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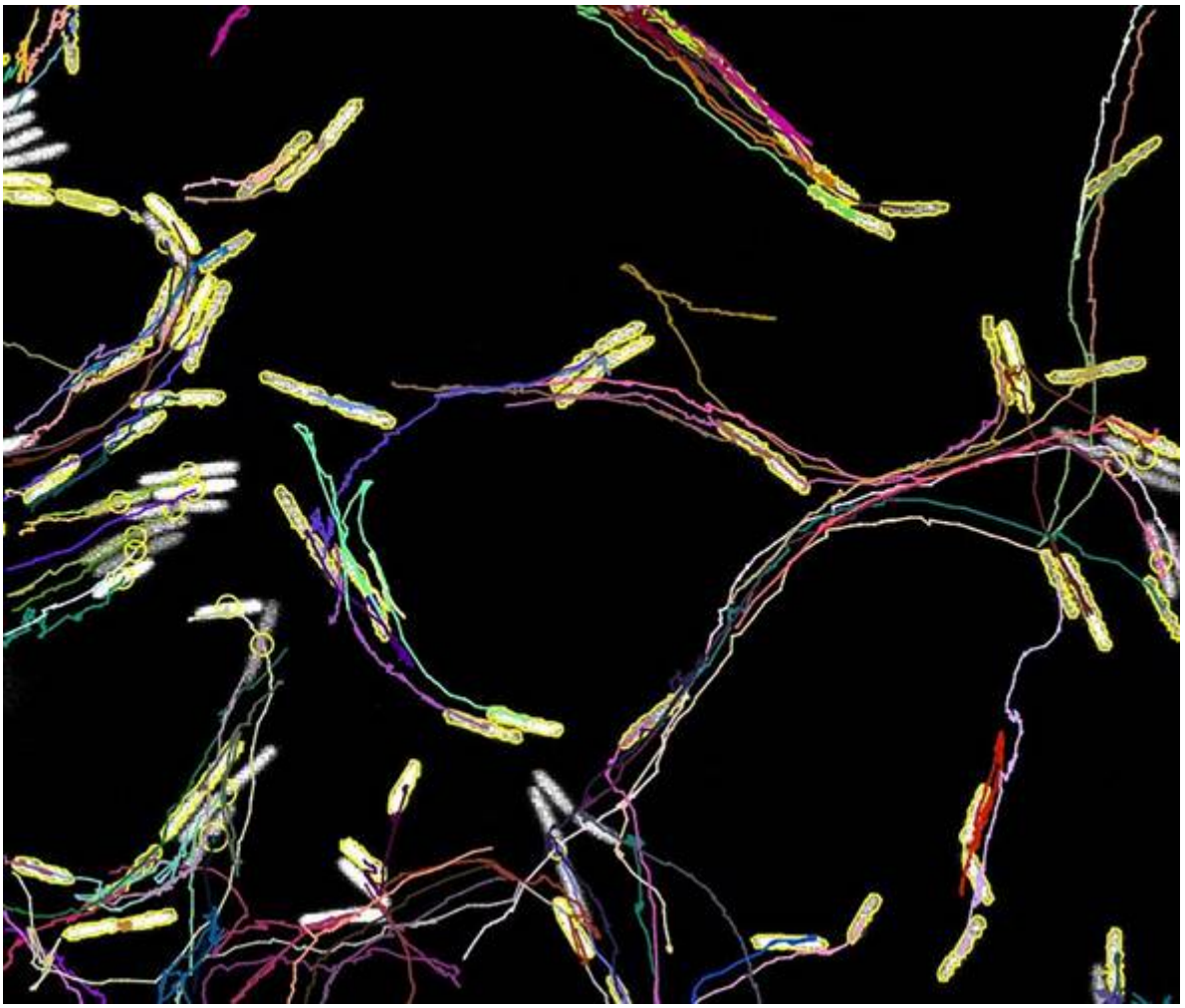

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

TAM SYSTEM IS INVOLVED IN PHOSPHOLIPID HOMEOSTASIS IN THE OPPORTUNISTIC PATHOGEN *Ochrobactrum anthropi*

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The precise assembly and remodeling of cell envelope structures in Gram-negative bacteria, especially the outer membrane (OM), is crucial for successful infection and resistance to diverse environments. The OM consists of an asymmetric lipid bilayer composed of lipopolysaccharide (LPS) on the outer leaflet and phospholipids (PLs) on the inner leaflet. The OM contains proteins (OMPs) with a ?-barrel structure and lipoproteins, some of which interact with the peptidoglycan. Despite considerable advances in understanding the mechanisms underlying OM biogenesis, certain processes are still unknown. Evidence was presented indicating that in gammaproteobacteria, the TAM system would be involved in the correct translocation of a subset of OMPs. TAM consists of TamA, a member of the Omp85 superfamily, and TamB, a large protein of the AsmA-*like* family that is inserted in the inner membrane (IM) by an N-terminal non-cleavable signal peptide while the rest of the protein is immersed in the periplasm. *Brucella* is an intracellular pathogen from the Alphaproteobacteria group that exhibits unique cell surface characteristics, making it highly resistant to various disruptive agents. While we found that certain OMPs may not be efficiently translocated to the OM in a mutant of the TamB homologue (MapB) of *Brucella suis*, several pieces of evidence indicate that MapB plays a crucial role in OM integrity. Due to the envelope features of *Brucella*, separating the IM and OM has not been possible, which hinders the identification of altered membrane components in a *mapB* mutant. For this reason, to understand the role of TAM in envelope biogenesis, *Ochrobactrum anthropi* was used as a closely related bacterial species. A mutant in the *mapB* homologue locus of *O. anthropi* was generated and envelope-related phenotypes were analyzed. This mutant showed increased sensitivity to lysozyme, SDS and vancomycin, confirming that MapB of alphaproteobacteria is required for cell envelope stability. Recent findings in *E. coli* suggested a possible function of TamB and other AsmA-*like* proteins in phospholipid (PL) translocation. To give insight into that hypothesis, we analyzed the PL composition of the IM and OM in the wt and ?*mapB* strains of *O. anthropi*. Highly enriched IM and OM fractions were successfully obtained by spheroplast preparation, French press disruption and sucrose gradient ultracentrifugation. To confirm IM and OM enrichment, NADH dehydrogenase activity and Western blotting with anti-OMPs were performed. The lipids were extracted by Bligh and Dyer method and organic fractions were analyzed by 1D and 2D NMR spectroscopy. Comparison of the lipid spectra from the IM and OM fractions of

the wt and the *mapB* mutant revealed significant differences in the relative amount and composition of PLs between both strains. These results provide evidence supporting the hypothesis that TAM plays a role in PL homeostasis in Alphaproteobacteria.

Palabras clave: Keywords: Outer membrane, Ochrobactrum anthropi, AsmA-like proteins, subcellular fractionation, phospholipid.