**Phospho-dependent assembly of**

**DNA double strand break repair machineries in mitosis**

Supervisor: Sophie Zinn-Justin, co-head of the IntGen team in the B3S department.

Website of the IntGen team: https://www.i2bc.paris-saclay.fr/nuclear-enveloppe-telomeres-and-dna-repair/

Contact: sophie.zinn@i2bc.paris-saclay.fr

To mitigate the threat of DNA double strand breaks (DSBs), human cells rely on the activity of multiple DNA repair machineries, that are tightly regulated throughout the cell cycle. Timely assembly of these machineries depends on phosphorylation of disordered and conserved motifs in DNA repair proteins. However, phospho-dependent interactions are still poorly characterized. In interphase, DSBs are mainly repaired by non-homologous end joining (NHEJ) and homologous recombination (HR). In mitosis, it was recently shown that the cell is also able to repair DSBs through a new pathway that involves polymerase Polθ. This polymerase is a new target for drugs against cancers. However, the mechanisms through which it contributes to DSB repair are yet unknown. The central region of Polθ is large and disordered; it is phosphorylated upon DNA damage. Our team, in collaboration with the team of R. Ceccaldi at Institut Curie, focuses on the characterization of phospho-dependent interactions of Polθ with its partners during DNA repair. The student will use real-time Nuclear Magnetic Resonance (NMR) coupled to Isothermal Titration Calorimetry (ITC) and X-ray crystallography in order to identify mitotic phosphorylation events in Polθ, and describe the associated binding events. More specifically, the student will analyze the NMR signals of Polθ disordered fragments, monitor their phosphorylation by NMR at the residue level, search for phospho-dependent partners together with our collaborators using affinity chromatography and mass spectrometry, reveal the residues involved in binding using NMR, quantify the affinity gain due to phosphorylation using ITC, and determine the 3D structure of the complexes using X-ray crystallography and/or cryo-EM in the case of large complexes. The general idea is to understand how disordered proteins are able to assemble complexes through interfaces composed of one or several low affinity contact sites, and how phosphorylation regulates these interactions, during the repair of highly toxic DSBs in mitosis. The student will benefit from the protein expression, protein-protein interaction, crystallography, NMR and cryo-EM facilities of the Institute for Integrative Biology of the Cell (I2BC), as well as from the beamline time dedicated to the IntGen team at the nearby Synchrotron SOLEIL.

Recent publications of the team on similar projects:

1. [BRCA2 binding through a cryptic repeated motif to HSF2BP oligomers does not impact meiotic recombination.](https://pubmed.ncbi.nlm.nih.gov/34326328/) Ghouil R, Miron S, Koornneef L, Veerman J, Paul MW, Le Du MH, Sleddens-Linkels E, van Rossum-Fikkert SE, van Loon Y, Felipe-Medina N, Pendas AM, Maas A, Essers J, Legrand P, Baarends WM, Kanaar R, Zinn-Justin S, Zelensky AN. Nat Commun. 2021 Jul 29;12(1):4605. doi: 10.1038/s41467-021-24871-6.
2. [Intrinsic Disorder and Phosphorylation in BRCA2 Facilitate Tight Regulation of Multiple Conserved Binding Events.](https://pubmed.ncbi.nlm.nih.gov/34356684/) Julien M, Ghouil R, Petitalot A, Caputo SM, Carreira A, Zinn-Justin S. Biomolecules. 2021 Jul 20;11(7):1060. doi: 10.3390/biom11071060.
3. [Di-phosphorylated BAF shows altered structural dynamics and binding to DNA, but interacts with its nuclear envelope partners.](https://pubmed.ncbi.nlm.nih.gov/33744941/) Marcelot A, Petitalot A, Ropars V, Le Du MH, Samson C, Dubois S, Hoffmann G, Miron S, Cuniasse P, Marquez JA, Thai R, Theillet FX, Zinn-Justin S. Nucleic Acids Res. 2021 Apr 19;49(7):3841-3855. doi: 10.1093/nar/gkab184.
4. [Proper chromosome alignment depends on BRCA2 phosphorylation by PLK1.](https://pubmed.ncbi.nlm.nih.gov/32286328/) 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. doi: 10.1038/s41467-020-15689-9.