Conservation and mechanism of the methionine reductase C family

empty vector

E.coli msrC

M. tuberculosis

M. tuberculosis

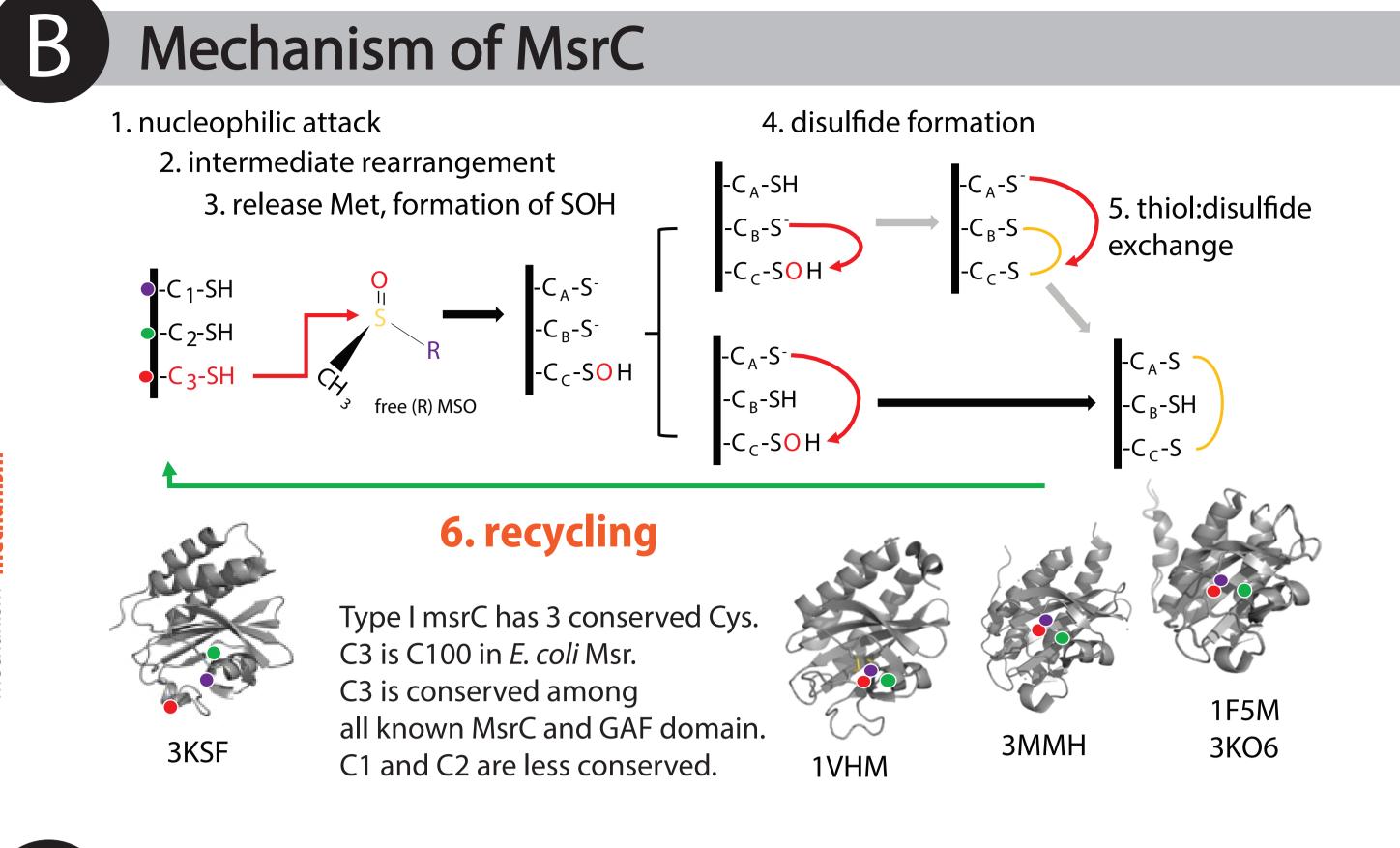
msrB

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Oxidation of methionine to methionine sulfoxide (MSO) is reversed by methionine sulfoxide reductases (Msr), a family of six evolutionarily-unrelated proteins that converge on this activity. Here we explore the mechanism of MsrC, an unusual Mrs active on free-MSO with evolutionary origins on ligand-binding GAF-domain containing proteins involved in signaling.

