Titre du stage (en français et en anglais):

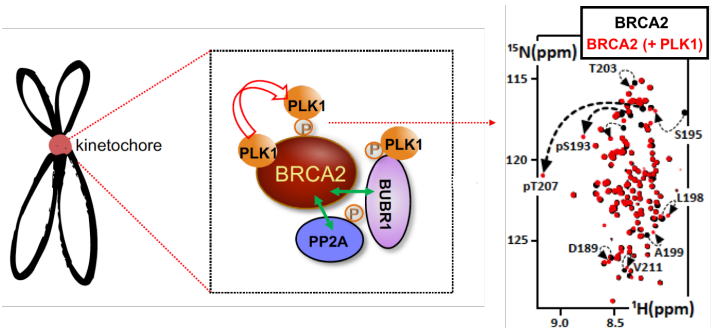
Régions intrinsèquement désordonnées de BRCA2 : phosphorylations et interactions

Intrinsically disordered regions in BRCA2: phosphorylations and interactions

Résumé du projet:

The BReast CAncer susceptibility 2 (BRCA2) protein is essential for DNA repair by homologous recombination (HR) during the S/G2 phases of the cell cycle: it interacts with the recombination proteins RAD51 and DMC1, thus facilitating proper HR in both mitotic and meiotic cells (Jensen et al., 2010; Martinez et al., 2016; Brandsma et al., 2019). In mitosis, it also binds to BUBR1, which is a component of the spindle assembly checkpoint. At the end of mitosis, it localizes to the midbody and facilitates cell division by serving as a scaffold protein for the central spindle components. Several mutations in the gene coding for BRCA2 cause genome instability and are associated with breast and ovarian cancers.

Despite the accumulated knowledge about BRCA2 functions, the molecular mechanisms associated to these functions are poorly described. This is in part due to the disordered character of BRCA2, which prevents the identification of well-folded regions with defined targets. Interactions involving disordered regions remain difficult to identify because the docking sites cannot be accurately predicted using bioinformatics tools. Yet, the lack of well-structured binding sites provides unique functional opportunities for BRCA2 to bind to a large set of partners in a tightly regulated manner. Within the 3418 aa of BRCA2, only one single globular domain has been identified, which binds to single-stranded DNA and the small disordered protein DSS1, as observed in the 3D structure of the domain bound to an oligo(dT)9 and DSS1 (Yang et al., 2002). Two small BRCA2 fragments with high predicted disorder propensity have been captured by X-ray crystallography when bound to PALB2 or RAD51 (Oliver et al., 2009; Pellegrini et al., 2002). Through these interactions, BRCA2 coordinates the activities of the recombination factors DSS1 and PALB2 to promote RAD51 loading onto ssDNA and activity during HR.



Our team has initiated the structural analysis of the interactions between several disordered and conserved regions of BRCA2 and their partners in mitosis (Julien et al., Bio Mol NMR Assign 2020; Alik et al., Angew Chem Int Ed Engl 2020). The aim is to identify the impact of BRCA2 variants detected in patients with breast and ovarian cancers (listed in the national UMD-BRCA2 database) on these interactions and the associated functional defects. We identified an interaction between BRCA2 and the kinase Plk1 that is regulated by phosphorylation (Ehlen et al., Nat Comm 2020). In collaboration with the team of Dr A. Carreira (Institut Curie, Orsay), the function of this interaction in mitosis was revealed, with important consequences on chromosome stability. The impact of several variants associated with breast cancer was described, which suggested an explanation for the aneuploidy observed in *BRCA2*-mutated tumors.

The Master 2 project will extend this first study, and focus on interactions of the disordered and conserved regions of BRCA2 with phosphatases in mitosis. Preliminary biophysical experiments were performed in the team validating an interaction between BRCA2 and the B56 subunit of the phosphatase PP2A. The PhD student will characterize its affinity, minimal interacting domains, regulation through phosphorylation, 3D structure at an atomic resolution, using a panel of biophysical (fluorescence, ITC, mass spectrometry) and structural biology (NMR, SAXS, X-ray crystallography) techniques. He/she will design mutations disrupting this interaction, which will be used by our collaborators to characterize the functional role of these interactions in cells. All the experiments will be carried out with the help of the protein expression, protein-protein interaction and structural biology platforms at I2BC, as well as through the team's regular access to the protein crystallography and SAXS beamlines of the synchrotron SOLEIL.

Recent references of the team of this topic:

1) Multiple Site-Specific Phosphorylation of IDPs Monitored by NMR.
Julien M, Bouguechtouli C, Alik A, Ghouil R, Zinn-Justin S, Theillet FX. Methods Mol Biol. 2020;2141:793-817.

2) Proper chromosome alignment depends on BRCA2 phosphorylation by PLK1.
Ehlén Å, Martin C, Miron S, Julien M, Theillet FX, Ropars V, Sessa G, Beaurepere R, Boucherit V, Duchambon P, El Marjou A, Zinn-Justin S, Carreira A. Nat Commun. 2020 Apr 14;11(1):1819.

3) ^{1}H, ^{13}C and ^{15}N backbone resonance assignment of the human BRCA2 N-terminal region.
Julien M, Miron S, Carreira A, Theillet FX, Zinn-Justin S. Biomol NMR Assign. 2020 Apr;14(1):79-85.

4) Combining Homologous Recombination and Phosphopeptide-binding Data to Predict the Impact of *BRCA1* BRCT Variants on Cancer Risk.
Petitalot A, Dardillac E, Jacquet E, Nhiri N, Guirouilh-Barbat J, Julien P, Bouazzaoui I, Bonte D, Feunteun J, Schnell JA, Lafitte P, Aude JC, Noguès C, Rouleau E, Lidereau R, Lopez BS, Zinn-Justin S, Caputo SM; UNICANCER Genetic Group BRCA network. Mol Cancer Res. 2019 Jan;17(1):54-69.