

DNA Replication *Junior Group*

Summary

We study DNA replication in mammalian cells using a combination of biochemistry, molecular biology, and mouse genetics. Our main goal is to understand the molecular events regulating replication origin activation and fork progression. We are particularly interested in the MCM complex – investigating its activity, regulation, and associated factors. We are also interested in the genome-wide regulation of DNA replication which correlates with chromatin structure, nuclear position, and developmental stage and have developed several mouse models to address the effects of deregulated DNA replication *in vivo*.

Strategic Goals

- Characterise proteins that activate human origins of replication, e.g. the MCM complex and its associated factors
- Elucidate the spatial-temporal programme of DNA replication at the genomic level in primary and transformed cells
- Evaluate the effects of deregulated DNA replication *in vivo* using genetically modified mice

Juan Méndez *Junior Group Leader*

Juan Méndez was born in 1967 and received his BSc degree in Biochemistry and Molecular Biology in 1990 from the *Universidad Autónoma de Madrid*. He was a graduate student in the laboratory of M. Salas at the *Centro de Biología Molecular "Severo Ochoa"*, where he studied the molecular mechanisms of bacteriophage DNA replication. His PhD Thesis (1995) received First Class Honours from the *Universidad Autónoma de Madrid*, the *Premio Juan Abelló* from the Spanish *Real Academia de Doctores*, and the Glaxo Wellcome Prize for Biomedical Research.

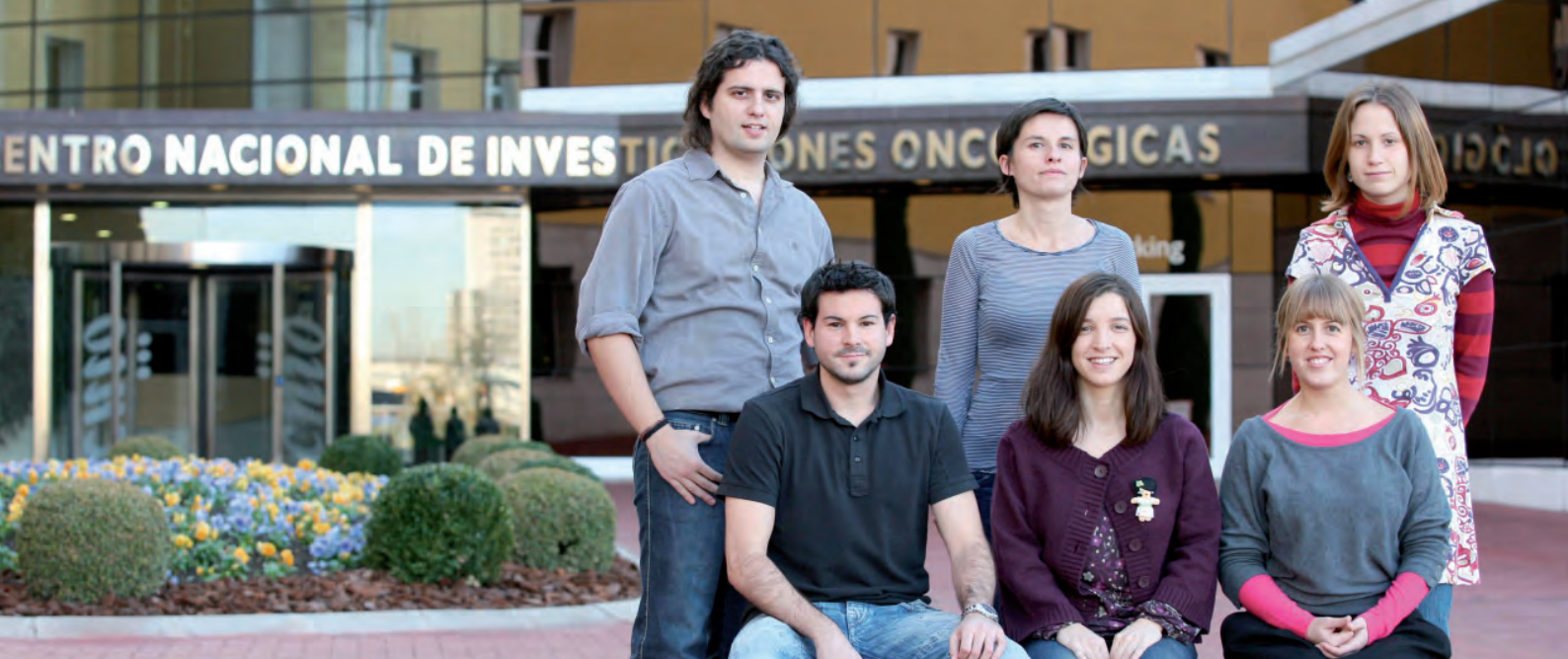
From 1997 – 2004 he conducted his postdoctoral research with B. Stillman at the Cold Spring Harbor Laboratory, New York, USA, during which time he characterised the function and regulation of human DNA replication proteins ORC, Cdc6 and MCM.

