Our research focuses on a protein complex named cohesin that is essential for chromosome organisation. Cohesin mediates sister chromatid cohesion and, thereby, ensures faithful DNA repair by homologous recombination and proper chromosome segregation during cell division. It 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, organisation of DNA replication factories and locus rearrangement by recombination. Mutations in cohesin have recently been found in several tumour types, most prominently in bladder cancer and acute myeloid leukaemia. Mutations in cohesin and its regulatory factors are also at the origin of a group of human 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 in somatic cells. We use mouse models carrying knock out alleles of genes encoding cohesin subunits to investigate their functional specificity, both at the cellular level and in the context of an organism. We also take advantage of the Xenopus egg cell-free system to explore additional aspects of cohesin regulation.

“We aim to define the specific contributions of cohesin-SA1 and cohesin-SA2 to genome organisation. Our work may uncover vulnerabilities in cancer cells carrying mutations in the gene encoding SA2, which is one of the twelve genes most mutated in cancer.”
Cohesin-S2A regulates transcription independently of CTCF

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. We have previously reported that cohesin-SA1 is required for telomere cohesion, while cohesin-SA2 plays a major role in centromeric cohesion. In terms of transcriptional regulation, much less is known about the potential differences between these two complexes. A study in HeLa cells reported a similar genomic distribution for cohesin-SA1 and cohesin-SA2 that largely overlapped with the distribution of the architectural protein CTCF. Our initial comparison of distribution and transcription profiles of wild type and SA1-null mouse cells suggested that cohesin-SA1 could be more important for the regulation of transcription than cohesin-SA2. First, we detected an almost double number of cohesin-binding sites genome wide in SA1 null cells, and the new sites displayed reduced overlap with CTCF and TSS (transcription start sites). Second, we found that in a number of genes whose expression was altered in SA1 null cells, cohesin-SA2 could not replace cohesin-SA1 efficiently since cohesin occupancy was clearly reduced in these cells (Remeseiro, Cuadrado et al., 2015). More recently, using a new and better SA2 antibody, we examined the binding of cohesin-SA1 and cohesin-SA2 along the genome of human mammary epithelial cells. We found more than 20,000 positions common for both complexes in which CTCF is also present, but also found around 10,000 cohesin-SA2-specific positions, lacking SA1, in which the overlap with CTCF is significantly lower. Importantly, these cohesin-SA2 specific positions are highly enriched in enhancers and promoters and contain transcription factor binding motifs other than CTCF. Proteomic analysis of immunoprecipitates obtained with SA1 and SA2 antibodies detected several transcription factors among the SA2-specific interactors. Functional analyses, in which we compare changes in gene expression, cohesin distribution as well as chromatin architecture changes after downregulation of SA1, SA2 or CTCF, further support a prominent role of cohesin-SA2 in promoting local interactions between cis-regulatory elements independently of CTCF, while cohesin-SA1 would instead collaborate with CTCF in the demarcation of domain boundaries (FIGURE 1).

Pds5 proteins regulate cohesin dynamics

Two factors are associated with chromatin-bound cohesin, Pds5 and Wapl. Wapl promotes cohesin unloading and in its absence there is an excess of cohesin on chromatin, and chromosome organisation is altered both in interphase and mitosis. The role of Pds5 is less clear. Moreover, there are two versions of Pds5 present in vertebrate cells: Pds5A and Pds5B. In order to explore their specific functions we previously generated murine Pds5 Knock out (KO) alleles for these two genes. We showed that both Pds5A and Pds5B contributed to cohesion establishment during S phase by promoting cohesin acetylation and Sororin binding, with Pds5B being specifically required for cohesion at centromeres (Carretero et al. 2013). Now we have observed that cells lacking Pds5A, Pds5B, or both, have distinct alterations in their transcription profiles when compared to wild type cells. Genome wide distribution of cohesin is not obviously altered in the absence of either Pds5 protein, but does change in the absence of both. Under this condition, the dynamic association of cohesin to chromatin, measured in Fluorescence Recovery After Photobleaching (FRAP) experiments, is significantly decreased. Much milder effects are observed in cells lacking only Pds5A or Pds5B. Abrupt accumulation of cohesin in axial structures, known as vermici, previously described in Wapl depleted cells, can be observed only in the absence of the two Pds5 proteins (FIGURE 2). Thus, Pds5 proteins are required for proper cohesin dynamics and cohesin distribution, although no clear specificities can be found for Pds5A and Pds5B, at least globally. The gene expression differences described above might therefore be caused by preferential interactions with chromatin regulators at specific loci. It is also possible that Pds5 proteins have functions independent of cohesin. We are currently exploring both these possibilities.

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