GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

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OVERVIEW

Safeguarding genetic information is essential to avoid malignant transformation. Two key cellular processes keep it free from errors: DNA replication and DNA repair. Importantly, when these 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 all hallmarks 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 goes wrong with these molecular machines, and when, so we can act on it to correct it and prevent it from happening.

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 (cryo-EM) and biochemistry in an integrative approach. Beyond fundamental research, this structural information provides the necessary detail for drug development.

"Macromolecules are like real-life machines. To understand how they work, we study their structures using cryo-EM. Beyond basic research, this provides the necessary information for drug development."

Technician
Araceli Grande (TS)*

**Titulado Superior* (Advanced Degree)

Students in Practice
Emma Areán (Bachelor's Degree Final Project; Master's Thesis, *Univ. Complutense de Madrid*, Spain), Carlos Chacón (until June) (Bachelor's Degree Final Project, *Univ. Autónoma de Madrid*, Spain), Raquel Cuesta (Bachelor's Degree Final Project; Master's Thesis, *Univ. Complutense de Madrid*, Spain), Marta Seijo (*Univ. Complutense de Madrid*, Spain)

RESEARCH HIGHLIGHTS

DNA replication & repair - focus on mitochondria

Mitochondrial DNA (mtDNA) replication is critical for human health. Deficiencies in the operation of mtDNA replication machinery underlie various devastating multi-systemic mitochondrial disorders. Importantly, mtDNA defects have been linked to other prominent diseases, including Parkinson's and Alzheimer's disease, autism spectrum disorders, diabetes, and several cancer types. 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, particularly their relationship to cancer.

Genome integrity - focus on telomeres

Telomeres are essential nucleoprotein structures that protect the end of our chromosomes. These structures are shaped by the protective shelterin complex that specifically binds to telomeric TTAGGG DNA repeats. Shelterin is composed of 6 proteins – TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 – and their proper arrangement and function protect telomeres from degradation and activation of a persistent DNA damage response. Shelterin function is therefore crucial for telomere and genome integrity. Despite the key role of the shelterin complex in cell viability and tissue homeostasis, as well as its potential use as a target for anti-cancer therapeutic strategies, its mechanistic details and architecture are poorly understood.

Biochemistry & cryo-EM

By combining *in vitro* reconstitution and native purification of protein-DNA complexes and by taking advantage of the recent developments in cryo-EM imaging, we can capture these protein machineries in different functional states to study their structures. With this information we will be able to unveil their molecular mechanisms, rationalise pathological mutations and their physiological consequences, and aid in the development of future cancer therapeutic strategies. ■

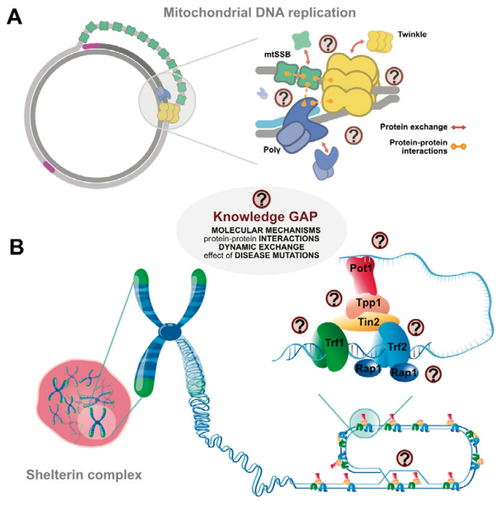

FIGURE 1 (**A**) Mitochondrial DNA replication machinery. (**B**) The shelterin complex shapes, protects, and regulates enzymatic activities at telomeres. Several key aspects of their regulation, and the molecular mechanisms through which both protein complexes exert their activities, remain unknown.

PUBLICATION

- Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver A.W, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.

PATENT

- Lamers MH, Fernández-Leiro R (2022). Device and method for cryogenic electron microscopy sample preparation. *Dutch Patent Application No. 2033291*.