Methionine sulfoxide reductases *E. coli* gene name substrate methionine methionine protein-bound and free MSO sulfoxide protein-bound MSO only reductases free MSO only (Msr) free MSO? protein-bound and free MSO



in-cell activity assay

 $\Delta 6$ is a Met-auxotroph *E.coli* strain with no MSRs. NO growth with MSO as the only source of Met. Growth recovered when an ACTIVE Msr is expressed from plasmid

methionine

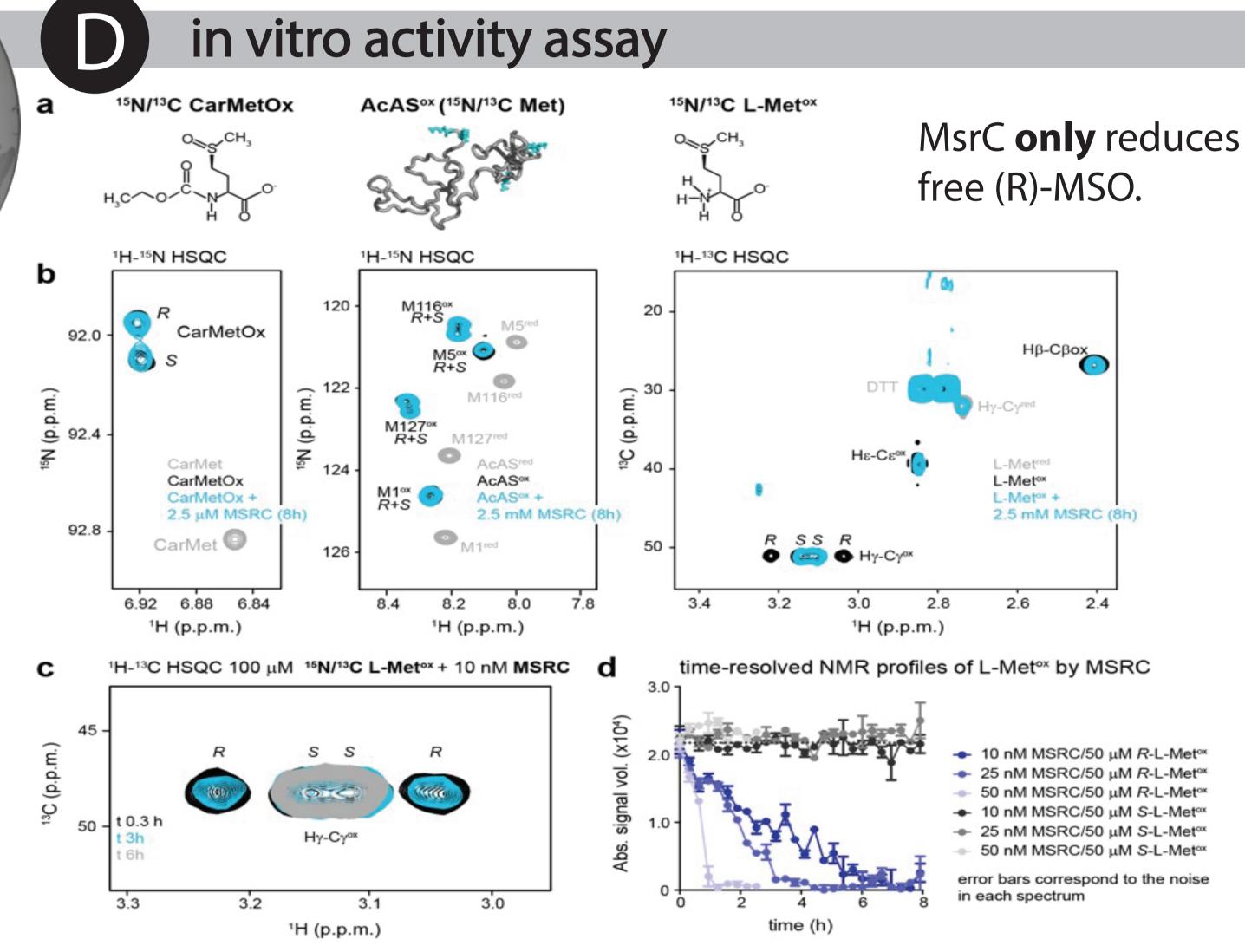
sulfoxide

| Empty vector | | | | | | | 490 | |
