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.

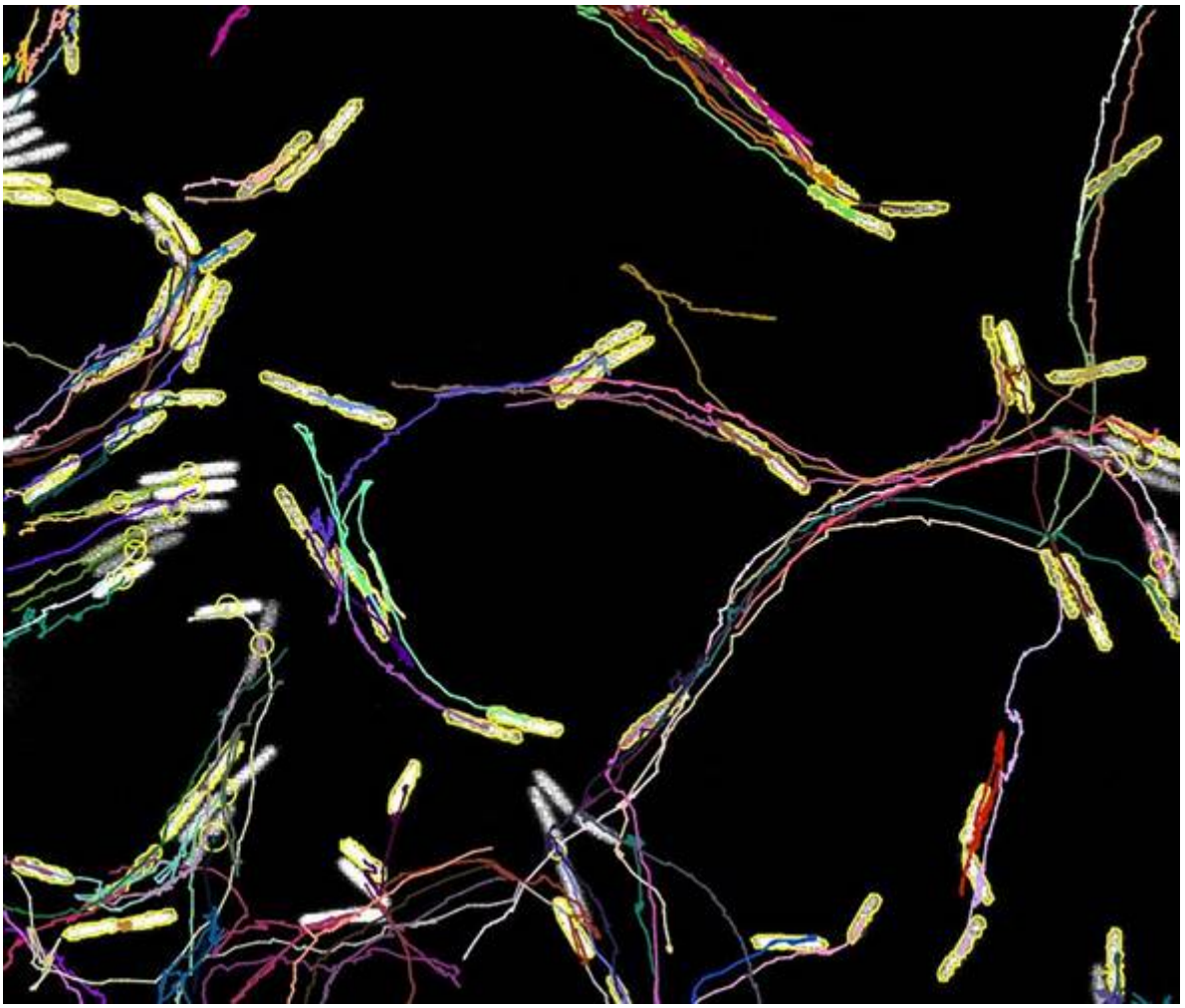

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

PERIPLASMIC PROTEIN HOMEOSTASIS AND ITS IMPACT ON BACTERIAL RESISTANCE: A STUDY AT ATOMIC RESOLUTION IN LIVING CELLS.

González, Lisandro J [1]- Hita, Francisco J [1] - Pontoriero, Letizia [2] - Pierattelli, Roberta [2] - Binolfi, Andrés [1] - Vila, Alejandro J [1]

1) Instituto de Biología Molecular y Celular de Rosario (IBR), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (UNR), Ocampo y Esmeralda, Rosario S2000EZP, Santa Fe, Argentina.
2) CERM and Department of Chemistry Ugo Schiff, University of Florence, Via Luigi Sacconi6, 50019, Sesto Fiorentino, Italy.
Contacto: hita@ibr-conicet.gov.ar

The bacterial periplasm is a dynamic and permeable compartment that functions as a sensitive interface between the cell and environmental signals. In this space, various proteins operate, participating in nutrient absorption, cell wall metabolism, virulence mechanisms, and antibiotic resistance. The homeostasis of these proteins depends on a specific protein quality control (PQC) mechanism that ensures the proper functioning of the periplasm. However, the small size of this compartment has hindered the use of tools to study these processes at the molecular level in their native state, so current knowledge about periplasmic PQC has been mainly based on in vitro biochemical studies. In this work, we used general biochemical techniques, confocal microscopy, and nuclear magnetic resonance in living cells (In-cell NMR) to describe the PQC mechanism of a periplasmic protein at the atomic level, locating and dissecting the role of the proteases that degrade it. As a model system, we studied NDM-1, a clinically relevant periplasmic membrane-associated metallo-?-lactamase. This enzyme becomes destabilized after zinc metal deprivation and is degraded by the periplasmic proteases Prc and DegP. NDM is distributed homogeneously in the membranes, while the proteases DegP and Prc show node and polynode localization in the bacterial periplasm, respectively. The In-cell NMR experiments allowed us to identify the recognition and cleavage patterns of each protease, which differ from those obtained in in vitro experiments. Structurally, it was identified that Prc's substrate recognition mechanism depends on the presence of ?-sheets in the substrate and their pairing with a specific domain of the protease. Coincidentally, a lack of cleavage sites in and around ?-helices was observed for both enzymes. Moreover, our results show a concerted mechanism in which Prc initiates the degradation of NDM at one of its membrane proteolytic nodes, and then DegP completes the degradation in the periplasm, further hydrolyzing the peptides produced by Prc. This work provides a novel approach to studying complex systems within the bacterial periplasm in living cells with atomic resolution. It details the localization and mechanism of action of the

proteases Prc and DegP, in living bacteria, and expands knowledge of the degradation pathway of a key enzyme involved in antibiotic resistance. As a research tool, it offers detailed and physiologically relevant structural information that could contribute to the discovery of new therapeutic targets to combat infectious diseases.

Palabras clave: Antibiotic Resistance - Metallo-?-lactamases - Metal Deprivation - Protein Quality Control – In-cell NMR