

CHROMOSOME DYNAMICS GROUP

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OVERVIEW

Our research focuses on a protein complex named cohesin that embraces DNA to mediate sister chromatid cohesion, a process essential for chromosome segregation and faithful DNA repair by homologous recombination. Cohesin also plays a major role in the spatial organisation of the genome by promoting long-range DNA looping, which in turn contributes to transcriptional regulation. Mutations in cohesin have been found in several tumour types, most prominently in bladder cancer, Ewing sarcoma and acute myeloid leukaemia. Germ line mutations in cohesin and its regulatory factors are also at the origin of human developmental syndromes collectively known as cohesinopathies.

Our goal is to understand how cohesin works, how it is regulated and how its dysfunction contributes to cancer and other human diseases. In particular, we are intrigued by the existence of different versions of the cohesin complex. We use human cells and mouse models carrying *knock out* alleles of genes encoding variant cohesin subunits to investigate their functional specificity.

“We are dissecting the functional specificity of cohesin variant subunits to better understand how their mutation promotes carcinogenesis.”

RESEARCH HIGHLIGHTS

Cohesin consists of four core subunits, SMC1, SMC3, RAD21 and SA. There are two versions of the SA subunit in vertebrate somatic cells, SA1 and SA2. Loss of function mutations in the *STAG2* gene encoding SA2 have been identified in bladder cancer, Ewing sarcoma and myeloid malignancies, among others. In cells lacking cohesin-SA2, cohesin-SA1 performs the essential functions of cohesin related to cohesion. We suspect, however, that cohesin-SA1 cannot accomplish other functions of cohesin-SA2 related with chromatin organisation and gene regulation. Importantly, lack of cohesin-SA2 may also generate vulnerabilities that could be exploited in cancer therapy. We aim to identify the specific functions of the two variant complexes in chromatin architecture and gene regulation.

Dissecting the role of cohesin-SA1 and cohesin-SA2 in human cells

We analysed the genome-wide distribution of the two variant cohesin complexes in several human cell lines and applied functional genomics to assess their enrichment in different regulatory elements as well as their co-localisation with other factors involved in genome organisation such as CTCF. We then addressed how this distribution changes when one or the other variant is missing and the subsequent alterations in the transcriptome and in chromatin organisation, analysed by Hi-C in collaboration with M. A. Marti-Renom (CNAG-CRG). Our results show that the two complexes fulfil different functions (FIGURE 1). Cohesin-SA1 is important for the organisation of the topological domains or TADs, which make up the global structure of the genome, and works always alongside the CTCF protein. In contrast, cohesin-SA2 is more versatile and is capable of interacting with diverse transcription factors to form local chromatin loops that bring together enhancers and promoters. Cohesin-SA2 is also more dynamic in its chromatin association, and a larger fraction of cohesin-releasing factor Wapl is found associated with SA2 than with SA1.

In the absence of cohesin-SA1, cohesin-SA2 can still cooperate with CTCF to demarcate contact domains although border strength is decreased. In the absence of SA2, however, cohesin-SA1 cannot occupy the non-CTCF sites present at many enhancers. As a result, short-range, intra-domain contacts disappear while new, aberrant contacts are formed between neighbouring domains. These changes have more noticeable consequences for gene expression. Moreover, cohesin-SA2 is enriched at superenhancers, regulatory elements that control cell identity. Thus, the absence of this cohesin variant in tumour cells most likely alters their identity and/or affects cell differentiation.

Dissecting the role of cohesin-SA1 and cohesin-SA2 in mice

We have generated a conditional *Stag2* knockout allele in collaboration with Francisco X. Real (CNIO). Embryos lacking cohesin-SA2 die by mid-gestation and we are currently addressing the cause of this lethality. We are also using mouse embryo fibroblasts (MEFs) to further understand the specific contribution of cohesin-SA2 to cohesion and genome organisation. We observed loosened centromere cohesion and slower proliferation in the *Stag2* deficient MEFs, consistent with reports in other cell lines (FIGURE 2). However, the defects are milder than expected and are unlikely to be the sole cause of the embryonic lethality. Complementary to previous observations in *Stag1* deficient MEFs, in which the distribution of cohesin changed to include new non-CTCF positions, the number of cohesin binding sites detected in *Stag2* deficient MEFs is restricted to those overlapping with CTCF. This result is in line with the idea that cohesin-SA1 cannot replace cohesin-SA2 at many non-CTCF sites, as described in human cells. Experiments aimed to identify the molecular determinants of the distinct behaviour of the two cohesin variants are underway. ■

