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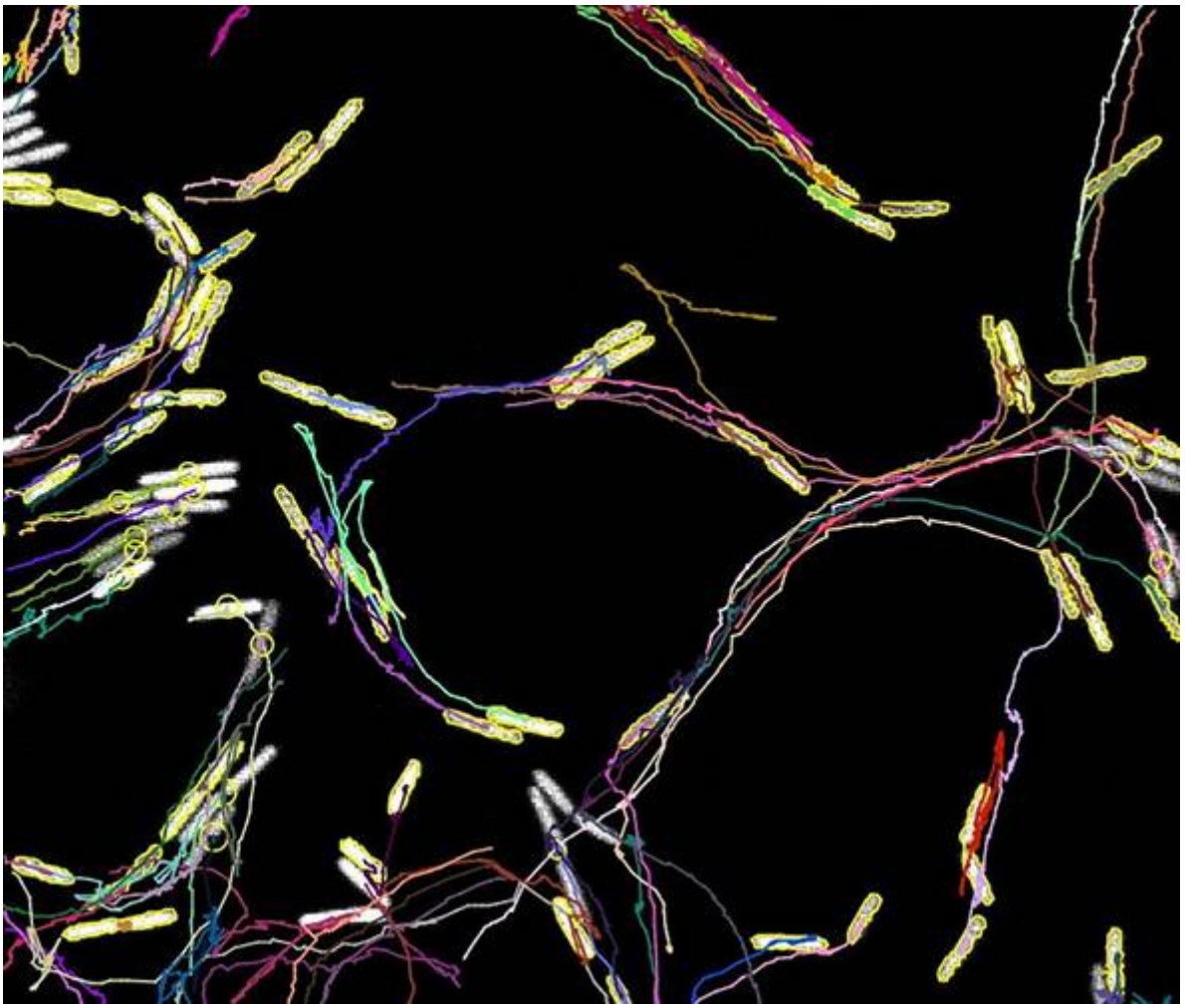

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

CHARACTERIZING RcgA-DOMAINS AND THEIR IMPACT ON RHIZOBIAL CONJUGATIVE TRANSFER

Cabrera, María Delfina - Castellani, Lucas - Luchetti, Abril - D'addona, Catalina - Torres Tejerizo, Gonzalo

Instituto de Biotecnología y Biología Molecular, CONICET - UNLP, La Plata, Buenos Aires, Argentina
Contacto: delfinacabrera@biol.unlp.edu.ar

Rhizobia are gram-negative bacteria known for their symbiotic relationship with leguminous plants. These bacteria often carry plasmids that can be transferred through conjugation. The conjugative transfer (CT) of plasmids is one of the primary mechanisms contributing to bacterial adaptation and diversification. Regulation of this process is important because plasmid transfer imposes a significant energetic cost on the bacteria, as it must express a large number of genes encoded in the DNA transfer and replication (Dtr) and mating pair formation (Mpf) regions. There are two well-studied systems regulating CT in rhizobia. One of them is mediated by Quorum Sensing (QS), where a signal molecule (AHLs, acyl homoserine lactones) produced by the *traI* (*luxI*-like) gene binds to the TraR regulator, which then activates the expression of conjugative genes in response to bacterial density.

According to their TraA (relaxase) evolutionary branch, rhizobial plasmids are categorized into groups I, II, III and IV. Group I comprises plasmids primarily regulated by QS and is subdivided into I-A, I-B, I-C, and I-D. Within Group I-C, plasmid pLPU83a from *Rhizobium favelukesii* LPU83 serves as a model. The regulatory network governing its transfer has been studied recently. pLPU83a contains a *traR* gene within its conjugation locus, yet it lacks a traI gene in the conjugative region. In addition, CT does not respond to AHLs, suggesting the presence of a novel regulatory system for rhizobial plasmid transfer. One of the newly actors identified in pLPU83a for this CT mechanism is *rcgA*, which is essential for CT. It is located between the Dtr and Mpf regions, and it is organized in tandem with another gene, *rcgR*. RcgA was predicted to be a hypothetical protein with transmembrane domains, although its function remains unknown.

In this work, we used bioinformatics and molecular biology tools to study whether the whole gene is necessary or if certain domains of RcgA are enough for the CT of plasmid pLPU83a. After protein alignment studies, we observed two conserved regions. Thus, we generated a neomycin-tagged pLPU83a *rcgA* mutant and three plasmids for complementation assays: pBBR-1MCS5 carrying an entire copy of the deleted gene, its N-terminus, or its C-terminus, respectively. Plasmid pLPU83a is conjugative, but the *rcgA* mutant is no longer conjugative. However, the phenotype is restored when complemented with the entire copy of the *rcgA* gene. This is not the case when complemented with either of its conserved regions; in both cases, the phenotype is not restored.

The results of the CT frequency assays indicate that the rcgA gene must be complete to restore the conjugative phenotype, suggesting that it is essential in its entirety for conjugative transfer. Upcoming work will focus on further elucidating the molecular system regulating CT in Group I-C plasmids.

Palabras clave: Conjugation – Plasmids – Rhizobia – Regulation