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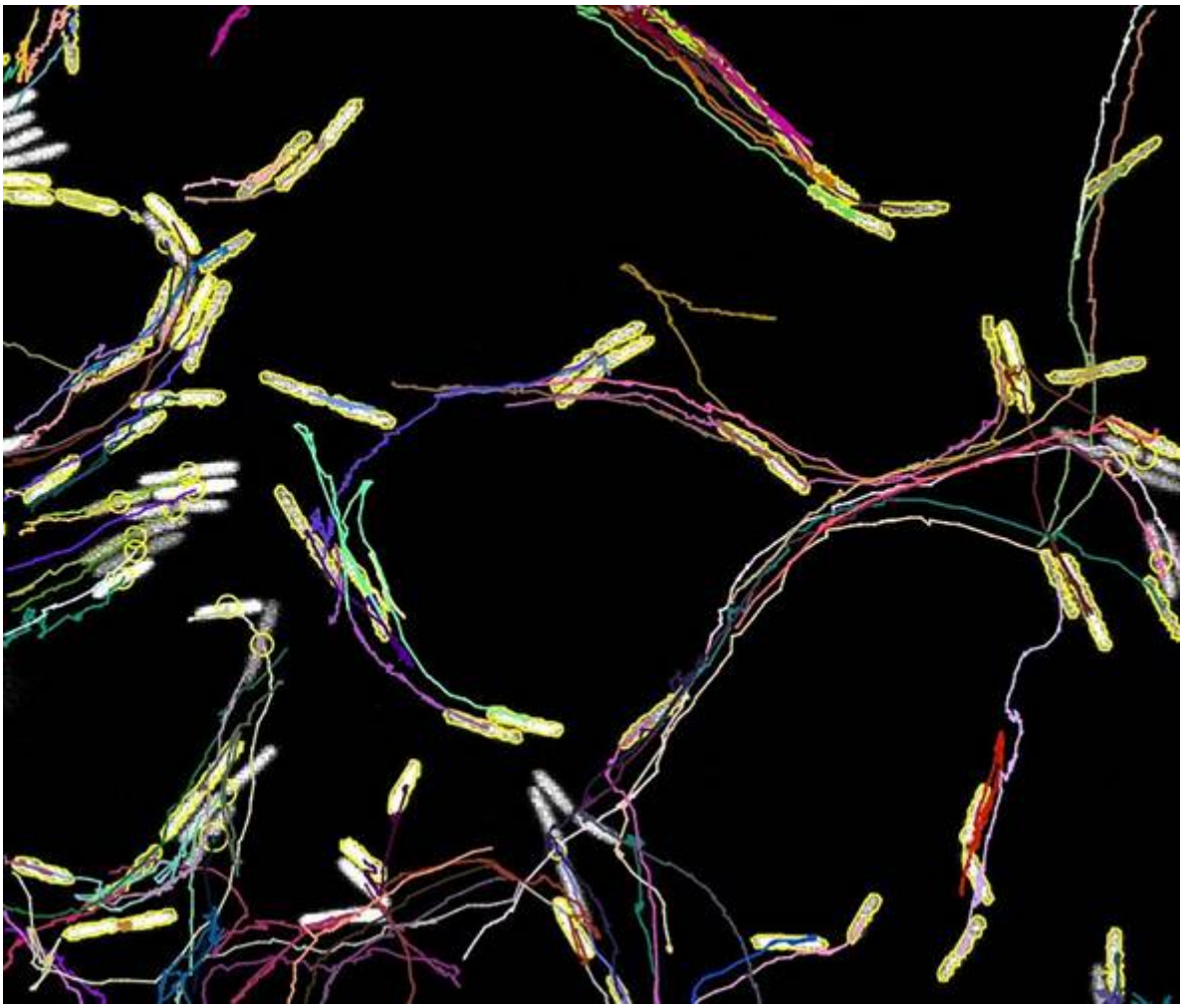

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

A NOVEL INNER-MEMBRANE TRANSPORTER INVOLVED IN COPPER HOMEOSTASIS IN Salmonella

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Salmonella enterica serovar *Typhimurium* (*S. Typhimurium*) is a Gram-negative, facultative anaerobic bacillus belonging to the *Enterobacteriaceae* family. This bacterium causes gastroenteritis in healthy individuals and systemic disease in immunocompromised or elderly people. Copper (Cu) homeostasis plays a crucial role in the interaction of *Salmonella* with the host. This metal, as a redox cofactor of enzymes, is essential for many biological processes but it is toxic in excess due to its ability to generate reactive oxygen and nitrogen species and to displace other metal ions from their binding sites. The host immune system exploits copper toxicity to combat infections in the phagosomes containing invading bacteria. Previously, our laboratory identified and characterized several components involved in *Salmonella* copper homeostasis, such as the periplasmic chaperone CueP, the inner membrane exporters CopA and GolT, and the transcriptional regulators CueR, GolS and CpxRA. Currently, we aim to elucidate how copper is mobilized from the periplasm to the cytoplasm. Recently, a *Pseudomonas aeruginosa* gene coding for a major facilitator superfamily (MFS) protein, was described. This gene is repressed in the presence of copper. We identified a homologue in *Salmonella*, that we named *cuiT*, and we setup a series of experiments to elucidate whether CuiT is involved in Cu import across the inner membrane. A *cuiT* mutant strain was generated using one-step mutagenesis method. Additionally, *cuiT* was cloned into an IPTG-inducible expression vector, with and without a 3XFlag tag. CuiT expression was confirmed by western blot analysis. To analyze the role of CuiT in Cu homeostasis, we tested the cuiT mutant or the CuiT-overexpressing strains for their sensitivity to CuSO?, both in liquid and solid media, and under different growth conditions. Although the ?*cuiT* did not show any growth defect, the strain overexpressing CuiT showed increased sensitivity to Cu ions compared to the wild-type strain, and this phenotype was more pronounced at lower incubation temperatures. This suggests that CuiT-overexpression promotes entry of the metal ion into the cytoplasm, increasing its toxic effects. Using atomic absorption spectroscopy, we found that in fact the CuiT-overexpressing strains accumulates more intracellular Cu than the wild-type strain. Accordingly, the mutant deleted in *cuiT* exhibited less Cu content than the wild-type, or the CuiT-complemented ? *cuiT* strain. These results suggest that CuiT acts as the inner-membrane Cu importer of *Salmonella*. How this transporter integrates into the *Salmonella* copper homeostasis and the particularities of its regulation are matters of current

investigation in our laboratory.

Palabras clave: SALMONELLA - COPPER - TRANSPORTER - GRAM NEGATIVE