|--------------|----|---|---|---|-----|-----|------|--|
| EcMsrC WT | 0, | 0 | 0 | 0 | 0 | 2 | | |
| C66S | 0 | 0 | 0 | 9 | 4. | 1.4 | , | |
| C76S | 0 | 0 | 0 | 9 | (ii | :) | | |
| C100S | | | | | | | | |
| C66/76S | 0 | 0 | 0 | 0 | 48 | 1.4 | | |
| C66/100S | | | | | 77 | - 5 | 1907 | |
| C76/100S | | | | | | | | |
| C66/76/100S | | | | | | | | |



C100 is the catalytic residue.

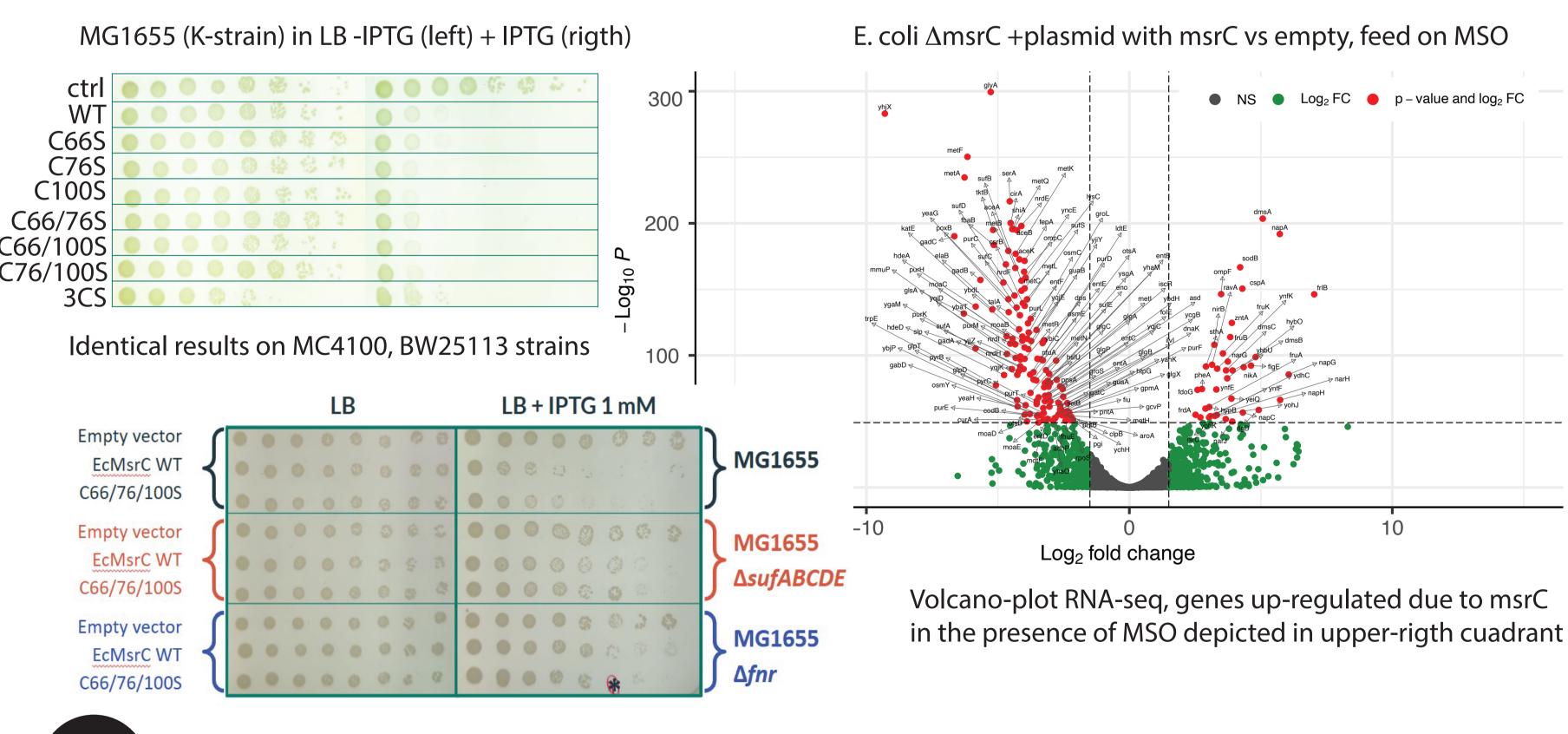
Both Trx and/or GSH/Grx system can support redox activity.



