal, from oxidative damage.

MsrQ had been described elsewhere as the putative redox partner for MsrP; however, a non-thioredoxin redox partner would be unique among all known Msrs. Deletion of *trxA* confirmed that thioredoxin is not required for Met-O reduction by MsrP. Furthermore, whereas thioredoxin obtains electrons from NADPH, MsrQ shares a haem cofactor with cytochrome *b*, suggesting that MsrQ might acquire electrons from the respiratory chain, rather than from NADPH. Mutants unable to transfer electrons from the respiratory chain supported this hypothesis, establishing that MsrPQ uses an electron source that has not previously been observed in protein repair. The mechanistic novelty of MsrPQ was further highlighted by experiments showing that, unlike other Msrs, MsrP has no substrate stereospecificity.

Thus, protein repair in the periplasm is induced by an agent of oxidative damage, HOCl, and mediated by a wholly novel type of Msr system, MsrPQ, which differs from other systems in its redox partner, use of respiratory chain electrons and lack of substrate stereospecificity.

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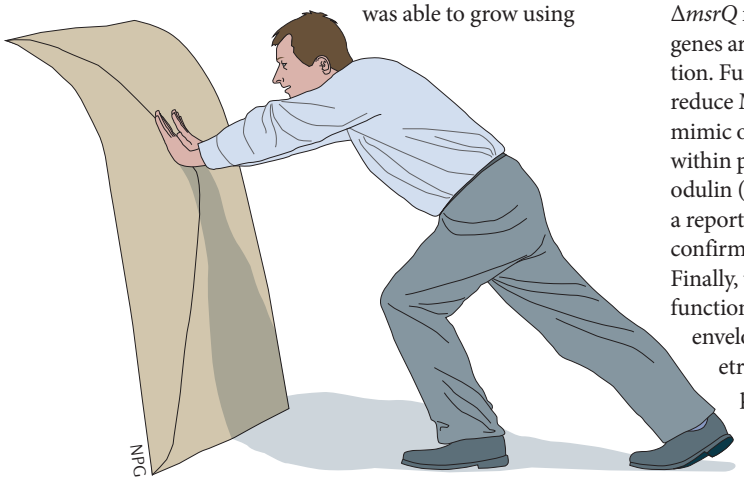

ORIGINAL ARTICLE Gennaris, A. *et al.* Repairing oxidized proteins in the bacterial envelope using respiratory chain electrons. *Nature* **528**, 409–412 (2015)