« PROPOSITION DE STAGE ET/OU DE THESE »

Laboratoire : I2BC

Adresse : CEA Saclay

Directeur du laboratoire : F BOCCARD

Équipe de recherche (si pertinent) : INTGEN

Responsable de l'équipe : JB Charbonnier

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N° et intitulé de l'Ecole Doctorale de rattachement : ED569 Innovation Therapeutique

Profil recherché : Biophysique/Biochimie/Biologie Structurale

Possibilité de poursuite en thèse : OUI

Si oui financement envisagé : Ecole doctorale ED569, Ligue contre Cancer, FRM, CIFRE

Titre du stage : Molecular mechanisms of the main human double-strand break repair pathway : from cryoEM to characterization of potent inhibitors for Cancer therapies

Résumé : Our team at I2BC is interested in the networks of protein-protein interactions controlling the stability of the genome using an integrative structural biology approach. One of the focus of the team is the study of the molecular mechanisms taking place in the primary pathway for repairing double-strand breaks (DSB) in DNA in vertebrates, called Non-homologous end Joining (NHEJ). The heterodimer Ku70/Ku80 recognizes DBS rapidly and coordinates the recruitment of the different activities of this NHEJ repair pathway (ligation, ends processing, and polymerization). The DSBs are deleterious lesions for cell homeostasis. If they are not repaired or incorrectly repaired, they can induce cell death or chromosomal rearrangements. Our team study the interactions between Ku70/Ku80 and several DNA substrates representing the different types of DNA ends that exist in the cell when a DSB is formed by exogenous or endogenous stress. We also analyse the recruitment by Ku with its NHEJ partners. Our aims are to solve the three-dimensional structure of major complexes formed by Ku using crystallography or cryoEM, and to quantify these interactions by biophysical methods in tight collaborations with NHEJ biologists.
The M2 student will perform the expression in insect cells or in E coli of the NHEJ proteins and their purification. The candidate will characterize the different recombinant NHEJ pathway proteins, alone or in complex with Ku70/Ku80 and DNA. He / she will perform the structural analyses by combining Xray crystallography and cryoEM. He / she will work in the preparative biochemistry laboratory for the purification of these proteins, and for the preparation of crystallisation screening, then for the freezing of the crystals during the collection campaigns before the analyses by X-ray diffraction performed at Synchrotron SOLEIL. The candidate will analyse by cryoEM the Ku complexes when possible. He / she will also quantify the different interactions using several biophysical approaches ITC, MST and swithSENSE approaches. All these complementary methodologies are available in the laboratory and will allow us to characterize the thermodynamic and kinetic properties of these interactions as well as to assess in a second step the impact of DNA modifications in the recognition of DSB by Ku70/80.

References:

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Ropars V, Drevet P, Legrand P, Baconnais S, Amram J, Faure G, Márquez JA, Piétrement O, Guerois R, Callebaut I, Le Cam E, Revy P, de Villartay JP, Charbonnier JB. Structural characterization of filaments formed by human Xrcc4-Cernunnos/XLF complex involved in nonhomologous DNA end-joining. **Proc Natl Acad Sci U S A**. 2011 Aug 2;108(31):12663-8. doi: 10.1073/pnas.1100758108. Epub 2011 Jul 18. PMID: 21768349; PMCID: PMC3150875.