

GENOME INTEGRITY AND STRUCTURAL BIOLOGY JUNIOR GROUP

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**Titulado Superior* (Advanced Degree)



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 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 what and when something goes wrong with these molecular machines, so that we can act 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

“Using cryo-EM, we have captured the DNA mismatch repair machinery in multiple functional steps and studied conformational changes that these proteins undergo to recognise the mismatch and license the events that lead to repair.”

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

Students in Practice
Emma Arean (since September)
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Visiting Scientist
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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. Recognition of an error in 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-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 T. Sixma

(*Netherlands Cancer Research Institute*) and M. Lamers (*Leiden University*).

DNA replication & 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, particularly 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. ■

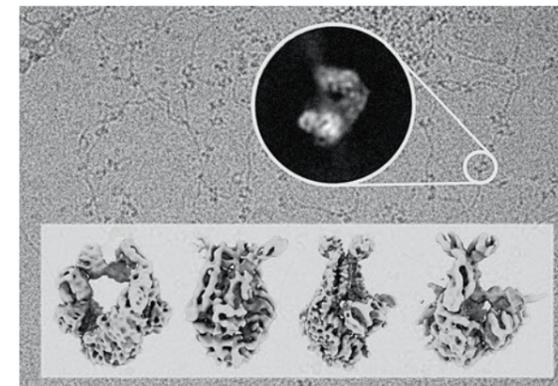


FIGURE 1 Mismatch repair studies. The background of the image shows a cryo-EM micrograph of MutS protein (white circle) on DNA (long strings). Circular inset shows a 2D class average of the protein after image processing. The bottom rectangular insert highlights the multiple structures solved in the

successive steps of the DNA repair process: mutS loading and DNA scanning, mismatch binding, clamp formation, and mutL recruitment and sliding clamp formation. These steps control the licensing of the DNA mismatch repair pathway.

PUBLICATIONS

► Fernández-Leiro R*, Bhairosing-Kok D, Kunetsky V, Laffebler C, Winterwerp HH, Groothuizen F, Fish A, Lebbink JHG, Friedhoff P, Sixma TK*, Lamers MH* (2021). The selection process of licensing a DNA mismatch for repair. *Nat Struct Mol Biol* 28,

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