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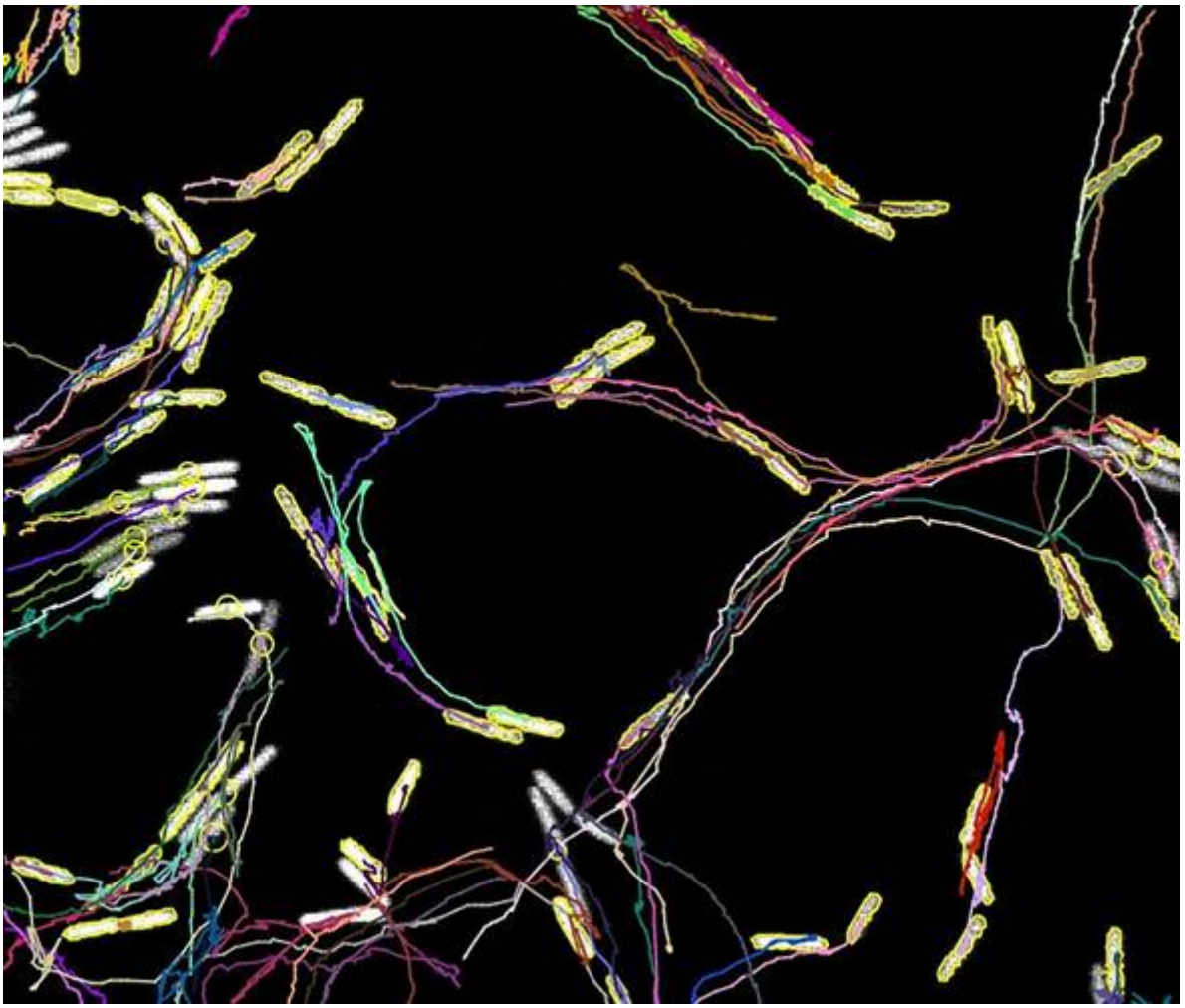

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

ROLE OF THE ScsABCD SYSTEM IN THE METAL/REDOX HOMEOSTASIS OF THE *Salmonella enterica* ENVELOPE

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Foodborne diseases are among the most prevalent health problem worldwide, including those linked to *Salmonella enterica*, a pathogen causing from self-limited gastroenteritis to severe invasive illness in susceptible hosts. The emergence of *Salmonella* strains carrying resistance genes against different antibiotic and other biocides such as Cu, has generated the need to investigate novel therapeutic strategies. Like other bacterial pathogens, the interaction of *Salmonella* with the host is influenced by redox stress and transition metals, especially by copper (Cu). In fact, Cu ions exacerbate redox stress, primarily in the bacterial cell envelope, where many of the Cu distribution proteins and cuproproteins reside. ScsABCD, a putative thioredoxin system encoded in a single operon, contributes to both Cu and redox stress tolerance in *Salmonella*. This system, absent in *Escherichia coli* but present in other enteropathogens, may play an important role in the formation of virulence factors, and therefore emerge as a putative anti-virulence target. ScsB, ScsC and ScsD carry putative Cu-binding motifs in their periplasmic thioredoxin-like domains, while ScsA carries a peroxidase motif in its periplasmic domain. Regarding the importance of periplasmic copper homeostasis for *Salmonella* virulence, we tested the intracellular survival of both WT and a *scsABCD* deleted mutant in macrophages invasion assay, both in the presence and absence of Cu. We found that this system is important for survival inside macrophages in the presence of Cu. To verify this system´s expression under copper treatment, we generated a transcriptional reporter fusing gfp to the scs promoter and transformed it in the wild-type (WT) strain and in a mutant in the two-component system that controls *scsABCD* transcription, ?*cpxR*/*A*. Cu induction was confirmed in the WT, both in rich and minimal media, and as expected, fluorescence was barely detected in the ?*cpxR*/*A* strain. Concerning the Scs proteins, our studies focused on the characterization of ScsA and ScsD. ScsA contains a putative lipobox at its N-termini that predicts the protein could be an outer membrane lipoprotein, whereas ScsD is predicted to be an integral inner membrane protein. By adding a 3xFLAG epitope at the 3' end of the chromosomal copy of scsA and scsD genes, we were able to verify the intracellular localization of these proteins by western-blot. ScsD-3xFLAG was detected at 1 h after exposure of 1 mM Cu and remaining constant over time. By contrast, the expression of ScsA-3xFLAG is transient, since it was detected at 1.5 h after Cu treatment and became undetectable after 5 h. This indicates that ScsA, the only member of the system not linked to Cu-tolerance, could be the target of a periplasmic protease and

suggests that it may have a regulatory role on Scs activity. These results contribute to understanding the role of the Scs system in the metal/redox managing in the *S. enterica* envelope and its importance for virulence.

Palabras clave: Salmonella - copper - redox stress - thioredoxins