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.

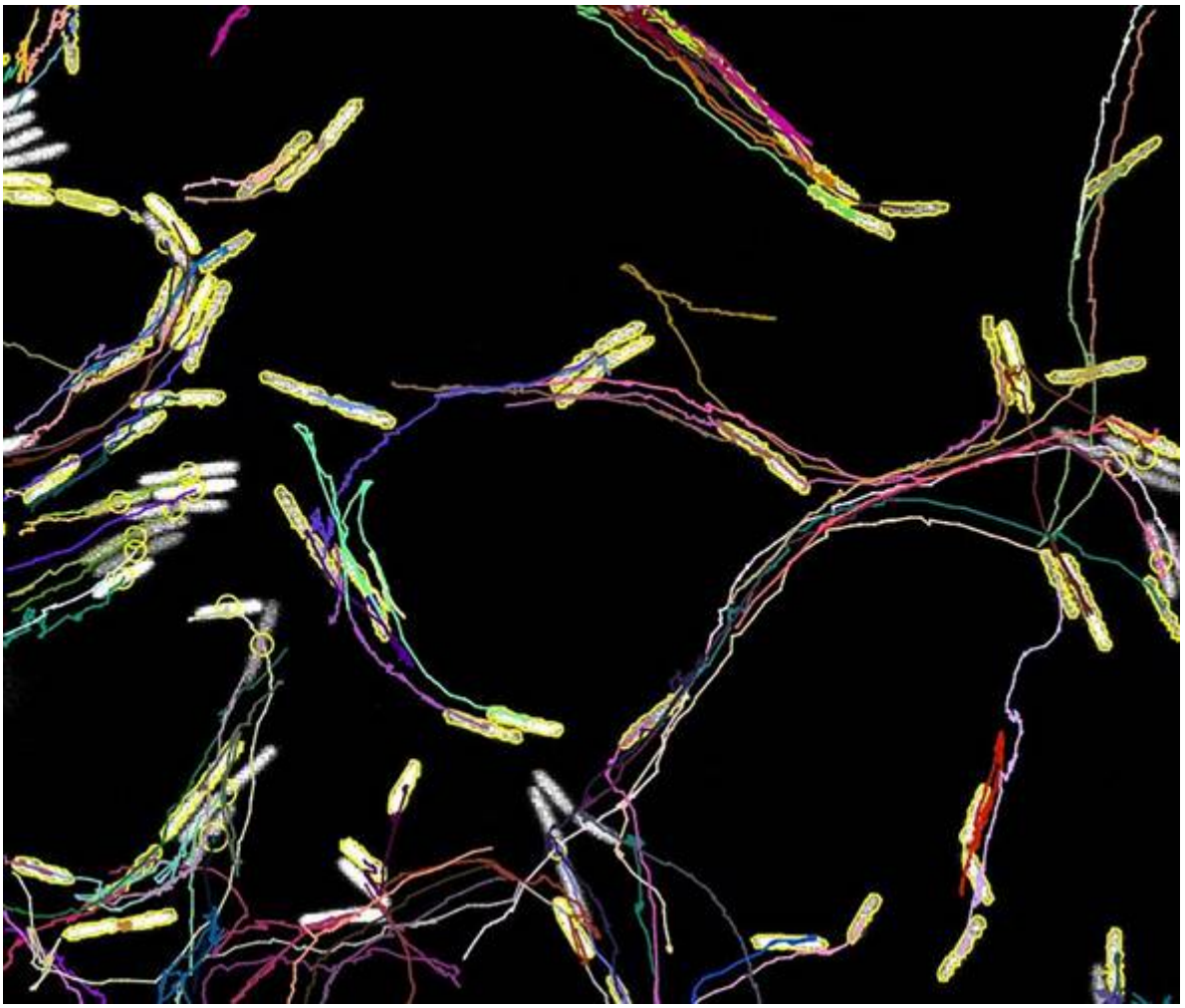

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

RoI of internal dynamics on metal homeostasis by AztC, a solute binding protein from Paracoccus denitrificans

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Zinc is an essential micronutrient for all domains of life. In zinc-limited environments, such as those encountered by bacterial pathogens during the host's nutritional immunity response, pathogens acquire zinc by expressing ATP-binding cassette (ABC) transporters. These transporters rely on solute binding proteins (SBPs) to deliver zinc to the membrane-bound ABC machinery with high affinity and specificity. Understanding how these proteins ensure metal homeostasis is, therefore, crucial. In Paracoccus denitrificans, zinc homeostasis is partially modulated by AztC, the SBP of the AztABCD transport system. Upon zinc-binding, AztC switches between "open" and "closed" conformations in the apo and zinc-bound state, respectively. Interaction with the ABC membrane complex triggers allosteric changes that allow the release of zinc from AztC, though the exact mechanism remains unclear.

In this work, we aim to study whether dynamic changes in the loop elements modulate zinc binding and delivery in AztC. NMR triple-resonance $^{1}H/^{13}C/^{15}N$ experiments were carried out to assign AztC, and ^{15}N relaxation measurements were performed to obtain backbone dynamic parameters, including longitudinal relaxation (T_1), transverse relaxation (T_2), and ^{15}N-{^{1}H} NOE. Our results indicate that holo-AztC maintains a significant degree of loop flexibility on the sub-nanosecond time scale, while rigidification upon Zn-binding occurs in other structural motifs rather than the loops. Based on these observations, we designed and biochemically characterized a novel point-mutant of AztC (D279S) lacking one of the zinc ligands from the C terminal domain, where most of the structural changes occur upon zinc binding. This mutant still binds zinc with high affinity, but with an exceptionally fast off rate, and presents altered fluorescent behavior relative to the wild type. Currently, we are characterizing the impact of this mutation on the internal dynamics, as well as the physiological impact of this mutation in vivo. We hope that these results will elucidate the mechanism by which SPBs ensure metal homeostasis in bacteria.

Palabras clave: Solute-binding protein – ABC transporters – Zinc – Metal homeostasis