Chromosome Dynamics *Junior Group*

Summary

Our Group is interested in the mechanisms that regulate 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 also in finding out the consequences of its malfunction in the context of a mammalian organism. We have chosen to study two aspects that are central to chromosome segregation: the specification of centromeres and the regulation of sister chromatid cohesion.

Strategic Goals

- Dissect the mechanisms of CENP-A deposition at centromeres
- Understand the function and regulation of Cohesin
- Address the role of cohesion factors in development and cancer

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 the 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. In 2006, her Group was selected by the New Established Team (NET) Programme of the EU-funded Epigenome Network of Excellence.

Post-doctoral fellow: Rafael Bernad. Graduate students: María Carretero, Silvia Remeseiro, Teresa Rivera, Patricia Sánchez, Ángel Serrano. Technician: Miriam Rodríguez.

Highlights

Centromere specification by CENP-A

In the active centromeres of most organisms a histone H3 variant known as CENP-A replaces histone H3. Little is known about how and when CENP-A is incorporated specifically into centromeric chromatin. We have developed an assay that measures CENP-A incorporation at centromeres of chromosomes assembled in *Xenopus* extracts. We have found that CENP-A deposition in this *in vitro* system is subject to the same spatial and temporal restrictions that apply in human cells and that the major players involved are highly conserved. The assay is currently being used to dissect the molecular mechanisms underlying CENP-A loading in detail.

The role of Shugoshin proteins in chromosome segregation

We previously characterised the role of Sgo1 – a member of the Shugoshin family of proteins – in the regulation of cohesion through its protection of centromeric cohesin in mitosis. We have now identified a second Sgo protein in *Xenopus*, Sgo2, and found that it is not involved in cohesion but instead participates in mitotic spindle assembly (in collaboration with H. Funabiki, Rockefeller University, New York, USA). Our results indicate that Sgo2 modulates the activity of Aurora B, one of the major mitotic kinases, towards proteins involved in microtubule dynamics such as MCAK and Op18.

Mouse models of cohesion factors

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 SA1-null mice die during embryogenesis. Cells derived from SA1-null embryos show low proliferation and increased aneuploidy. Our data suggest that SA1 is specifically required for telomere cohesion (Figure). We are currently analysing the effect of reduced dosage of SA1 on telomere function and tumourigenesis.

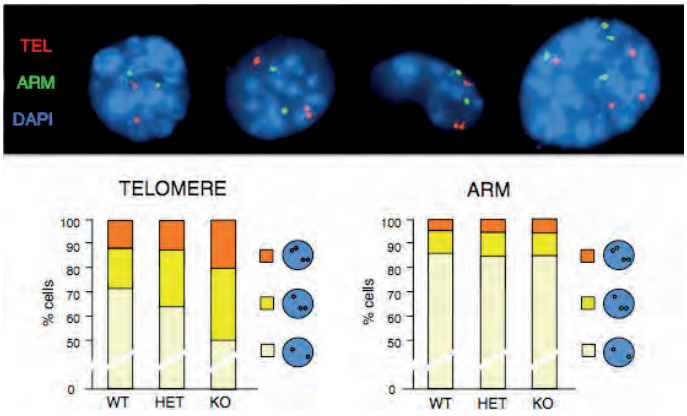

Figure: Defects in telomere cohesion – but not arm cohesion – can be observed in MEFs from SA1-KO embryos as judged by the separation of signals obtained by fluorescent *in situ* hybridisation with probes from the telomere (TEL, red) and arm (green) regions of chromosome 8. The analysis was carried out in MEFs from wild-type (WT), SA1 heterozygous (HET) and SA1 knock-out (KO) mice.

Publications

Errico A, Cosentino C, Rivera T, Losada A, Schwob E, Hunt T, Costanzo V (2009). Tipin/Tim1/And1 protein complex promotes Polalpha chromatin binding and sister chromatid cohesion. *EMBO J* 28, 3681-3692.

Rivera T, Losada A (2009). Shugoshin regulates cohesion by driving relocalization of PP2A in *Xenopus* extracts. *Chromosoma* 118, 223-233.

Bernad R, Sánchez P, Losada A (2009). Epigenetic specification of centromeres by CENP-A. *Exp Cell Res* 315, 3233-3241.

Serrano A, Rodríguez-Corsino M, Losada A (2009). Heterochromatin protein 1 (HP1) proteins do not drive pericentromeric cohesin enrichment in human cells. *PLoS ONE* 4, e5118.