New mechanisms and new models of DNA replication and repair.
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Description and research lines.
We stablished our independent research group in 2018 at Instituto de Investigaciones Biomédicas Alberto Sols, but we still maintain a close relationship with the former laboratory of Prof. Margarita Salas in the Centro de Biología Molecular Severo Ochoa

We are devoted to the functional and biochemical characterization of DNA replication and repair enzymes. For that, we prefer to work with simple systems, like bacterial viruses or genetic mobile elements. Our current efforts are focused in two models, bacteriophages from the genus **Betatectivirus** and **pipolins**, a widespread genetic mobile element recently characterized in our lab.

1. Molecular characterization of betatectiviruses.
Tectiviruses are tailless, icosahedral bacterial viruses, containing an inner membrane that can infect Gram-negative (*Alfatectivirus*) or Gram-positive (Beta- and Gammatectivirus) bacteria. This group of viruses has gained relevance in recent years, thanks to various studies highlighting their ecological distribution and evolutionary relationship with eukaryotic DNA viruses. During the last years, we have characterized the viral DNA polymerase and SSB as well as the mechanism of protein-primed

genome replication initiation of Bam35 virus, the model virus of the genus Betatectivirus. More recently, aiming to characterize and understand our working model, we also got involved in the use of yeast two-hybrid (Y2H) to analyze protein-protein interactions (PPIs). We combined the traditional Y2H with high-throughput sequencing for increased sensitivity. Thus, we generated and thoroughly analyzed a genomic library of Bam35′s host B. thuringiensis HER1410 and screened interactions with all the viral proteins using different combinations of bait–prey couples. Initial analysis of the raw data enabled the identification of over 4000 candidate interactions, which were sequentially filtered to produce 182 high-confidence interactions that were defined as part of the core virus–host interactome. This work resulted in a better understanding of the Bam35–B. thuringiensis interaction at the molecular level and holds great potential for the generalization of the Y2H-HTS approach for other virus–host models.

2. Primer-independent DNA polymerases (piPolB) as the hallmark of, pipolins, novel genetic mobile elements in bacteria.

The piPolB group comprises enzymes with unexpected features, like the capacity to initiate DNA replication "de novo", without the requirement of a pre-existing primer. Overall, piPolB holds great promise for developing novel biotechnological applications that we are currently exploring.

Moreover, piPolBs are encoded by pipolins, highly flexible and dynamic elements present in a wide range of bacteria, including several opportunistic pathogens, such as *Escherichia coli* or *Staphylococcus* sp. We are very interested in the mobilization and biological role of pipolins as well as in their occurrence and diversity of pipolins both (meta)genomic databases and in circulating pathogens.

Selected publications (last 5 years).

1: Lechuga A, Lood C, Berjón-Otero M, Del Prado A, Wagemans J, van Noort V, Lavigne R, Salas M, Redrejo-Rodríguez M. Unraveling Protein Interactions between the Temperate Virus Bam35 and Its *Bacillus* Host Using an Integrative Yeast Two Hybrid-High Throughput Sequencing Approach. Int J Mol Sci. 2021 Oct 14;22(20):11105. doi: 10.3390/ijms222011105.

2: Lechuga A, Kazlauskas D, Salas M, Redrejo-Rodríguez M. Unlimited Cooperativity of *Betatectivirus* SSB, a Novel DNA Binding Protein Related to an Atypical Group of SSBs From Protein-Primed Replicating Bacterial Viruses. Front Microbiol. 2021 Jun 29;12:699140. doi: 10.3389/fmicb.2021.699140.

3: Ordóñez CD, Lechuga A, Salas M, Redrejo-Rodríguez M. Engineered viral DNA polymerase with enhanced DNA amplification capacity: a proof-of-concept of isothermal amplification of damaged DNA. Sci Rep. 2020 Sep 14;10(1):15046. doi: 10.1038/s41598-020-71773-6.

4: Flament-Simon SC, de Toro M, Chuprikova L, Blanco M, Moreno-González J, Salas M, Blanco J, Redrejo-Rodríguez M. High diversity and variability of pipolins among a wide range of pathogenic *Escherichia coli* strains. Sci Rep. 2020 Jul 27;10(1):12452. doi: 10.1038/s41598-020-69356-6.

5: Redrejo-Rodríguez M, Ordóñez CD, Berjón-Otero M, Moreno-González J, Aparicio-Maldonado C, Forterre P, Salas M, Krupovic M. Primer-Independent DNA Synthesis by a Family B DNA Polymerase from Self-Replicating Mobile Genetic Elements. Cell Rep. 2017 Nov 7;21(6):1574-1587. doi: 10.1016/j.celrep.2017.10.039.

6: Berjón-Otero M, Lechuga A, Mehla J, Uetz P, Salas M, Redrejo-Rodríguez M.

Bam35 Tectivirus Intraviral Interaction Map Unveils New Function and Localization of Phage ORFan Proteins. J Virol. 2017 Sep 12;91(19):e00870-17. doi: 10.1128/JVI.00870-17.

PhD Thesis.

- Caracterización Molecular del bacteriófago Bam35. Mecanismo de replicación del DNA y estudio del interactoma proteico.
  Mónica Berjón Otero, UAM 2017
- Disclosing Bacillus virus Bam35 and its host. Identification and characterization of the viral SSB, host genomic characterization and phage-bacteria interactome.
  Ana Lechuga Mateo, UAM 2020

Funding

- Insights into Pipolins diversity and dynamics in a wide range of pathogenic bacteria.
  PI: Modesto Redrejo Rodríguez.
  Founder: UAM, 1/1/2022 – 31/12/2023 .
  Grant Ref. SI3-PJI-2021-00271.
- Comprehensive virus-host protein interactome by the use of yeast-two-hybrid system coupled to next-generation sequencing analysis (VirHost-omics).
  PI: Modesto Redrejo Rodríguez (since November 2019).
  Founder: Fundación Ramón Areces, 1/4/2019-31/3/2022.
  Grant Ref.: CIVP19A5940.
- Primer-independent DNA polymerases and their applications in biotechnology and biomedicine.
  PI: Modesto Redrejo Rodríguez
  Founder: Agencia Estatal de Investigación, 1/1/2019-30/9/2022
  Grant Ref.: PGC2018-093723-A-I00.