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Sociedad Argentina de Microbiología General

# XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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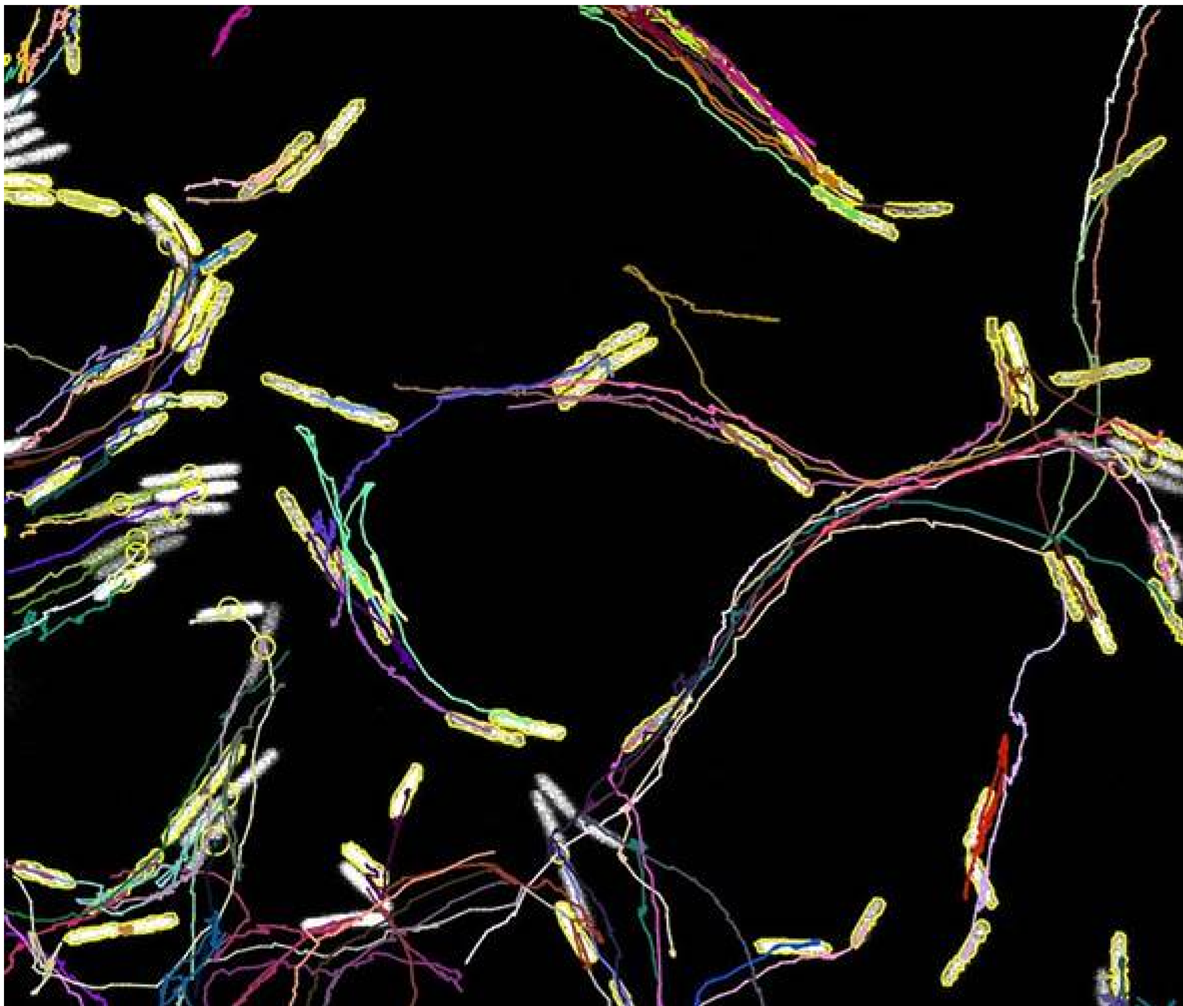
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.



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79 - Microbiología Molecular y Fisiología

## EXPLORING THE EVOLUTION AND MECHANISM OF METHIONINE SULFOXIDE REDUCTASE C

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Oxidation of methionine (Met) to methionine sulfoxide (MSO) is reversed by enzymes known as methionine sulfoxide reductases (Msr), a family of six evolutionarily unrelated proteins that converge on this activity. Among them, MsrC (previously known as fRMsr) constitutes an oddity. While most of the known Msrs, like MsrA and B, have proven roles in repairing oxidized proteins, MsrC is exclusively active on free MSO, the biological relevance of which is still unknown. The activity of MsrC on this small molecule may be a reminiscence of its evolutionary origin, as MsrCs are GAF-domain containing proteins. GAF domains are abundant in prokaryotic membrane-bound multidomain proteins such as cyanobacteriochromes, that use conserved cysteines to bind pigments and alter gene expression in response to light. MsrC, on the contrary, are stand-alone globular, cytosolic, single-domain GAF proteins that, while conserving the cysteine residues relevant for pigment binding, gain a new, family-conserved cysteine residue essential for the redox activity. Here, we explored the conservation of MsrC across the tree of life, aiming to provide a rationale to how and when a new family of Msr enzymes evolved in the context of multiple redundant Msr activities. The presence of this gene is scattered among prokaryotic groups and only present in a few unicellular eukaryotes, where it has no known biological function. To gain insight into its mechanism we used in vitro and in vivo approaches. The E. coli recombinant protein and cysteine mutants were analyzed for reaction with MSO, proving to be a very effective reductase that relies on the MsrC-conserved Cys residues. Using a Met-auxotroph, Msr-deficient E. coli strain which is unable to grow on minimal media with MSO as the only Met source, we tested the in vivo mechanism as the growth phenotype of this strain is recovered by msrC expression from plasmid. In addition, to gain insight into substrate/ligand binding, we performed structural analysis using published and modeled MsrC structures, while protein crystallization trials are ongoing. Interestingly, phenotype recovery suggests that the enzymatic mechanism relies only on the catalytic cysteine present in redox-active GAF

domains and absent in canonical GAF domains. Hence, we hypothesize that glutathione (GSH) is the most probable reducing agent, contrary to what's known for MsrA/B proteins, that rely on thioredoxin (Trx). To tackle this question, we knocked down the GSH or Trx system by deleting key genes gor and trxB, respectively, in order to study the in vitro reducing system of MsrC. Taken together, our results suggest that MsrC may be a bifunctional enzyme, capable of binding and reducing MSO using a mechanism that resembles MsrA, or via thiol:disulfide exchanges with the GAF-conserved cysteines, leading to a conformational change that may/could transduce signals downstream using a yet unknown MSO-dependent redox-relay.

Palabras clave: methionine sulfoxide - reductases - GAF - redox - cysteine