PUBLICATIONS

- Kojic A, Cuadrado A, De Koninck M, Giménez-Llorente D, Rodríguez-Corsino M, Gómez-López G, Le Dily F, Marti-Renom MA, Losada A (2018). Distinct roles of cohesin-SA1 and cohesin-SA2 in 3D genome organization. *Nat Struct Mol Biol* 25, 496-504.
- Morales C, Losada A (2018). Establishing and dissolving cohesion during the vertebrate cell cycle. *Curr Op Cell Biol* 52, 51-57.
- Mata-Garrido J, Tapia O, Casafont I, Berciano MT, Cuadrado A, Lafarga M (2018). Persistent accumulation of unrepaired DNA damage in rat cortical neurons: nuclear organization and ChIP-seq analysis of damaged DNA. *Acta Neuropathol Commun* 6, 68.

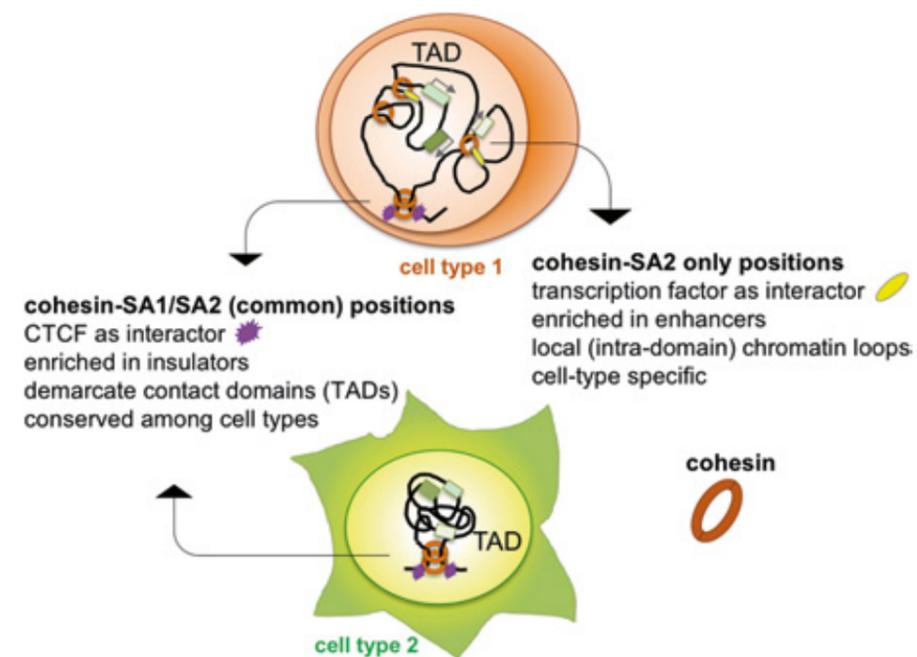


Figure 1 Graphical summary of the distinct features of cohesin variant distribution and function in genome organization in two cell types. TAD, topological associating domain encompassing several genes and demarcated by CTCF bound sites.

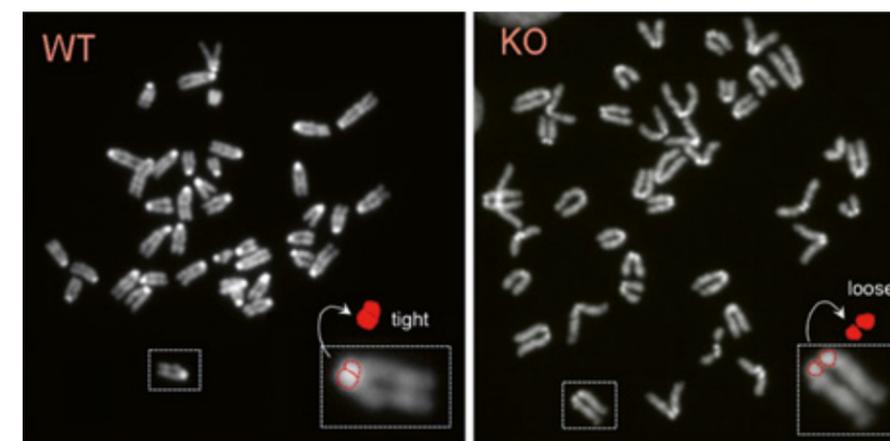


Figure 2 Metaphase spreads of mouse embryo fibroblasts with (WT) or without (KO) cohesin-SA2 reveal loosened centromere cohesion in the latter. The “tight” and

“loose” configuration of the sister centromeres in terms of cohesion is shown in the magnified chromosome at the bottom right.