

Chromosome Dynamics *Junior Group*

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Ana Losada

Junior Group Leader

Ana Losada, born in León in 1967, joined the CNIO as Junior Group Leader of the Chromosome Dynamics Group in October 2004. She obtained her PhD in Biochemistry and Molecular Biology in the laboratory of A. Villasante at the *Centro de Biología Molecular "Severo Ochoa"* (CSIC-UAM), Madrid. Her research aimed at identifying the DNA sequences that specify the centromeres of higher eukaryotes, using *Drosophila melanogaster* as a model system.

In October 1996 she joined T. Hirano's group at Cold Spring Harbor Laboratory in New York (USA) as a postdoctoral fellow supported first by a fellowship from the *Ministerio de Educación y Ciencia* (1997-1999) and later by a Special Fellowship from the Leukemia and Lymphoma Society (2001-2004). Using the *Xenopus* egg cell-free system, she identified the first cohesin complex from vertebrate cells. The importance of her studies on cohesin and the molecular mechanism of sister chromatid cohesion has been widely recognised in the field of chromosome dynamics.

In 2003 she obtained a Research Contract from the *Ramón y Cajal* Programme (funded by the *Ministerio de Educación y Ciencia*). A year later she returned to Spain to establish her own research Group at the CNIO where she has continued to work on the regulation of chromosome segregation.

Summary

Our Group is interested in the mechanisms that contribute to faithful chromosome segregation and prevent aneuploidy, a condition that is found in many human tumours and is the major cause of miscarriages and birth defects.

We are interested in understanding this regulation at the molecular level and the consequences of its malfunction in the context of a mammalian organism. We study two aspects that are central to chromosome segregation: the specification of centromeres and the regulation of sister chromatid cohesion.

Strategic Goals

- Elucidate the mechanisms of CENP-A incorporation at centromeres
- Understand the function and regulation of cohesin
- Establish mouse models to address the role of cohesin in development and cancer





Staff scientist: Ana Cuadrado (since April). **Post-doctoral fellow:** Rafael Bernad (until August). **Graduate students:** María Carretero, Iva Krizaic (since November), Silvia Remeseiro, Teresa Rivera (until October), Patricia Sánchez and Ángel Serrano (until March). **Technician:** Miriam Rodríguez.

Highlights

The mechanism of CENP-A incorporation. Centromeric Protein A (CENP-A), the epigenetic mark of the centromeres, must be replenished each cell cycle to compensate for the dilution associated with DNA replication. Little is known however about how this is achieved mechanistically. We have developed an assay using *Xenopus* egg extracts that recapitulates the spatial and temporal specificity of the CENP-A deposition observed in human cells. This deposition depends on xHJURP, a member of the HJURP/Scm3 family of CENP-A chaperones recently identified in yeast and human cells (Figure). In collaboration with G. Almouzni's group at the *Institut Curie* (France), we have demonstrated that human HJURP can substitute for xHJURP even though there is little sequence homology between the two proteins. We have also

found that condensin, a protein complex involved in chromosome condensation, plays a key role in CENP-A nucleosome dynamics, affecting both the incorporation and stability of CENP-A.

Cohesin Mouse models. Cohesin mediates sister chromatid cohesion and is essential for chromosome segregation. Increasing evidence suggests that cohesin is also involved in the organisation of chromatin within the interphase nucleus, thereby regulating key processes such as gene expression and DNA replication. In humans, mutations in cohesin and its chromatin loader cause the Cornelia de Lange Syndrome (CdLS), characterised by a variety of developmental anomalies that include mental retardation and growth delay. Cohesin consists of four subunits: Smc1, Smc3, Rad21 and SA. There are three versions of the SA cohesin subunit in vertebrates: SA1, SA2 and SA3 – the latter being meiosis-specific. To date, the distinct functions of SA1 and SA2 remain unclear. We have found that mice lacking SA1 die during late embryogenesis. On the other hand, heterozygous mice do not show CdLS-like phenotypes. Instead, they have shorter life spans and a higher incidence of spontaneous tumour formation, compared to their wild type litter mates. Decreased SA1 also has no significant effect on tumour development upon p53 or PTEN inactivation. In contrast, tumourigenesis induced by carcinogens such as 3-MC or DEN is clearly delayed, most likely due to a decreased proliferative capacity of the tumour cells.

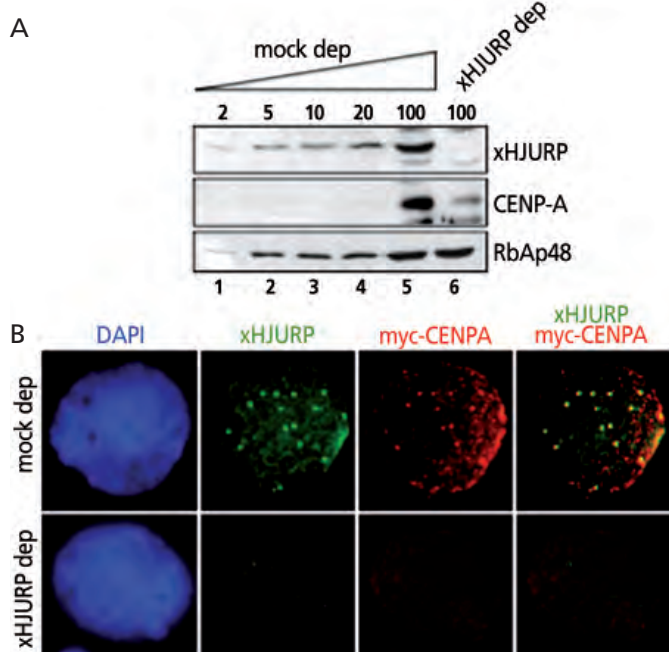


Figure: *Xenopus* HJURP (xHJURP) is required for CENP-A deposition at the centromeres. (A) Immunoblot analysis of egg extracts xHJURP immunodepleted (lane 6) or mock depleted (lanes 1-5). Partial co-depletion of CENP-A is observed. (B) Interphase nuclei assembled in mock depleted (top) and xHJURP depleted extracts (bottom) containing myc-CENPA were stained with antibodies against xHJURP and myc. No incorporation of myc-CENPA is observed in extracts lacking xHJURP.

Publications

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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.

Pié J, et al. (2010). Mutations and variants in the cohesion factor genes *NIPBL*, *SMC1A*, and *SMC3* in a cohort of 30 unrelated patients with Cornelia de Lange syndrome. *Am J Med Genet A* 152, 924-929.