Biotin tagging knock-in approach for decoding NER in development and disease

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DNA damage, caused by endogenous and exogenous sources, interferes with DNA replication and transcription machinery. To preserve genomic integrity, cells have evolved multiple repair mechanisms which counteract the adverse consequences of DNA lesions and prevent their transmission to daughter cells, but also maintain the transcriptional status of the genome. Nucleotide Excision Repair pathway, a principal repair mechanism to this process, is able to repair both transcription-blocking lesions and distortions throughout the genome that might interfere with replication. Several NER deficient knock-out mouse models exist, showing either pronounced cancer predisposition or a more complex phenotype, like growth failure, cachexia, neuronal abnormalities but not cancer (e.g $Ercc1^{-/-}$, $Xpf^{-/-}$, $Xpg^{-/-}$, $Xpa^{-/-}/Csb^{m/m}$ mice). ERCC1-XPF, an essential component of NER pathway, forms an obligate heterodimer as a structure specific endonuclease to make the incision at the 5' end of damaged DNA strand, together with XPG that functions at the 3' end. Nonetheless, NER factors role in DNA repair cannot fully explain the phenotypic heterogeneity observed in the respective deficient mouse models. To address this issue, we generated a biotin-tagged XPF knock-in mouse that allows the identification of proteomic components associated with XPF *in vivo* by recovering proteins from streptavidin-bound nuclear extracts derived from tissues/cells, followed by mass spectrometry. We targeted the C-terminus of the endogenous Xpf gene and added a Flag-Tev-Avidin tag that is specifically recognized and biotinylated by a birA biotin ligase transgenic mouse. *In* vivo, birA ligase efficiently biotinylates tagged XPF in several embryonic and adult tissues, allowing us to understand the role of XPF in processes outside NER during mouse development and disease.