STRUCTURAL AND FUNCTIONAL STUDY OF THE COMPLEX FORMED BY THE WERNER PROTEIN (WRN) AND THE KU70/KU80 HETERODIMER

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Chemotherapy or radiobiology treatments aim at generating DNA double-strand breaks (DSBs). Tumor cells are more sensitive to DSBs than healthy cells due their phenotype and genotype[1]. One important axis in radiobiology is to combine radiation therapy with inhibitors of the DNA repair pathways to increase radio-sensitivity and overcome radiation resistance of some cancer cells[2]. Our objectives is to unveil the molecular mechanism of the NHEJ (Non Homologous End Joining) pathway and to characterize new specific inhibitors of this pathway. Ku70/Ku80 heterodimer (Ku) is a central player of the NHEJ for DSB recognition and in downstream DNA events (processing and ligation steps). Our team showed that Ku can recruit several enzymes of the NHEJ pathway through direct interactions and thus acts as a hub that coordinates the whole NHEJ[3][4][5]. Many interactions involve motifs called KBM (Ku Binding Motif). One of the enzymes is Werner (WRN) that has two KBM motifs. This protein is part of the helicase RecQ family. It is the only member of this family to have in addition to its helicase domain, an exonuclease domain. It is particularly studied in the case of Werner Syndrome, a rare autosomal-recessive inherited disease, characterised by an early aging[6]. It was also identified as a promising drug target for frequent cancers presenting local DNA instability[7], but little is known about its role in the NHEJ pathway. During my PhD, I successfully produced several WRN constructs and full-length protein and studied them by combining multiple structural approaches. I showed that WRN can adopt several oligomeric state using SAXS and SEC-MALS, and that WRN interacts directly with Ku through two peptidic motifs, located in N-terminal and C-terminal extremity. I determined the CryoEM structure of a Ku-DNA complex bound to the exonuclease domain of WRN at 3Å of resolution. We showed that Ku recruits WRN through its KBM motif and position the DNA in the exonuclease site of WRN. Our collaborators analysed by life cell imaging and enzymatic assays the Ku-WRN interaction and observed a stimulatory effect of Ku on WRN function in good agreement with our data.

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Multiprotein assemblies of DNA DSBs repair pathway