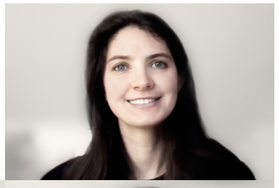
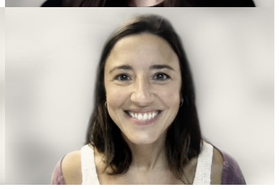
GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

Rafael Fernández Leiro
Junior Group Leader

Post-Doctoral Fellow
Maria Dolores Moreno

Graduate Students
Ester Casajús, Samuel Míguez



OVERVIEW

Safeguarding genetic information is essential to all forms of life. Two key cellular processes keep it free from errors: DNA replication and repair. Importantly, when they do not work correctly, genetic information may be damaged or lost, ultimately leading to disease. Deregulation and malfunction of the protein machinery that safeguards our genome are a hallmark of cancer, but it remains unclear how this happens at the molecular level. The devil is in the detail, and we aim to understand what and when something goes wrong with these molecular machines, so that we can act on it to correct it as well as to prevent it from happening.

"Combined with other approaches already established at the CNIO, we use cryo-Electron Microscopy to study diverse macromolecular complexes involved in cancer to an unprecedented level of detail."

These macromolecules are like real-life machines, with intricate mechanisms that allow them to perform their activities. To understand how they work, we use cryo-electron microscopy and biochemistry in an integrative approach. Beyond fundamental research, this structural information provides the necessary detail for drug development.

Technician
Araceli Grande (TS)*

Titulado Superior (Advanced Degree)

Students in Practice
Alba De Haro (until July) (*Universidad Autónoma de Madrid*, Madrid, Spain), Alberto López Francos (February-July) (*Universidad Autónoma de Madrid*, Madrid, Spain), Anna Martina Lippert (since April) (*Universidad Internacional Menéndez Pelayo*, Madrid, Spain)

Visiting Scientist
Svein Isungset Stove (University of Bergen, Bergen, Norway)

RESEARCH HIGHLIGHTS

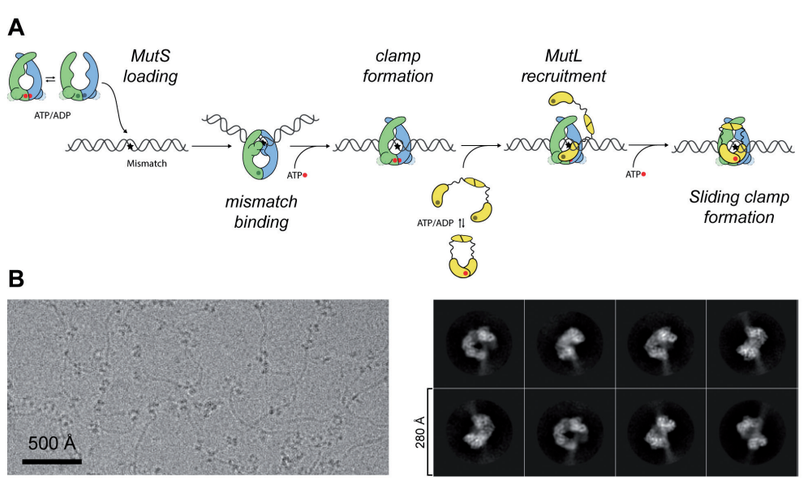
Mismatch repair

The DNA mismatch repair machinery (MMR) corrects the errors introduced by DNA polymerases during DNA replication and is critical for genome stability. The MutS protein loads onto newly synthesised DNA and searches for mismatches. The recognition of an error in the DNA leads to an ATP-dependent conformational change that transfers MutS into a sliding clamp state. Only this MutS state can activate the MutL ATPase, which, in turn, promotes the removal of the DNA for repair. These protein complexes are incredibly dynamic and flexible. Because of this, critical steps of this process have remained elusive to structural analysis. Using cryo-Electron Microscopy (cryo-EM), we captured multiple functional steps and studied the conformational changes that these proteins undergo to recognise the mismatch and license the downstream events that lead to repair. These studies were carried out in collaboration with Titia Sixma (Netherlands Cancer Research Institute) and Meindert Lamers (Leiden University).

DNA replication and repair - focus on mitochondria

Eukaryotic cells have 2 genomes: nuclear and mitochondrial. However, how the mitochondrial genome's integrity is maintained through the equilibrium between DNA replication, repair and degradation, and organelle dynamics, remains unclear. We are interested in understanding these pathways because of their implications for ageing and disease and, in particular, their relationship to cancer. By combining *in vitro* reconstitution of DNA replication complexes with cryo-EM imaging, we aim to capture the replication machinery at different functional stages, allowing us to understand in detail its mechanisms and how it is regulated. ■

FIGURE Mismatch repair studies. (**A**) Scheme representing the initial stages of the DNA mismatch repair pathway: mutS loading and DNA scanning, mismatch binding, clamp formation, mutL recruitment, and sliding clamp formation. These steps control the licensing of DNA repair. (**B**) Cryo-EM micrograph of MutS protein on DNA (long strings) (left) and 2D class averages of the protein after image processing (right). These images are used for high-resolution structural analysis.



PUBLICATIONS

- Zimmermann F, Serna M, Ezquerra A, Fernandez-Leiro R, Llorca O, and Luders J (2020). Assembly of the asymmetric human γ-tubulin ring complex by RUVBL1-RUVBL2 AAA ATPase. *Sci Adv* 6, eabe0894.
- Dodd T, Botto M, Paul F., Fernandez-Leiro R, Lamers MH, and Ivaylo Ivanov I (2020). Polymerization and editing modes of a high-fidelity DNA polymerase are linked by a well-defined path. *Nat Commun* 11, 5379.
- López-Perrote A, Hug N, González-Corpas A, Rodríguez CF, Serna M, García-Martín C, Boskovic J, Fernandez-Leiro R, Cacres JF, Llorca O (2020). Regulation of RUVBL1-RUVBL2 AAA-ATPases by the nonsense-mediated mRNA decay factor DHX34, as evidenced by Cryo-EM. *ELife* 9, e63042.
- Míguez Amil S, Jiménez-Ortega E, Ramírez-Escudero M, Talens-Perales D, Marín-Navarro J, Polaina J, Sanz-Aparicio J, Fernandez-Leiro R (2020). The cryo-EM structure of *Thermotoga maritima* β-galactosidase: quaternary structure guides protein engineering. *ACS Chem Biol* 15, 179-188.