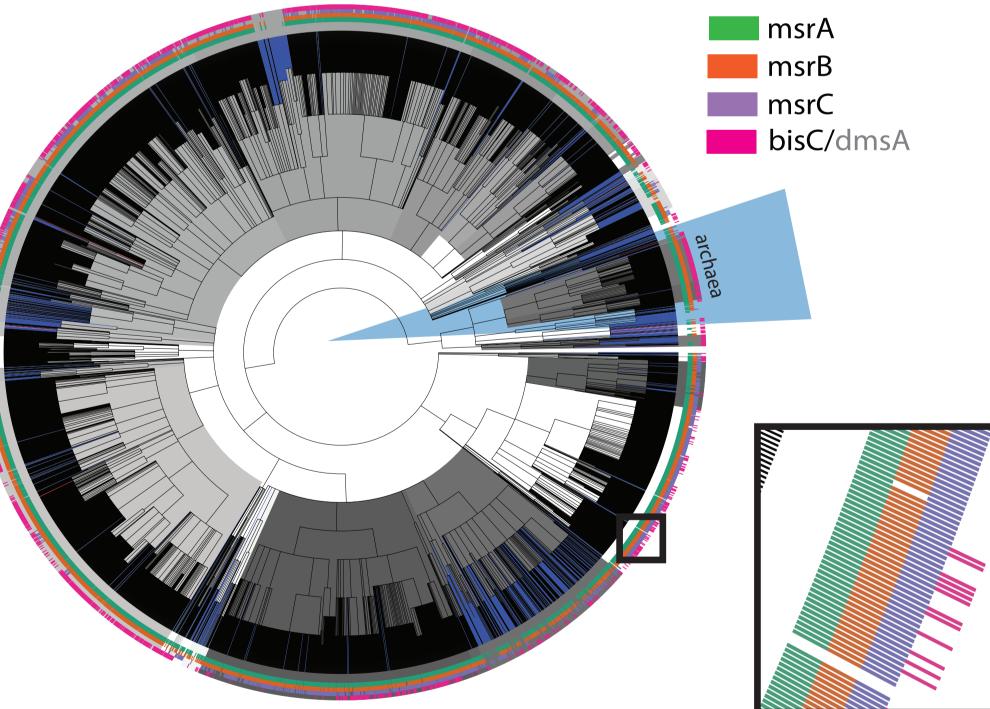
MsrC is a dimeric enzyme with 3 titrable thiols per unit, all of the with pKa around 8 (not shown). k2MSO similiar to msrA (not shown).

Expression of msrC changes gene expression pattern

Recombinant MsrC is produced with high-yield on B-type strains. However, when expressed on K-type *E. coli* cells, compromises cell growth. This phenomena is independent of its redox mechanism. Expression of msrC on a msrC/KO strain alters gene expression (RNAseq). The mechanism is related to SUF and/or FNR.



Conservation of msrC (GAF_2) in prokaryotes



the presence of msrC

is scattered among

most intracellular

parasites

domains (PFAM GAF_2)

prokaryotes, absent in

msrC is a subtype of redox-active GAF domain

MsrC"protein" is the effector MG1655 (LB+IPTG) The "toxic" phenotype is mediate by MsrC EcMsrC WT (cod. sin.) PROTEIN and it's not hidden in the RNA EcMsrC WT (cod. sin.) **Empty vector** squenece (cod.sin=same protein seq from (low MSO+IPTG) EcMsrC WT a different RNA seq). C66/76/100S

msrC genes cluster into a ubtype of GAF_2 containing proteins. They show lower sequence conservation than other GAF 2 **Z**3 domains from signaling proteins. **Z6**

In-vivo mechanism and effect of MsrC on gene expression may represent two sides of the same mechanism. MsrC relies on C100 to be active, and both Trx/Grx systems can reduce it. MsrC impacts gene expression in response to MSO. This phenotype is linked to SUF and FNR systems, FeS cluster proteins involved in stress response..

