GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

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OVERVIEW

Safeguarding the genetic information is essential to all forms of life. Two key cellular processes keep it free from errors: DNA replication and 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 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 to the highest level of detail what and when things can go wrong with these molecular machines, so we can act on it to correct it and prevent it from happening.

"The high-end cryo-electron microscopy setup at the CNIO allows us to look at every detail of the cell's protein machinery, so that we can understand how it works and intervene."

These macromolecules are like real life machines, with intricate mechanisms that enable 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.

RESEARCH HIGHLIGHTS

Mismatch repair

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

DNA replication & repair - focus on mitochondria

Eukaryotic cells have two genomes: nuclear and mitochondrial. However, how the integrity of the mitochondrial genome 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 relation to cancer.

Cryo-electron microscopy (cryo-EM)

Combined with many other approaches already established at the CNIO, we use cryo-EM to study diverse macromolecular complexes involved in cancer. Significant recent technological developments in microscopes, detectors and image processing tools have significantly improved the resolution of the technique, enabling the structural analysis of many elusive macromolecules to an unprecedented level of detail. Last year, we worked together with the Óscar Llorca Group and the EM Unit to bring the cryo-EM facility at the CNIO to a state-of-the-art level. Moreover, we have been awarded access to high-end microscopes at the Biological Electron Bio-Imaging Centre (eBIC) in Oxford (UK). We can now efficiently prepare samples and solve their structures, using the in-house facilities, to a high level of detail. ■

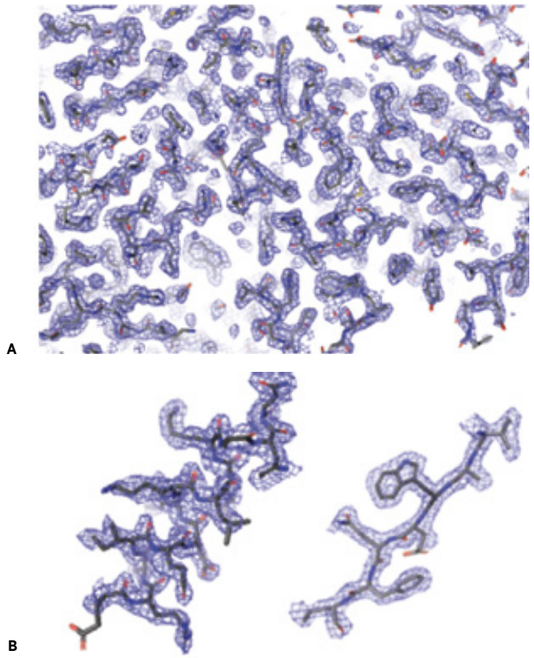

Figure High-resolution reconstruction of a protein structure from the lab to 2.1Å resolution (to be published). (**A**) Shows the overall atomic density of the protein, and (**B**) shows close-ups of density and model with clear signals for amino-acid side-chains.

AWARDS AND RECOGNITION

- Awarded with a *Ramón y Cajal* Fellowship, Spanish Ministry of Science, Innovation and Universities.