In collaboration with S.I. Reed's Group at The Scripps Institute, La Jolla, California, USA, he demonstrated that cyclin E deregulation – a common event occurring in cancer cells – interferes with the activity of replication origins, leading to inefficient DNA replication and chromosome instability.

During his postdoctoral tenure, Méndez was supported by fellowships from the *Fundación Ramón Areces*, Human Frontiers Science Programme Organization, and the US Department of Defense (Breast Cancer Programme).

In October 2004 he was appointed a *Ramón y Cajal* Investigator and joined the CNIO as Junior Group Leader, the Molecular Oncology Programme.





Post-doctoral fellow: Emmanuelle C. Guillou. **Graduate students:** Silvia Álvarez (since December), Tomás Aparicio, Sabela Búa, M. Fernanda Rodríguez (since December), Arkaitz Ibarra. **Technician:** Mónica López (until October).

Highlights

Identification of the human CDC45-MCM-GINS complex (CMG)

The GINS complex binds to replication origins before the onset of S phase and travels with the replication forks after initiation. Using new antibodies to detect endogenous hGINS in cells and tissues, we studied its expression, abundance, subcellular localisation, and association with other DNA replication proteins. During S phase, hGINS becomes part of a Cdc45-MCM-GINS (CMG) complex that assembles on chromatin. Down-regulation of hGINS destabilises CMG, causes a G1-S arrest, and slows down DNA replication, effectively blocking cell proliferation. Our working model illustrates that CMG is part of the replicative DNA helicase (Figure).

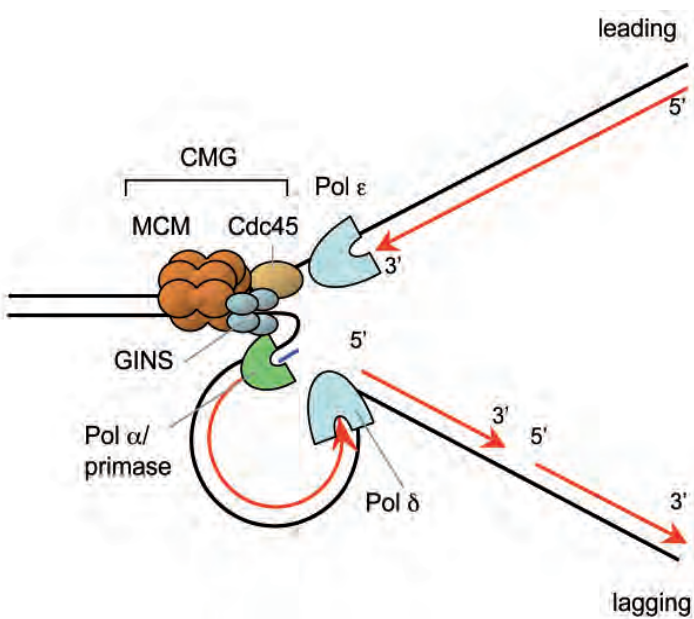


Figure: Proposed role for the CMG complex at the replication fork. The GINS tetramer is part of the CMG DNA helicase, acting as a bridge between the MCM hexamer and the Cdc45 monomer. hGINS also interacts with ssDNA.

Cohesin affects DNA replication

In a search for new MCM-interacting partners we identified three components of Cohesin, the complex that maintains sister chromatid cohesion and also functions as a transcriptional insulator. Down-regulation of Cohesin delays S-phase progression by reducing the number of active origins without affecting fork speed. We found that Cohesin binds to replication origins and mediates the formation of intra-chromosomal loops that likely serve as the replicon units. This observation provides new clues regarding how DNA replication is organised in the context of higher-order chromatin structure.

DNA replication timing correlates with association to the nuclear lamina

We have completed a map on the replication timing (RT) of the human genome using comparative genomic hybridisation. The RT programme is robust and conserved within different primary and tumour cell lines. We found that late-replicating chromosomal domains – characterised by an increased fragility and mutational rate – correlate almost perfectly with their association to the nuclear lamina. This observation provides a novel link between the RT programme and nuclear structure.

Publications

Chuang LC, Teixeira LK, Wohlschlegel JA, Henze M, Yates JR, Méndez J, Reed SI (2009). Phosphorylation of Mcm2 by Cdc7 promotes pre-replication complex assembly during cell-cycle re-entry. *Mol Cell* 35, 206-219.

Méndez J (2009). Temporal regulation of DNA replication in mammalian cells. *Crit Rev Biochem Mol Biol* 44, 343-351.

Aparicio T, Guillou E, Coloma J, Montoya G, Méndez J (2009). The human GINS complex associates with Cdc45 and MCM and is essential for DNA replication. *Nucleic Acids Res* 37, 2087-2095.