# The role of P97 segregase in the repair of DNA-protein crosslink in vivo using CRISPR/Cas9 gene editing in zebrafish

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DNA-Protein-Crosslinks (DPCs) are DNA lesions which occur when proteins become irreversibly covalently bound to DNA, thereby physically blocking all DNA transactions including chromatin remodeling, replication, transcription and repair. DPC repair (DPCR) is a specialized DNA damage repair pathway in which crosslinked proteins are proteolytically or nucleolytically cleaved. Recently, the specific roles of several enzymes in DPCR have been uncovered, and their dysfunction has been linked to ageing, cancer and neurodegenerative diseases. To quantify DPCs, we are developing a new method using stably transfected HeLa cells expressing a GFP-tagged Histone which can form model DPCs upon e.g. formaldehyde treatment. The presence of GFP in particular cellular fractions, which depends on the presence and activity of DPCR enzymes, can then be analyzed, either by fluorescence or by western blot. Once the method is established in cell culture, we aim to develop an *in vivo* assay in zebrafish. Currently, we are creating zebrafish mutants for DPCR factors using Cripsr/Cas9 gene editing with the aim of studying the function of target proteins in DPCR *in vivo* during embryonic development and in adults. In particular, we are generating a zebrafish mutant line by introducing the K524A mutation in the *p97* gene using the CRISPR/Cas9 *knock-in* method to elucidate the role of the segregase P97 specifically in DPCR. We will investigate the epistasis of P97 with Sprtn, which is a central component of DPCR and for which we are generating equivalent mutant zebrafish lines. Taken together, our results will unravel the role of P97 during the orchestration of DPC repair *in vivo*.