

DNA replication *Junior Group*

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Juan Méndez

Junior Group Leader

Juan Méndez obtained his BSc (1990) and PhD in Biochemistry and Molecular Biology (1995) from the *Universidad Autónoma de Madrid* (UAM). As a graduate student he worked with M. Salas at the *Centro de Biología Molecular "Severo Ochoa"*. His PhD Thesis on bacteriophage DNA replication received First Class Honours from the *Universidad Autónoma de Madrid*, the *Premio Juan Abelló* from the *Real Academia de Doctores* and the Glaxo Wellcome Prize for Biomedical Research.

From 1997-2004 he was a Postdoctoral Fellow at B. Stillman's Group, Cold Spring Harbor Laboratory, New York (USA). During this tenure he was supported by the *Fundación Ramón Areces*, Human Frontiers Science Program Organization and the US Department of Defense (Breast Cancer Programme) and studied the functions of human DNA replication proteins ORC, Cdc6 and MCM, as well as the impact of cyclin E deregulation in the DNA replication process in cancer cells.

In October 2004 he was awarded a Research Contract from the *Ramón y Cajal* Programme and appointed as Junior Group Leader in the CNIO's Molecular Oncology Programme.

Summary

We study the molecular mechanisms of DNA replication in mammalian cells using a combination of biochemistry, cell biology and mouse genetics. Our research interests cover three main areas. First, the characterisation of proteins that are recruited to origins of replication to assemble the DNA helicase machinery, such as the mini-chromosome maintenance (MCM), Cdc45 and the GINS complex. Second, the mechanisms behind the organisation of "DNA replication factories" during S phase and their spatio-temporal activation programme. Findings have recently unveiled the role of proteins, previously known to regulate sister chromatid cohesion, in DNA replication.

Thirdly we focus on the development of "gain of function" and "loss of function" mouse models in DNA replication genes. These models are used to study effects of deregulated DNA replication *in vivo*.

Strategic Goals

- Characterise the mini-chromosome maintenance (MCM) complex and its associated factors
- Elucidate the molecular organisation of DNA replication factories
- Study the effects of deregulated DNA replication *in vivo* using genetically modified mice





Staff scientist: Silvana Mouron (since September). **Post-doctoral fellows:** Christine Guillou (until June) and Sara Rodríguez (since September). **Graduate students:** Silvia Álvarez, Tomás Aparicio (until November), Sabela Búa, Arkaitz Ibarra (until October) and M. Fernanda Rodríguez (until July).

Highlights

Over the last year we have discovered that Cohesin, the complex required for sister chromatid cohesion which also functions as a transcriptional regulator, interacts with MCM proteins and is enriched at replication origins. Down-regulation of Cohesin in human cells slows down S-phase progression and reduces the efficiency of DNA synthesis at replication foci.

Using extended fibres to visualise replication in single DNA molecules, we found that cellular levels of Cohesin are proportional to the number of active origins without affecting fork speed. Our model is that Cohesin contributes to the architectural organisation of replication factories by bringing together clusters of origins and looping out inter-origin DNA. The size of

the chromosomal loops is proportional to the length of the replicon units.

We have also generated two mouse models with genetic alterations in key DNA replication proteins. The first model is a transgenic strain in which CDC6 is over-expressed in stratified epithelia under the control of the keratin 5 promoter. K5-CDC6-tg mice are viable and their life expectancy is similar to that of wild type animals but they also show an enhanced skin fitness at older ages (>20 months). This effect may be related to the increased loading of MCM complexes onto chromatin in the keratinocytes, which confers them a replicative advantage under situations of stress.

In the second model, a GFP-Luciferase reporter gene has been inserted at the 3' UTR of *MCM3*, allowing for the identification of tissues with MCM expression (Figure). In addition, given that exons 14-17 of *MCM3* are flanked by LoxP sites, we generated heterozygous *MCM3*^{+/-} mice by crossbreeding *MCM3*^{loxGFPLuc} mice with a tool strain expressing Cre recombinase under the CMV promoter. We are currently using both models to study the effects of MCM levels on the maintenance of genomic stability.

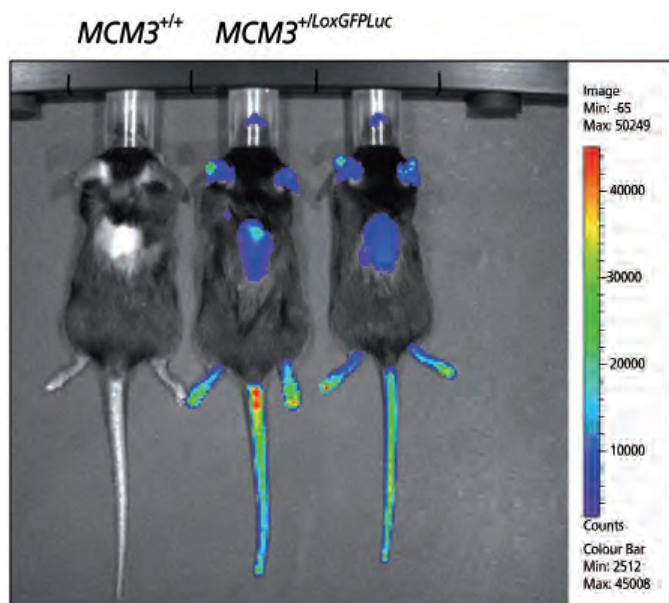


Figure: *In vivo* detection of luciferase activity in the skin in *MCM3*^{+/-LoxGFPLuc} mice. An area of back fur was shaved to expose the skin. Mice were photographed using an IVIS optical imaging device (CNIO's Molecular Imaging Core Unit) 10 min after an intraperitoneal injection of luciferin (150 mg/kg body weight). A wild type (*MCM3*^{+/+}) littermate is shown as negative control.

Publications

Guillou E, Ibarra A, Coulon V, Casado-Vela J, Rico D, Casal I, Schwob E, Losada A, Méndez J (2010). Cohesin organizes chromatin loops at DNA replication factories. *Genes Dev* 24, 2812-2822.

Méndez J (2010). Cyclin E goes nuts: a cell cycle regulator affects male fertility. *Cell Cycle* 9, 4782-4787.