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. Germline 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 investigating the contribution of mutations in cohesin STAG2 to the aggressive phenotype of Ewing sarcoma, the second most common bone cancer in children, in order to improve diagnosis and treatment of these tumours.”
The spatial organisation of the genome inside the nucleus is critical for transcription, DNA replication and repair. Cohesin mediates 3D genome organisation by binding to chromatin and extruding DNA loops that become stabilised at several locations along the genome, most notably CTCF-bound sites (FIGURE 1A). In this way, the complex facilitates contacts between promoters and distal enhancers while restricting such interactions within topologically associated domains (TADs). Loop extrusion by cohesin also promotes intermingling of active/inactive chromatin compartments. There are two variants of the cohesin complex in all somatic vertebrate cells that carry SMC1A, SMC3, RAD21 and either STAG1 or STAG2. The association of these complexes to chromatin is modulated by additional proteins: NIPBL, PID55A/B, WAPL and ESCO1/2 acetyltransferases. Our studies in human and mouse cells deficient for STAG1 or STAG2 have identified differential contributions of the two complexes to genome architecture and transcriptional regulation. Cohesin-STAG1 plays a more important role in the demarcation of TADs, together with CTCF, and in counteracting compartmentalisation. Cohesin-STAG2 promotes more local chromatin contacts that are relevant for tissue-specific transcription independently of CTCF. Consistent with this, STAG1 is found almost exclusively at CTCF-bound sites while a fraction of STAG2 can be also detected at non-CTCF, NIPBL-bound cohesin positions along the genome (FIGURE 1B). Salt extraction of chromatin fractions and fluorescence recovery after photobleaching (FRAP) experiments show that binding of cohesin-STAG2 to chromatin is more salt sensitive and more dynamic, respectively, than binding of cohesin-STAG1. One factor contributing to this behaviour is the preferential association of STAG2 with cohesin releasing factor WAPL. We continue to explore the molecular determinants underlying these preferences and how they contribute to shape chromatin architecture.

**Distinct contribution of variant subunits and regulators to genome-wide distribution and dynamics of cohesin**

We address the role of cohesin in disease in two different lines of research. In the first one, we are interested in the consequences of cohesin dysregulation during development. Our analyses of murine embryos lacking STAG1 or STAG2 have revealed their differential requirements during embryonic development, which lead to lethality by mid-gestation. We plan to complete these studies by examining early developmental stages *in vivo* (FIGURE 2). *Ex vivo*, we are dissecting the contribution of each cohesin variant to mouse embryonic stem cell differentiation using the auxin-dependent degron technology. We have also collaborated with the group of Miguel Maizananares (CIBERSAM) to show that CTCF is required to establish proper chromatin structure in early embryos. Finally, we are investigating the pathophysiology of Cornelia de Lange Syndrome (CdLS), the most prevalent cohesinopathy, in collaboration with the group of Ethel Queralt (IDIBELL). Consistent with our previous analyses in mouse cells deficient for the cohesin loader NIPBL, studies in fibroblasts from CdLS patients show altered distribution of cohesin and transcriptional dysregulation.

The second line of research addresses how mutations in STAG2 promote metastasis in Ewing sarcoma (EWS). This is the second most frequent type of bone cancer in children, and it is driven by a fusion protein that alters the gene expression programme of the cell initiating the tumour. It is a highly aggressive cancer with a 5-year survival below 30% in patients that present metastases. Among the few recurrent mutations identified in EWS, in addition to the oncogenic fusion, are those that inactivate cohesin STAG2. Importantly, STAG2 mutations are often present in the most aggressive EWS tumors. From the bioinformatic analysis of transcriptomic data from EWS patients and cell lines, we have identified a gene signature dependent on STAG2 mutation that correlates with poor survival. We are currently exploring the contribution of these genes to the metastatic phenotype of EWS cells and its potential use as a diagnostic tool.