Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

22 al 25 de octubre del 2024
Centro cultural y Pabellón Argentina de la Universidad Nacional de Córdoba, Córdoba, ARGENTINA.

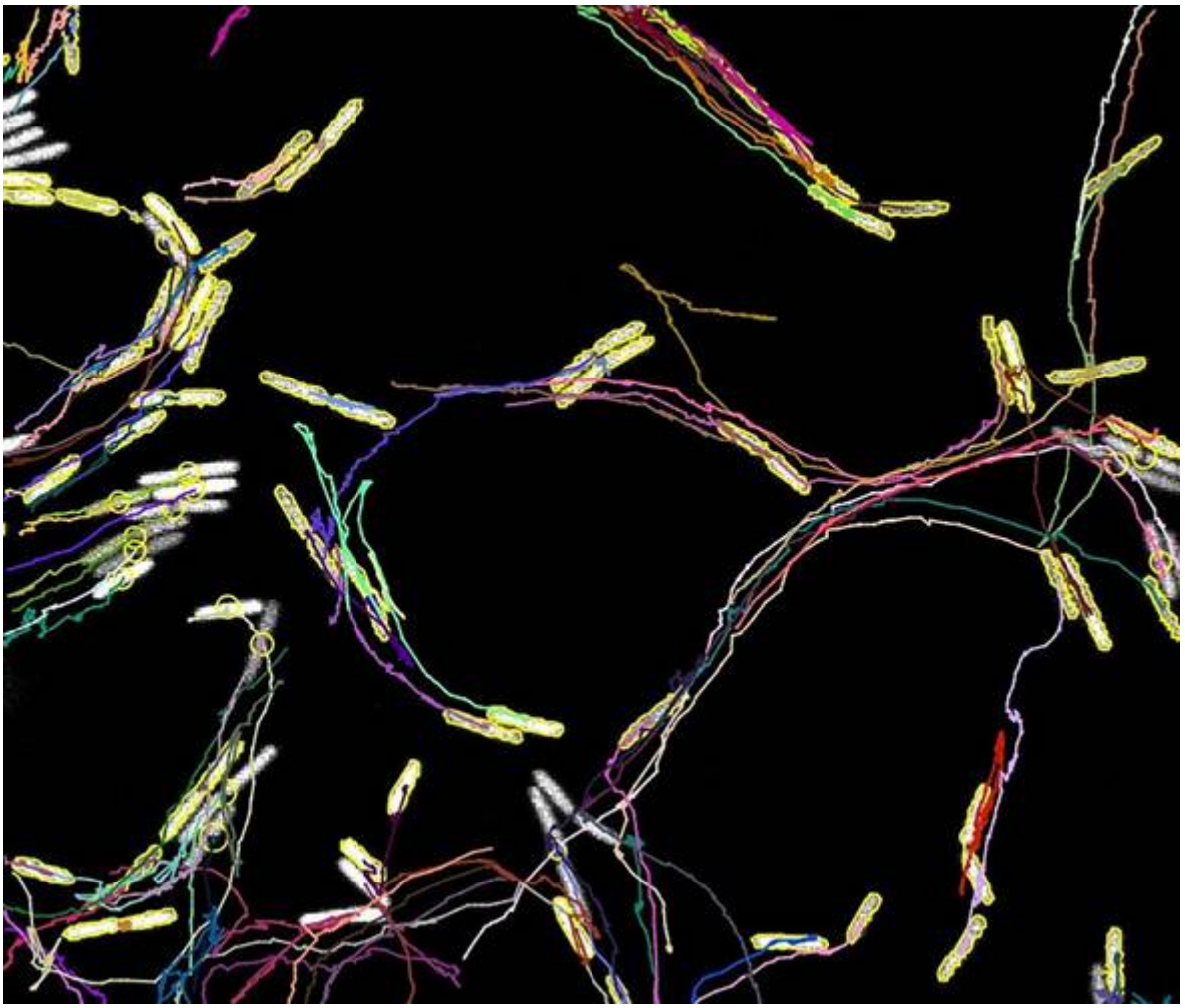

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

THE *Brucella abortus* T4SS EFFECTOR PROTEIN CYPB MODULATES HOST ACTIN DYNAMICS BY RECRUITING N-WASP TO THE *Brucella*-CONTAINING VACUOLE.

Hernández Oliva, Daleina-Pepe, María Victoria-Fernández, Agostina B.-Arias, Paula-Briones, Gabriel-Roset, Mara S.

Instituto de Investigaciones Biotecnológicas IIBIO-UNSAM-CONICET - San Martín - Buenos Aires - Argentina
Contacto: dhernandezoliva@iib.unsam.edu.ar

Brucella spp. is an intracellular pathogen responsible for brucellosis, a zoonotic disease with significant impacts on both human and animal health globally. The virulence of *Brucella* is closely associated with its ability to establish and maintain an intracellular niche within host cells. Previous research has demonstrated that the cyclophilins CypA and CypB are upregulated within *Brucella*'s intraphagosomal replicative niche, playing essential roles in stress adaptation, intracellular survival, and virulence. Cyclophilins, which are conserved protein-folding enzymes with peptidyl-prolyl cis-trans isomerase (PPIase) activity, are found in nearly all studied organisms. While CypA exhibits characteristics typical of Gram-negative bacterial cyclophilins, our findings reveal that CypB possesses features more closely resembling those of eukaryotic cyclophilins and is translocated into the host cell cytoplasm in a Type IV Secretion System (T4SS)-dependent manner upon *Brucella* internalization. This study identifies CypB as a critical bacterial effector protein for modulating host cell processes during infection. Through confocal microscopy and pull-down assays, we demonstrated that CypB interacts with N-WASP, a key regulator of the actin cytoskeleton. Furthermore, immunofluorescence analysis revealed that N-WASP is recruited to the *Brucella*-containing vacuole (BCV) upon infection in both bone-marrow-derived macrophages and J774.A1. This process was significantly impaired in the absence of a functional CypB, as shown in the *B. abortus ?cypAB* mutant strain. Our data also indicate that PPIase activity and CypB dimer formation are required for N-WASP recruitment. In contrast, CypA, which is not translocated into the eukaryotic cytoplasm, is not required for N-WASP recruitment to the BCV. To further elucidate the functional role of N-WASP during *Brucella* infection, we utilized the N-WASP inhibitor Wiskostatin, and a dominant-negative approach using a VCA-GFP fusion protein to sequester the Arp2/3 complex, thereby preventing actin polymerization. Both strategies effectively inhibited *Brucella* infection, highlighting the essential role of N-WASP in the bacterium's intracellular lifecycle. In summary, our results demonstrate that the *Brucella* T4SS effector protein CypB is necessary for the recruitment of N-WASP to the BCV, a process indispensable for the pathogen's successful infection and intracellular survival.

Palabras clave: Brucella-Cyclophilin B (CypB)-Effector protein-N-WASP-Intracellular infection